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Subacute manganese exposure in rats is a neurochemical model of early manganese toxicity

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ABSTRACT

Manganese (Mn) is an essential trace element, but excess exposure leads to accumulation in biological tissues, including the brain. Chronically high Mn levels in the brain are neurotoxic and can result in a progressive, irreversible neurological disorder known as manganism. Manganism has signs and symptoms similar to, but distinguishable from idiopathic Parkinson's disease, which include both psychological and motor disturbances. Evidence suggests that Mn exposure impacts neurotransmitter levels in the brain. However, it remains unclear if subacute, low-level Mn exposure resulted in alterations in neurotransmitter systems with concomitant behavioral deficits. The current study used high performance liquid chromatography to quantify neurotransmitter levels in rat striatum (STR), substantia nigra (SN), and hippocampus (HP). Subacute Mn exposure via i.p. injection of 15 mg Mn/kg as $MnCl_2$ caused significantly increased dopamine (DA) levels in the STR. The enhancement was accompanied by significantly elevated levels of the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the STR. In addition, levels of HVA were significantly increased in the SN and HP. These data indicate that subacute, low-level Mn exposure disrupts multiple neurotransmitter systems in the rat brain which may be responsible, in part, for observed locomotor deficits.

1. Introduction

Manganese (Mn) is an essential trace element required for normal function and development of many physiological processes. In the central nervous system, Mn functions as a cofactor for enzymes necessary for neuronal function and neurotransmitter biosynthesis, such as superoxide dismutase 2, pyruvate decarboxylase, and glutamine synthetase (Aschner et al., 2009; Bowman et al., 2011; Schneider et al., 2009). Normally, regulatory mechanisms carefully maintain homeostatic levels of Mn in the body by controlling absorption and elimination of excess metal via hepatobiliary excretion (Klassen, 1976). However, excess Mn exposure can overload these mechanisms (Schneider et al., 2009), leading to Mn accumulation in body tissues, including bone, liver, kidney, and pancreas (Dobson et al., 2004). Mn in the 2+ and 3+ states readily cross the BBB (Aschner et al., 1999) and preferentially accumulate in specific brain regions, such as striatum (STR), globus pallidus (GP), cerebellum, and hippocampus (HP) (Andersen et al., 2010; Fitsanakis et al., 2008).

Adequate levels of Mn are obtained from food, such as nuts, tea, and legumes, and water intake (Bowman et al., 2011). Mn dusts in the air, Mn-containing pesticides in soil, contaminated water, and occupational exposure during mining and agricultural processes, are potential sources of toxic exposure (Mergler et al., 1999). The highest levels of exposure are observed in the workplace, where workers chronically exposed to levels <5 mg/m^3 have reported neurological symptoms of Mn toxicity (Crossgrove and Zheng, 2004). These symptoms result from toxic Mn accumulation in the caudate putamen, GP, STR, and substantia nigra (SN) (Aschner et al., 1999) and are clinically diagnosed as manganese intoxication

Abbreviations: AAS, atomic absorption spectroscopy; Cu, copper; DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine; Fe, iron; GABA, gamma aminobutyric acid; HVA, homovanillic acid; GP, globus pallidus; HP, hippocampus; Mn, manganese; PD, Parkinson's disease; NE, noradrenaline; SN, substantia nigra; STR, striatum; TH, tyrosine hydroxylase; Zn, zinc; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindolacetic acid.

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or manganism. Manganism is a severe, progressive and largely irreversible disorder that resembles, but is distinguishable from idiopathic Parkinson's disease (PD) and other extrapyramidal motor disorders (Bowman et al., 2011).

The prevailing symptoms of manganism include poor hand–eye coordination, bradykinesia, changes in mood and memory deficits, abnormal gait, muscle and joint pain or weakness, bent posture, and a kinetic tremor (Bowman et al., 2011; Mergler et al., 1999). These symptoms exist as a slowly progressing biphasic continuum, with psychiatric symptoms appearing first in the "prodromal period" or early phase of the disease, while motor symptoms dominate the later, "established" phase (Bowman et al., 2011; Rodier, 1955) and persist even after the person is no longer exposed to Mn (Aschner et al., 2009; McMillian, 1999).

Mn accumulation in the basal ganglia may alter levels of neurotransmitters, such as dopamine (DA) and γ-aminobutyric acid (GABA), in the STR and in pathways postsynaptic to the nigrostriatal system (Bowman et al., 2011; McMillian, 1999) which produce the observed motor symptoms. Data from rodent studies generally offer conflicting reports on how Mn exposure affects neurochemistry and motor function. A general consensus regarding the effects of Mn on motor function is that hyperactivity is observed early in Mn toxicity in the young or after low cumulative doses, in both human and animal studies (Bouchard et al., 2007; Nachtman et al., 1986), and as the disease progresses and cumulative dose levels increase, patients and animals both tend to become hypoactive (Oszlanczi et al., 2010; Torrente et al., 2005).

It has been difficult to determine if a relationship exists between altered neurotransmitter levels and motor deficits after Mn treatment. The problem arises from the fact that behavioral changes can be observed in rodents at low levels (1–10 mg/kg) while changes in neurochemistry tend to be studied after exposure to higher levels (>10 mg/kg) (Gwiazda et al., 2007).

There is evidence that Mn exposure is associated with parkinsonism. While links between Mn and PD remain controversial, alterations in dopamine levels and dopamine signaling, mitochondrial dysfunction, and oxidative stress have all been observed in both diseases (Aschner et al., 2007). However, dopaminergic neurons in the SN and their terminals in the STR, which are selectively lesioned in PD, remain intact after Mn intoxication (Guilarte, 2010). Thus, changes in neurotransmission, as opposed to frank cell-loss, likely underlie behavioral observations. Most studies to date have focused on striatal DA levels and not regional differences in other monoamines and their metabolites, or GABA. Additionally, few studies have aimed to investigate changes in motor function in animals, particularly rodents, despite many of the prominent symptoms of manganism being motor deficits, including abnormal gait and postural impairment. Therefore, the present study was undertaken to identify alterations in multiple neurotransmitter systems resulting from subacute, low-level Mn exposure in rats. We have previously used a Mn exposure dose regimen, i.e., 6 mg/kg i.p. injection for 4–6 weeks to study Mn toxicity on iron (Fe) and copper (Cu) transport properties in brain barrier systems (Zheng et al., 1999, 2009).

To ensure a dose-related response, the current study used two dose levels, 6 mg/kg and 15 mg/kg, which bracket the commonly used doses to investigate changes in Mn levels with concomitant changes in regional neurochemistry, motor function, and changes at the cellular level. The cumulative doses used in this study (120 mg/kg and 300 mg/kg) are considered to be low in relation to other estimated cumulative doses (range 72 to more than 100,000 mg/kg) administered in rodent studies reviewed by Newland (1999). Only one study by Bonilla (1980) utilized a lower cumulative dose. The next lowest dose was used by Autissier et al. (1982) and the cumulative dose was higher than both used in the current study. Neurotransmitter levels were assessed in the STR, SN, and HP, an area implicated in learning and memory. It was necessary to study the HP because many of the earliest symptoms of manganism are due to impaired memory. Thus, examination of changes in 4 neurotransmitter (DA, NE, 5-HT, GABA) systems in multiple brain regions was expected to provide significant data on the complex neurochemical changes that result from Mn intoxication.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade, HPLC grade or the highest available pharmaceutical grade. HPLC grade water was obtained from a NANOpure Diamond Ultrapure Water System (Barnstead International, Dubuque, IA); Mn chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$) from Fisher Chemical (Fair Lawn, NJ); nitric acid, tripotassium phosphate (K_3PO_4) and DPX mountant from VWR International (Radnor or West Chester, PA); Mn, Cu, Zn, and Fe stock solutions from SCP Science (Champlain, NY); perchloric acid from RICCA (Arlington, TX); dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindolacetic acid (5-HIAA), noradrenaline (NE), γ-aminobutyric acid (GABA) standards, disodium hydrogen phosphate, anhydrous (Na_2HPO_4) triethylamine (TEA), octanesulfonicacid (OSA), acetonitrile, paraformaldehyde (PFA), sodium phosphate monobasic (NaH_2PO_4), Triton X-100, and porcine skin gelatin from Sigma (St. Louis, MO). Bovine serum albumin (BSA) was purchased from Thermo Scientific (Rockford, IL); methanol, ethylenediaminetetraacetic acid (EDTA), and sucrose from Macron Fine Chemicals (Center Valley, PA); *o*-phthalaldehyde (OPA) from Pickering Laboratories (Mountain View, CA); and BCA protein assay kit from Bio-Rad (Hercules, CA). Normal donkey serum was purchased from Jackson ImmunoResearch (West Grove, PA); mouse monoclonal anti-TH and rabbit polyclonal anti-DARPP-32 antibodies from Millipore (Temecula, CA); secondary donkey anti-mouse IR800 and donkey anti-rabbit IR800 antibodies from LICOR (Lincoln, NE); histoclear from National Diagnostics (Atlanta, GA); potassium permanganate from Amresco (Solon, OH); fluoro-jade C from Histochem (Jefferson, AR); and glacial acetic acid from Mallinckrodt Chemicals (Phillipsburg, NJ).

2.2. Animals

Male Sprague Dawley rats were obtained from Harlan Laboratories, Inc. (Indianapolis, IN) and allowed to acclimate to the holding room for 7 days after arrival. Rats were 8 weeks of age and weighed approximately 240–260 g at study initiation. Animals were randomized by drawing numbers for treatment designation. Sample size was $n = 5$ for all experiments unless otherwise noted. Rats were group housed in a temperature (22 ± 2 °C) and humidity (30–70%) controlled room on a 12 h light/dark cycle (lights on at 0600). Rats had ad libitum access to food (Harlan Teklad 2018 rodent diet) and reverse osmosis water.

All animal studies were approved by the Institutional Animal Care and Use Committee at Purdue University.

2.3. Mn administration and experimental design

Stock solutions of 6 mg Mn/ml (low dose) and 15 mg Mn/ml (high dose) were prepared by dissolving $MnCl_2{\cdot}4H_2O$ in saline and passing through a 0.2 μm syringe filter. Mn-exposed rats received i.p. injections equivalent to either 6 or 15 mg Mn/kg in a volume of 1 ml/kg once per day, 5 days per week, for 4 consecutive weeks. Control animals received the same volume of sterile saline.