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Manganese in Health and Disease

From Transport to Neurotoxicity

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1. INTRODUCTION

Manganese (Mn) is a mineral that is required in small amounts to manufacture enzymes necessary for the metabolism of proteins and fats. A partial list of Mn-dependent enzyme families includes oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Mn is involved in the function of numerous organ systems and is needed for normal immune function, regulation of blood sugars, production of cellular energy, reproduction, digestion, and bone growth. Mn works with vitamin K to support clotting of the blood. As a vital component of superoxide dismutase (SOD), Mn has important antioxidant properties since MnSOD is one of the body's main frontline defense mechanisms against damaging free radicals.

A deficiency in Mn intake can retard growth, cause seizures, lead to poor bone formation, impair fertility, and cause birth defects. At the other spectrum, excessive exposure to Mn is associated with an irreversible brain disease with prominent psychological and neurological disturbances, characterized initially by a psychiatric disorder (*locura manganica*) that closely resembles schizophrenia. Symptoms include compulsive or violent behavior, emotional instability, as well as hallucinations. Subsequently, ataxia ensues followed by an extrapyramidal syndrome, resembling several clinical disorders collectively described as "extrapyramidal motor system dysfunction," and in particular, Parkinson's-like disease and dystonia. This condition known colloquially as manganism is associated with elevated brain levels of Mn, primarily in those areas known to contain high concentrations of nonheme iron (Fe), including the caudate-putamen, globus pallidus, substantia nigra, and subthalamic nuclei. The condition is also associated with decreased regional cerebral blood flow in the caudate and thalamus.

This chapter provides a synopsis of issues pertinent to Mn exposure and health effects. It will commence with a brief discussion on the pharmacokinetics of Mn, followed by its mechanisms of transport into the CNS, its primary target tissue site. In addition, the chapter will discuss the potential mechanisms of neurotoxicity that are associated with excessive Mn exposure.

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2. PHARMACOKINETICS

2.1. *Exposure Sources*

Mn comprises approx 0.1% of the earth's crust and is readily found in soil, air, water, and food. In nonoccupationally exposed individuals, the major route of exposure is via food ingestion. Mn is an essential element, with average intake between 2–9 mg/d (for an average 70-kg person). Foods high in Mn include avocados, blueberries, nuts and seeds, seaweed, egg yolks, whole grains, legumes, dried peas, teas, and green leafy vegetables. The typical English winter diet (with substantial tea intake) provides up to 8.8 mg of Mn/d, while studies of women in Japan, Canada, New Zealand, and the United States suggest average daily intakes from 2.5–4 mg/d.

There are relatively few reports of Mn toxicity arising from water or dietary sources. Water Mn concentrations typically range from 1–100 µg/L with most values being below 10 µg/L. In 1941, Kawamura and coworkers *(1)* reported an outbreak of Mn toxicity in Hiratsuka, Japan due to the consumption of water from several wells heavily contaminated with Mn (~14 mg/L) from discarded dry-cell batteries. In contrast to most cases of airborne Mn toxicity, the outbreak described by Kawamura and coworkers occurred after only a relatively short exposure to the Mn-contaminated drinking water. In general, milk is low in Mn with levels found in human and cow's milk ranging from 3–10 to 30–50 µg/L, respectively *(1a)*.

Human health risks have been associated with exposure to organic Mn-containing pesticides, such as manganese ethylene-bis-dithiocarbamate *(2)*. In addition to occupational exposure, a common source of Mn is found in the street drug "Bazooka." It is a cocaine-based drug contaminated with Mn-carbonate from free-base preparation methods *(3)*. Mn is also found in the ambient air *(4)*. The main sources are industrial emissions associated with ferroalloy production, iron and steel foundries, and power plant and coke-oven combustion emissions. Wind erosion of dusts and soils is another important source of atmospheric Mn. Manganism primarily arises in humans as a consequence of the chronic inhalation of high amounts of respirable airborne Mn (>5 mg/m^3) as may occur in Mn mining, steel manufacturing, or welding *(5–7)*.

Mn is also present in methylcyclopentadienyl manganese tricarbonyl (MMT); an octane-enhancing fuel additive used in unleaded automotive gasoline. Since MMT is extremely unstable in light and rapidly degrades in air, exposure to its combustion products is of special concern. The combustion of MMT by the automobile engine results in the formation of a complex mixture of Mn salts. Different MMT combustion products are produced depending on the fuel composition and engine and catalytic converter thermodynamics. Modern automobiles equipped with catalytic converters emit Mn primarily in the phosphate form, although smaller amounts of sulfates and oxides may also be discharged *(8,9)*. Using various environmental modeling approaches, it was estimated that air levels of Mn in most urban areas in the United States would increase less than 0.02 µg/m^3 if MMT were used in all unleaded gasoline *(8)*. Actual air Mn concentrations from Canadian cities in which MMT has been widely used for over 10 yr remain well below the current inhalation reference concentration (0.05 µg Mn/m^3) for respirable Mn set by the US Environmental Protection Agency (EPA) *(10–12)*.

2.2. *Route of Exposure as a Critical Determinant of Neurotoxicity*

The route of exposure can influence the distribution, metabolism, and neurotoxicity of Mn *(13,14)*. The oral route is considered to be less important for risk assessment purposes, since oral Mn is poorly absorbed from the gastrointestinal tract (net absorption < 5%), and brain and other tissue Mn levels remain relatively constant despite large fluctuations in oral Mn intake. In contrast, inhalation is more efficient than ingestion at delivering Mn to the brain. Pharmacokinetic factors that may contribute to the increased efficiency in brain Mn delivery observed after inhalation exposure include increased Mn absorption from the pulmonary tract and slower blood clearance of absorbed Mn *(13)*.

The liver plays a key role in maintaining normal organ Mn concentrations. Mn absorbed from the gastrointestinal tract is first transported to the liver where it is removed from the blood. Dose-dependent biliary excretion of divalent Mn serves to regulate the percentage of ingested Mn retained by the body and to limit increases in systemic tissue Mn concentrations. Liver Mn concentrations are commonly elevated following high-level oral exposure. In contrast, liver Mn concentrations often remain normal following Mn inhalation exposure. For example, Ulrich and coworkers *(15)* found no increases in rat or monkey liver Mn concentrations following subchronic inhalation exposure to Mn_3O_4 (11.6–1152 μg Mn/m^3, 24 h/d, for 9 mo). Similarly, Vitarella and coworkers *(16)* did not observe increased liver Mn concentrations in rats following short-term (2-wk) inhalation exposure to Mn phosphate. These investigators did, however, report increased fecal Mn elimination rates and enhanced whole-body Mn clearance suggesting that enhanced biliary excretion was occurring. Under certain exposure conditions, inhalation exposure to Mn may result in increased liver Mn concentrations. For example, Morganti and coworkers *(17a)* reported increased liver Mn concentrations in male Swiss mice following subchronic (16–32 wk) inhalation exposure to much higher Mn concentrations (49–85 mg MnO_2/m^3, 7 h/d).

Mn is capable of existing in a number of oxidation states, and Mn may undergo changes in valence states within the body. The valence of Mn in most enzymes is Mn^{+3} while most Mn taken into the body exists as either the Mn^{+2} or Mn^{+4} form. In vitro, ceruloplasmin can oxidize Mn^{+2} to Mn^{+3}. Mn oxidation can result in a shift of Mn binding from α_2-macroglobulin to transferrin. Clearance of Mn^{+2} bound to α_2-macroglobulin is more rapid than clearance of Mn^{+3} bound to transferrin *(17)*. The rate and extent of Mn oxidation or reduction in the body and associated protein binding is a key determinant of Mn retention in the body and brain Mn delivery *(18)*. Depending on the liver's ability to excrete excess Mn, exposure may or may not result in elevated liver concentrations of manganese. Chronic liver disease can interfere with first-pass biliary excretion of Mn in two ways: first, through cholestasis, and second, by portacaval shunting. Some patients with chronic liver disease with no unusual Mn oral intake or inhalation exposure were observed to have pallidal MR T1-weighted hyperintensities and increased sleep disturbance correlated with elevated erythrocyte Mn concentrations *(19)*. Additionally, Mn exposure can exacerbate liver dysfunction: an iv bolus of bilirubin followed by Mn causes cholestasis in rats *(20)*. Therefore, liver function is an important consideration in the monitoring and treatment of individuals possibly affected by Mn exposure.

2.3. Species and Age-Related Differences in Manganese Pharmacokinetics and Neurotoxicity

A substantial literature exists regarding significant species differences in the neurotoxicity of Mn. Therefore, extrapolation among Mn-induced effects in rats, nonhuman primates, and human beings must be made with caution. The use of T1-weighted magnetic resonance imaging (MRI) techniques has revealed that similar to human beings, macaques given high doses of Mn develop elevated brain Mn concentrations localized to the striatum, globus pallidus, and substantia nigra *(21,22)*. Furthermore, Mn-exposed monkeys have reduced levels of striatal and pallidal dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) and demonstrate dopaminergic neuronal losses analogous to Mn-poisoned humans *(23)*. Monkeys also develop gait and other motor abnormalities that mimic those observed in affected human beings. In contrast, rats do not demonstrate selective Mn accumulation in the striatum *(24)*. Only a limited number of studies have been conducted that evaluated the effect of Mn on neonatal or adult rat behavior. None of these studies, however, confirmed development of a behavioral syndrome comparable to that seen in Mn-poisoned humans and monkeys *(25)*.

The human substantia nigra becomes depigmented as the result of Mn neurotoxicity. One possible explanation for this selective depigmentation is the observation that Mn has a high affinity for neuromelanin and that its deposition is highest in melanin-containing tissues *(26)*. The interplay between Mn and melanin may also explain some of the known species differences in Mn neurotoxicity. Neuromelanin levels increase considerably with higher phylogeny, and brain levels are highest in primate brain regions where the dopaminergic pathways are most active. Rats, including pigmented strains, have extremely low levels of substantia nigra neuromelanin and thus may not demonstrate the same patterns of Mn accumulation or extrapyramidal symptomatology.

A wealth of evidence indicates that neonates are more susceptible than adults to many neurotoxicants. Neonatal rats appear to be at an increased risk for Mn-induced neurotoxicity due to their ability to develop higher brain Mn levels and more pronounced brain pathology than do adults in the face of equivalent or lesser Mn exposures *(27,28)*. Factors influencing this increased susceptibility include increased Mn absorption from the gastrointestinal tract, an incompletely formed blood-brain barrier (BBB), and the virtual absence of biliary Mn excretory mechanisms until weaning. The brain takes up a significant proportion of the Mn retained during the early neonatal period. For example, Keen and coworkers *(1a)* report that approx 8% of the total oral Mn dose is retained, in rats, by the brain. In addition, Mn crosses the placental barrier and accumulates in the brain following gestational exposure *(29)*.

2.4. Distribution to Other Tissues

In humans, most tissue Mn concentrations range between 0.1 and 1 μg Mn/g wet weight. Tissues with high Mn concentrations include the liver, pancreas, and kidney. Lung Mn concentrations demonstrate dose- and time-dependent increases following inhalation exposure. Plasma and red blood cell samples typically have low Mn concentrations normally and even following short-term Mn exposure. The bone can account for up to 25% of the total Mn found in the body. In addition to its role in normal osteoblast metabolism, bone also effectively sequesters Mn. Dramatic elevations in bone Mn concentration may occur following excessive Mn exposure. For example, an

80–90-fold increase in bone Mn concentration is observed in Sprague-Dawley rats given intraperitoneal injections of 11 mg $MnCl_2 \cdot 4H_2O$/kg/d for 1 mo *(30)*. Animals given high levels of Mn also had decreased zinc and magnesium bone concentrations, suggesting that Mn interacts with other trace minerals critical for bone formation. When compared to liver, kidney, and other organs that eliminate Mn quickly (half-life 10–15 d), bone demonstrates prolonged retention of elimination half-lives > 50 d *(31)*. Another tissue showing prolonged Mn retention is the brain where an apparent half life of elimination is estimated to be on the order of 50–75 d *(32,33)*. Oral supplementation with a combination of Ca, Mn, Cu, and Zn has been shown to reduce loss of spinal bone-mineral density in postmenopausal women *(34)*. However, it has not been shown that Mn supplementation alone has this effect, so toxicity concerns should outweigh possible benefits of supplementation with Mn alone for this indication.

3. TRANSPORT OF MANGANESE INTO THE CENTRAL NERVOUS SYSTEM

3.1. *Olfactory Transport*

There is evidence that uptake of Mn into the brain may occur by direct olfactory axonal-transport mechanisms. Olfactory transport of Mn has been demonstrated to occur in the rat, mouse, and pike following intranasal instillation *(35,36)*, and in rats following inhalation exposure *(37)*. In rats, intranasal instillation of Mn has been shown to result in direct movement of Mn to the olfactory bulb and the telencephalon via transport in secondary olfactory neurons. Once in the brain, Mn can continue to move across synaptic connections between neuronal-cell bodies and continue to be transported along their cell processes to sites distantly connected to the olfactory pathway *(36)*. It is this neuronal-transport characteristic of Mn that has led to its use as a neuronal tracer *(38,39)*. This route has been shown to be a rapid route of transport of Mn to brain structures in the olfactory pathway, but a slow route of delivery to the rat striatum.

Several observations indicate that Mn undergoes active axonal transport. Mn transport to the olfactory bulb demonstrates concentration-dependent and saturable transport kinetics *(40)*. Mn transport kinetics is altered following colchicine treatment suggested that the metal uses microtubule-associated fast axonal transport *(41)*. The maximal transport velocity determined for ^{54}Mn in pike exposed to the chloride salt was determined to be approx 70 mm/d which is consistent with a fast axonal-transport system *(36)*. In mammals, fast axonal transport occurs at a rate of approx 200–400 mm/d and is used by the neuron to transport organelles, lysosomes, nerve-growth factor, and selected small molecules.

Until recently, little was known regarding the olfactory transport of Mn following inhalation exposure. Brenneman and coworkers *(37)* conducted studies in rats using short-term (90-min) inhalation exposure to radiolabeled $^{54}MnCl_2$ aerosols (0.5 mg Mn/m^3). These investigators used an animal model in which one nostril was occluded thus preventing olfactory transport of Mn to one side of the rat brain. Interestingly, these investigators showed that the olfactory route contributed the majority (>90%) of the ^{54}Mn found in the olfactory pathway of the brain up to 8 d following acute inhalation exposure. Their conclusion was further supported by data using longer-term rat inhalation to a less soluble form of Mn *(16)*. In the Vitarella study *(16)*, rats were exposed

6-h/d for 7 d/wk (14 exposures) to Mn phosphate at 0, 0.03, 0.3, or 3 mg Mn/m^3. The Mn concentration achieved in the olfactory bulb following the end of this 2-wk inhalation exposure was significantly higher than that observed in either the striatum or cerebellum, lending credence to the direct olfactory-transport theory. These findings suggest that the olfactory route may indeed be a significant pathway by which Mn may gain access to the brain. However, neither of these inhalation studies clearly demonstrated that direct olfactory uptake contributes significantly to increased striatal Mn concentrations. It is possible that repeated exposure or a longer postexposure delay might be required for detection of Mn at a site so distant from the olfactory pathway.

The relevance of these findings to human Mn inhalation exposure and the risks for neurotoxicity are not known and are complicated by interspecies differences in nasal and brain anatomy and physiology. In the rat, the olfactory bulb accounts for a relatively large portion of the central nervous system (CNS), and the nasal olfactory mucosa covers approx 50% of the total nasal epithelium. These structures are proportionately smaller in humans, suggesting that this route of brain delivery may be less important in humans as compared to the rat. In addition, total airflow to the olfactory mucosa is much lower in humans than in rats. These differences likely predispose the rat, more so than humans, to olfactory deposition and potential olfactory transport of Mn. Additional research will be required to better clarify the potential significance of the olfactory route of delivery of Mn to the brain in humans exposed via inhalation.

3.2. *Brain Uptake of Manganese*

Mn^{2+} does not exhibit high affinity for any particular endogenous ligand. It has almost no tendency to complex with-SH groups or amines, and it does not possess much variation in its stability constants for endogenous complexing ligands ($\log_{10}k$ = 3, 4, 3, and 3, for glycine, cysteine, riboflavin, and guanosine, respectively, where k is the affinity constant). Approximately 80% of Mn in the plasma is bound to β_1-globulin and albumin *(42)*. A small fraction of Mn in plasma is in the trivalent oxidation state and bound to transferrin (Tf) *(43)*. At normal plasma Fe concentrations (0.9–2.8 µg/mL), Fe binding capacity (2.5–4 µg/mL), and Tf concentration (3 mg/mL, with 2 metal-ion-binding sites per molecule [M_r 77000], of which only 30% are occupied by Fe^{3+}), Tf has 50 µ mole/L of unoccupied Mn^{3+} binding sites, and has therefore been implicated as a potential transporter for Mn across membranes. It is noteworthy that Tf receptors have been localized on the surface of the cerebral capillaries *(44–46)* and that endocytosis of Tf in capillaries of the BBB has been noted *(46)*. Endocytosis of a Mn-Tf complex in cultured neuroblastoma cells (SHSY5Y) provides further support for receptor-mediated transport of Mn across membranes *(47)*.

The distribution of Tf receptors in the CNS vis-à-vis Mn accumulation is noteworthy. The thalamic nuclei, the pallidum, as well as the substantia nigra contain the highest brain Mn concentrations *(48)*, as well as appreciable levels of Fe *(49)*. The areas with dense Tf distribution *(50)* do not correspond to the distribution of brain Mn (or Fe). Yet, the fact that Mn-accumulates in brain areas that are efferent to high Tf-receptor density raises the intriguing possibility that Mn-rich brain sites accumulate Mn via axonal transport *(35,39,51)*. Indeed, the nucleus accumbens and the caudate-putamen—two areas that are abundantly rich in Tf receptors—provide efferent fibers to areas that are Mn rich, such as ventral-pallidum, globus pallidus, and substantia nigra *(52,53)*.

The competition between Fe and Mn for the same transport carrier is also noteworthy. For example, plasma Fe overload, significantly decreases the uptake of Mn across the BBB, whereas Fe deficiency is associated with increased CNS burden of Mn *(54–56)*. High dietary Fe intake reduces the concentration of Mn in the CNS *(57)*. In vivo, 6 h of intravenous administration of ferric-hydroxide dextran complex significantly inhibits Mn brain uptake as compared with its uptake in Fe-free dextran-treated rats *(55,56)*. In an additional study, the transport of Mn across the rat BBB was characterized by a single capillary-pass technique *(58)*. Initial rate measurements (at 15 s) of Mn accumulation in rat brains after intra-arterial injections indicated saturation kinetics. Common carotid injection of freshly mixed Mn^{2+} with Tf at a 1:10 molar ratio did not lead to a significant change in the initial rate of Mn brain levels compared with injection of Mn^{2+} alone. However, when Mn^{2+} was incubated at 25ºC in the presence of Tf at a 1:10 ratio for up to 5 d prior to common carotid injection, the initial rate of Mn uptake by brain was incubation-time-dependent, increasing linearly with prolonged incubations. These findings suggest that the saturable component of divalent Mn transport into brain represents but one of the transport mechanisms for Mn across the BBB, and that a second transport system for Mn may occur via a Tf-conjugated Mn-transport system *(58)*. The increase in initial rate of Mn uptake in samples equilibrated with excess Tf for days (therefore presumably oxidized to Mn^{3+} and complexed in an iron-binding site of Tf) probably reflects binding of MnTf to brain capillary endothelial TfR. However, the internalization of MnTf into brain-capillary epithelia has yet to be demonstrated.

Mn shares numerous similarities with Fe. For example: (1) Fe^{3+} and Mn^{2+} share a d5 electron configuration, allowing for both to adopt a wide variety of coordination geometries. (2) Both carry similar valence charges (2+ and 3+) in physiological conditions. (3) Both have similar ionic radius. (4) Both strongly bind Tf *(47,55,56,59)*. (5) Intracellularly, both preferentially accumulate in mitochondria *(60,61)*. (6) Both Fe- and Mn can catalyze autoxidation of dopamine in the presence of L-cysteine *(62)*. Given these similarities, it is not surprising that Mn (at least in high concentrations) can interfere with Fe-regulated processes, and in particular certain mitochondrial enzymes (aconitase, NADH-ubiquinone reductase, and succinate dehydrogenase) that require Fe as a cofactor in their active catalytic center.

A limited number of studies have addressed the transport kinetics of blood Mn into the CNS. Collectively, these studies suggest that Mn ($MnCl_2$) enters the brain from the blood either across the cerebral capillaries and/or the cerebrospinal fluid (CSF). At normal plasma concentrations, Mn enters the CNS primarily across the capillary endothelium, whereas at high plasma concentrations, transport across the choroid plexus predominates *(64,65)*. These findings are consistent with observations on the rapid appearance and persistent elevation of Mn in the choroid plexus *(66,67)*. Radioactive Mn injected into the blood stream is concentrated in the choroid plexus within 1 h after injection, and 3 d postinjection, Mn is localized to the rat dentate gyrus and CA3 of the hippocampus *(68)*.

3.3. *Choroid Plexus Transport of Manganese*

The choroid plexus is potentially an important site for the homeostasis of Mn. This structure is where ^{54}Mn from injected doses appears first in rodent brain *(41,69)*. The

regulation of substrate entry into the brain via choroid plexus synthesis of CSF is different from that at the BBB. Capillaries in the choroid plexus are fenestrated, and substances must first be taken up by choroid plexus epithelium. This epithelium then secretes CSF. Eventual neuronal uptake of substances from CSF must proceed via the cells of ependyma. It is thought that tanycytes of the ependyma may play an important role in delivery of nontransferrin bound iron to specific nuclei in the hypothalamus *(70)*. Further study is required on this potential mechanism of delivery of Fe and Mn to neurons.

3.4. *Transport of Manganese in a Hypotransferrinemic Mouse Model*

It has long been appreciated that total body and specifically brain Mn concentrations are elevated with decreased stores of Fe *(54,69,71,72)*. There is evidence for competition between Mn and Fe for intestinal absorption *(73,74)*, probably by way of the transporter DMT-1 (otherwise known as DCT-1 or nramp-2)*(75)*. However, brain Mn is elevated in some disorders of Fe overload, and transferrin receptor is elevated following Mn exposure *(63)*.

Because Mn^{3+} may be an important physiological oxidation state of this metal, and Mn^{3+} binds with reasonable affinity to Tf, we sought to explore the role of Tf in Mn transport. The hypotransferrinemic mouse (hpx/hpx) provides a unique model to study the role of transferrin in Mn transport in the brain. The hpx/hpx mouse is the result of a spontaneous mutation *(76)*, and has a mRNA splicing defect resulting in virtually no synthesis of Tf protein *(77)*.

No difference was found in total brain uptake of sq, ip, or iv injections of $^{59}FeCl_3$ or $^{54}MnCl_2$ between +/+ and hpx/hpx mice *(78–80)*. However, striking differences in regional distribution were noticed for ^{59}Fe, but only subtle differences were noticed in the case of ^{54}Mn *(80)*. This experiment demonstrated a striking effect of Tf on brain distribution of ^{59}Fe: in hpx/hpx mice, iron remained in the choroid plexus 7 d after an iv injection, whereas in +/+ mice Fe was distributed to hippocampus, thalamus, and striatum *(80)*. This observation is consistent with the Tf/TfR system being the predominant mechanism for Fe delivery to the brain, with a minor contribution coming from non-Tf-mediated Fe uptake by the choroid plexus.

Although one report exists of low Tf mRNA in human choroid plexus *(81)*, we hypothesize that choroid plexus synthesizes and secretes Tf to the CSF. Slightly lower levels of ^{54}Mn in cerebral cortex and corpus collosum were observed in the hpx/hpx mice relative to +/+ controls. These subtle differences may be due to differences in the brain development of the hpx/hpx mice *(78)* and not strictly a Tf effect. Clearly Tf is not required for parenchymal Mn delivery to the extent that it is for Fe, at least in the hypotransferrinemic mouse. The most parsimonious interpretation of these data is that Tf is required for delivery of Fe to brain parenchyma, but that the Tf/TfR system is less important for delivery of Mn to brain parenchyma in mice. The data suggest that non-Tf dependent mechanisms for Mn transport exist and that these mechanisms are unmasked in the hpx/hpx mutant.

At this writing, no unique mammalian transporters are known for Mn. It remains to be seen what role, if any, iron transporters play in Mn homeostasis. There has been an explosion in recent years in candidate genes implicated in regulating iron transport

(69,82). The near future may bring similar breakthroughs for the field of Mn neurotoxicity.

4. MECHANISMS OF MANGANESE NEUROTOXICITY

While many studies have suggested that industrial chemicals and pesticides may underlie idiopathic Parkinson's disease (IPD) *(2,7,83–87)*, its etiology remains elusive. Among the toxic metals, the relationship between Mn intoxication and IPD has long been recognized *(5,48,54,84,88–90)*. The neurological signs of manganism have received close attention because they resemble several clinical disorders collectively described as "extrapyramidal motor system dysfunction," and in particular, IPD and dystonia. Unlike Parkinsonism, manganism also produces dystonia, a neurological sign associated with damage to the globus pallidus *(21,91)*. A comprehensive survey of patients afflicted by PD or manganism concludes that although similar in many respects, there are distinct differences between the two neurological disorders. Similarities between PD and manganism include the presence of generalized bradykinesia and widespread rigidity. Dissimilarities between Parkinson's disease and manganism were also recognized, notably the following in manganism: (1) A less frequent resting tremor; (2) more frequent dystonia; (3) a particular propensity to fall backwards; (4) failure to achieve a sustained therapeutic response to levodopa; and (5) failure to detect a reduction in fluorodopa uptake by positron emission tomography (PET; for further details *see* ref. *21*). Given these differences, it has been proposed that Mn intoxication is associated with preservation of the nigrostriatal dopaminergic pathway, and that chronic Mn intoxication causes parkinsonism-like effects by damaging output pathways downstream of the nigrostriatal dopaminergic pathway *(21,91)*.

The literature is replete with a number of potential mechanisms for Mn-induced neurotoxicity. These include the following: (1) a direct toxic effect of Mn in its divalent oxidation state (or perhaps Mn in a higher oxidation state; *92,93,93a*) to dopamine-containing cells *(94)*; (2) a Mn-induced decrease in the content of peroxidase and catalase within the substantia nigra *(95)*; (3) production of superoxide (SO; $O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), or hydroxyl free ($^{\cdot}OH$) radicals by Mn, which in turn, "attack" dopamine, dopaminergic cells, and dopamine receptors *(96–99)*; (4) production of 6-hydroxydopamine or other toxic catecholamines by Mn^{2+} and a decrease in protective thiols (*96,100–102*); (5) auto-oxidation of dopamine, leading to formation of toxic (semi) quinones, concomitantly depleting tissue dopamine *(92,101,103–106)*; and (6) an excitotoxic mechanism in which the activation of glutamate-gated cation channels contributes to neuronal degeneration *(107)*.

As recently proposed by Verity *(108)*, understanding of the pathogenesis of Mn neurotoxicity likely will have to incorporate a number of considerations/mechanisms: (1) the factors controlling Mn^{2+} uptake and distribution into the brain; (2) account for the apparent selectivity of dopaminergic neurons. (3) account for the role of mitochondrial dysfunction; and (4) account for the role of oxidative injury in the genesis of toxicity. Mn-induced neurotoxicity is likely a multi-factor process that in addition to coincident transport disturbances of iron may also affect the transport of aluminum and perhaps other metals as well. As evident from the previous discussion on mechanisms of injury, the selectivity of dopaminergic neurons will also need to be considered.

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