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Gender and manganese exposure interactions on mouse striatal neuron morphology

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ABSTRACT

Gender differences in sensitivity and toxicokinetics of multiple metals have been identified in humans. A recent study suggested that young girls performed worse on intellectual exams than young boys exposed to manganese (Mn) in the environment. Animal studies have shown that Mn exposure causes differential effects on behavior in male compared to female mice. We hypothesized that in response to Mn exposure striatal Mn accumulation and/or striatal medium spiny neuron (MSN) morphology show gender-dependent effects. We evaluated the contribution of gender to neuropathology by examining striatal MSN morphology in male and female mice exposed to Mn. We found that gender played a significant role in alterations of striatal MSN morphology in mice exposed to Mn. Gender-dependent changes were strongest when striatal Mn levels were elevated 24 h following the final Mn exposure. Nevertheless, gender-dependent alterations in neuron morphology were still present 3 weeks after the final Mn exposure. Gender differences in neuron morphology were not due to differential striatal Mn accumulation between genders. We conclude that although gender does not affect striatal Mn accumulation, MSN morphology is differentially sensitive to elevated Mn levels.

1. Introduction

Gender differences in the toxicokinetics of xenobiotics have been previously reported (Clewell et al., 2002; Fletcher et al., 1994; Nicolson et al., 2010). These differences can be due to multiple factors; from differences in cytochrome P450 (CYP450) expression, transporter expression and influences of sex hormones on expression of these enzymes and transporters (Bonate, 1991; Harris et al., 1995). A limited number of studies have been conducted to examine gender differences in Mn toxicokinetics but none have examined morphological alterations as a function of gender.

Mn exposure typically occurs in occupational settings such as welding, smelting, Mn mining and working in a dry-cell battery factory (Bowler et al., 2006, 2007a,b; Huang et al., 1989; Jiang et al., 2007; Ono et al., 2002; Wang et al., 1989; Zayed et al., 2003). Studies conducted in the general population, unexposed to high levels of Mn, have shown conflicting results. Men have been shown to have lower ferritin levels, reduced Mn absorption from the gastrointestinal tract and slower Mn clearance than women (Finley et al., 1994). Some studies indicate that in a healthy unexposed population, serum Mn levels are higher in men than women (Davis and Greger, 1992; Greger et al., 1990). However, more recent reports indicate that there is no gender difference in baseline serum Mn levels (Diaz et al., 2001; Rukgauer et al., 1997). One recent study explored intellectual function of children living in a Mexican Mn mining district. Children exposed to Mn had elevated levels (20×) of Mn in hair and blood than children unexposed to high Mn levels. Young girls exposed to Mn performed worse on the revised Wechsler Intelligence Scale for Children than Mn exposed boys (Riojas-Rodriguez et al., 2010). Although there were no gender differences in Mn levels of exposed children, this suggests that cognitive performance may be differentially affected in boys and girls. Gender differences in response to metal exposure are not unique to Mn. Human studies have found gender differences in accumulation, storage and sensitivity to metals including cadmium (Cd), lead (Pb), Mn, mercury (Hg) and nickel (Ni) (Akesson et al.,

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2002, 2005; Bjorkman et al., 2000; Grandjean et al., 1998; Gulson et al., 1998; Jin et al., 2004; Kobayashi et al., 2006; Manton et al., 2003; McKeown-Eyssen et al., 1983; Meding et al., 2001; Nielsen et al., 2002; Pounds et al., 1991; Uno et al., 2005; Vahter et al., 2007, 2002). Animal studies also show gender differences in metal toxicokinetics and sensitivity. For example, male and female rats accumulated Mn differentially across body tissues following Mn exposure by inhalation; however, there was no difference in Mn accumulation in the striatum or other regions of the basal ganglia (Dorman et al., 2004). Gender differences in toxicokinetics following a single oral dose of the gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT) showed that female rats accumulated higher MMT levels than male rats due to slower clearance of MMT (Zheng et al., 2000). Erikson et al. (2004a) exposed juvenile rats to airborne Mn for 13 weeks and found gender-specific changes in protein and mRNA expression of glutamine synthetase, metallothionein mRNA and glutathione levels in multiple regions of the brain. Gender differences in the toxicokinetics of Mn are present regardless of route of administration and Mn speciation. As with humans, rodents show gender differences upon exposure to other metals such as Pb and Hg (Fortoul et al., 2005; Magos et al., 1981; Sager et al., 1984).

Mn overexposure results in motor symptoms that closely resemble Parkinson's disease (PD) (Calne et al., 1994; Lucchini et al., 2009). Acute high-level Mn exposure results in manganism, symptoms include cognitive, psychiatric and motor deficits. Motor symptoms of late-stage manganism are similar but distinct from idiopathic PD (Calne et al., 1994). Shared symptoms include rigidity and bradykinesia; however, manganism has a distinct cock-walk compared to the festinating gait observed in PD (Lucchini et al., 2009). Additionally, patients with manganism rarely show a sustained response to dopamine (DA) replacement therapy and fail to show reduced striatal DA uptake (Calne et al., 1994; Huang et al., 2003). More recently, studies have shown that chronic low level exposure to Mn is a risk factor for PD (Gorell et al., 1997, 1999; Lucchini et al., 2009; Perl and Olanow, 2007). Mn accumulates preferentially in the basal ganglia, specifically the striatum, globus pallidus and substantia nigra (SN) (Aschner et al., 2005; Dobson et al., 2004; Dodd et al., 2005; Erikson et al., 2002, 2004b; Fitsanakis et al., 2008; Olanow et al., 1996; Williams et al., 2010). Rodent, non-human primate and human post-mortem research has found that the striatum shows damage following exposure to Mn (Aschner et al., 2007; Milatovic et al., 2009; Olanow, 2004; Perl and Olanow, 2007). Furthermore, the nigrostriatal DA system is impacted by Mn exposure (Guilarte et al., 2008, 2006; Huang et al., 2003; Kessler et al., 2003; Kim et al., 2002; Stanwood et al., 2009). In PD, the pigmented DAergic neurons of the SN degenerate resulting in damage to striatal medium spiny neurons (MSN) (Day et al., 2006; Garcia et al., 2010; Wright et al., 2009; Zaja-Milatovic et al., 2005). Thus overlapping, but not identical brain regions are involved in Mn neurotoxicity and PD, with the striatal MSNs altered in both conditions.

Gender differences in prevalence, age of onset and progression have been described for PD (Haaxma et al., 2007; Miller and Cronin-Golomb, 2010; Shulman, 2007), however the influence of gender on behavior or neuropathology in Mn neurotoxicity has not been a major focus in the field of Mn toxicology. Females tend to have a later age of onset and less severe symptoms of PD. Gender studies in animal models of PD have also shown that estrogen plays a protective role in neuropathology and behavior. Survival of DA neurons following exposure to DA neurotoxicants, used in animal models of PD, is sensitive to gender (Richardson et al., 2008; Tamas et al., 2005). A study examining gender effects of 6-hydroxydopamine (6-OHDA) lesions on behavior and neuropathology showed that male rats are more sensitive to this toxicant than female rats. Male rats showed a greater loss of DA neurons in the SN and had greater deficits in locomotor activity than female rats (Tamas et al., 2005). This suggests that the nigrostriatal DA system is more sensitive to toxicants in male than female rodents. Animal models of manganism and PD share common features of neuropathology. Striatal neurodegeneration and damage to the nigrostriatal DA pathway is present in both diseases. A recent behavioral study suggests that male mice are more susceptible to Mn exposure than female mice and that the behavioral deficit may be related to reduced striatal DA following Mn exposure (Moreno et al., 2009; Simon et al., 1994). A similar behavioral deficit has been found in a PD mouse model (George et al., 2008). Mn is known to cause damage to the nigrostriatal DA system and alter striatal MSN morphology (Guilarte et al., 2008, 2006; Milatovic et al., 2009; Stanwood et al., 2009), but gender specific changes in neuron morphology following Mn exposure have not been previously reported.

Previous studies carried out in our laboratory on female mice showed that their MSNs are vulnerable to Mn toxicity (Milatovic et al., 2009). Based on the literature of gender differences in Mn exposure, we hypothesized that gender may be a factor in Mn neuropathology. Therefore, we examined striatal MSN morphology and striatal Mn content 24 h and 3 weeks following Mn exposure in mice of both genders to determine (1) if gender has an effect on MSN morphology in the presence or absence of Mn exposure, (2) if gender is a factor in striatal Mn accumulation and (3) if gender differences in MSN morphology are present when Mn levels return to baseline.

2. Methods

2.1. Chemical reagents

Osmium tetroxide and glutaraldehyde were obtained from Electron Microscopy Sciences (Hatfield, PA), $MnCl_2$ from Alfa Aesar (Ward Hill, MA), paraformaldehyde from Fisher Scientific (Pittsburgh, PA), Phosphate Buffered Saline (PBS) from Mediatech Inc. (Manassas, VA), isoflurane from Phoenix Pharmaceutical Inc. (St. Joseph, MO) and all other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

2.2. Animal housing and manganese exposure

All animal protocols were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee (IACUC) and strictly adhered to in order to minimize pain in the animals. All procedures followed NIH laboratory animal care and use guidelines. Animals were distributed into exposure groups across multiple litters and the gender in each of the groups was balanced. The Mn exposure paradigm was adapted from the previously published protocol that showed a significant increase in striatal Mn levels without significant motor impairment (Dodd et al., 2005; Williams et al., 2010). Twelve-week-old wild-type FVB mice (from the FVB-Tg(YAC128)53Hay/J mouse line, JAX #004938) were subcutaneously (s.c.) injected at the hind leg with vehicle (water, Veh) or $MnCl_2$–$4H_2O$ (50 mg/kg) on exposure day 0, 3, and 6.

2.3. Golgi impregnation

On day 7 (24 h post-exposure, 13 weeks old, $N = 2$–3 mice per exposure group for each gender) or day 28 (3 weeks post-exposure, 16 weeks old, $N = 2$–3 mice per exposure group for each gender). The animals were deeply anesthetized with isoflurane, and transcardially perfused with 15 mL 0.1 M phosphate buffer (PB) followed by 40 mL of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M PB. The brains were removed from the skulls and post-fixed for 3 h at room temperature. Vibratome sections were