

Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination

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ABSTRACT

This review attempts to summarize and clarify our basic knowledge as to the various factors that potentially influence the risks imposed from chronic exposure to high atmospheric levels of manganese (Mn). The studies describe the interrelationship of the different systems in the body that regulate Mn homeostasis by characterizing specific, biological components involved in its systemic and cellular uptake and its elimination from the body. A syndrome known as manganism occurs when individuals are exposed chronically to high levels of Mn, consisting of reduced response speed, intellectual deficits, mood changes, and compulsive behaviors in the initial stages of the disorder to more prominent and irreversible extrapyramidal dysfunction resembling Parkinson's disease upon protracted exposure. Mn intoxication is most often associated with occupations in which abnormally high atmospheric concentrations prevail, such as in welding and mining. There are three potentially important routes by which Mn in inspired air can gain access the body to: 1) direct uptake into the CNS via uptake into the olfactory or trigeminal presynaptic nerve endings located in the nasal mucosa and the subsequent retrograde axonal transport directly into the CNS; 2) transport across the pulmonary epithelial lining and its subsequent deposition into lymph or blood; and/or 3) mucociliary elevator clearance from the lung and the subsequent ingestion of the metal in the gastrointestinal tract. Each of these processes and their overall contribution to the uptake of Mn in the body is discussed in this review as well as a description of the various mechanisms that have been proposed for the transport of Mn across the blood-brain barrier which include both a transferrin-dependent and a transferrin-independent process that may involve store-operated Ca channels.

Key terms: manganese, iron, lung, Parkinson's disease, divalent metal transporter (DMT1), transferrin, transferrin receptor

Manganese (Mn) is one of the most prevalent metals on the earth, being the fifth most abundant metal and twelfth most abundant element on earth. It is an essential component for the production of steel, batteries, welding metals, ceramics, and pigments. In nature, Mn exists in 11 different oxidation states ranging from -3 to +7, although within biological systems the +2 and +3 valences are the most dominant species. Within recent years, the U.S. has developed considerable interest in Mn because of the potential health risk associated with increased atmospheric levels prompted by the impending use of the gas additive methylcyclopentadienyl manganese tricarbonyl (MMT).

Mn is an essential trace metal required for a variety of enzymatic and cellular processes within the human body (Schroeder et al., 1966; Hurley, 1981; Grieger, 1999; Keen et al., 1999). Under normal dietary consumption, systemic homeostasis of Mn is maintained by both its rate of transport across enterocytes lining the intestinal wall and by its efficient removal within the liver (Papavasiliou et al., 1966). At the cellular level, Mn balance is proficiently managed by processes controlling cellular uptake, retention, and excretion. These processes are in delicate balance to maintain essential levels of Mn that fulfill nutritional requirements of the different organelles within the body. This

normally efficient system of checks and balances that regulate Mn levels *in vivo*, however, appear to fail under conditions of exposure to chronic high doses of the metal, and thus, alterations in the elaborate homeostatic mechanisms are no longer adaptable to maintaining the status quo required.

Mn intoxication, a syndrome known as manganism, is most often associated with prolonged occupational exposure to abnormally high atmospheric concentrations of the metal. Neurological symptoms consist of reduced response speed, irritability, intellectual deficits, mood changes, and compulsive behaviors in the initial stages of the disorder to more prominent and irreversible extrapyramidal dysfunction resembling Parkinson's disease upon protracted exposure (Mena et al., 1969; Huang et al., 1993; Olanow et al., 1996; Mergler and Baldwin, 1997; Pal et al., 1999). Classic symptoms include mask-like face, limb rigidity, mild tremors, gait disturbance, cock-like walk, slurred speech, excessive salivation and sweating and a disturbance of balance. Although Mn intoxication and Parkinson's disease are both associated with neurological changes in the basal ganglia, the latter disorder correlates with loss of dopaminergic neurons within the nigro-striatal pathway, whereas manganism is associated with the preferential degeneration of GABAergic neurons within the globus pallidus, at least in the acute exposure paradigm and in the initial stages of the disorder (Olanow et al., 1996; Pal et al., 1999).

The strongest evidence indicating the sparing of neurons within the nigro-striatal pathway in manganism comes from studies demonstrating that fluorodopa scans are normal in individuals with high brain Mn levels (Olanow et al., 1996; Kim et al., 1998). Fluorodopa is a dopamine derivative that is taken up selectively within the presynaptic dopaminergic nerve endings in the striatum. The intensity of fluorodopa scans is depressed significantly in individuals with Parkinson's disease, indicative of the loss of dopaminergic neurons in the nigro-striatal pathway, whereas these scans are normal, for the

most part, in individuals with high Mn content in blood and brain. Abnormal fluorodopa scans, however, have been reported recently in a patient with manganism, suggesting that overexposure to Mn may manifest in a more diffuse injury within the basal ganglia than generally acknowledged (Racette et al., 2005). These findings are consistent with studies demonstrating loss of neurons in the substantia nigra in rats treated with Mn for 18 months (Gupta et al., 1980) and with recent studies demonstrating Mn-induced injury to the substantia nigra subsequent to degeneration of neurons within the globus pallidus of rats (Wright et al., 2004). Thus, the central nervous system (CNS) injury caused by Mn actually manifests in a diverse set of symptoms reflecting upon the fact that the basal ganglia is one of the most complex areas of the brain and associated with many positive and negative feedback loops in an attempt to preserve normal function. A disturbance in any one of these components, whether it occurs in the globus pallidus as in manganism or the nigro-striatal neurons as in Parkinson's disease, may manifest with insidious neurological deficits that are remarkably similar. In fact, there is not always a clear distinction in the expressed symptoms between the two disorders since the observed neurotoxic responses to Mn tend to vary greatly between individuals. Differences in response to Mn overexposure, most likely, are due to underlying genetic variability, which ultimately manifests in variation in both susceptibility and in the characteristics of the neurological lesions and symptoms expressed. The similarities in symptoms between the two disorders are not totally unexpected, since exposure to high levels of Mn is associated with elevated levels in both the globus pallidus and the substantia nigra, as seen in T1-weighted magnetic resonance imaging (MRI) scans (Hauser et al., 1994; Burkhard et al., 2003). Moreover, there also is increasing evidence that chronic exposure to elevated levels of Mn correlates with increased vulnerability to develop Parkinsonism, again implicating a possible common genetic component regulating these disorders (Pal et al., 1999;

Gorell et al., 1999; Hudnell, 1999; Kim et al., 2002; Racette et al., 2001; Racette, 2005). This is consistent with the observation that chronic asymptomatic exposure to miners manifests in a detectable late-life abnormalities of movement (Hochberg et al., 1996). Similarly, markedly decreased D2 receptor density using F¹⁸-methylspiperone positron emission tomography (PET) scans have been reported during the very late stages of chronic manganese toxicity, suggesting that manganese intoxication may trigger a neurodegenerative disease process (Kessler, et al., 2003).

Actually Mn overexposure can manifest in a broad spectrum of symptoms not necessarily confined to or associated with the degeneration of the basal ganglia. For example, tremor and seizures were shown to develop in a 2-year-old girl receiving total parenteral nutrition (Komaki et al., 1999). In her case, blood Mn levels were elevated, and T1-weighted MRI images revealed areas of hyperintensity in the basal ganglia, brainstem, and cerebellum. Other studies suggested that Mn also may play a role in the pathogenesis of encephalopathy in patients with liver failure (Kreiger et al., 1995).

ROUTES OF EXPOSURE TO MANGANESE

As noted above, Mn intoxication generally is considered to be an occupational disorder confined to individuals mining the metal or those individuals working at or near industrial settings in which Mn is used in production or processing of other materials. Thus, welders and those working in or living near ferroalloy processing plants are at particular risk for developing manganism. The common feature in each of these exposure settings is elevated atmospheric levels of the metal that initially enter the body via the inspired ambient air. The Occupational Safety and Health Administration (OSHA) has set the permissible occupational airborne exposure limit (PEL) to 5 mg/mm³ based on an eight-hour total weighted average exposure, whereas the current threshold limit value

(TLV) in the U.S. is set at 0.2 mg/mm³. There is strong sentiment, however, in much of the scientific community that these concentrations are actually set too high, since there is the potential risk that these concentrations are sufficient to provoke neurological damage in particularly susceptible individuals (Roels et al., 1987).

This raises an important question as to how Mn in inspired air actually enters the body and ends up in the brain. There are three potentially important routes by which Mn can gain access to the body: 1) direct uptake into the CNS via transport into the olfactory or trigeminal presynaptic nerve endings located in the nasal mucosa and the subsequent retrograde axonal delivery directly into the CNS; 2) transport across the pulmonary epithelial lining and its subsequent deposition into lymph or blood; and/or 3) mucociliary elevator clearance from the lung and the subsequent ingestion of the metal in the gastrointestinal tract. The relative contribution of each of these to increasing the body burden of Mn is difficult to determine, although chances are that all three components factor into the final accumulated toxic levels observed.

Since the lung is the first organ exposed to Mn, the events that take place within the respiratory tract will determine, without question, its biological availability. Deposition within the pulmonary airway of the inspired particulates containing Mn is dependent on the size, mass, and density of the particle. In general, ultrafine particles that are breathed in are rapidly exhaled without being deposited in the lung, whereas particles ranging from 0.02 to 1 μ are preferentially deposited in the lower airway (Pityn et al, 1989; Yu et al, 2000). Larger particles, (> 5 μ) in general, fail to penetrate the smaller branches of the respiratory tract and are mainly deposited in the upper airway. In addition to the site of deposition, several other factors ultimately govern biological availability of inspired Mn and include the composition of Mn within the particulate matter, the time of exposure within the lung, and how efficiently the metal is solubilized by macrophage within the lung.

The first potential site for entry of inspired Mn into the body is a relatively unique process that allows for the direct transport of Mn into the brain, bypassing the blood-brain barrier. Numerous studies confirm that Mn is readily taken up within the nasal cavity in presynaptic nerve endings of axonal projections leading from the olfactory (Tjalve and Henriksson, 1999; Vitarella et al., 2000; Fechter et al., 2002; Dobson et al., 2003; Normandin et al., 2004; Dorman et al., 2002) and trigeminal nerves (Lewis et al., 2005). Mn that is taken up into these axons undergoes retrograde transport up the axons to the perikarya of the respective neurons within the CNS, where it is subsequently released into the interstitial space. The amount of Mn that is transported into brain via this route is dependent on its composition in the inhaled air with the more soluble forms being more efficiently taken up via this process. There also is evidence that extremely small ultrafine particles can be taken up by the nerve endings and subsequently delivered to the CNS via a similar retrograde transport process (Oberdorster et al., 2004). The majority of the studies examining this process have utilized the rat as a model, and therefore, the significance of this delivery system in humans should be viewed with some caution since the rat may not be a suitable paradigm for comparison in humans. The caveat lies in the fact that the nasal surface of the rat is almost 20 times greater than that for the human, and thus, the overall contribution of neuronal uptake process in primates may likely be attenuated.

The second process responsible for transport of Mn, and that which probably represents the major factor contributing to its uptake, is transport across the pulmonary epithelial lining. Mn that is taken into the lung from inspired air is probably a complex mixture of soluble and insoluble Mn oxides along with aggregates of other metals. The particulate matter deposited in the lung is normally cleared by a process involving engulfment and the subsequent solubilization by pulmonary macrophage (Antilla, 1986; Lehnert, 1992; Antonini et al., 2004). Pulmonary macrophages

represent approximately 3-5% of all cells in the alveolar region (Lehnert, 1992), and the fundamental process of metal solubilization is facilitated by the acidic environment within the macrophage (Kreyling, 1992; Heilmann et al., 1992; Lundborg et al., 1992, 1995). Once solubilized, one of two things can happen: either Mn or some other potentially cytotoxic component within the particulate can provoke degeneration of the pulmonary macrophage (Antonini et al., 1999; Wesselius et al., 1999) or a specific transport processes can expel the solubilized metal back into the pulmonary fluid. Particulates that are liberated intact can again be rephagocitized, and the process of resolubilization will be resumed. The solubilized metal that is released is presumably transported across the epithelial lining of the lung by a transferrin-independent or transferrin-dependent process that is subject to the valence state of solubilized Mn. If Mn^{+3} is released, it will bind avidly to transferrin present within the pulmonary fluid, whereas Mn^{+2} will bind to other proteins such as albumin (Wang et al., 2002; Yang et al., 2002). The transport proteins, divalent metal transporter 1 (DMT1) and ferroportin, are both present within the alveolar epithelial cells lining the pulmonary/blood partition, presumably to aid in the transport of metals across the alveolar cells for deposition in the lymphatic system or directly into blood (Wang et al., 2002; Yang et al., 2002). In addition to this carrier-mediated system, there is also the possibility that Mn, which is complexed to transferrin in the pulmonary fluid, can be directly transported across the epithelium by the process of transcytosis, as recently reported for the GCSF-transferrin complex (Widera et al., 2003a,b). In this case, the Mn-TfR complex, located within the endosomal or pinocytotic vesicles that are formed, transverses directly from the apical-to-basolateral surface of the alveolar cell lining the pulmonary/blood interface. It also is feasible that over an extended period of time the accumulated Mn and other noxious components of particulates can lead to disruption of the epithelial cells, allowing the direct access of the metals into the

circulatory system (Pascal and Tessier, 2004). It is difficult to quantitate the extent to which pulmonary uptake contributes to the overall increase of Mn in blood and brain seen in miners and welders, but it is likely to represent the major route for entry of metals into the body. It is important to realize the fact that much of the Mn that accumulates in the lung persists for extended periods of time, having a biphasic half-life in the lung of 2 and 125 days (Kalliomaki et al., 1983), thus allowing the biological systems that are in place to eventually solubilize the metal within the particulates, thus favoring its entry into the fluids of the body.

The remaining Mn within the pulmonary fluid, whether it represents particulate or solubilized Mn that is bound to proteins or present in macrophage, will undergo mucocilliary escalator transport up the bronchioles, where it is eventually ingested and enters the digestive system. It is estimated, at least in the rat, that approximately 2.4% of the alveolar macrophage are transported to the upper respiratory tract daily (Fritsch and Masse, 1992). The fate of Mn ingested via the mucocilliary escalator likely will behave within the intestinal tract in a similar fashion as dietary Mn. The stomach, being approximately pH 2, readily dissolves complexed metal allowing for its subsequent uptake within the intestinal tract. The emptying time for the stomach varies considerably from 1 to 5 hrs and is largely dependent on content of the stomach. Thus, the extent to which Mn is solubilized within the stomach is a function of its concentration and composition as well as the presence of other ingested foods, which directly impacts on emptying time. Solubilized Mn that is released from the stomach into the duodenum is transported across the microvilli into blood via the transport protein, DMT1 and ferroportin (Cannon-Hergaux et al., 1999; Trinder et al., 2000; Knopfel et al., 2005). The percentage for Mn entering the intestines from mucocilliary escalator is not known nor is it known what percentage is actually absorbed into the body via this route. Assuming it behaves like dietary Mn, we

anticipate uptake to only be around 5% suggesting that a small percentage of Mn from inspired air actually will enter the blood stream via the gastrointestinal tract (Davidsson et al., 1989; Johnson et al., 1991).

Although the mechanism responsible for the delivery of Mn from inspired air into the body is still under investigation, the literature is unequivocal about the fact that the levels of Mn are elevated significantly in blood and brains of individuals exposed to high levels of Mn in the environmental workplace. Regardless of the route of entry, its prevalence is brought about by its protracted accumulation on a daily basis to high levels of the metal in particulate matter. The accumulated Mn in the lung more than likely reaches a steady state in which increased stores are met with an equal rate of elimination ultimately leading to enhance uptake and the creation of noxious levels within the body.

BLOOD – BRAIN BARRIER

Other than the direct uptake of Mn via the olfactory or trigeminal nerves, Mn must cross the blood-brain barrier to get into the brain. Unfortunately, the mechanism for transport of Mn across the endothelial cells lining the cerebral vasculature has not been identified satisfactorily, as there is considerable conflicting data in the literature as to the actual biochemical processes involved. Substantial evidence has been presented in recent years indicating that Mn is transported into these cells via DMT1, in a transferrin-dependent or possibly a transferrin-independent process (Burdo et al., 2001, 2004; Zheng et al., 2003; Wu et al., 2004; Roth and Garrick, 2003). Arguments in favor of DMT1 being the major transport protein responsible for Mn uptake is based on several observations including the fact that DMT1 is present in the vasculature wall along with the transferrin receptor (Burdo et al., 2001) and ferroportin 1 (Wu et al., 2004), the protein assumed to be involved in export of Mn out of the vascular endothelial cell. DMT1 expression has been

reported to be regulated by iron status with low iron increasing DMT1 expression (Oates et al., 2000; Moos et al., 2002; Erikson et al. 2004). Several studies have reported that Mn accumulation in the CNS is, in fact, elevated during iron deficiency, supporting the hypothesis that DMT1 is involved in this uptake process (Erikson et al. 2002, 2004). In addition, the Belgrade rat, which has a mutation in DMT1 that essentially makes it inactive, has decreased brain levels of Mn and other divalent metals known to be transported by this protein (Burdo et al., 2001). The major arguments against DMT1 playing a role in Mn transport into the CNS come from the studies of Moos and Morgan (2004), Takeda et al. (2000) and Yokel and Crossgrove (2004). Their findings reveal that the Mn/transferrin-complex is not the most favorable species of the metal capable of crossing the blood-brain barrier and that DMT1 is not present in capillary endothelial cells. Yokel and Crossgrove (2004) have further demonstrated that the neutral pH optimum observed for Mn transport into the CNS is inconsistent with the expected acidic pH optimum found for DMT1-mediated uptake. These investigators propose that store-operated Ca channels, as well as other mechanisms, are responsible for the transport of Mn across the blood-brain barrier (Crossgrove and Yokel, 2005). The evidence against a transferrin-DMT1-dependent process, however, is not sufficient to exclude the possibility that DMT1 plays a role in Mn transport across the blood brain barrier. The studies of both Takeda et al., (2000) and Yokel and Crossgrove (2004) may be explained by the fact that transferrin-dependent transport into the endothelial cell may not be the rate-limiting step for Mn transfer into the CNS. Instead, we also have to consider the possibility that transport of Mn out of the cell may be rate limiting, and this may occur at optimal pH near neutrality. This suggestion recently has been introduced for Fe transport across enterocytes cells (Beutler, 2004). It is also feasible that Mn can cross the blood-brain barrier complexed to transferrin by a process involving transcytosis (Fenart and

Cecchelli, 2003; Moroo et al., 2003). Obviously, further studies are necessary to adequately characterize the specific mechanisms responsible for the transport of Mn across the cerebral vasculature.

MANGANESE HOMEOSTASIS: NUTRITIONAL REQUIREMENTS, BLOOD SPECIATION AND LIVER

As noted above, Mn is an essential trace mineral required for normal growth and development. Deficiency of Mn is extremely rare in the adult, although neurological and behavioral deficits as well as other abnormalities have been observed during development (Hurley, 1981; World Health Organization, Environmental Health Criteria, 1981; Keen et al. 1999; Grieger, 1999). The total body burden of Mn for the standard 70 kg man is estimated to be approximately 10 to 20 mg of which 25 to 40% is present in bone. Concentrations in most adult tissues range between 3 and 20 μM .

Other than occupational exposures, the major source of Mn is from our diet. Foods that are particularly rich in Mn include cereal, vegetables, fruits, nuts, spices and beverages such as wine, tea and coffee (Wenlock et al., 1979; Gillies and Birkbeck, 1983). The ubiquitous presence of Mn in a variety of foods accounts for the fact that Mn deficiency in the adult is practically nonexistent. The average daily intake is estimated to be between 3 and 9 mg/day which is greater than the recommended daily allowance of 2.3 mg/day set by the FDA in the U.S. Overdoses from dietary intake of Mn are unlikely, as approximately 5% of the Mn ingested actually ends up in the body via the intestines (Davidsson et al., 1989; Johnson et al., 1991).

The ionic species of Mn that enters the blood stream from the intestines is not known but will have a major impact on the fate of Mn retained in the body. As noted above, the liver plays a vital role in regulating Mn levels in vivo principally because it is the major route for its elimination. Hepatic elimination of Mn, however, is greatly dependent on the Mn-protein complex present in serum. Mn^{+3} ,

the predominant form in blood, is bound tightly to transferrin (Gibbons et al., 1976) and, under normal conditions, is not in direct competition with bound Fe^{+3} , since only 30% of the available binding sites on transferrin are typically bound by iron. This raises an interesting question as to the fate of Mn in blood in conditions such as sickle cell anemia or hemochromatosis where transferrin may be totally saturated with iron. Mn^{+3} has a high affinity for transferrin (Gibbons et al., 1976), but its ability to compete with iron under these conditions has not been examined. Because Mn^{+3} forms a stable complex with transferrin, hepatic removal is relatively slow and comparable to that for Tf- Fe^{+3} complex. Mn^{+2} in blood is preferentially bound to α_2 -macroglobulin, although because of the significantly greater abundance of albumin in serum, a large percentage also is presumed to be bound to this latter protein, as well. The ligand interaction between Mn^{+2} and serum proteins is rather weak accounting for the rapid hepatic elimination from blood. Unlike Fe^{+2} , oxidation of Mn^{+2} to Mn^{+3} does not occur spontaneously in vivo and probably requires the copper-containing enzyme, ceruloplasmin, or the equivalent to support this conversion. Importantly, Mn^{+2} is essentially the only oxidation state of Mn that is seen intracellularly.

Thus, by regulating the body burden of Mn, the liver can directly influence brain levels upon chronic exposure to abnormally high levels of the metal (Papavasiliou et al., 1966). Since welders and miners experience substantially elevated Mn blood and brain levels, the assumption must be made that, under these conditions, the liver is incapable of keeping up with the demands to maintain normal homeostatic concentrations. This may partially be explained by the fact that much of the Mn from inspired air that is transported across the pulmonary epithelium is absorbed into tissues prior to it ever being encountered by the liver.

Mn toxicity is not only restricted to clusters of occupational exposed workers but also is seen in individuals with cirrhosis of the liver and related hepatic dysfunction

(Krieger et al., 1995; Hauser et al., 1994, 1996; Spahr et al., 1996; Burkhard et al., 2003). These individuals express both elevated blood and brain levels of Mn and display many of the behavioral and neurological symptoms associated with this disorder. Based on T1-weighted MRI scans, Mn levels were elevated in both the globus pallidus and substantial nigra (Hauser et al., 1994). The observation of elevated Mn in patients with liver failure is not unexpected, given the fact that hepatic elimination of Mn is the normal route for its removal from the body and anything that disrupts normal hepatic function has the potential to provoke elevated systemic levels of the metal. A similar situation occurs for individuals receiving parenteral nutrition, since they also display classical symptoms of Mn overexposure (Reimund et al., 2000; Fok et al., 2001; Suzuki et al., 2003; Iinuma et al., 2003). Thus, when evaluating the contribution of the various factors that potentially influence Mn toxicity, we must not only include the different routes of delivery but also the ability of the liver to remove the various species of Mn that exist in blood.

TRANSPORT OF MN

Within the past decade, various transport mechanisms that are responsible for cellular uptake of Mn^{+2} and other divalent transition metals have been identified. These studies reveal that Mn is transported into cells via a mechanism similar to that for iron, as evidenced by the fact that both Mn and iron, as well as several other divalent transition metals, compete for uptake into a number of different cell systems (Gunshin et al., 1997; Roth et al., 2002, 2003). As illustrated in figure 1, there are two distinct but related mechanisms responsible for the transport of Mn and ferrous ion: a transferrin-dependent and a transferrin independent pathway. As illustrated, the transferrin dependent pathway proposes that the transferrin- Mn^{+3} complex initially binds to the transferrin receptor (TfR) on the cell surface similar to that which occurs for iron. After attachment of Tf to the TfR, endosomal vesicles that are

formed at the cell surface are internalized and subsequently acidified by a hydrogen ion ATPase pump causing release of the metal from the Tf/TfR complex. Mn^{+3} released into the vesicles is presumably reduced to Mn^{+2} although a specific reductase responsible for this reaction has not been identified. Mn^{+2} is subsequently transported across the endosomal membrane via the transport protein, divalent metal transporter 1 (DMT1; also named Nramp2, DCT1 or SLC11A2) (Gunshin et al., 1997; Fleming et al., 1997; Fleming et al., 1998). Since Mn^{+2} forms a relatively weak complex with either α_2 -macroglobulin or serum albumin, there is also the possibility that transport of Mn^{+2} released from this complex can be taken up directly at the cell surface by DMT1, independent of transferrin. Which of the two uptake mechanisms functionally predominates is likely to be cell specific and dependent on the presence of TfR on the cell surface. In addition to cellular uptake by DMT1, there is also evidence in the literature, as illustrated in figure 1, for transport of Mn via the voltage regulated and store-operated Ca^{+2} channels, as well as the ionotropic glutamate receptor Ca^{+2} channels (Lucaciu et al., 1997; Kannurpatti et al., 2000; Riccio et al., 2002). In each of these processes, Mn transport requires an external signal to initiate its entry into the cell such as membrane depolarization, agonist mediated depletion of Ca^{+2} stores, or glutamate attachment to its receptor, respectively. The extent to which these processes contribute to the overall cellular uptake of Mn is not known, however as noted above, recent evidence suggests that the store-operated Ca^{+2} channels may play an important role in the transport of Mn across the blood-brain barrier (Crossgrove and Yokel, 2005).

CYTOTOXIC MECHANISMS OF MANGANESE

Considerable progress has been made within the past decade regarding the mechanism by which Mn induces cell death. Both apoptosis and necrosis have been implicated in provoking Mn-induced cell toxicity with differences in the mechanisms reflecting

variation in the signaling processes and disposition of Mn in different cells. There is strong evidence that apoptosis contributes to Mn toxicity, since many of the classical markers and signaling pathways associated with programmed cell death are activated in cells treated with Mn. These include: increased TUNEL staining; internucleosomal DNA cleavage; activation of the JNK and p38 (stress-activated protein kinase); activation of caspase activity; cytochrome c release; and caspase-dependent cleavage of PARP (Desole et al., 1996, 1997; Hirata et al., 1998; Schrantz et al., 1999; Roth et al., 2000; Chun et al., 2001; Latchoumycandane et al., 2005). In addition, Bcl-2 overexpression is capable of preventing Mn-stimulated toxicity (Schrantz et al., 1999; Kitazawa et al., 2005).

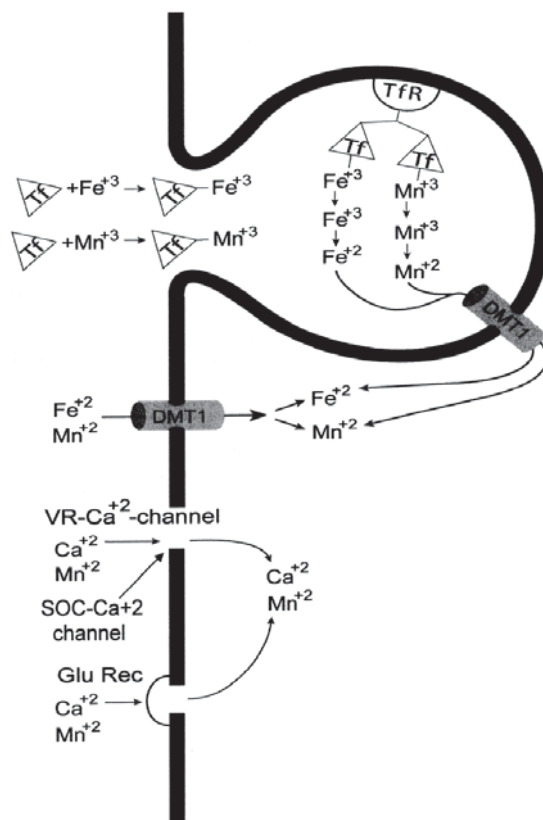


Figure 1. Transport mechanisms responsible for the uptake of manganese. Tf – transferrin; TfR transferrin receptor; DMT1 – divalent metal transporter 1; VR – voltage gated Ca^{+2} channel; SOC – store operated Ca^{+2} channel; Glu Rec – glutamic acid ionotropic receptor.

In contrast, other studies have suggested that Mn is, in fact, a potent antioxidant capable of suppressing oxidative-initiated events within the cell (Sziraki et al., 1995, 1998, 1999; Talavera et al., 1999; Roth et al., 2000). Studies in our laboratory have shown that Mn toxicity is probably not solely due to apoptosis, since inhibitors of several classic apoptotic markers fail to prevent cytotoxicity (Roth et al., 2000). For example, selective inhibitors of both the caspase family of proteases and the stress-activated protein kinase, p38, do not prevent Mn toxicity in PC12 cells, even though these apoptotic markers are stimulated upon treatment with Mn. Thus, other cytotoxic events must be responsible for the observed cell death initiated by Mn. Several studies have reported that Mn is capable of inducing mitochondrial membrane transients leading to disruption of mitochondrial function (Gavin et al., 1992, 1999). These investigators have proposed that Mn promotes calcium accumulation within the mitochondria by inhibiting both the sodium-dependent and sodium-independent exporter, resulting in the activation of the permeability transition pore (PTP) and loss of mitochondrial function. In addition, Mn also can interfere with oxidative phosphorylation by inhibiting both mitochondrial F1-ATPase (Gavin et al., 1992, 1999) and complex I (Galvani et al., 1995), causing a depletion of ATP (Brouillet et al., 1993; Roth et al., 2000; Chen and Liao, 2002; Zhang et al., 2005). Thus, either apoptosis or necrosis may lead to the ultimate demise of the cell integrity with the predominant cytotoxic pathway most likely being cell specific.

Because of the similarities between the neurological symptoms associated with manganism and Parkinson's disease, most research efforts in the past have focused on model cell systems in which dopamine is the principal neurotransmitter. Although considerable information has been profited from these studies, it is reasonable to assume that signaling events controlling Mn cytotoxicity may be considerably different than those provoking cell death within the neurons of the globus pallidus, since these neurons do not contain dopamine (Olanow

et al. 1996; Mergler and Baldwin, 1997). Pallidal neurons are GABAergic and receive glutaminergic input from the subthalamic nuclei. Because of this, there have been a number of studies that have attempted to examine the actions of the excitatory neurotransmitter, glutamate, on Mn-induced toxicity (Plenz et al., 1998; Rouse et al., 2000). Results of these studies implicate glutamate as a potential contributing factor to the toxicity of Mn within pallidal neurons and suggest that toxicity is not caused by any one factor but, instead, result from an amalgamation of several events occurring simultaneously, the center of which includes accumulation of glutamate in this area of the brain. These factors include: 1) excess accumulation of Mn in the globus pallidus; 2) Mn inhibition of glutamate transport leading to increase synaptic levels of glutamate (Hazell and Norenberg, 1997; Erikson and Aschner, 2002); and 3) increased uptake of Mn in pallidal neurons by activated glutamate channels (Kannurpatti et al., 2000). This hypothesis is supported by the studies of Brouillet and coworkers (1993) demonstrating that the noncompetitive NMDA receptor antagonist, MK-801, is capable of blocking lesions produced by intrastriatal injections of Mn.

CONCLUSIONS

The studies above attempt to summarize and clarify our basic knowledge as to the various factors that potentially influence the risks imposed from chronic exposure to high atmospheric levels of Mn. These studies endeavored to define the interrelationship of the different systems in the body that regulate Mn homeostasis by characterizing specific biological components involved in its systemic and cellular uptake, transport in blood and delivery to organelles, retention in the brain and other target organs, and its elimination from the body. Results from these studies have begun to define environmental and dietary conditions, such as iron deficiency, that alter the biological parameters that maintain normal iron balance and to explain

how these changes may result in adverse conditions that actually support and accelerate Mn toxicity. It is reasonable to hypothesize that genetic and environmental factors regulating susceptibility to develop Mn toxicity also may influence vulnerability to acquire Parkinson's disease, since physiological processes controlling the two disorders are multifaceted, contingent on the interdependence of many overlapping biochemical and molecular systems within the body. To fully understand these relationships, future studies will need to focus on genetic differences in the population that regulate vulnerability to develop Mn toxicity and that control the expressed symptoms in this disorder.

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