

Impairment of glutamine/glutamate- γ -aminobutyric acid cycle in manganese toxicity in the central nervous system

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Abstract

Manganese (Mn) is an essential trace element that is required for maintaining the proper function and regulation of many biochemical and cellular reactions. Despite its essentiality, at excessive levels Mn is toxic to the central nervous system. The overdose accumulation of Mn in specific brain areas, such as the substantia nigra, the globus pallidus and the striatum, triggers neurotoxicity resulting in a neurological brain disorder, referred to as manganism. Manganese toxicity is associated with the disruption of glutamine (Gln)/glutamate (Glu) GABA cycle (GGC). The GGC represents a complex process, since Gln efflux from astrocytes must be met by its influx in neurons. Mn toxicity is associated with the disruption of both of these critical points in the cycle. In cultured astrocytes, pre-treatment with Mn inhibits the initial net uptake of Gln in a concentration-dependent manner. Manganese added directly to astrocytes induces deregulation in the expression of SNAT3, SNAT2, ASCT2 and LAT2 transporters and significantly decreases in Gln uptake mediated by the transporting Systems N and ASC, and a decrease in Gln efflux mediated by Systems N, ASC and L. Further, Mn disrupts Glu transporting systems leading to both a reduction in Glu uptake and elevation in extracellular Glu levels. Interestingly, there appear to be common signaling targets of Mn in GGC cycling in glial cells. Namely, the PKC signaling is affected by Mn in Gln and Glu transporters expression and function. Additionally, Mn was identified to deregulate glutamine synthetase (GS) expression and activity. Those evidences could triggers depletion of Gln synthesis/metabolism in glia cells and consequently diminish astrocytic-derived glutamine, while disruption of Glu removal/transport can mediate dyshomeostasis in neurotransmission of functioning neurons. Overdose and excessive Mn accumulations in astrocytes not only culminate in pathology, but also affect astrocytic protective properties and defect or alternate astrocyte-neuronal integrity. Here we highlight the mechanistic commonalities inherent to Mn neurotoxicity related to the astrocyte pathology and GGC impairment.

Key words: manganese, neurodegeneration, neurotoxicity, transporter, Gln, Glu, GABA.

Manganese

Essentiality and toxicity

Manganese (Mn) is an essential trace metal commonly found in the environment. It is an important dietary nutrient, and as such is required for growth,

development, and maintenance of normal physiological function. Manganese is necessary for a variety of metabolic functions, including lipid, protein, and carbohydrate metabolism. It is a critical component of numerous metalloenzymes, including Mn superoxide dismutase, arginase, glutamine synthetase and

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phosphoenolpyruvate decarboxylase [42], to name a few. However, excessive and prolonged exposure to Mn may lead to a neurological disorder that shares many similarities with Parkinson's disease (PD), and is referred to as manganism [15]. Exposure to Mn in the general population occurs from the automobile combustion of gasoline containing methylcyclopentadienyl manganese tricarbonyl (MMT) or from organic Mn-containing pesticides, such as manganese ethylene-bis-dithiocarbamate [8]. Chronic exposure to high levels of inhalable Mn (1-5 mg Mn/m³), which is commonly associated with several occupations (e.g. mining, battery manufacturing, welding, ferroalloy smelting, and steel production), is believed to be the most frequent cause of Mn neurotoxicity [32]. Individuals with some medical conditions such as liver failure or total parenteral nutrition exhibit elevated Mn blood level and neurological dysfunctions [1]. The accumulation of Mn in basal ganglia is responsible for a form of parkinsonism with clinical features overlapping with, but distinct from, those observed in idiopathic PD [18]. Manganese-induced neurotoxicity affects mainly the globus pallidus as well as the cortex and hypothalamus [48,50], distinct from the striatal changes associated with PD. Excessive Mn deposition in the central nervous system (CNS) contributes to the pathogenesis of PD, causing loss of dopamine in the striatum, death of non-dopaminergic (DAergic) neurons in the globus pallidus, and damage to glutamatergic and GABAergic projections [25,39]. Manganese has also been shown to mediate fibril formation by α -synuclein along with its expression and aggregation [47]. A role for Mn has also been advanced in the etiology of a few neurodegenerative disorders, e.g. Huntington's disease, amyotrophic lateral sclerosis, prion diseases and Alzheimer's disease (AD), all of which rely on similar intracellular mechanisms involving oxidative stress, mitochondrial impairment, and protein aggregation.

Mitochondrial dysfunction was found to be a common effect in Mn toxicity [7]. A previous study showed that primary astrocytic cultures are highly sensitive to Mn and undergo apoptotic cell death involving mitochondrial dysfunction [51]. Manganese was found to be preferentially and rapidly transported into the mitochondrial matrix via the calcium (Ca²⁺) uniporter, with relatively slow clearance, leading to the accumulation of Ca²⁺ [16]. Elevated matrix calcium increases formation of reactive oxygen species (ROS) by the electron transport chain and mediates

inhibition of aerobic respiration [22]. Manganese was found to induce mitochondrial respiratory dysfunction *in vitro*, induce ROS, and inhibit the antioxidant system by depleting glutathione and glutathione peroxidase [26,52]. Additionally, activation of oxidative stress-sensitive kinases and transcription factors including nuclear factor (NF)- κ B has been identified in cell lines exposed to Mn [31,51].

Manganese-mediated dysfunction of astrocytes and astrocyte-neuron integrity

Astrocytes play an essential role in the CNS by regulating and maintaining the extracellular environment and protective barriers, which include glia scars, the glia limitans and the blood-brain barrier. Astrocytes ensure trophic, energy and metabolic support to neurons. Glial cells actively participate in neuronal excitability and survival by modulating synaptic homeostasis and formation [38]. Astrocytes accumulate Mn up to 50-fold greater than neurons through a high-affinity transport mechanism [2] and hence are considered as an initial target for Mn-induced neurotoxicity. Chronic overexposure to Mn is associated with glial cell activation and is manifested with gliosis. As in neurons, albeit at higher concentrations, Mn is sequestered in mitochondria and disrupts astrocytic energy metabolism [6]. Importantly, the level of glutathione (GSH), the main cellular antioxidant, is significantly lower in neurons than in astrocytes. In general, neuronal stores of GSH are largely dependent upon astrocytic stores, and neurons are more sensitive than astrocytes to oxidative stress [33]. Several studies have demonstrated that Mn promotes failure of astrocytes to maintain antioxidant defense mechanisms via disruption of GSH synthesis [49]. *In vivo*, interneuron injury in striatal and pallidal regions of Mn-exposed mice is associated with an increased number of reactive astrocytes expressing inducible form of nitric oxide synthase (iNOS) in the same brain areas [41]. Treatment of astrocytes with Mn increases uptake of *L*-arginine, an iNOS substrate, leading to increased ROS generation as a consequence of nitric oxide (NO) production and deterioration of cellular antioxidant capacity and energy metabolism. Furthermore, as discussed below, Mn is believed to exert toxicity via disruptive effects on the synthesis of neurotransmitters. While Mn, at higher concentrations than in neurons, might be toxic to astrocytes themselves and the astrocytes

are crucial for its brain transport and accumulation, they are extremely important from a neuroprotection point of view. For example, Mn-induced neuronal injury in the striatum and the globus pallidus is associated with primary dysfunction of astrocytes via mechanisms involving NO [45]. In addition, Mn inhibits the ability of astrocytes to promote neuronal differentiation by a mechanism that involves oxidative stress and a reduction of the extracellular matrix protein, such as fibronectin [17].

Glutamine in general and in the glutamine/glutamate- γ -aminobutyric acid cycle

Glutamine (Gln) content in the extracellular fluid (microdialysates) (ECF) or in the cerebrospinal fluid (CSF) (~0.5-1 mM) exceeds, by at least one order of magnitude, the extracellular contents of other amino acids in these compartments. Glutamine is the most abundant amino acid in the plasma (at 600-800 μ mol) and exhibits extremely rapid cellular turnover rates [9,10]. This amino acid serves multiple roles in the mammalian brain: as an essential precursor in nucleotide, glucose and amino sugar biosynthesis, glutathione homeostasis and protein synthesis. Glutamine transport across cell membranes has been extensively studied. In the past few years a number of glutamine carriers have been cloned, and their molecular and functional properties have been characterized. Glutamine transport systems in the mammalian brain are classified into two distinct groups termed sodium-dependent (systems A, ASC and N) and sodium-independent (system L). All of the glutamine transport systems has been identified in brain, indicating that the CNS possesses multiple pathways for Gln transportation across the plasma membrane [5].

In the mammalian brain, Gln functions via the complex glutamine/glutamate- γ -aminobutyric acid cycle (GGC), where Gln efflux from astrocytes must be met by its influx in neurons. After exocytotic release at synaptic terminals, glutamate (Glu) is taken up by surrounding astrocytes via the glia specific Glu transporter 1 GLT-1 and the Glu-aspartate transporter GLAST [29,43]. In astrocytes Glu is converted to Gln by glutamine synthetase (GS). Astrocytic Gln transporters with the predominant role of SNAT3 (system N) mediate its release into the extracellular space. In turn, Gln is transferred into the neurons mainly by SNAT1 and SNAT2 transporters belonging to system A. In neurons Gln is catalyzed to Glu via

phosphate-dependent glutaminase (PAG). Glutamate is subsequently converted into GABA via decarboxylation by glutamic acid decarboxylase (GAD). In both astrocytes and neurons Glu is used for the synthesis of alpha-ketoglutaric acid, a substrate for the tricarboxylic acid cycle, by oxidative deamination mediated by Glu dehydrogenase (GDH) [3]. Furthermore, Glu is stored in synaptic vesicles at presynaptic terminals by the vesicular glutamate transporters VGluT1, VGluT2 and VGluT3 (SLC17A6, SLC17A7 and SLC17A8, respectively).

The glutamate- γ -aminobutyric acid cycle plays an essential role in recycling the excitatory neurotransmitter glutamate. High affinity glutamate transporters play essential roles in removing released glutamate from the synaptic cleft and for maintaining the extracellular glutamate concentration of the cerebrospinal fluid below neurotoxic levels. Glutamine transporters mediate uptake and release of glutamine and supply neurons with the primary neurotransmitter precursor. Disruption of GGC has been reported in numerous pathological conditions, such as epilepsy, cerebral ischemia, AD, PD and manganese [3].

Manganese and glutamine/ glutamate- γ -aminobutyric acid cycle

Manganese and glutamine turnover

Studies have established that the effects of Mn inhalation on GGC vary greatly among different mammalian brain regions. For example, in the brain of monkeys exposed to airborne Mn, GS protein levels were found to be altered, with a strong decrease of GS protein expression in the cerebellum and the globus pallidus [11]. Monkeys exposed to Mn also had several other alterations including decreased caudate, globus pallidus, olfactory cortex, and cerebellum GLT-1 protein, decreased olfactory cortex and cerebellum GLAST protein. Other rodent models of manganese exposure have also pointed to alterations in GABA and GAD in the caudate and globus pallidus [19,46].

Manganese toxicity is associated with disruption of critical points in the GGC, such as glutamine translocation and glutamate uptake by astrocytes. In cultured astrocytes, pre-treatment with Mn inhibits the initial net uptake of Gln in a concentration-dependent manner. Manganese added directly to astrocytes induces deregulation in the expression of SNAT3,

SNAT2, ASCT2 and LAT2 transporters [34]. Corroborating the changes in transporter protein expression levels, astrocytes treated with Mn displayed a significant decrease in Gln uptake mediated by the transport systems N and ASC, and a decrease in Gln efflux mediated by systems N, ASC and L. PKC(s) signaling, with the predominant role of the PKC δ isoform, has been invoked as a potential mechanism leading to transporters inactivation and glial dysfunction in Mn-mediated disruption of Gln transport. Manganese exposure has been demonstrated to mediate the specific phosphorylation of PKC δ isozyme, and significantly increase PKC(s) activity. In addition, astrocytes transfected with shRNA against PKC δ are significantly less sensitive to Mn compared to those transfected with control shRNA [36]. Treatment of primary astrocyte cultures with a PKC(s) stimulator reduces Gln uptake mediated by systems ASC and N, and reduces expression of ASCT2 and SNAT3 protein levels in cell lysates and in plasma membranes [37]. Furthermore, increased binding of PKC δ to ASCT2 and SNAT3 upon exposure to Mn has been identified by a co-immunoprecipitation study. It is noteworthy that both Mn-affected transporters contain putative PKC phosphorylation sites, which are conserved in the human, rat and mouse [28,30].

SNAT3 (system N), selectively expressed on astrocytic processes ensheathing synapses, mediates release of glutamine and supplies neurons with the primary neurotransmitter precursor. Thus, functional integrity of SNAT3 may be a key regulator for the recycling of glutamate and GABA. SNAT3 exhibits a highly differential cellular and subcellular localization and the highest sensitivity to Mn exposure among all investigated Gln transporters. A recent *in situ* study revealed that PKC(s) activation induces phosphorylation of SNAT3 and regulates its membrane trafficking and protein degradation [28]. Notably, the study demonstrated that Mn-dependent PKC activation induces hyper-ubiquitination, and increases the association of SNAT3 with ubiquitin-protein ligase E3, Nedd4-2 (neuronal precursor cell expressed, developmentally down-regulated 4-2) in primary culture of astrocytes. In addition, internalization of SNAT3 upon PKC-dependent phosphorylation is corroborated by evidence from an *Xenopus laevis* oocyte model in which PKC activation reduced V_{\max} of the Gln uptake activity [4]. Together, this evidence could represent a link of manganese to PKC(s) regulation in the GGC cycle, since phosphorylation by PKC stimulates

transporters' internalization and/or ubiquitination and further lysosomal or proteasomal degradation.

Manganese and the glutamate transport system

The Glu transport system is essential for maintaining optimal extracellular Glu concentrations that do not activate Glu transporters and receptors. Rapid removal of glutamate from the extracellular space is required for survival and proper neuronal function. Impaired uptake of glutamate by astrocytes mediates excitotoxicity, slows the synthesis of glutathione and leaves the brain vulnerable to oxidative damage [24]. Several studies have demonstrated the propensity of Mn to disrupt the Glu transport system and other components of the GGC, leading to both a reduction in Glu uptake and elevation of extracellular Glu level [23]. Manganese has been widely reported to mediate the impairment of glutamate transporters, GLAST and GLT-1 expression and function [12,27]. Furthermore, Chinese hamster ovary cells transfected with GLAST or GLT-1 were found to fail to transport Glu after Mn exposure [27]. A recent finding has identified that PKC signaling is involved in Mn-induced deregulation of Glu turnover [35]. The study revealed that PKC(s) stimulation significantly reduces astrocytic Glu uptake, while treatment with a general PKC(s) inhibitor protects astrocytes from Mn-induced deregulation of Glu transport. Interestingly, co-immunoprecipitation studies demonstrated association of GLT-1, but not GLAST, with the PKC δ and PKC α isoforms and a Mn-induced specific increase in PKC δ -GLT-1 interaction [28,35]. This evidence combined with findings on Mn's influence on Gln transport is consistent with abnormal GGC cycling function caused by Mn at two key steps, including Gln and Glu transport, via a homologous, PKC(s)-dependent pattern.

A more recent study revealed the important role of the transcription factor Yin Yang 1 (YY1) in Mn-mediated disruption of GLT-1. Manganese increases YY1 promoter activity and, consequently, its mRNA and protein levels [20]. Manganese enhances YY1 binding to its consensus sites in the GLT-1 promoter, and, accordingly, mutations of YY1 binding sites attenuate the Mn-induced decrease in GLT-1 promoter activity, indicating that YY1 is a critical transcriptional mediator in Mn-induced repression of GLT-1. Manganese likely activates YY1 via proinflammatory mediators, such as tumor necrosis factor α (TNF- α), interleukin

(IL)-6 and IL-1 β [13,14]. TNF- α and IL-1 β are negative regulators of GLT-1, and they reduce GLT-1 mRNA and protein levels in astrocytes [21,40,44]. Recent studies have indicated that Mn increases production of TNF- α , which, in turn, increases YY1 promoter activity, and mRNA and protein levels [20], suggesting that TNF- α mediates Mn effects on reduction of GLT-1 expression via YY1 in astrocytes.

Concluding remarks

Manganism has been considered as a metabolic syndrome related to impairment of glutamate transport and, more recently, the glutamine/glutamate- γ -aminobutyric acid cycle. *In vivo* and *in vitro* studies have demonstrated that Mn evokes mitochondrial abnormalities, oxidative/nitrosative stress and morphological/functional changes of astrocytes, major players of the GGC. Manganese effectively increases abnormalities in the glutamine metabolism and turnover between glia and neurons. There appear to be common PKC(s) signaling targets of Mn in GGC cycling in glial cells. The astrocytic glutamate and glutamine transporters may be targeted by these regulatory mechanisms as a response to certain synaptic activity synergistically inhibiting glutamate recycling. The evidence discussed here not only contributes to understanding the mechanism by which Mn disrupts astrocyte function and astrocyte-neuron intercommunication but may potentially lead to the development of novel therapeutic interventions in animal models of manganese toxicity.

Disclosure

Author reports no conflict of interest.

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