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Review

Modulation of cholinergic systems by manganese

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Abstract

Information on changes in the central nervous system (CNS) cholinergic systems following exposure to manganese are considerably less extensive than that associated with other neurotransmitter systems. However, experimental and clinical evidence support the notion that cholinergic activity plays a key role in the pathophysiology of manganese-induced neurotoxicity. Manganese acts as a chemical stressor in cholinergic neurons in a region-specific manner causing breakdown of the cellular homeostatic mechanisms. In fact, a number of cholinergic synaptic mechanisms are putative targets for manganese activity: presynaptic choline uptake, quantal release of acetylcholine into the synaptic cleft, postsynaptic binding of acetylcholine to receptors and its synaptic degradation by acetylcholinesterase. Moreover, manganese significantly influences astrocytic choline transport systems and astrocytic acetylcholine-binding proteins. Thus, manganese exerts its effect on the highly dynamic reciprocal relationship between astrocytes and cholinergic neurons. Cholinergic afferents are crucial in the physiology of locomotion, cognition, emotion and behavioral response, and therefore, it is not surprising that the anatomical selectivity of most manganese-induced cholinergic effects is compatible with the clinical correlates of manganism, which involves impairment of emotional response, decline in higher cortical functions and movement disorder. Manganism, also referred to as Parkinson's-like disorder, is initially manifested by a neuropsychiatric syndrome (locura manganica), the most frequent symptoms and signs of which are compulsive behavior, emotional lability, visual hallucinations and flight of ideas, cognitive decline and memory loss. These signs and symptoms are followed by an extrapyramidal syndrome, which shares numerous clinical and pathophysiological characteristics with idiopathic Parkinson's disease (PD). This natural history of disease could be a clinical reflection of the preferential involvement of the cholinergic systems, initially in the septo-hippocampus and later in the basal ganglia. These observations highlight the importance of studying the role of the CNS cholinergic systems in manganese-mediated neurotoxicity.

Keywords: Manganese; Acetylcholine; Choline uptake; Acetyl cholinesterase; Muscarinic receptors; Parkinsonism

Contents

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1. Manganism—historical background

Neurotoxicity of low-level, long-term exposure to manganese (Mn) holds special clinical relevance in both occupational and environmental medicines. Clinically, manganism is a central nervous system disease which is manifested following exposure to high concentrations of manganese oxides, first described in the 19th century. However, after the turn of the 20th century, manganese compounds were still used as remedies for ''anemia, syphilis, scrofula, enlargement of spleen and chlorosis'' (Merck's Manual, 1899). Until the 1960s, most manganism cases were occupational and were diagnosed in miners. Later on, cases were described in workers engaged in the ferromanganese-alloy industry and the manufacturing of dry-cell batteries (NAS, 1973).

It was estimated that 68,000–185,000 workers in the US were being exposed to the potential health hazard caused by Mn and its compounds (Tanaka, 1994).

During the last three decades, welding has come gradually into focus as a high-risk occupational factor for the development of manganism (Bowler et al., 2003; Chandra et al., 1981; Racette et al., 2001, 2005). However, it must be acknowledged that not all studies have demonstrated such a linkage. A recent Swedish study (Fored et al., 2006) failed to find a relationship between welding and Parkinson's disease (PD). This was a record linkage study which, unfortunately, provided no information on individual welders' exposure to manganese and failed to account for potential confounding factors such as smoking (linked to reduced risk of PD). Most cases were identified through a hospital discharge registry, and only cases of PD serious enough to require hospitalization were ascertained. Thus, whether welding represents a major occupational risk factor for PD clearly requires additional well-designed studies.

2. Manganese—common sources and routes of exposure

In adults, dietary manganese concentrations influence the amount of manganese absorbed from the gastrointestinal (GI) tract and the amount eliminated in the bile. When dietary manganese levels are high, adaptive changes reduce GI absorption, enhance liver metabolism and increase its biliary excretion (Britton and Cotzias, 1966; Davis et al., 1993; Mahoney and Small, 1968; Malecki et al., 1999). Thus, despite large fluctuations in oral manganese intake, brain and other tissue manganese levels remain relatively constant, at least in healthy adults. Increased oral exposure to manganese leading to clinical signs and symptoms has been reported in human in association with maneb-adulterated food (Ferraz et al., 1988). Organ and systemic effects of manganese were also observed in an experimental study in rats fed with the manganese superoxide dismutase mimetic, EUK-8 (McDonald et al., 2003). Recent studies suggest that high levels of manganese in drinking water (>300 μg/L) are associated with reduced intellectual function in children (Bouchard et al., 2007; Wasserman et al., 2006). The current U.S. EPA drinking water health advisory standard for manganese is set at 300 μg Mn/L (US EPA, 2004). As a trace element and an essential co-factor for enzymes (e.g. hexokinase, superoxide dismutase and xanthine oxidase), manganese is an ingredient of hyperalimentation formulae (Hsieh et al., 2007).

The principal organic manganese compound is methylcyclopentadienyl manganese tricarbonyl (MMT), an anti-knock additive. Combustion of gasoline containing this additive releases submicron particles of Mn_3O_4, which may penetrate deeply into the bronchial areas of the respiratory system. High exposure to airborne manganese has been associated with severe neurotoxic effects (Iregren, 1999). It has been proposed that even showering could also contribute to manganese intake by inhalation of aerosols (Elsner and Spangler, 2005). Exposure to manganese through welding fumes is associated with more aerosolized and smaller (ultrafine) particles of manganese. Metal particles generated from experimental welding fume exposure have been shown to be taken up by olfactory neurons and transynaptically transported in the brain (Elder et al., 2006).

3. Manganism and locura manganica (manganese madness)—clinical description

Manganese, usually in the form of dust from mining or grinding operations, can cause a neurological syndrome encompassing a broad spectrum of neurological deficits. Neuropsychiatric syndrome following prolonged exposure to manganese has been extensively described (Kessler et al., 2003; Nelson et al., 1993; Rudell et al., 1985; Sjogren et al., 1996; Tanaka and Lieben, 1969; Whitlock et al., 1966). The medical term, *locura manganica* or manganese madness, was coined to characterize this initial neuropsychiatric syndrome in miners of manganese ores in Chile, Australia and Taiwan (Halliwell and Gutteridge, 2005). In the early stages of the disease, workers may complain of anorexia, lassitude and excessive tiredness, apathy, joint pains and muscular cramps. These nonspecific complaints are followed by signs and symptoms of organic psychosis including disorientation, impairment of memory and judgment, acute anxiety, emotional lability, compulsive behavior, flight of ideas, visual hallucinations, illusions and delusions. Psychomotor slowing and cognitive decline evolve later. This organic mental syndrome is usually followed by

disturbances of gait and excessive salivation, which are the first manifestations of a movement disorder, an extrapyramidal syndrome clinically resembling PD (Archibald and Arch, 1987; McMillan, 1999), with peculiar neurological features (Bleich et al., 1999). The organic psychosis frequently disappears when parkinsonian signs supervene. Manganism may be reversible if diagnosed and treated in its early stages. Development of extrapyramidal syndrome denotes permanent damage to the CNS.

Unlike the full-blown syndrome of manganism (Bleich et al., 1999), which is well characterized, little is known about the relationship between manganese and its early neuropsychological effects (Mergler, 1996; Mergler and Baldwin, 1997). Similarities between signs and symptoms of idiopathic PD and manganism include generalized bradykinesia and rigidity. Clinical differences include more frequent retropulsion and dystonia in PD patients, in contrast to more frequent anteropulsion in manganism. Often there is no intention tremor or resting tremor present in manganism as is the case in PD (Lee, 1987). Other common differences between idiopathic PD and manganism are the earlier age of onset in manganism and elevated manganese levels in the blood and urine of workers who are still being exposed. Also, little or no response to L-dopa and other anti-parkinsonian dopaminergic agents (e.g. amantadine) is observed in patients with manganism (Bowler et al., 2006; Feldman, 1999; Koller et al., 2004; Lu et al., 1994).

Magnetic resonance imaging (MRI) abnormalities have been observed in individuals with manganese intoxication, including those who have been exposed through mining and hyperalimentation. Manganese may cause increased T1-weighted signal hyperintensities in the globus pallidus, striatum and, to a lesser extent, the substantia nigra (Shinotoh et al., 1995; Dietz et al., 2001; Kim, 2004; Kim, 2006; Kim et al., 1999; Lucchini et al., 2000; Nelson et al., 1993), while PD affects mainly the substantia nigra pars compacta and does not cause MRI abnormalities. Autopsies have confirmed that the most extensive damage in manganese-exposed patients is observed in the striatum and the pallidum. The substantia nigra, which is the main site of cell damage observed in autopsies of PD patients, is less damaged by chronic exposure to manganese (Barceloux, 1999). Given the overall picture of the pathologic disruption in the globus pallidus with sparing of the dopaminergic nigrostriatal pathway (Normandin et al., 2004) and a specific clinical pattern, manganese-induced neurotoxicity appears to be a well-defined nosologic entity.

4. Relevant epidemiological studies

The environmental and occupational impacts of manganese exposure have become increasingly important. Recently, neurological diagnostics have been directed toward the early detection of symptoms and abortive clinical forms of manganism. In a recent study, 62 welders with clinical histories of exposure to manganese were compared to 46 matched regional controls. Mood disturbances including anxiety, depression and confusion showed very high odds ratios (estimated adjusted odd ratios were 16.9, 8.4 and 35.4, respectively). The scores of the Beck Anxiety Inventory (BAI) (Beck and Steer, 1993) indicated workers were significantly more anxious (Whites = 85%, non-Whites = 86%) than the norms. Although the neurological exams and the neuropsychological tests complemented one another, neuropsychological methods may be more sensitive in detecting early signs of manganism (Bowler et al., 2006).

The effects of manganese on higher brain functions have been studied in workers exposed to this metal in the ship and electrical industries in Poland (Sinczuk-Walczak et al., 2001). The study covered a selected group of 75 male workers including 62 welders and fitters, as well as 13 workers involved in battery production. Their mean employment duration was 17.5 years. The control group consisted of 62 men who were non-occupationally exposed to manganese, matched by sex, age and work shift distribution. Emotional irritability, memory impairment, concentration difficulties, sleepiness and limb paresthesia were the predominant clinical symptoms in the workers chronically exposed to manganese. Medical physical examinations provided no evidence for toxic encephalopathy or polyneuropathy. Generalized and paroxysmal changes were the most common abnormalities seen in electroencephalography. Visual evoked potentials indicated optic neuron lesions which could be related to cumulated exposure. These findings imply that long-term low-level manganese exposure induces subclinical effects on the nervous system (Sinczuk-Walczak et al., 2001).Long-term exposure of miners to exceedingly high levels of manganese oxides leads first to an increase in the activity of acetylcholinesterase (AChE) and then, correspondingly, a decline in acetylcholine (ACh). Upon exposure to a manganese concentration of 5.9–16.3 mg/m^3, the adaptation exhaustion reactions in the sympathoadrenal system and the cholinergic system develop 7–9 years earlier than with an exposure of only 0.4–2.8 mg/m^3 of manganese oxides. Moreover, the cholinergic system responds sooner than the sympathoadrenal system to manganese exposure—specifically about 10 years earlier under high concentrations of manganese, and 5–6 years earlier under lower concentrations. These changes are believed (by the same authors) to be the underlying reason why nervous system pathologies develop reaching their maximal incidence after 13 or more years of exposure to manganese and 21 or more years of exposure to manganese in concentrations exceeding the maximal permitted levels (MLPs) of 19.5–54- and 1.3–9.3-fold, respectively (Iashchenko, 1998). The link between continuous occupational exposure in metallurgy workers and cholinergic changes was examined in a clinical occupational study in a group of metallurgy workers producing iron–manganese alloys. Acetylcholinesterase activity in the red blood cells (RBC-AChE) of these workers was decreased as compared to RBC-AChE in the control group (Misiewicz and Karmolinski, 1992). The values of RBC-AChE serve in occupational medicine as a routine marker of exposure of workers to AChE inhibitors (e.g. for periodical screening of agricultural workers exposed to organophosphorous pesticides). Currently, the risks of low-level long-term exposure to manganese in both industrial and environmental settings are being evaluated with regard to the development of subclinical neuropsychological changes.

Therefore, the American Conference of Governmental and Industrial Hygienists lowered the TLV-TWA for manganese compounds and inorganic manganese compounds to 0.2 mg Mn/m^3 (Barceloux, 1999). A lower TLV of 0.03 mg Mn/m^3 was proposed in 2003 (Bowler et al., 2006).

5. The possible role of cholinergic systems in manganese neurotoxicity

The cellular, intracellular and molecular mechanisms underlying neurotoxicity of manganese compounds are numerous, as manganese impacts many biological activities depending on levels and routes of exposure, dosage, age of the exposed individual and duration of exposure (Villalobos et al., 1994). Many of these mechanisms are not well understood. Most mechanisms of manganese neurotoxicity have been related to the brain dopaminergic systems (Eriksson et al., 1992). Free radicals generated by Mn^{2+} ions have been proposed to increase the oxidation of catecholamines with consequent oxidative stress and neurotoxicity (Villalobos et al., 2001). Mn^{2+} has also been shown to inhibit lipid peroxidation in the cellular membrane and acting as a scavenger of superoxide radicals produced during spontaneous dopamine auto-oxidation (Coassin et al., 1992). Manganese-induced changes have also been demonstrated in additional neurochemical systems: serotonin, GABA, norepinephrine and ACh. Manganese-induced changes have been described in the release of these neurotransmitters to the synaptic gap or their binding to the postsynaptic receptors (Bhargava, 1987; Bonilla and Diez-Ewald, 1974; Chandra et al., 1981; Villalobos et al., 1994). Indeed, some salient features of manganism, like the intensity of mood disturbances and the early timing of the intellectual decline (Bowler et al., 2006) cannot be explained by disruption of brain dopaminergic systems alone. The complex clinical picture and natural history of manganism may be consistent with the effects of manganese on additional neurochemical systems, first and foremost CNS cholinergic systems which are crucial in modulating emotional response, locomotion and higher cognitive functions. However, the role of cholinergic systems in the neurotoxicity of manganese and the potential of manganese to affect central cholinergic systems are not fully appreciated aspects of manganism. Most research on this topic has been focused on the involvement of dopaminergic and adrenergic systems, while relatively little attention has been given to involvement of cholinergic systems. Therefore, this paper is aimed to review the role of brain cholinergic systems in the toxicity of manganese. The next paragraphs correlate the anatomy and physiology of the cholinergic systems potentially involved in the manganese-induced impairment of emotional response, decline in higher cortical functions and movement disorder. This specific pattern of the effects of manganese on the brain cholinergic systems may be clinically reflected by the syndrome of manganism. Presentation of the clinical correlates of cholinergic neuroanatomy may help making the clinical signs and symptoms of manganism more comprehensible.

6. The cholinergic synapse

The activity of the cholinergic synapse is characterized by several dynamic mechanisms which can be measured quantitatively (Fig. 1). Choline is taken up by the presynaptic neuron via a Na^+-dependent high-affinity system (Fig. 1, point 1) (Jope, 1979). Within the neuron, acetylcholine is synthesized from choline and acetyl-coenzyme A (AcCoA) and is mediated by cholinacetyltransferase (ChAT) (Fig. 1, point 2). Acetylcholine is stored in the presynaptic vesicles and undergoes quantal release (Fig. 1, point 3) which depends upon the intensity of the depolarization (Deutch and Roth, 1990). Once released in the synaptic cleft, ACh diffuses, binds to pre- and postsynaptic muscarinic and nicotinic receptors (Fig. 1, point 5) and is hydrolyzed by cholinesterase (Fig. 1, point 4). In spite of acetylcholinesterase's efficiency, small amounts of ACh can be detected in the extracellular fluid even in the absence of cholinesterase inhibitors. Glial cells actively participate and

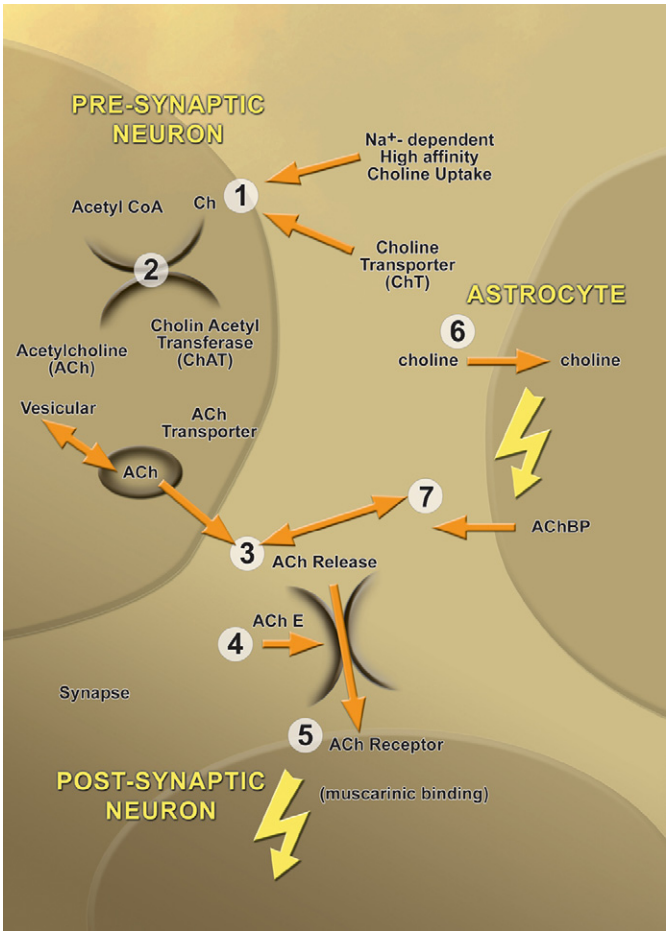

Fig. 1. The effect of manganese on cholinergic neurotransmission. Manganese exerts its effects along the chain of synaptic cholinergic activity: (1) high-affinity sodium-dependent choline uptake into the presynaptic cell; (2) acetylcholine (ACh) synthesis from choline and acetyl-coenzyme A (AcCoa), mediated by cholinacetyltransferase (ChAT); (3) ACh release into the synaptic gap; (4) ACh hydrolysis by acetylcholinesterase (AChE); (5) postsynaptic binding of ACh to muscarinic receptors; (6) high-affinity sodium-dependent choline uptake into the glial cell; (7) glia-derived soluble acetylcholine-binding protein (AChBP) release into the synaptic cleft.

modulate synaptic neurotransmission (Araque et al., 1999; Smit et al., 1991). Choline is taken up by glial cells in a Na^+-dependent high-affinity system (Fig. 1, point 6). Presynaptic release of ACh induces glial secretion of glia-derived soluble acetylcholine-binding protein (AChBP) into the synaptic cleft (Fig. 1, point 7), thus actively regulating cholinergic neurotransmission, producing AChBP and releasing it in response to ACh.

These cholinergic neuronal dynamics are actively involved in the behavioral response. The septo-hippocampal cholinergic synapse may be activated by different external inputs, serving as a final common pathway in the behavioral response (Finkelstein and Hod, 1991). This is characterized by a reduction in high-affinity choline uptake, increased release of newly synthesized acetylcholine into the synaptic gap and increased postsynaptic muscarinic-binding capacity in the rat hippocampus during periods of acute and chronic intermittent stress (Finkelstein et al., 1985). Hippocampal cholinergic changes are more pronounced in rat strains which are more reactive to stress (McCarty and Kopin, 1978).

7. Manganese, choline transporters and choline uptake at the blood–brain barrier

While manganese is able to bind to the choline transporter and inhibit choline uptake, there is no evidence of transport by this mechanism across the blood–brain barrier (BBB). However, the BBB is a possible mechanism of entry, considering that choline transporters have been shown to transport other compounds into lymphoblasts (Goldenberg and Begleiter, 1979) and into the CNS (Allen, unpublished data cited by Lockman et al., 2001). Furthermore, the choline transporter is not saturated at physiologic levels of plasma choline (Klein et al., 1990). A study evaluating cationic competition at the BBB choline transporter by physiologic ions indicates that this competition might occur with cationic metal compounds. To further characterize choline transport inhibition by Mn^{2+}, a stepwise addition of varying concentrations of $MnCl_2$ was added to the perfusion buffer, showing that Mn^{2+} inhibits choline uptake in the presence of physiologic cations, which also inhibit choline transport (e.g. Na^+ and K^+). These findings imply that manganese has at least equal or greater affinity for the choline transporter as other metals evaluated. Mn^{2+} may also inhibit choline permeability in the absence of physiologic inhibiting cations, suggesting that manganese interaction with the transporter is a result of the inhibition of choline binding and not an interaction with the other physiologic ions. Data suggests that a possible mechanism for divalent manganese brain entry occurs via the BBB choline transporter. There is significant reduction in choline uptake in the frontal and parietal lobes of the cerebral cortex, hippocampus, caudate and putamen (Lockman et al., 2001).

8. The effect of manganese on choline uptake

Choline transport across the BBB requires high- and low-affinity systems. Each mechanism of choline transport has a unique location and possesses specific characteristics, yet similarities do exist. Several studies have demonstrated cationic competition by divalent manganese in the high-affinity system. Using the *in situ* brain perfusion technique in rats, brain choline uptake was inhibited in the presence of Mn^{2+}. Furthermore, manganese caused significant regional choline uptake inhibition in the hippocampus, frontal and parietal cortices, the caudate and the putamen. These results suggest that choline uptake across the BBB is likely inhibited by Mn^{2+}. The acute effect of manganese is characterized by a mixed competitive–noncompetitive inhibition of synaptosomal Na^+-dependent choline uptake at different manganese concentrations (Eriksson et al., 1984).

Most of the experimental data in rodents were obtained after relatively short-term exposure to manganese – a few weeks – in most of the studies. Therefore, it is difficult to interpret these biological data or to establish their clinical relevance to the natural history of low-level long-term human exposure over many years. In an exceptional long-term study, the effects of manganese on choline uptake were measured in the rat brain following chronic and life-span (over 2 years) exposure to manganese in drinking water (Lai et al., 1984). Chronic manganese toxicity selectively affected the CNS cholinergic mechanisms, while its long-term effects on brain development and the aging of cholinergic systems were found to be different. During development, manganese treatment led to transient, age-dependent decreases in synaptosomal choline uptake in the hypothalamus but increased the synaptosomal choline uptake in the striatum (Lai et al., 1984). This observation may point to the differential effect of manganese on the locomotor extrapyramidal system.

As a result of chronic manganese treatment of rats from conception onwards, a decrease was observed in choline uptake by hypothalamic synaptosomes obtained from animals aged 70 to 90 days old. In manganese-treated rats aged 100–120 days old, the only difference observed was increased choline uptake by striatal synaptosomes. All comparisons were with age-matched controls (Lai et al., 1982). These observations, which are consistent with views of a dopaminergic and cholinergic involvement in manganese neurotoxicity, indicate that changes in these two neurotransmitter systems are observable in the locomotor extrapyramidal system only at specific stages of manganese intoxication.

The effects of Mn^{2+} on human erythrocyte choline transport, Na^+–K^+-ATPase, Ca^{2+}–Mg^{2+}-ATPase and intracellular K^+ levels were also examined. The concentrations used were below the levels which cause significant haemolysis (less than or equal to 300 μM). Mn^{2+} inhibited concentrative choline accumulation over a 3 h exposure period. The effects of Mn^{2+} on choline accumulation were reversed by removing the cations from the extracellular medium. Mn^{2+} also inhibited the efflux of choline. This inhibition of choline accumulation and efflux in erythrocytes by Mn^{2+} is not explicable solely in terms of either inhibition of Ca^{2+}–Mg^{2+}-ATPase or inhibition of Na^+–K^+-ATPase, which causes reduced intracellular K^{2+}. These findings are similar to those previously obtained using synaptosomes (King et al., 1983), demonstrating the ubiquity

of manganese-induced effects on choline uptake in different cells and organ systems.

9. The effect of manganese on ChAT activity

Cholineacetyltransferase (ChAT) is a specific enzyme of the cholinergic system and serves as a marker of cholinergic activity (Fig. 1, point 2). ChAT activity was not affected in any of the rat brain regions studied on the first, third and eighth month of continuous treatment with manganese chloride. From the first month, the manganese-treated rats ingested an amount of water significantly lower than controls (Martinez and Bonilla, 1981). In a long-term study, rats were chronically treated with manganese chloride from conception onward for a period of more than 2 years in order to study the effects of manganese and aging on the activity of choline acetyltransferase (ChAT) in the hypothalamus, cerebellum, pons and medulla, striatum, midbrain and cerebral cortex (which included the hippocampus). Manganese-treated 2-month-old and 24–28-month-old rats and age-matched controls were studied. Changes in ChAT activities during aging were observed only in the striatum and life-long treatment with manganese abolished partially these decreases in striatal ChAT activity (Lai et al., 1981). *In vivo* decrease of ChAT (in the striatum) is remarkable considering that ChAT is characterized by a high turnover rate (Jope, 1979). This finding may indicate the intensity of the manganese-induced cholinergic effect in the striatum, which plays a crucial role in the locomotor extrapyramidal system. The effect of manganese on ChAT activity during development is different. Treatment with manganese during development led to small decreases in ChAT activities in the cerebella and midbrains of 2-month-old rats (Lai et al., 1984). As ChAT serves as a specific marker of the cholinergic activity, these observations may point to a greater neurotoxic effect of manganese and to the greater vulnerability of cholinergic neurons in the developing brain.

10. The effect of manganese on ACh release

Manganese acts at presynaptic levels within the striatum by blocking release of the neurotransmitter (Fig. 1, point 3) thus creating a localized, relative deficit in caudate function.

The injection of manganese into the caudate nucleus of the rat brain resulted in a predominant ipsilateral turning behavior, accompanied at higher doses by an intermittent, alternating and dose-related incidence of contralateral turning and stereotypes. Tegmental serotonergic and intrastriatal cholinergic pathways were involved in the production of the basic postural asymmetry resulting in turning (Inoue et al., 1975). The amount of interference with the nigrostriatal and mesolimbic dopaminergic pathways may determine the concurrent inhibition of locomotion (Acquas and Chiara, 2001; Fenu et al., 2001). This impairment of postural asymmetry was more pronounced after bilateral injections of manganese into the caudate nucleus (Inoue et al., 1975). Most probably, these effects of manganese are partially mediated by intrastriatal cholinergic pathways. The effects of Mn^{2+} on quantal ACh release have been studied with conventional microelectrode techniques. Mn^{2+} led to increased miniature end-plate potential (MEPP) frequency at low levels. Stimulation of the motor nerve caused substantial increases in MEPP frequencies, with maximal frequency attained in the presence of Mn^{2+}; further stimulation led to a fall in frequency. Thus, Mn^{2+} is able to enter the nerve terminal through a voltage-gated channel. Once within the terminal, Mn^{2+} may stimulate quantal release by releasing Ca^{2+} within the terminal (Kita et al., 1981). Thus, manganese exerts its effect on the quantal release of Ach via divalent ion interactions. This effect of manganese was also studied in the peripheral nervous system. The effects of Mn^{2+} and the conditioning stimulus intervals on facilitation of acetylcholine release from parasympathetic nerve terminals were studied in quiescent guinea-pig auricles by electrophysiological methods. A maximum facilitation occurred at intervals of ~50 ms. The half-time of decay of facilitation after a conditioning stimulus was ~500 ms. When conditioning trains of stimuli were applied, a second, much longer-lasting component of facilitation was noted with a half-time of 4 s. Mn^{2+} initially exerted an inhibitory effect, followed later by an increase in ACh release, the development of which was dependent on frequent stimulation of the nerve fibers. This potentiation was accompanied by an apparent loss of facilitation. The decay to the control level displayed a relatively long half-time of ~20 min and may also be accelerated by frequent stimulation of the parasympathetic nerve fibers. It was suggested that Mn^{2+} ions not only inhibit a Ca^{2+} inward current, but may also act on intracellular Ca^{2+}-binding sites in the nerve terminal. When these sites are blocked, even a reduced Ca^{2+} influx can be more effective in the process of ACh release (Bechem et al., 1981).

In an earlier study of the cholinergic parasympathetic nervous system, the effects of changes in external Mn^{2+} concentrations and electrical stimulation on ACh release from Auerbach's myenteric plexus were measured. The responses of the longitudinal muscle were recorded in the presence of physiostigmine. Mn^{2+} in concentrations up to 125 μM depressed only ACh release, but not the contractile response (Cowie et al., 1978). The effect of Mn^{2+} on ACh release from vagus nerve terminals was studied in quiescent guinea-pig auricles. ACh release was induced by stimulus trains subthreshold for excitation of atrial cells. ACh release evoked hyperpolarization of the atrial cell membrane. Changes in ACh sensitivity of the atrial cell membrane were tested by the application of ACh-containing solutions. ACh release increased with the external Ca^{2+} cation concentration. Mn^{2+} cations strongly inhibited the stimulus evoked by ACh release (Glitsch and Pott, 1978). In a study on the cholinergic preganglionic component of the sympathetic nervous system, the enhancement of asynchronous muscarinic ganglionic firing following preganglionic nerve stimulus volley by high-frequency repetitive conditioning stimuli was studied in the isolated rat superior cervical ganglion. Muscarinic afterdischarge occurring in chlorisondamine-blocked ganglia was enhanced for up to 1 h after a 40 Hz conditioning volley lasting 7.5–30 s. Firing enhancement did not occur when ACh release was blocked by

$MgCl_2$ added to the saline during the conditioning period (McIsaac, 1978). The characteristics of the late response of the superior cervical dog ganglion were studied by close-arterial injection of catecholamines and divalent cations to the ganglion. $MnCl_2$ may inhibit ganglionic transmission by suppressing ACh release from presynaptic nerve terminals. The late response represents the late discharges of ganglion cells, which are very sensitive to inhibition by $MnCl_2$ (Chen, 1975).

The effects of Mn^{2+} cations and acidic pH on ACh release were studied in the frog neuromuscular junction, using intracellular recording techniques. Acidic pH reduced the amplitude of the end-plate potentials, resulting in a decrease in the number of quanta of ACh liberated by the nerve impulse. This effect of low pH was blocked by low concentrations of Mn^{2+}. These results indicate that H^+ and Mn^{2+} ions bind to an acidic site which regulates Ca^{2+}-mediated ACh release. It was suggested that protonation of the acidic site evoked transmitter release by blocking the influx of Ca^{2+} cations into the nerve terminal following the nerve action potential (Landau and Nachshen, 1975). The common denominator for most of these findings is the inhibitory effect of manganese on evoked ACh release in the presynaptic neuron.

11. The effect of manganese on AChE activity

Significant inhibition AChE activity (Fig. 1, point 4) was observed following lengthy periods of exposure to manganese. Life-long treatment with manganese partially abolished the aging-associated decrease in AChE activities in the hypothalamus, cerebellum and striatum (Lai et al., 1981). In another study, rats were chronically treated with manganese chloride from conception onward for a period of over 2 years. AChE activity was examined in the hypothalamus, cerebellum, pons and medulla, striatum, midbrain and cerebral cortex (which included the hippocampus). Manganese-treated 2-month-old and 24–28-month-old rats and age-matched controls were studied. In the control group, AChE activities decreased in all regions, particularly in the striatum, during aging. Following treatment with manganese chloride, AChE activity was not affected in any of the rat brain regions studied on the first, third and eighth month of treatment. Later on, AChE activity diminished in the caudate nucleus on the eighth month of treatment with manganese chloride but was not altered in any other region throughout the life-long study (Martinez and Bonilla, 1981). In a later study, AChE activities were measured in different brain regions of rats chronically treated with a high concentration of manganese chloride in drinking water throughout development until adulthood. Large increases in manganese accumulation were found in all brain regions of manganese-treated adult rats. AChE activities were increased in the striatum and cerebellum (Lai et al., 1992). However, earlier study of the same research group showed that treatment with manganese during development did not affect the regional distribution of AChE (Lai et al., 1984). Thus, manganese exerts different effects on AChE activity in different stages of the life cycle: AChE activity is unaffected by manganese during development, but increased in a long-term exposure to manganese throughout development until adulthood. During aging, the physiological decrease in AChE activity is abolished by manganese.

AChE was shown to be associated with increased oxidative and nitrosative stress, alterations in energy metabolism (Milatovic et al., 2006) and consequent degeneration by pyramidal neurons from the CA1 hippocampal region of rat brain. Ultimately, the additive or synergistic mechanisms of cellular disruption caused by manganese and other toxicants may lead to cellular dysfunction and cellular neurodegeneration (Gupta et al., 2007). Effects of manganese exposure on brain development in postnatal mice were also studied. In the Morris water maze test, the average latency for discovering the hidden platform was remarkably increased every day during the first 5 days in the high manganese-exposed group, but the latency for discovering the quadrant in which the hidden platform was located previously was reduced at the sixth day. In high manganese-exposed mice, the activity of AChE in the brain was decreased. The immunoreactivity of glial fibrillary acid protein (GFAP) and the average relative density of GFAP-positive products in the hippocampus (area CA3) of both the low and high manganese-exposed groups, especially in the high-dose manganese group, were significantly higher than those of the control group. The gain of body weight, brain weight and the ratio of brain weight to body weight in the high-dose manganese-exposed group was significantly decreased (Zhang et al., 2001).

12. Glia

Glial cells actively participate and modulate synaptic neurotransmission (Araque et al., 1999; Smit et al., 2001). Synaptic function basically involves bipartite synapses consisting of presynaptic and postsynaptic components and a synaptic cleft, in which a presynaptically released neurotransmitter binds to cognate receptors in the postsynaptic cell membrane. However, for several types of synapses, the glial cells complete a tripartite configuration, in which they can feed modulatory signals back to the neuronal synaptic components by releasing an endogenous neurotransmitter (Smit et al., 2001). The glia-derived soluble acetylcholine-binding protein (AChBP) is a naturally occurring analogue of the ligand-binding domains of the nicotinic acetylcholine receptors (nAChRs). Presynaptic release of acetylcholine induces the secretion of AChBP through the glial secretory pathway. Glial cells release AChBP in the synaptic cleft and actively regulate cholinergic transmission between neurons in the central nervous system. Glial cells may suppress cholinergic transmission, produce AChBP and release it in response to ACh. The release of this decoy glial receptor protein into the synaptic cleft provides a mechanism by which glial cells can modulate the efficacy of cholinergic transmission in the CNS (Smit et al., 2001).

Although, *in vitro*, manganese is capable of activating a number of enzymes, its low concentration within the CNS ($<10^{-5}$ M) is likely to preclude any *in vivo* function as an enzyme activator. A significant function of manganese within

the CNS is typified by the mitochondrial enzyme superoxide dismutase (SOD) that catalyzes the disproportionation of superoxide (O_2^-), the univalent reduction product of dioxygen. SOD functions in mitochondrial oxygen radical metabolism, catalyzing the formation of hydrogen peroxide from reactive oxygen species. Another manganoprotein is glutamine synthetase. Glutamate, an excitotoxic amino acid released into the synaptic cleft is carried by a high-affinity uptake system into astrocytes, where glutamine synthetase, an enzyme found exclusively in the astrocytes catalyzes its conversion to glutamine. Glutamine synthetase contains four Mn ions per octamer and accounts for approximately 80% of total Mn in brain. Accordingly, glia, and specifically astrocytes may be looked upon as a ''sink'' for CNS manganese.

Several studies showed specific modulation of the efficacy of cholinergic synaptic transmission by glial cells, which is apparent only after high-frequency stimulation of the presynaptic cell. When neurons were cultured in a triplet configuration in which the presynaptic neuron forms two synapses with its postsynaptic partners, only the synapse co-cultured with glia showed synaptic depression, leaving the non-glia-bearing synapse unaffected. As such, glial and neuronal cells can form integral modulatory components of synaptic function (Theodosis and Poulain, 1993). In the cholinergic neuromuscular junction, ACh released by activated perisynaptic glia (Jahromi et al., 1992; Robitaille et al., 1997) probably feeds back onto the presynaptic nerve terminal (Robitaille et al., 1997). Perisynaptic glial cells of a molluscan cholinergic synapse respond to ACh by releasing a soluble ACh-receptor-like protein into the synaptic cleft (Brejc et al., 2001). AChBP may act as a synaptic ACh buffer by capturing presynaptically released ACh, thus causing the immediate suppression of synaptic neurotransmission. The release of this decoy glial receptor protein into the synaptic cleft provides a mechanism by which glial cells can modulate the efficacy of neurotransmission in the CNS (Smit et al., 2001). The effect of manganese on AChBP has not yet been elucidated. The interaction between AChBP and ACh release into the synaptic gap plays a role in the chain of synaptic cholinergic activities. ACh release mechanisms are tightly linked to cognitive, behavioral and motor functions in CNS systems and may serve as a sensitive marker of overall cholinergic activity, as detailed above. Future studies are therefore required to investigate the effects which manganese might exert directly on AChBP and indirectly on ACh release via AChBP. Given the role of glia in modulating cholinergic activity in the synapse, and the effects of manganese on cholinergic activity (see above), studies on the interaction between manganese and cholinergic systems represent a worthy area for future research.

13. Possible implications of manganese-induced cholinergic changes on behavior and cognition

All these data provide evidence that manganese acts as a neurotoxicant on the cholinergic mechanisms at the anatomical sites which are involved in emotional response, modulation of behavior and various learning tasks. These cholinergic systems typically derive from small nuclei which provide diffuse projections to wide brain areas (Mesulam et al., 1983). The septo-hippocampal cholinergic system is an important part of a neuronal network in the brain which controls the physiological and behavioral response to stress (Finkelstein et al., 1985). This cholinergic pathway is the mainstay of mechanisms of adaptation, maintaining the affective balance and modulating the appropriate behavioral response to environmental stimuli (Finkelstein and Hod, 1991). Stressor stimuli, such as prolonged handling (Nilsson et al., 1990), immobilization (Finkelstein and Hod, 1991), and fear (Acquas et al., 1996) strongly activate the septo-hippocampal and frontal cortical cholinergic systems. Indeed, manganism at its initial stage of *lucura manganica* (manganese madness) is clinically characterized by impairment of adaptation, lack of affective balance and inappropriate behavioral response. As shown above, pertinent studies showed these systems to be affected by manganese. Therefore, the septo-hippocampal cholinergic system should be considered as a potential target organ for manganese-induced toxicity, clinically manifested by a specific neurobehavioral syndrome with prominent affective features.

Manganese may change the presynaptic activity in forebrain cholinergic neurons, as shown above. The cortical cholinergic input system is activated by projections of mesolimbic structures to the basal forebrain cholinergic system. In prefrontal regions, increases in cholinergic activity are thought to contribute to the activation of the anterior attention system and associated executive functions, particularly the top-down optimization of input processing in sensory regions (Sarter et al., 2006). Sustained cholinergic activation, demonstrated by the high levels of extracellular ACh observed in the behavioral paradigms, indicates that many behaviors occur within or require the facilitation provided by the cholinergic system for the normal operation of pertinent neuronal pathways (Pepeu and Giovannini, 2004). This activation appears to be diffused throughout the forebrain cholinergic network, possibly with different regional intensity (Inglis and Fibiger, 1995), and is a prerequisite of sustained attention (Sarter et al., 2006). In turn, sustained attention is the prerequisite of information acquisition, recall and correct responses to environmental stimuli. These observations are supported by studies with positron emission tomography in volunteers subjected to a visual working memory task in which cholinergic enhancement improves memory performance, likely by augmenting the selectivity of perceptual processing during encoding (Furey et al., 2000). Decline of these higher cortical functions is evident in dementing processes. Neuropsychological testing may detect cognitive deficits even at the earliest stages of exposure to manganese. These cognitive deficits include memory and intellectual loss (Feldman, 1999; Iregren, 1999; Tanner, 1992; Wennberg et al., 1991). Decreased visual reaction time, deficits in audio-verbal short-term memory and impaired hand–eye coordination were measurable on early testing of workers exposed to manganese (Mergler et al., 1994). Manganese-induced cortical cholinergic dysfunction is compatible with these cognitive deficits as well as with the full-blown dementia observed later in the clinical course of manganism.

14. The clinical significance of manganese-induced cholinergic effects

Integrating the above observations, it is evident that manganese exerts various effects in multiple sites within the cholinergic systems, both in the central and peripheral nervous systems. Manganese perturbs the CNS cholinergic mechanisms not only in the basal ganglia but also in cortical brain regions such as the hippocampus, frontal cortex and parietal cortex. Within the PNS, manganese exerts its effect on motor nerves and neuromuscular junctions. Manganese also affects both branches of the autonomic nervous system: the parasympathetic cholinergic branches (e.g. Auerbach's myenteric plexus) originating from the vagal nerve and the sympathetic branches originating from the hypothalamus (e.g. the presynaptic cholinergic synapse in the superior cervical ganglion). Thus, manganese changes the dynamics of the cholinergic systems, which are the mainstay of cognitive, emotional and motor activities. These neurochemical observations are compatible with the manganese-induced syndromes described in the literature. The time-honored clinical entities, ''manganism'' and ''locura manganica,'' comprise a wide array of cognitive, emotional and motor impairments. Thus, human exposure to manganese might cause CNS signs and symptoms manifested as movement disorder (extrapyramidal syndrome), affective disorder (mood instability) and behavioral changes.

Manganese-induced changes observed in the septo-hippocampal cholinergic system shed light on the pathophysiology of manganese-induced affective disorder (manganese madness). There exists a delicate balance between cholinergic activity (in the medial septum) and dopaminergic activity (in the lateral septum). Damage to the cholinergic neurons, as well as to serotoninergic and other interneurons, may interfere the cholinergic–dopaminergic balance, leading to instability of mood and even to a manic-depressive psychotic state. In a like manner, reported cognitive signs of inattention, poor concentration and slow mental rotation may be caused by manganese-induced damage to cholinergic pathways connecting the prefrontal and parietal cerebral cortices with other structures as described above. The posited hypothesis is consistent with numerous studies where changes in the cholinergic systems were accompanied by hyperactive behavior, suggesting that cholinergic mechanisms underlie specific information-processing abnormalities that occur in Attention Deficit Hyperactivity Disorder (ADHD) (Rowe and Hermens, 2006).

These observations are compatible with the dynamics of the septo-hippocampal cholinergic system during lengthy stress periods (Finkelstein et al., 1985). It may be assumed that manganese acts as a chemical stressor at both an anatomical site, the septo-hippocampus, and on a neurotransmitter system, the acetylcholine system, which plays a critical role in the modulation of the emotional responses. Many of the neurotoxic effects of manganese could possibly be related to the ability of manganese to interfere with the action of calcium as a regulator of cell function. Both mood and cognitive disorders are clinically manifested prior to the extrapyramidal manganese-induced disorder. These cumulative data provide evidence for multifocal damage occurring in multiple anatomical sites in both the CNS and PNS, encompassing diverse neurochemical pathways and mainly cholinergic systems.

An analogous cholinergic–dopaminergic relationship exists in the locomotor system within the basal ganglia. Indeed, the most extensively investigated manganese-induced disorder is the locomotor syndrome, although the overall clinical picture is multi-faceted. This evidence-based observation, supported by numerous clinical and research assessment data, necessitates additional research. The effect of manganese on the extrapyramidal system has been extensively investigated. The Parkinson-like movement disorder derives from the manganese-induced imbalance between the dopaminergic (DA) and cholinergic (ACh) systems of the basal ganglia. The delicate functional neurochemical balance is disturbed upon damage to both DA and ACh systems, caused either directly to their neurons or indirectly via GABAergic and glutamatergic interneurons. MRI demonstrates a specific pattern of pallidal lesions, corroborating the clinical observations of a specific manganese-induced extrapyramidal disorder which is differentiated from idiopathic PD on both clinical and imaging bases. The pathophysiology of idiopathic PD is related to the substantia nigra and therapy with dopaminergic agents is effective in PD cases, but is usually ineffective in manganese-induced extrapyramidal cases. The specific biochemical pattern of changes, along with the specific anatomical sites of manganese-induced damage within the basal ganglia, may explain the different natural history of manganese-induced disease and its poor response to dopaminergic therapy.

The cumulative data suggest that chronic manganese poisoning exerts its early effects first and foremost on the cholinergic septo-hippocampal system, manifested clinically by the neurobehavioral syndrome. The etiology of this reversible clinical picture may be cholinergic, comparable to the delayed neurobehavioral syndrome clinically manifested in severe cases of acute organophosphate (OP) poisoning which exclusively disrupts the cholinergic systems (Finkelstein et al., 1989). Moreover, a clinical neuropsychiatric and extrapyramidal picture, similar to the clinical picture of manganism, has been described in cases of chronic exposure to OP compounds. Chronic neuropsychiatric disorder (Jamal, 1997) with symptoms of anxiety and depression, memory and attention deficit has been described in workers exposed to OP compounds. In addition, dystonic reactions, cog-wheel rigidity and chorioathetosis have been reported after high-dose exposures to OP. These symptoms are related to the cholinergic syndrome caused by inhibition of AChE in cholinergic systems. Psychosis, delirium, aggression, hallucination and depression may also be observed during recovery from the cholinergic syndrome (Behan, 1996; Levin et al., 1976).

The importance of laboratory animal studies is evident. However, their relevance to human exposure to manganese cannot be done by a direct quantitative comparison to occupational or environmental settings of exposure. Therefore, the reasoning should be done from qualitative laboratory results to general principles.

Cholinergic dysfunction was not corroborated by several studies which showed that exposure to manganese did not change QNB binding to postsynaptic muscarinic receptors (Eriksson et al., 1992; Villalobos et al., 1994). Presynaptic cholinergic parameters were not examined in these studies. Clinically, the anti-cholinergic drug trihexyl phenidyl was ineffective in treating the extrapyramidal movement disorder of manganism (Cook et al., 1974; Spencer, 2000). Considering the clinical importance and possible therapeutic implications of the cholinergic mechanisms of manganese-mediated neurotoxicity, further research is clearly warranted. Additional studies are therefore required both at the molecular and cellular levels, in conjunction with clinical studies, to better delineate the relationship between dyshomeostasis in cholinergic innervation and manganese-associated neuropathology.

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