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Effect of olfactory manganese exposure on anxiety-related behavior in a mouse model of iron overload hemochromatosis

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ABSTRACT

Manganese in excess promotes unstable emotional behavior. Our previous study showed that olfactory manganese uptake into the brain is altered in *Hfe*$^{-/-}$ mice, a model of iron overload hemochromatosis, suggesting that Hfe deficiency could modify the neurotoxicity of airborne manganese. We determined anxiety-related behavior and monoaminergic protein expression after repeated intranasal instillation of $MnCl_2$ to *Hfe*$^{-/-}$ mice. Compared with manganese-instilled wild-type mice, *Hfe*$^{-/-}$ mice showed decreased manganese accumulation in the cerebellum. *Hfe*$^{-/-}$ mice also exhibited increased anxiety with decreased exploratory activity and elevated dopamine D1 receptor and norepinephrine transporter in the striatum. Moreover, Hfe deficiency attenuated manganese-associated impulsivity and modified the effect of manganese on the expression of tyrosine hydroxylase, vesicular monoamine transporter and serotonin transporter. Together, our data indicate that loss of HFE function alters manganese-associated emotional behavior and further suggest that HFE could be a potential molecular target to alleviate affective disorders induced by manganese inhalation.

1. Introduction

Manganese (Mn) is an essential metal required for proper brain function, serving as a cofactor of several critical enzymes, including superoxide dismutase, glutamine synthetase and arginase (Horning et al., 2015; Takeda, 2003). However, when overdeposited in the brain, manganese promotes neurotoxicity, which is characterized by memory loss, impaired motor coordination and psychotic behavior resembling Parkinson's disease (Avila et al., 2013). Manganese absorption from the gastrointestinal tract is limited due to first-pass elimination via biliary excretion (Roth, 2006). Hence, the enteral route provides a protective barrier against manganese poisoning by ingestion, such as drinking water or acute intoxication of dietary manganese. However, manganese bioavailability after inhalation is much greater than that after oral exposure due to lack of presystemic (hepatic) clearance mechanism (Brenneman et al., 2000). Moreover, the close proximity of the olfactory tract to the brain enables inhalation to represent the primary route of exposure for manganese neurotoxicity (Brenneman et al., 2000; Tjalve et al., 1996). This has raised significant concerns about Mn toxicity in human health, in particular for people in occupational settings (Avila et al., 2013), such as workers employed in mining and Mn ore processing (Park et al., 2005) and agricultural workers exposed to Mn-containing pesticide (Lucchini et al., 2009).

Neurological problems resulting from Mn intoxication are associated with altered monoaminergic signaling pathways (Guilarte, 2013; Subhash and Padmashree, 1990), which are involved in controlling emotional behavior (Kern et al., 2010; Li et al., 2011). For example, the enzymatic activity of tyrosine hydroxylase (TH), a critical enzyme for catecholamine synthesis, is impaired upon Mn exposure (Zhang et al., 2011). Norepinephrine transporter (NET) is differentially regulated in different brain regions upon Mn exposure (Anderson et al., 2009). In addition, it is well-documented that Mn exposure significantly affects dopaminergic function in the striatum (Kim et al., 2012; Subhash and Padmashree, 1990). Moreover, increasing evidence indicates that abnormal emotion state and general activity in several affective disorders are associated with altered dopaminergic pathway in the striatum (Fusar-Poli et al., 2012; Krause et al., 2000). These results suggest that striatal monoamine homeostasis is impaired by airborne manganese, which could be a potential risk for the development of psychiatric disorders.

Abbreviations: COMT, catechol-O-methyltransferase; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DAT, dopamine transporter; DMT1, divalent metal transporter 1; EPM, elevated plus maze; ICP-MS, inductively coupled plasma mass spectrometry; Mn, manganese; NET, norepinephrine transporter; SERT, serotonin transporter; TH, tyrosine hydroxylase; VMAT, vesicular monoamine transporter.

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A large body of evidence has indicated that manganese absorption is enhanced in iron-deficient anemia due to iron-responsive up-regulation of metal transporters (Erikson et al., 2002; Kim et al., 2012; Thompson et al., 2007), suggesting that altered body iron status can influence manganese transport and toxicity. However, the information about the role of iron overload in Mn's neurotoxic effects is scarce. This question is particularly important because of a high prevalence of the iron overload disorder hemochromatosis, which is one of the most common genetic diseases in the North American Caucasian population (Merryweather-Clarke et al., 2000; Pietrangelo, 2004). Mutations of the HFE (High iron or Fe) gene are the primary cause of this disease; the two most prevalent HFE missense variants are C282Y (7–17%) and H63D (10–32%) in the US population (Zhang et al., 2010). While airborne Mn exposure provides the greatest neurotoxic effects, whether or not HFE is the genetic determinant for manganese neurotoxicity has yet to be examined.

Our previous studies demonstrated that iron-loaded Hfe-deficient mice display increased olfactory uptake of Mn to the brain after a single intranasal dose of $^{54}MnCl_2$, suggesting an increased vulnerability of Mn neurotoxicity in HFE-related hemochromatosis (Kim et al., 2013). However, we also found that the steady-state levels of Mn in blood are decreased in humans with HFE variants, as well as in Hfe-deficient mice (Claus Henn et al., 2011). These results suggest that HFE deficiency could increase the clearance of Mn from blood, thereby protecting the body against Mn toxicity. Therefore, it is necessary to directly determine if loss of HFE function could modify the neurotoxic effects of Mn. Since Mn exposure is associated with psychiatric and mood disorders (Avila et al., 2013; Tran et al., 2002), in the present study we tested emotional behavior after repeated intranasal instillations of manganese using the *Hfe*$^{-/-}$ mouse model that recapitulates iron overload hemochromatosis in humans (Levy et al., 2000). Our data demonstrate that olfactory exposure to manganese increases an impulsivity-like response, which is partly reversed by Hfe deficiency, suggesting that HFE could be a potential therapeutic target to alleviate emotional dysfunction induced by manganese inhalation.

2. Materials and methods

2.1. Ethics statement

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Northeastern University Animal Care and Use Committee.

2.2. Animals and manganese exposure

Breeders for Hfe-deficient (*Hfe*$^{-/-}$) mice (Levy et al., 1999) and wild-type control (*Hfe*$^{+/+}$) mice were kindly provided by Dr. Nancy Andrews (Duke University, NC, USA). All mice used for these studies were on the 129S6/SvEvTac background (Levy et al., 1999). One-month old male mice were fed facility chow (Prolab Isopro RMH 3000, LabDiet; 96 mg manganese and 380 mg iron per kg diet) and given water ad libitum. Neither manganese nor iron was detected in drinking water. Male mice were chosen because estrogen affects iron metabolism (Hou et al., 2012; Yang et al., 2012). For olfactory exposure to manganese, mice were intranasally-instilled daily with manganese chloride (5 mg $MnCl_2$/kg body weight; 0.08 mL/kg) or double-distilled water as vehicle control for 22 days. This dose was chosen because we previously showed behavioral effects of intranasal Mn at 10 mg/kg of $MnCl_2$ twice a week in rats (Kim et al., 2012).

2.3. Elevated plus maze test

After the last dose, all animals were subject to elevated plus maze (EPM) test in order to examine anxiety/impulsivity-like behavior (n = 10–13 per group). The EPM apparatus (Med Associates, St. Albans, VT, USA) was composed of two open arms (35 L cm × 6 W cm) and two closed arms (35 L cm × 6 W cm × 15 H cm, height of the wall), which extended from the central zone platform (6 L cm × 6 W cm). The maze was 70 cm above the floor. Each mouse was placed in the center zone facing an open arm and allowed to explore the maze for 5 min. The latency of the first entry into an open arm, the number of entries and time spent in the open arms, distance traveled in the open arms and total distance in the maze were recorded and analyzed by ANY-maze (Stoelting, Wood Dale, IL, USA). The maze was cleaned thoroughly with Quatricide TB (Pharmacal Research Laboratory Inc., Naugatuck, CT, USA) after each test.

2.4. Tissue collection

After the EPM test, mice were euthanized by isoflurane overdose, followed by exsanguination to harvest brain and liver tissues. The brain samples were microdissected to collect different brain regions, including prefrontal cortex, striatum, hippocampus and cerebellum. All tissues were flash-frozen in liquid nitrogen and stored at −80 °C until analysis.

2.5. Western blot analysis

The striatum was selected for western blot analysis because intranasal Mn alters dopamine-related proteins in the striatum (Kim et al., 2012), a brain region involved in emotional behavior (Fusar-Poli et al., 2012; Krause et al., 2000). Striatum tissues (n = 4 per group) were homogenized, electrophoresed on 10% SDS-polyacrylamide gels (30 μg proteins) and transferred to polyvinylidene difluoride membranes. After blocking with 5% non-fat milk, the membrane was incubated with different antibodies, including goat anti-DAT (1:200; Santa Cruz Biotech, Dallas, TX, USA), rabbit anti-D1R (1:500, Abcam, Cambridge, MA, USA), mouse anti-D2R (1:200, Santa Cruz), mouse anti-TH (1:200, Santa Cruz), rabbit anti-VMAT (1:200, Santa Cruz), rabbit anti-COMT (1:200, Santa Cruz), goat anti-serotonin transporter (SERT; 1:200, Santa Cruz) and rabbit anti-NET (1:200, Santa Cruz). As a loading control, the immunoblot was incubated with mouse anti-actin (1:5000, MP Biomedicals, Solon, OH, USA). The blots were incubated with secondary antibodies conjugated with HRP, including donkey anti-goat IgG (1:1000, Santa Cruz), goat anti-rabbit IgG (1:1000, Santa Cruz) or sheep anti-mouse IgG (1:1000 or 1:5000, GE Healthcare, Piscataway, NJ, USA). Immunoreactivity was detected using ECL West Dura substrate (Thermo Scientific, Tewksbury, MA, USA). Protein bands were visualized by ChemiDoc XRS (Bio-Rad, Hercules, CA, USA) and intensities of protein bands were quantified using Image Lab (version 4.1, Bio-Rad).

2.6. Analysis of metals in the brain

Liver (n = 6–8 per group) and microdissected brain tissues (n = 6–7 per group) were digested in 0.5 mL of 20% nitric acid (Trace grade, Fisher Scientific; Pittsburgh, PA, USA) at 125 °C for 1 h. After a complete digestion, the samples were diluted with metal-free double-distilled water up to a volume of 5 mL. Metal concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Varian 810/820MS, Bruker, Billerica, MA, USA) and calculated as μg/g tissue.