9 Regulation of Neuroactive Metals by the Choroid Plexus

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CONTENTS

9.1 INTRODUCTION

The choroid plexus plays a pivotal role in maintaining the homeostasis of essential metal ions in the central nervous system (CNS), which embraces the cerebrospinal fluid (CSF) compartment, the interstitial fluid (ISF) or extracellular fluid compartment, and the intracellular compartment. The choroid plexus, where the blood–CSF barrier is located, separates the CSF compartment from the systemic blood compartment. Research in the last several decades has revealed that at least 11 metals—lead (Pb), mercury (Hg), cadmium (Cd), manganese (Mn), arsenic (As), iron (Fe), copper (Cu), zinc (Zn), silver (Ag), gold (Au), and tellurium (Te)—accumulate in the choroid plexus (Zheng, 2001a, 2002), making the tissue a major target in brain for toxicities associated with environmental exposure to heavy metals.

Metals acting on the choroid plexus can be categorized into three major groups. The first group of metals directly damages the choroid plexus structure, such as Pb, Hg, and Cd—the name-direct choroid plexus toxicants. Metals in the second group can impair specific plexus regulatory pathways that are critical to brain development and function, but they do not necessarily induce massive pathological alteration as do the metals in the first group. Thus, metals in the second group are called selective choroid plexus toxicants. Typical examples include Mn, Cu, and aluminum (Al). The third group of metals can be sequestered in the choroid plexus. Sequestration may represent an essential defense mechanism for the barrier system to protect against insults from the blood circulation. Metals in this group, including Zn, Fe, Ag, and Au, are thus called sequestered choroid plexus toxicants.

Trace metals in the CNS compartment are essential to brain development and function. For example, Zn, Cu, and Mn are required for optimal CNS function. They play important roles as catalysts, second messengers, and gene expression regulators. Being essential cofactors for functional expressions of many proteins, these elements are needed to activate and stabilize enzymes, such as superoxide dismutase (SOD), metalloproteases, protein kinases, and transcriptional factors containing zinc finger proteins. Consequently, the concentrations of these metal ions in the CNS compartment must be maintained at an optimal level, for both deficiency and excess can result in aberrant CNS function.

Other metals, such as Pb, Hg, and Al, have no known beneficial utility to the brain. The presence of these metals at even a low level causes defects in brain development and function or even degeneration. Thus, preventing their entry into the CNS compartment at the brain barriers is fundamental to the chemical stability of the CNS.

This chapter will review the current understanding of the role of choroid plexus in metal-associated neurotoxicity. The anatomical location in the brain and the structural characteristics of the choroid plexus, which render the tissue vulnerable to metal insults, will be briefly discussed. Since extensive reviews on the topic of metals in the choroid plexus have been done previously (Zheng, 1996, 2001a,b; Zheng et al., 2003), this chapter will focus exclusively on three neuroactive metals (i.e., Cu, Zn, and Al), their CNS homeostasis, their neurotoxicities, and the possible relationship to the choroid plexus. Finally, the implications of the choroid plexus in neurotoxicology and future research needs concerning the toxicology of the choroid plexus are discussed.

9.2 VULNERABILITY OF THE CHOROID PLEXUS TO METAL INSULTS

As a barrier between the blood and CSF, several unique anatomical and physiological features of the choroid plexus render the tissue more vulnerable to insults from the circulating bloodstream or from the CSF. As discussed earlier in this volume, the choroid plexus occupies all the brain ventricles. The large surface area of the choroidal microvilli increases the chance of this tissue being exposed to toxic metals from either side of the barrier.

The leaky endothelial layer in the choroid plexus allows metals to readily gain access to the choroidal epithelial cells. A faster blood flow at the choroid plexus than elsewhere in the brain brings toxic metals in the blood circulation to the choroid plexus.

The tight junctions between the epithelial cells in the blood–CSF barrier are less tightly connected than those between endothelial cells of the blood–brain barrier. The looseness of the choroidal barrier not only may permit metals to leak into the CSF, but it may also provide a pathway for metals present in the CSF to enter the choroid plexus, serving as a cleansing mechanism in the CNS.

It is unclear if the location of the choroid plexus within the cerebral ventricles is an important factor in metal-induced neurotoxicities. For example, the choroid plexus in the lateral ventricle is adjacent to hippocampal formation and other neuronal structures. Toxic Pb is known to accumulate in the choroid plexus and have access to the hippocampus. Whether or not there is a connection between these two seemingly separate events is unknown.

Because of these anatomical and physiological characteristics, the choroid plexus is a vital functional compartment that readily accumulates toxic metals in the brain.

Cu and Zn, under normal physiological conditions, are present in the serum and CSF. The concentrations of Cu and Zn in the CSF are about 30- and 80-fold less than those in the serum, respectively (see Table 9.1). Both metals are known to accumulate in the choroid plexus. Normal human brains contain Al at a concentration

TABLE 9.1
Concentration of Copper, Zinc, and Aluminum in Serum, Brain Tissue, CSF, and Choroid Plexus of Normal Human Subjects

Metals	Serum	Brain	CSF	Choroid Plexus
Cu	1060 μg/L (1)	0.1–0.5 mM (2)	33.9 ± 2.88 μg/L (3)	0.70 μg/g (1)
Zn	897 ± 110.5 μg/L (4)	0.1–0.5 mM (2)	10.4 ± 2.60 μg/L (4)	39.0 μg/g (1)
	975 μg/L (&rat) (5)		7 ± 5.9 μg/L (6)	
	920 μg/L (1)			
	850 ~1100 μg/L (7)			
Free Zn:	$6.5 \times 10^{-6} \sim 10^{-5}$ μg/L (5)			
Al	<10 μg/L) (8)	0.1–0.5 mM (2)	< 270 μg/L (8)	Unknown
		0.017 mM (8)		

Source: (1): Reviewed in Zheng, 1996; (2): Lovell et al., 1998; Atwood et al., 1999; (3): Burhanoglu et al., 1996.; (4): Palm and Hallmans, 1982.; (5): Bradbury, 1992; (6): el-Yazigi et al., 1986; (7): Goyer, 1998; (8): Markesbery et al., 1984; Yokel, 2002.

of 2 μg/g of tissue weight. In one Al intoxication case, the Al concentration in the cortex and subcortex reached as high as 9.3 μg/g along with a significant accumulation in the choroid plexus (Reusche et al., 2001). While it is postulated that Al may enter CSF to gain access to the brain parenchyma, the exact concentration of Al in the CSF remains uncertain.

Table 9.2 summarizes the binding species for Cu, Zn, and Al in the blood and in the CSF compartment. All three metals are bound to large molecular weight proteins in plasma, which represent the predominant forms of three metals in blood circulation. In the CSF, however, the metals are bound to ligands of much smaller molecular weight, such as histidine, citrate, or metallothionein.

9.3 COPPER (CU)

9.3.1 Cu in Brain Function and Dysfunction

Cu is an essential trace metal element in all living organisms. Inadequate or excessive intake of Cu can be pathogenic and life-threatening. Cu serves as a cofactor for a variety of proteins, including more than 30 enzymes involved in biological reactions, such as photosynthesis and respiration, free radical eradication, connective tissue formation, iron metabolism, and so on. In mammals the balance between Cu supply and consumption is maintained at both the cellular and tissue levels. Genetic factors that affect systemic levels of Cu, either excess or deficiency, cause clinically well-defined syndromes.

TABLE 9.2
Binding Species/Ligands for Copper, Zinc, and Aluminum in Plasma, Brain ISF, and CSF

Metals	Plasma	Brain ISF/CSF
Cu	Exchangeable:	Cu-histidine
	Cu-albumin (88%)	
	Cu-transferrin (11%)	
	Cu-histidine and cysteine (1%)	
	Nonexchangeable:	
	Cu-ceruloplasmin (15 ~ 20%) (1)	
Zn	Exchangeable:	
	Zn-albumin (70%)	Zn proteins/metallothioneins (2)
	Zn-histidine and cysteine (3)	
	Unknown:	
	Zn-α_2-macroglobulin (50%) (1)	
	Zn-transferrin	
Al (4)	Al-transferrin (81 ~ 91%)	Al-citrate (90%)
	Al-citrate (7 ~ 20%)	Al-transferrin (4%)
		Hydroxide (free) (5%)

The numbers represent the percentage of metals predicted to be associated with that ligand.

Source: (1): Bradbury, 1992; (2): Frederikson, 1989; St Croix et al., 2002; (3): Harris and Keen, 1989; (4): Öhman and Martin, 1994; Yokel, 2002.

9.3.1.1 Wilson's Disease

Wilson's disease is a genetic disorder that affects one in 50,000 to 100,000 people worldwide. A mutation in a Cu-transport ATPase gene, which is located on the long arm (q) of chromosome 13 (13q14.3), causes an overload of Cu in the body, initially mainly in the liver. When the capacity of the liver to handle Cu is exceeded, the metal is then released from the liver and begins to accumulate in other organs of the body, particularly the brain, eyes, and kidneys. In essence, Wilson's disease is the dysfunction of Cu transport in the body.

The build-up of Cu in the brain causes neurological symptoms, including tremor of the head, arms, or legs; generalized, impaired muscle tone and sustained muscle contractions that produce abnormal postures, twisting, and repetitive movements (dystonia); and slowness of movements (bradykinesia), particularly those of the tongue, lips, and jaw. Patients may also experience clumsiness, difficulty with balance, and impaired coordination of voluntary movements, such as walking (ataxia), or slowness of finger movements and loss of fine motor skills (Kodama, 1996).

Wilson's disease may be associated with a damaged blood–brain barrier. In one clinical study with four patients who had cerebral manifestation of Wilson's disease, the ratio of albumin concentrations in the CSF and serum was used as the indicator of barrier intactness in addition to other parameters such as Cu concentrations in the CSF and serum. The investigators reported that in all cases there was an increase

in the albumin ratio, suggesting a disturbed blood–brain barrier. All patients showed an initial worsening of the neurological condition, which corresponded to a rise in the albumin ratio. The maximal rise in albumin ratio was reached after about seven months. The ratio declined and finally returned to the normal level during and after therapeutic treatment (Stuerenburg, 2000). These authors conclude that blood–brain barrier permeability may play a role in the early stages of disease development. The normalization of the CSF Cu concentration in patients is a slow process, even if the therapy is sufficient (Stuerenburg, 2000).

9.3.1.2 Menkes' Disease

Menkes' disease and a related disorder, occipital horn syndrome (OHS), result from a significant Cu deficiency due to a failure to transport Cu across membranes of intestinal enterocytes and across cerebrovascular cells of the blood–brain barrier. Deprivation of Cu directly affects the activity of intracellular cuproenzymes, such as cytochrome c oxidase, CuZnSOD, lysyl oxidase, tyrosinase, ascorbic acid oxidase, ceruloplasmin, and dopamine β-hydroxylase (Harris, 2003). The Menkes gene is located on the long arm of the X chromosome at Xq13.3; the gene product is a 1500-amino-acid P-type adenosine triphosphatase (ATPase), which has 17 domains: six Cu binding, eight transmembrane, one phosphatase, one phosphorylation, and one ATP binding. The predominant sites of Menkes gene expression are the placenta, gastrointestinal tract, and blood–brain barrier. As the gene is an X-linked gene, the disease primarily affects male infants.

Patients with Menkes' disease, primarily infants, have abnormally low levels of Cu in the liver and brain, but higher than normal levels in the kidney and intestinal lining. Affected infants may be born prematurely. Symptoms appear during infancy, including seizures, psychomotor deterioration, failure to thrive, temperature instability (hypothermia), and strikingly peculiar hair. There can be extensive neurodegeneration in the gray matter of the brain. Arteries in the brain can also be twisted with frayed and split inner walls. This can lead to rupture or blockage of the arteries (Dexter et al., 1991).

The clinical manifestations of Menkes' disease are due to a lack of Cu in certain regions; this could, in turn, be due to a deficient transport of Cu by the blood–brain barrier. By using reverse transcription-polymerase chain reaction (RT-PCR), it has been shown that cerebrovascular endothelial cells that comprise the blood–brain barrier express the gene for the Cu-ATPase. Functional analysis using an ATP7A inhibitor also reveals that Cu efflux can be blocked by p-chloromercuribenzoate (p-CMB), a potent inhibitor of ATP7A (Qian et al., 1998). Thus, these results provide strong evidence that a Cu-ATPase is present at the blood–brain barrier, and the transport of Cu at the barrier may control the entrance of Cu into brain parenchyma. Understandably, a genetic disorder in the expression of the Cu-ATPase transporter in cerebrovascular endothelial cells would lead to a low brain Cu level in Menkes' disease. However, whether the mutation of the Menkes gene in the choroid plexus contributes to the progress of the disease is unknown.

9.3.1.3 Alzheimer's Disease

Alzheimer's disease (AD) is characterized by the chronic deposition of β-amyloid peptides (Aβ) in senile plaques and hyperphosphorylated Tau protein in neurofibrillary tangles. Essential metals such as Cu, Zn, and Fe accumulate in Aβ deposits in the cortex along with sugar-derived glycation end products (Waggoner et al., 1999). Aβ peptide is generated from amyloid precursor protein (APP) by the proteolytic activity of Aβ- and γ-secretase (Checler, 1995). The presence of binding sites for Cu and Zn on the precursor Aβ partly explains the enrichment of these metals in the plaques (Atwood et al., 2000).

The levels of Cu in the CSF and serum, as well as in brain tissues in Alzheimer's patients are rather inconsistent and sometimes controversial (Basun et al., 1991; Cuajungco and Lees, 1997; Cuajungco and Fagét, 2003). Some investigators report a significant decrease of brain Cu concentrations in AD patients (Deibel et al., 1996), while others present a 2-fold increase of Cu levels in the CSF (Basun et al., 1991), serum (Gonzalez et al., 1999), and amyloid plaque rim (Lovell et al., 1998), as well as an increase in brain and CSF ceruloplasmin levels. The latter is a known Cu-binding/transporting protein (Loeffler et al., 1996) that is synthesized by the choroid plexus. This discrepancy may result from the differences in analytical approaches, technical variations during tissue sampling and processing, and the limited sample sizes.

$A\beta_{1-40}$ has two binding sites for Cu: the higher affinity site of log Kapp 10 and lower affinity site of log Kapp 7.0. But the binding affinity of Cu to $A\beta_{1-42}$ is much greater than that to $A\beta_{1-40}$ (Atwood et al., 2000). Binding of Cu to Aβ can lead to an augmented oxidation of Aβ (Huang et al., 1999) and accelerate the formation of covalently crosslinked glycation end products (Loske et al., 2000).

Cu can also directly bind to amyloid precursor protein (APP). Specific and saturable binding sites for copper (APP 135–155; $K_D = 10$ nM) have been identified within the cysteine-rich region of the APP-695 ectodomain (Hesse et al., 1994). While Zn binding to APP is believed to perform a structural role, binding of Cu to APP reduces Cu(II) to Cu(I), which, in turn, results in an oxidation of Cys144 and Cys155. The formation of ensuing intramolecular disulfide bridge renders the APP-Cu(I) complex more prone to redox reactions, leading to a random APP fragmentation (Multhaup et al., 1996).

The choroid plexus contains a high amount of Cu. The extensive precipitation of amyloid plaques in the AD choroid plexus (Miklossy et al., 1999) may be associated with Cu accumulation in this tissue.

9.3.1.4 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of motor neurons in the CNS. While the cause remains unsolved, a connection of the ALS to Cu has been established through CuZnSOD. A clinical study has observed a dominant mutation in the copper/zinc superoxide dismutase (SOD1) gene on chromosome 21 in 15 to 20% of familial ALS (FALS) cases (Reinholz et al., 1999). In brains of ALS patients, most neurons stained weakly, or not at all, with both anti-Cu/Zn- and

Mn-SOD antibodies, whereas the pia mater and the epithelial cells of choroid plexus stained intensely (Uchino et al., 1994).

In another study with a transgenic ALS mouse model having a point mutation in the gene encoding Cu/Zn SOD, continuous subcutaneous administration of polyamine-modified catalase, which increases the permeability of the blood–brain barrier, delays the onset of symptoms, and increases animal survival rate, suggested a possible role of brain barriers in ALS disease (Poduslo et al., 2000).

9.3.1.5 Prion Disease and Other Brain Disorders

Prion disease has been linked to an abnormal form of the prion protein (PrP) in neurons. PrP molecules are believed to bind Cu at multiple sites along the peptide chain (Hornshaw et al., 1995), serving to stabilize the PrP. Excess brain Mn, due to rising environmental levels of Mn, may replace Cu and causes malfunction of PrP, which clinically manifests itself as Creutzfeldt-Jakob disease. Current evidence has shown that both the blood–brain barrier and the blood–CSF barrier are target sites for Mn toxicity, at least in cases of Fe-associated neurotoxicities. However, whether or not overexposure to Mn can lead to an altered Cu transport at brain barriers is unknown and deserves further investigation.

Cu may be involved in congenital hydrocephalus. Mori and colleagues (1993) report that the amount of Cu/Zn-SOD in the brain of a congenitally hydrocephalic rat model is less than in the control, especially in the choroid plexus. The authors postulate that a congenitally reduced SOD activity may impair the function of choroid plexus as a result of increased oxygen species in the plexus tissue. A combination of reduced SOD in the choroid plexus, hippocampus, ependymal cells of ventricles, and aqueduct may promote the development of hydrocephalus.

9.3.2 Transport of Cu by Brain Barriers

Under normal physiological conditions, brain barriers are impermeable to Cu. Movement of Cu across brain barriers between two fluid compartments requires specific Cu transport system(s). However, under certain pathological conditions, where the barrier's permeability is increased, Cu may enter the CSF via passive diffusion. For example, in children with meningitis, the elevation of CSF Cu levels is associated with an elevated protein level in the CSF, suggesting that the high CSF Cu is a result of the breakdown of the blood–brain barrier and subsequent leakage of the trace element along with proteins from serum to the CSF (Burhanoglu et al., 1996).

The levels of CSF-Tau protein also correlate significantly with the serum levels of Cu in Alzheimer's disease patients treated with clioquinol; the latter is a chelator that crosses the blood–brain barrier and has greater affinity for Zn and Cu ions than for Ca and Mg ions (Regland et al., 2001). Again, the damaged blood–brain barrier may permit the passage of the trace elements, including Cu, into the subarachnoid space (Kapaki et al., 1989).

Cu transport at brain barriers is achieved by a coordinate series of interactions between passive and active transport proteins. There are several membrane Cu

TABLE 9.3
Possible Transporters Involved in the Influx and Efflux of Copper, Zinc, and Aluminum at Brain Barriers

Metal	Fluxes	Metal Transporter	Present at BBB	Present at BCB
Cu	Influx	Ctr1 (1)	Unknown	Yes (2)
		Nramp2//DMT1/DCT1(3) (energy-independent)	Yes (4)	Yes (4)
		ATP7A	Yes (5)	Yes (2)
		ATP7B (6)	Yes	Unknown
	Efflux	MTP1 (2)	Yes	Yes (7)
Zn		PHT1[a]	Unknown	Yes (8)
		DMT1[b]	Yes (4)	Yes (9)
		ZIP (ZRT1. IRT1-like protein) (10)	Unknown[e]	
		ZnT-1 through ZnT-4 (11)	Unknown[e]	
Al	Influx (15)	TfR-ME (3)[c]	Yes (12)	Yes (13)
		MCT1[d]	Yes	Yes (14)
		MCT7	Unknown	Unknown
		MCT8	Unknown	Unknown
	Efflux (16)	MCT	Yes	Yes (14)
		Oatp	Yes	Yes (17)

[a]peptide/histidine transporter; [b]divalent metal transporter; [c]TfR-ME: transferring-receptor-mediated endocytosis; [d]MCT: monocarboxylate transporter—Na independent, pH independent, and energy dependent, but not dependent on transporter Na/K-ATPase; [e]hZIP-1 mRNA and hZnT-1 mRNA present in prostate epithelial cell line LnCap, PC3, and CRL2220; hZnT-4 present in LnCap (Beck et al., 2004).

Source: (1): Dancis et al., 1994; (2): Nishihara et al., 1998; (3): Rolfs and Hediger, 1999; (4): Burdo et al., 2001; (5): Qian et al., 1998; (6): Hamza, 1999; (7): Wu et al., 2004; (8): Yamashita et al., 1997; (9): Burdo et al., 2001; Siddappa et al., 2002 (10): Grotz et al., 1998; Gaither and Eide, 2000; (11): Ebadi et al., 1995; (12): Roskams and Connor, 1990; (13): Zheng, 2002; (14): Leino et al., 1999; (15): Yokel et al., 2002; (16): Ackley and Yokel, 1997, 1998; Koehler-Stec et al., 1998; (17): Choudhuri et al., 2003.

transporters present in the brain barriers, including copper transporter 1 (Ctr1), divalent metal transporter 1 (DMT1), and Cu transport ATPases (see Table 9.3). In addition, Cu is transported by certain vesicles and soluble peptides such as chaperones (Harris, 2001).

9.3.2.1 Copper Transporter 1

The yeast *Ctr1* gene was the first eukaryotic gene discovered that codes for a Cu transport protein (Dancis et al., 1994). A Cu transport gene in human cells (*hCtr1*) can transport Cu as Cu(I) in a reaction that does not require the energy of ATP, but is stimulated by K ions and an acidic pH (Lee et al., 2002). Human fibroblasts transfected with *hCtrl* cDNA favorably take up Cu (Moller et al., 2000). By inactivating

the *Ctr1* gene in mice via targeted mutagenesis, Kuo and his colleagues (2001) also provide the evidence that *Ctr1* is essential for embryonic growth and development and is required for Cu transport into the brain. *Ctr1* expression is abundant in a variety of epithelial-derived tissues, including the choroid plexus (Nishihara et al., 1998).

9.3.2.2 Divalent Metal Transporter 1

DMT1 (Slc11a2), also known as Nramp2 (natural resistance-associated microphage protein) and DCT1 (divalent cation transporter), was originally identified as the transporter responsible for intestinal nonheme iron apical uptake and trafficking and also believed to mediate influx of Cu via an energy-independent mechanism (Rolfs and Hediger,1999; Arredondo et al., 2003). Yet its role in Cu uptake is probably less specific (Harris, 2003; Garrick et al., 2003).

Using the branched DNA signal amplification method, Choudhuri et al. (2003) demonstrated that DMT1 mRNA was expressed in the choroid plexus at a higher level than in liver, kidney, and ileum of rats. During the perinatal period, DMT1 is expressed in the choroid plexus epithelial cells, the cerebral blood vessels, and ependyma in developing rat brain (Burdo et al., 2001; Siddappa et al., 2002). However, Moos and Morgan (2004) recently failed to detect DMT1 in brain capillary endothelial cells, although they identified DMT1 in neurons and choroid plexus.

9.3.2.3 ATPases Involved in Cu Transport

Two diseases, Menkes' syndrome and Wilson's disease, have led to the discovery of the Cu-transport ATPases in humans. ATP7A (coded by 8.5 kb mRNA) and ATP7B (coded by 7.5 kb mRNA), belonging to a subclass of ATPases, conduct ATP-dependent transport of Cu across brain barriers in mammals (Solioz and Vulpe, 1996). ATP7A is a component of Cu efflux from brain endothelial cells. A shutdown of ATP7a gene expression can result in Cu accumulation in brain capillaries of brindled and macular mutant mice (Yoshimura et al., 1995), suggesting that a dysfunction in Cu transport from blood to brain via the blood–brain barrier may contribute to the etiology of Menkes' disease. Qian et al. (1998) also provide further evidence to support the view that a lesion to the Cu-ATPase at brain barriers may be the primary cause of low brain Cu levels in Menkes' disease. In Wilson's disease, the overload of Cu in brain may be associated with the mutated ATP7b in the blood–brain barrier (Stuerenburg, 2000).

ATP7A is highly expressed in the choroid plexus (Nishihara et al., 1998); whether ATP7B is also present in the choroid plexus remains unknown.

9.3.2.4 Export via Vesicles

The movement of Cu within the cells through the cytosol is controlled by Golgi-derived vesicles and soluble polypeptides called chaperones. Vesicles containing embedded Cu-ATPase protein are believed to pinch off from the trans-Golgi membrane and move to the outer membranes (Voskoboinik et al., 1998). The movement is seen as an emergency response to a toxic threat and is consistent with cells in the

act of releasing Cu. The intracellular vesicle movement is also viewed as a necessary event in transporting Cu across the blood–brain barrier, but the event has not been identified in the blood–CSF barrier. The mobility of vesicles depends upon the availability of heavy metal binding (Hmb) domains in the protein (Harris, 2003).

9.3.2.5 Chaperones

The Cu chaperones are small Cu-binding peptides. As part of a larger family of metallochaperones, they are structurally adapted to bind Cu, recognize recipients, and conduct facile exchange by ligand exchange reaction with target proteins. Thus, chaperones typically have the Cu-binding motif that is found in the N-terminus of ATP7A and ATP7B (Larin et al., 1999). To date, at least four chaperones are known to perform Cu transport functions in eukaryotes. The chaperone ATOX1 (or HAH1/ATX1) is capable of transporting Cu to membrane-bound CuATPases. The transfer of cytosolic Cu to the mitochondria is a function of a small peptide called COX (cytochrome oxidase enzyme complex) (Glerum et al., 1996). CCS (copper chaperone for superoxide dismutase)/LYS7 is the chaperone required to insert Cu into the Cu_2Zn_2 superoxide dismutase (CuZnSOD) in mammals (Culotta et al., 1997; Harris, 2003). Besides these target-specific chaperones, glutathione (GSH) is a general Cu mobilizer and a nonspecific Cu transport factor.

By in situ hybridization, Ctr1, ATX1, and ATP7A have been found to have a high expression highly in the choroid plexus (Nishihara et al., 1998). Immunostaining for COX shows a remarkable signal in choroidal epithelial cells (Taskinalp et al., 2000). In addition, staining for cytochrome oxidase activity displays an intense staining in the mitochondria of choroidal epithelium (Kim et al., 1990). Thus, it seems reasonable that the choroid plexus, by adapting the Cu intracellular trafficking from uptake to export, may participate in the regulation of Cu in the CSF.

9.3.2.6 Metallothionein in the Choroid Plexus

Metallothionein (MT) is abundantly expressed in the choroid plexus, much more so than in the rest of brain (Nishimura et al., 1992). MT is a low molecular weight protein inducible by metals such as Cu, Cd, and Zn. MT functions to regulate Cu and Zn homeostasis and to sequester metals so as to reduce the cytotoxicity induced by metals. A study on a Cu-poisoned sheep showed that elevated brain Cu is associated with increased MT immunoreactivity in astrocytes, pia mater, choroid plexus, and ependyma. The authors suggest that these sites may sequester Cu and possibly modulate CNS Cu homeostasis (Dincer et al., 1999).

Cu as Cu(I) may enter the cells through the Ctr1 transporter and distribute intracellularly to enzymes (CuZnSOD), storage proteins (metallothionein), or organelles (mitochondria) via GSH and Cu chaperones (CCS, COX, and ATOX1). Efflux from nonhepatic cells is via the trans-Golgi network, which uses Cu-ATPase ATP7A and vesicles that cycle between the trans-Golgi and the plasma membrane (Harris, 2003). Since the genes for many Cu regulatory proteins are intensely expressed in the choroid plexus (Iwase et al., 1996; Murata et al., 1997), this tissue may serve as an important Cu port for the brain (Murata et al., 1997; Nishihara et al., 1998).

9.3.3 Toxicity of Cu at Brain Barriers

Based on the study of Cu and angiogenesis, some researchers have proposed that Cu may selectively target endothelial cells during the developmental period (Harris, 2003). While the mechanism is not completely understood, Cu appears to have the capacity to mobilize endothelial cells during the development of brain blood vessels. A 48-hour exposure to 500 μM Cu in serum-free medium nearly doubled the number of human endothelial cells derived from umbilical artery and vein, but it had no effect on the growth of dermal fibroblasts or arterial smooth muscle cells (Hu, 1998). In addition, Cu appears to induce the synthesis of vascular endothelial growth factor, thereby promoting wound healing through an angiogenesis process in the wound area (Sen et al., 2002). Damage by Cu to the brain barrier system, particularly the blood–CSF barrier, is not well documented.

Cu itself is not a barrier destroyer, but the presence of Cu may alter the transport of Fe. This was seen in rats fed a Cu-containing diet, where the influx of Fe into brain was significantly decreased compared to that of rats fed a control diet (Crowe and Morgan, 1996).

Exposure to other metals may interfere with Cu transport by brain barriers. Qian et al. (1999) demonstrated that Pb accumulation in C6 rat glioma cells altered the membrane transport properties for Cu, leading to an increased uptake and a decreased efflux of Cu. However, in another study on rats exposed to Pb, Cd, or combination of these two metals, no changes in brain Cu levels were observed (Skoczynska et al., 1994). The interaction of Cu and Pb in the choroid plexus has not been explored.

9.4 ZINC (Zn)

Zn, second only to Fe, is one of the most needed essential elements in the human body (Vallee and Falchuk, 1993; Choi and Koh, 1998). The normal plasma Zn level ranges from 85 to 110 μg/dL (Goyer, 1998). Zn in blood binds primarily to albumin, constituting the largest component of exchangeable Zn pool (see Table 9.2). Zn also binds to other plasma proteins, such as transferrin and α_2-macroglobulin. Albumin-bound Zn is not essential for Zn transport into brain (Takeda et al., 1997), while the function of α_2-macroglobulin-bound Zn remains unknown.

In addition to protein binding, Zn also forms complexes with amino acids such as histidine and cysteine, which makes up the second largest pool of exchangeable Zn (Harris and Keen, 1989). Evidence has also shown that L-histidine may play a role in the transport of Zn into the brain at brain barrier systems. The transfer of Zn from plasma proteins to histidine determines the brain permeability to Zn (Buxani-Rice et al., 1994; Keller et al., 2000).

9.4.1 Zn in Brain Function and Dysfunction

Zinc is essential for the normal growth, development, and function of the CNS. Involvement of Zn in enzymatic reactions has been known for more than half a century. Zn-requiring/containing enzymes whose three-dimensional (3D) structures are well

defined number more than 200 (Maret, 2001). Zn performs three major functions in Zn enzymes: catalysis, coactivity, and structure (Vallee and Falchuk, 1993).

9.4.1.1 Zn Deficiency

Deficiency in the dietary supply of Zn causes alteration of Zn homeostasis in the brain, leading to brain dysfunctions, e.g., mental disorders (Golub et al., 1995). Zn deficiency during brain development in experimental animals causes permanent malformations; the activities of Zn metalloenzymes (e.g., glutamate dehydrogenase and 2'-3'-cyclic nucleotide 3'-phosphohydrolase) are reduced in brains of Zn-deficient rats (Dreosti et al., 1981); animals with a decreased hippocampal Zn develop convulsive seizures (Fukahori et al., 1988). In humans, Zn deficiency in young or mature individuals can lead to abnormal neuromotor and cognitive function (Keen et al., 1993).

Brain dysfunction due to zinc deprivation may be indirectly related to a generalized decrease in zinc-dependent processes, such as protein synthesis, DNA and RNA synthesis, or cell membrane stability (Keen et al., 1993). More recently, Zn was found to be involved in the release of synaptic neurotransmitters (Frederickson et al., 2000; Huntington et al., 2001).

Systemic Zn deficiency can also affect the permeability of brain barriers. Noseworthy and Bray (2000) reported that Zn deficiency significantly increases the permeability of the blood–brain barrier and further suggested that the diminished brain barrier integrity may be a free radical-mediated process, as the ratio of oxidized to reduced glutathione (GSSG:GSH) is significantly elevated.

9.4.1.2 Zn in Alzheimer's Disease

Involvement of Zn in Alzheimer's disease has been recognized since the early 1990s. Both APP and Aβ can be characterized as Cu/Zn metalloproteins, with APP in the CSF being more easily precipitated than Aβ peptides (Brown et al., 1997). Zn can bind to a specific, cysteine-rich region of the APP-695 ectodomain (Bush et al., 1994b; Cuajungco and Fagét, 2003). Noticeably, Zn(II) is the only physiologically available metal ion capable of precipitating Aβ at pH 7.4. The consequence of Zn binding to APP or Aβ remains controversial. Some suggest that binding results in Zn-induced oxidative stress or Aβ-mediated oxidative stress or both in affected brain regions (Moreira et al., 2000), while others indicate that Zn may inhibit Aβ-mediated cytotoxicity, particularly at low concentrations, playing a protective role against Aβ cytotoxicity (Yoshiike et al., 2001).

Reports on Zn concentrations in the systemic circulation and brain among Alzheimer's patients have been contradictory (Basun et al., 1991; Cuajungco and Lees, 1997; Gonzalez et al., 1999; Rulon et al., 2000; Tully et al., 1995). Earlier studies suggested no observable changes in CSF concentrations of Zn in patients (Basun et al., 1991). A more recent study indicated that CSF Zn level is significantly decreased in AD as compared to the age-matched controls (Molina et al., 1998). When Zn was supplemented at 200 to 400 mg/day for 1 year, Alzheimer's patients

showed a significant slowing of cognitive decline (Van Rhijn et al., 1990; Potocnik et al., 1997).

Thus, an altered brain homeostasis of Zn appears to contribute to the etiology of Alzheimer's disease (Bush et al., 1994a,b). Brain turnover of Zn is rather slow, with a half-life ($T_{1/2}$) of 7 to 42 days. The half-life lasts even longer in brain regions that contain vesicular Zn (Kasarskis, 1984). Zn concentrations above 300 nmol/L can rapidly precipitate synthetic human $A\beta_{1-40}$ (Bush et al., 1994a). Since Zn in the brain is regulated by the transport mechanisms located at the blood–brain barrier, any disruption of the barrier's function and permeability to Zn could conceivably lead to an increased influx of Zn into the brain. With regard to Zn regulation by the blood–CSF barrier in the choroid plexus, it is known that certain Zn transporters are located in the choroid plexus, e.g., PHT1 and DMT1; whether Zn is transported via the choroid plexus and in which direction the choroid plexus modulates Zn fluxes remain unknown.

9.4.1.3 Protective Effect of Zn-Containing Enzymes at Brain Barriers

Following trauma, ischemia, and reperfusion injury, the permeability of the blood–brain barrier can be altered, resulting in pathogenesis of brain edema. The oxygen-derived radicals, particularly superoxide, may contribute to barrier damage. The cytosolic antioxidant Cu/Zn superoxide dismutase (CuZnSOD) may be an important factor in protecting against oxidative damage to barriers. In transgenic mice with overexpression of CuZnSOD, thromboembolic cortical ischemia causes much less oxidative DNA damage, less DNA fragmentation, and much less ensuing blood–brain barrier disruption compared to wild-type mice (Kim et al., 2001). Studies on a reperfusion-ischemia model using transgenic mice deficient in CuZn-SOD provide the opposite results and thus reach the similar conclusion (Gasche et al., 2001). Chan and colleagues (1991) have also proved that an increased level of superoxide dismutase activity in the brain reduces the development of vasogenic brain edema and infarction.

9.4.2 Transport of Zn by Brain Barriers

9.4.2.1 Brain Regional Distribution

The concentration of Zn in the CSF of normal subjects is about 1% that of plasma, i.e., 0.15 μM (Franklin et al., 1992). Zn concentration in brain extracellular fluid is nearly the same as that in the CSF (Palm et al., 1986). The half-life of ^{65}Zn in rat brain tissue ranged between 16 and 43 days (Takeda et al., 1995). A considerable difference exists in elimination of ^{65}Zn from various brain regions. Zn may be eliminated from the arachnoid villi or arachnoid granulations, or both via the CSF.

The levels of Zn in the brain vary during the developmental stage. The brain concentration of Zn increases with growth after birth and is maintained constant in the adult brain (Sawashita et al., 1997). Concentrations of Zn along with Cu and Fe in gray matter are in the same order of magnitude as that of magnesium (0.1 to 0.5

mM) (Lovell et al., 1998). Because of its relatively high concentration in the brain, Zn is hardly regarded as a "trace" element in the brain.

In the brain, Zn exhibits a regional specific distribution, with the highest level found in the limbic system, including the hippocampus and amygdala. Autoradiographic study using in vivo ^{65}Zn shows a low level of radioactivity in the white matter and a high level in choroid plexus and cerebral cortex, particularly in the dentate gyrus (Franklin et al., 1992). Approximately 90% of the total brain Zn is bound in Zn proteins in neurons and glial cells. The rest appears to be present in presynaptic vesicles as a neuro-modulator in synaptic neurotransmission (Howell and Frederickson, 1990).

9.4.2.2 Transport by Blood–Brain Barrier

Zn in the blood circulation is transported to the brain via the blood–brain barrier. With prolonged perfusion (>30 min) in rats, the uptake of Zn is unidirectional with an influx rate constant (K_{in}) of approximately 5×10^{-4} mL/min/g. If the perfusion time is more than 30 minutes, ^{65}Zn fluxes between blood and brain become bidirectional, with an influx K_{in} greater than 5×10^{-4} mL/min/g (Pullen et al., 1991).

In a Transwell model using cultured brain capillary endothelial cells (BCEC), treatment with low concentrations of Zn in culture media (3 μmol/L) results in an increased rate of Zn uptake into BCEC and an increased rate of Zn transport across the BCEC. The same experiment with a high Zn concentration (50 μmol Zn/L), however, decreases the rate of Zn transport across BCEC, although the uptake of Zn into BCEC is indeed increased (Lehmann et al., 2002). Apparently, at high concentration, Zn ions are sequestered by intracellular binding proteins, which prevent the metal from further entering the other side of the compartment. It is well known that Zn induces metallothionein.

9.4.2.3 Transport by Blood–CSF Barrier

Zn can also be transported to the CSF compartment via the blood–CSF barrier in the choroid plexus. One hour after intravenous injection of $^{65}ZnCl_2$ to rats, ^{65}Zn is highly concentrated in the choroid plexus. It subsequently accumulates in brain parenchyma with an apparent decrease in choroidal ^{65}Zn. The time sequence of radioactive Zn in brain suggests that Zn ion gradually reaches the brain mainly via the CSF secreted by the choroid plexus (Takeda et al., 1997). The permeability of choroid plexus to Zn, with a K_{in} of 61×10^{-4} mL/min/g, is about 12 times higher than that of the cerebral capillaries ($K_{in} = 5 \times 10^{4}$ mL/min/g). However, overall influx of Zn to brain via the choroid plexus is less than 5% that across cerebral capillaries. Thus, some authors suggest that the primary supply of Zn to the brain is perhaps via the blood–brain barrier, while the choroid plexus may contribute to the slow, prolonged flux to the brain (Franklin et al., 1992).

The main contribution of the choroid plexus to brain Zn regulation may come from mediation of efflux, i.e., by transporting Zn out of the CSF compartment (Kasarskis, 1984). Brain Zn is maintained at a fairly stable level; it does not change significantly even if plasma Zn concentrations rise 10 times above the normal level

(Blair-West et al., 1990). In Zn-deficient animals, brain Zn levels are also scarcely affected (O'Dell et al., 1989). The controlled influx at both barriers and efflux by the choroid plexus may explain the tight regulation in the brain homeostasis of Zn.

9.4.2.4 Mechanism of Zn Transport

The mechanisms by which Zn is transported from brain capillary endothelial cells or choroidal epithelial cells to the brain extracellular fluid and the CSF remain unclear. Several transport systems are suggested to be responsible for Zn transport at the cell membrane or inside of the cells (see Table 9.3). ZnT-1, 2, 3, and 4 (originally known as MT-I through MT-IV) are Zn-binding proteins whose sequences have been cloned (McMahon and Cousins, 1998). ZnT-1/MT-I and ZnT-2/MT-II can be induced by Zn and Cu in astrocytes (Hidalgo and Carrasco, 1998). Functionally speaking, ZnT-1/MT-I may be associated with Zn efflux. ZnT-3/MT-III is expressed in Zn-containing glutamatergic neurons and is supposedly involved in Zn transport from the cytosol to synaptic vesicles in neurons (Palmiter et al., 1996; Wenzel et al., 1997; Tsuda et al., 1997; McMahon and Cousins, 1998; Cole et al., 1999). These proteins are involved in intracellular Zn homeostasis (Ebadi et al., 1995). It is unclear if all these Zn-binding proteins exist at both barriers. The other transporter that may be involved in Zn transport across the brain barrier is ZIP (zipper) (ZRT1, IRT1-like protein, also named ZIRTL, zinc-iron regulated transporter-like gene) (Grotz et al., 1998; Gaither and Eide, 2000).

9.5 ALUMINUM

9.5.1 Al in Brain Dysfunction

Al is found throughout the environment (Flaten et al., 1996). It apparently serves no essential biological function and is a known neurotoxicant potentially associated with a number of neurodegenerative disorders (Yokel, 2000; Spencer, 2000). Potential sources of Al exposure include food, vaccines, antiperspirants, drinking water, antacids, and intravenous solutions. A direct Al neurotoxicity seen in clinics has been dialysis encephalopathy, where Al is a major source of toxicity (Flaten et al., 1997). During the disease development, patients display Al toxicity-associated symptoms from neurological, skeletal, and hematological systems and in most cases with advanced renal failure (Alfrey, 1991).

One of the major Al-induced neurotoxicities that remains debatable is the role of Al in Alzheimer's disease (Flaten et al., 1997; Savory et al., 1997; Spencer, 2000). In particular, the evidence from human studies of AD patients has been highly controversial. Reusche (1997) reported an increased level of Al in neurons of an AD patient who suffered from a dialysis-associated encephalopathy (DAE). Zatta et al. (2003) also found that Al was more often associated with the neurofibrillary tangles (NFT) than with the plaques. Using a laser microprobe mass analysis technique, Good et al. (1992) demonstrated a significant increase of Al and Fe in NFT among AD patients. However, Landsberg et al. (1992) failed to reveal the presence of Al in senile plaques from AD brains by autopsy. Lovell et al. (1993) found no significant

increase of Al in NFT in AD brain; Murray et al. (1992) further reported a diminished Al concentration in NFT. Several epidemiological studies also failed to establish the associations between AD occurrence and Al contamination in drinking water (Forster et al., 1995; Martyn et al., 1997), although some studies suggested a small, yet not statistically significant, increase in the risk of dementia illness for people living in areas with higher Al concentrations in drinking water (Neri and Hewitt, 1991; McLachlan et al., 1996).

Deposition of amyloid β polypeptide in cerebral vessels is a common phenomenon in β-amyloid diseases, including Alzheimer's disease. Al is known to alter the structure and function of Aβ by inhibiting the enzymes (metalloproteases) associated with the processing and degradation of Aβ. For example, Banks et al. (1996) found that Al altered the enzymatic degradation of Aβ through a calcium-sensitive process. A reduced degradation of Aβ by Al treatment as demonstrated in vitro could alter the entry of Aβ to brain. Al can also change the permeability of the blood–brain barrier to peptides with the molecular weight similar to Aβ. Kaya et al. (2003) found that exposure to a high level of Al results in an additional increase in blood–brain barrier permeability to Evans blue (EB) dye in chemical-induced chronic hypertensive rats. These studies indicate that Al is able to alter the access of bloodborne Aβ to the CNS either by increasing the permeability of the BBB or by affecting its enzymatic degradation.

Al also induces other neurotoxicities. Reusche et al. (2001) reported a case of Al intoxication. The patient underwent a bone reconstruction with an Al-containing cement, which had about 30 mg of Al. Autopsy data revealed the presence of Al-containing intracytoplasmic argyrophilic inclusions in choroid plexus epithelia, neurons, and cortical neuroglia. Atomic absorption spectrometry to analyze Al showed an increase of Al concentrations in the cortex and subcortex up to 9.3 μg/g (normal range < 2 μg/g). Apparently Al in blood passes across the brain barriers to gain access to brain parenchyma via a CSF leakage.

9.5.2 Transport of Al by Brain Barriers

Even though Al is the third most ubiquitous element on earth, very little Al is absorbed into the human body. Two major barrier systems may be designed to rigorously exclude Al from entering the systemic circulation and targeted tissues such as brain. The barrier in the gastrointestinal tract forms the first defense line for Al toxicity; the blood–brain barrier serves as the second line to protect the brain from Al insults (Banks et al., 1996).

9.5.2.1 Toxicokinetics of Al

Approximately 80% of plasma Al is bound to transferrin. Another 11% of plasma Al forms Al-citrate complex, which is the predominant small molecular weight Al species in plasma (Öhman and Martin, 1994). In contrast, about 90% of Al in brain extracellular fluid may be in the form of Al-citrate complex and only 4% of Al bound to transfer (Yokel, 2002).

Oral ingestion of Al leads to an increased amount of Al in brain tissue. Rats receiving oral doses of ^{26}Al for 1 to 2 weeks showed a significant accumulation of ^{26}Al in brain tissues (Walton et al., 1995). Determination of ^{26}Al in rat brain at various times after intravenous ^{26}Al suggested a prolonged brain ^{26}Al half-life. The half-life of brain ^{26}Al was estimated to be about 150 days (Yokel, 2002). Microdialysis allows one to sample brain extracellular fluid in different brain compartments and to detect unbound Al in brain extracellular fluid. Using this method, Allen and Yokel (1992) reported that systemically administered Al-citrate can be quickly detected in brain extracellular fluid, indicating that Al in the blood circulation is capable of penetrating the blood–brain barrier to enter the brain and accumulate there. It also suggests that the blood–brain barrier is the primary site for Al-citrate transport.

9.5.2.2 Influx of Al

Like many chemicals, Al in the blood circulation may enter brain parenchyma through either the blood–brain barrier or the blood–CSF barrier in the choroid plexuses. Yokel et al. (1999) suggested that Al enters the brain primarily through brain microvessels, rather than through choroid plexuses, because Al has a much more rapid appearance in the frontal cortex than in other brain regions following injection. In addition, the Al concentration is always lower in the lateral ventricle than in the frontal cortex, suggesting a lack of concentration gradient for Al to distribute from the choroid plexus in the ventricles to other brain regions.

There appear to be at least two mechanisms mediating Al distribution across the blood–brain barrier (see Table 9.3). One involves receptor-mediated brain influx of Al, which is bound to transferrin; the other involves transport via small molecular weight species, such as Al-citrate complex.

Roskams and Connor (1990) have shown that transferrin-bound Al can be detected in brain less than one hour following dose administration. These authors suggest that a TfR-mediated endocytosis may be responsible for fast influx of Al into the brain, and that the mechanism is similar to Fe transport via TfR at the blood–brain barrier. The assumption is reasonable, because Al binds extensively to plasma transferrin and because there are many similarities between the chemistry of Fe and Al. In vitro uptake studies further support the TfR-mediated mechanism, since the presence of transferrin in the culture media increases in vitro Al uptake into neuroblastoma cells and oligodendroglia but not into astrocytes (Golub et al., 1999).

Transport of Al into brain by Al-citrate conjugates appears to be much faster than TfR-mediated endocytosis or other diffusion processes (Yokel et al., 1999). Akeson and Munns (1989) suggest that Al uptake in the presence of citrate may be carrier mediated, because the process is energy dependent and proton driven.

The monocarboxylate transporter (MCT) may be a candidate carrier for brain Al-citrate influx. The MCT is located at both the luminal and abluminal surfaces of the blood–brain barrier (Gerhart et al., 1997) and is believed to mediate bidirectional transport of lactate, pyruvate, and other monocarboxylates. It has the second highest V_{max} (60 nmol g^{-1} brain min^{-1}) of all known carriers at the blood–brain barrier

(Laterra et al., 1999). Al-citrate conjugates are formed by coordinative binding of Al with the hydroxyl group and the two terminal carboxylates of citrate (Gregor and Powell, 1986). This leaves a free carboxylate, which is dissociated at physiological pH, perhaps serving as the binding site for the MCT. This hypothesis, however, has not been tested.

Other candidate carriers for Al-citrate include the anion-exchanger family (SCL4A), the organic anion transporter (OAT), also known as multidrug resistance-associated protein (MRP) family (ABC subfamily C), or the organic anion transporting polypeptide (Oatp) family (SCL21). The question as to whether and how these carriers mediate Al influx at the blood–brain barrier remains a topic for further investigation.

The other potential route for Al to enter brain may be via olfactory absorption from the nasal cavity. In Alzheimer's disease, it has been recognized that there is a tendency for the development of NFTs among neurons of cortical regions associated with the olfactory system. Perl and Good (1991) found that neurofibrillary tangle–bearing neurons in cortical regions contained highly elevated levels of Al. This not only indicates that Al may enter the central nervous system via the olfactory pathways, it also implies an association between inhaled Al and the risk of AD.

Although Al is known to accumulate in the choroid plexus (Reusche et al., 2001), whether and to what extent the blood–CSF barrier may contribute to the homeostasis of Al in the brain is unclear.

9.5.2.3 Efflux of Al

Clearance of Al out of the brain is a rather slow process. In human, brain Al concentration increases with age (Markesbery et al., 1984; Ehmann et al., 1986). The concentration of Al in normal adult brains is 17 μM, which is 42 times higher than the normal blood Al concentration (<0.4 μM) (Markesbery et al., 1984). Results from animal studies also support prolonged retention of Al in brain. Following systemic ^{26}Al injection to rats, brain ^{26}Al concentrations decreased only slightly even 35 days after dose administration. These results suggest that Al may be intracellularly distributed in the brain, possibly due to tight tissue binding. Alternatively, Al may be very slowly cleared or removed from the brain compartment.

The efflux of Al from the brain likely takes place at the blood–brain barrier rather than at the blood–CSF barrier (Allen et al., 1992). The efflux of Al occurs shortly after the Al enters the brain in a small quantity. The efflux is energy-dependent (Allen et al., 1995; Ackley and Yokel, 1997); it is unlikely to be TfR mediated (Yokel et al., 1999). Other studies suggest that the MCT is more efficient in efflux than influx of Al at the blood–brain barrier (Deguchi et al., 1997). It has been demonstrated that at least one MCT isoform, MCT1, is expressed at the blood–brain barrier (Yokel, 2002). Ackley and Yokel (1997, 1998) found that Al has been transported out of brain extracellular fluid by a proton-dependent MCT at the blood–brain barrier, probably MCT1.

The choroid plexus occupies the entire brain ventricles. The large surface area of the choroidal epithelia makes the tissue an ideal place to remove unwanted materials from the CSF. Many transporter proteins for Al have been identified in the

choroid plexus. It is surprising, however, that the role of the choroid plexus in clearance of CSF Al has never been addressed.

9.5.3 Toxicity of Al at Brain Barriers

As discussed earlier, Al exposure increases the permeability of the blood–brain barrier. The effect is rapid in onset, quickly reversible, and largely dependent on the metal species expressed by the different physicochemical properties of the various metal ligands (Banks et al., 1996). Despite a reported increase of Al in the choroid plexus of an Al-intoxicated patient (Reusche et al., 2001), no study so far has been conducted to identify and characterize the specific damage cause by Al.

9.6 SUMMARY AND PERSPECTIVES

The choroid plexus, as a barrier between the blood and CSF, possesses numerous transporters for metals, metal–amino acid conjugates, and metal–protein complexes. It is clear that dysregulation of metal transport by brain barriers will lead to a significant change in metal homeostasis in the cerebral compartment. This could further contribute to the etiology of metal-associated neurodegenerative disorders. While an effort has been made to understand the underlying mechanisms and therefore to structure the strategy of prevention and therapy, many questions remain. For example, how do these metals get into the brain? Are these metals removed from the CNS by the choroid plexus, or by other mechanisms? Does the disease status become significant only after the barrier loses its protective effect? Our knowledge about the molecular and cellular mechanisms associated with the transport of these metals across brain barriers, as well as the properties and kinetics of molecular movement at the barriers, remains incomplete.

In addition, there are many channels, transporters, carriers, and receptors at the brain barriers. Too little has been learned about how Cu, Zn, and Al interact with endothelial and epithelial cells and to what degree these interactions contribute to neurological disorders. The mechanism whereby the choroid plexus interacts with toxic metals remains largely unknown. The significance of these metals acting on the brain barrier system for the etiology and pathology of CNS disease also needs to be clarified.

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