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Vanadium exposure induces olfactory dysfunction in an animal model of metal neurotoxicity

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ABSTRACT

Epidemiological evidence indicates chronic environmental exposure to transition metals may play a role in chronic neurodegenerative conditions such as Parkinson's disease (PD). Chronic inhalation exposure to welding fumes containing metal mixtures may be associated with development of PD. A significant amount of vanadium is present in welding fumes, as vanadium pentoxide (V_2O_5), and incorporation of vanadium in the production of high strength steel has become more common. Despite the increased vanadium use in recent years, the neurotoxicological effects of this metal are not well characterized. Recently, we demonstrated that V_2O_5 induces dopaminergic neurotoxicity *via* protein kinase C delta (PKCδ)-dependent oxidative signaling mechanisms in dopaminergic neuronal cells. Since anosmia (inability to perceive odors) and non-motor deficits are considered to be early symptoms of neurological diseases, in the present study, we examined the effect of V_2O_5 on the olfactory bulb in animal models. To mimic the inhalation exposure, we intranasally administered C57 black mice a low-dose of 182 μg of V_2O_5 three times a week for one month, and behavioral, neurochemical and biochemical studies were performed. Our results revealed a significant decrease in olfactory bulb weights, tyrosine hydroxylase (TH) levels, levels of dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) and increases in astroglia of the glomerular layer of the olfactory bulb in the treatment groups relative to vehicle controls. Neurochemical changes were accompanied by impaired olfaction and locomotion. These findings suggest that nasal exposure to V_2O_5 adversely affects olfactory bulbs, resulting in neurobehavioral and neurochemical impairments. These results expand our understanding of vanadium neurotoxicity in environmentally-linked neurological conditions.

1. Introduction

Metal exposure has been considered a major chemical risk factor in the pathogenesis of chronic neurodegenerative conditions such as Parkinson's disease (PD) (Dobson et al., 2004; Aschner et al., 2009; Furbee, 2011; Caudle et al., 2012; Kanthasamy et al., 2012). PD imposes an estimated economic burden of $23 billion per year in the United States alone (Weintraub et al., 2008). Multifactorial etiology is associated with progressive and substantive degeneration of nigral dopaminergic neurons and extra-nigral neurons underlying PD (Anglade et al., 1997; Braak and Braak, 2000; Braak et al., 2000; Allam et al., 2005; Przedborski, 2005; Przedborski and Ischiropoulos, 2005). The cause and mechanism of the disease's progression are poorly understood and have not yet been exhaustively explored. Recently, many non-motor symptoms have been determined to precede the onset of motor symptoms and are considered hallmark in the early stages of PD. A non-motor, early stage symptom of PD is impaired olfactory function (Ansari and Johnson, 1975; Langston, 2006; Goldstein et al., 2010). However, the effect of environmental neurotoxic metals on the olfactory system has not been well characterized.

Case-control and epidemiological studies have linked metal exposure to the increased incidence of PD (Fleming et al., 1994; Schulte et al., 1996; Gorell et al., 1997; Liou et al., 1997; Marder et al., 1998; Smargiassi et al., 1998; Taylor et al., 1999; Priyadarshi et al., 2000; Ritz and Yu, 2000). Studies have shown that welders

Abbreviations: V_2O_5, vanadium pentoxide; Mn, manganese; PD, Parkinson's disease; TH, tyrosine hydroxylase; OB, olfactory bulb; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; PFA, paraformaldehyde; PKCδ, protein kinase C delta.

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have an increased risk of developing PD (Racette et al., 2001; Park et al., 2005). Manganese (Mn), which is typically present in welding fumes mixed with other metals including vanadium, is the major metal that has been studied with respect to PD (Aschner et al., 2007, 2009; Guilarte, 2010). Mn mainly targets the basal ganglia comprising the caudate nucleus, putamen, globus pallidus, substantia nigra, and subthalamic nucleus (Eriksson et al., 1992; Calne et al., 1994; Brenneman et al., 1999; Nagatomo et al., 1999; Aschner et al., 2009). Neurotoxicity resulting from excessive Mn exposure is distinct from sporadic PD in that the globus pallidus appears to be the most severely affected of all of the basal ganglia regions (Verity, 1999). However, studies have shown that dopamine (DA), which is the principal neurotransmitter in the striatum that is severely depleted in PD patients, is also decreased by Mn, with both *in vivo* (Parenti et al., 1986) and *in vitro* (Vescovi et al., 1991) exposure paradigms in animals. Dorman et al. (2001) reported the accumulation of $MnSO_4$ in the olfactory bulb and striatum of inhalation-exposed rats relative to controls.

The rapid growth and modernization of U.S. cities are dependent on ever-changing infrastructures. Central to the evolution of these structures is welding, one of the primary anthropogenic sources of environmental metals. Vanadium, typically present in welding fumes as vanadium pentoxide (V_2O_5), is emitted by welding rods commonly used in construction. Vanadium is also widely used in various steelmaking industrial applications, such as plane and ship building, in the production of temperature-resistant alloys and glass, and in pigment and paint manufacturing (McNeilly et al., 2004). Also, large quantities of vanadium compounds are released into the environment mainly through the burning of fossil fuels, with vanadium reported as the most abundant trace metal in petroleum samples (Amorim et al., 2007). Vanadium accumulates in soil, groundwater, and plants, and is consumed by animals and humans (Pyrzynska and Weirzbicki, 2004). The processing of vanadium slag (about 120 g/kg of vanadium pentoxide) generates dust, with vanadium concentrations ranging from 30 to 120 mg/m^3 (IARC, 2006). Crude oil from Venezuela is believed to have the highest vanadium concentration, ranging up to 1400 mg/kg. Fifty percent vanadium pentoxide has been discovered in flue-gas deposits from oil-fired furnaces (IARC, 2006). Elevated levels of vanadium (4.7 mg/m^3) have been found in the breathing air of steel industry workers (Kiviluoto et al., 1979). Vanadium exposure to humans has been shown to cause motor deficits (Done, 1979; WHO, 2000). Thus, the growing use of vanadium in a wide variety of applications warrants the full characterization of its neurotoxicological properties.

Chronic exposure to environmental toxicants, including herbicides, pesticides, solvents, and heavy metals, can alter the ability to smell (Doty and Hastings, 2001), with the best documented metal in this regard being cadmium, chromium, nickel, and manganese. Further, Avila-Costa et al. (2004) observed that inhaled V_2O_5 damages the nigrostriatal dopaminergic systems in rodent models. In a recent study, we showed that vanadium is neurotoxic to dopaminergic neurons in cell culture models (Afeseh Ngwa et al., 2009). In the present study, we further examine the neurotoxic properties of vanadium, specifically focusing on its effects on the olfactory bulb to determine whether subchronic nasal exposure impairs neurobehavioral and neurochemical processes associated with olfactory function.

2. Materials and methods

2.1. Chemicals

Vanadium pentoxide (V_2O_5) salt, protease cocktail inhibitor, phosphatase inhibitors and anti-β-actin antibody were purchased from Sigma (St. Louis, MO). A Bradford protein assay kit was purchased from Bio-Rad Laboratories (Hercules, CA). Mouse monoclonal antibodies against tyrosine hydroxylase (TH) and GFAP were obtained from Millipore (Upstate, Billerica, MA, USA) and Cell Signaling Technology, Inc. (Danvers, MA), respectively. The anti-mouse and anti-rabbit secondary antibodies (Alexa Fluor 680 conjugated anti-mouse IgG and IRdye 800 conjugated anti-rabbit IgG) were purchased from Invitrogen and Rockland Inc., respectively.

2.2. Treatment paradigm

Six to eight week old male C57BL/6 mice were housed at room temperature under a 12 h light/dark cycle. The control and treatment animals were age-matched. Food and water were provided *ad libitum* and animal weights were monitored. Animals were cared for in accordance with institutional animal care guidelines. A previous study exposed mice to 5–20 mM V_2O_5 through inhalation route and examined neurotoxic effects of the metal (Avila-Costa et al., 2005; Fleming et al., 2008). In the present study, we used a low dose of 182 μg of V_2O_5 in 50 μL of de-ionized water and administered intranasally three times a week for period of one month. The vanadium pentoxide was administered to mice intranasally using micropipettes after briefly anaesthetizing the mice with isoflurane to prevent a gag reflex. The control animals received equal volumes of pH-adjusted deionized water (pH ~2.0 for V_2O_5 solution). Following the treatment, mice were subjected to behavioral and neurochemical tests one week following last dose of V_2O_5. Intranasal delivery was chosen because vanadium exposure mostly occurs through inhalation route. Intranasal delivery of chemicals takes advantage of an incomplete blood brain barrier in the olfactory epithelium (Graff and Pollack, 2005). The olfactory nerves bypass the blood brain barrier, therefore chemicals can be taken up by these neurons and transported directly into the brain (Graff and Pollack, 2005).

2.3. Olfaction test

The ability of mice to detect pheromones from female bedding by sniffing was used as a measure of olfaction, as described in previous studies (Fleming et al., 2008; Kim et al., 2011). This test combines the principle behind the wooden block test, which relies on the ability of mice to discriminate between self and non-self odors (Fleming et al., 2008; Kim et al., 2011), and on the knowledge that female body odor and urine attract males (Lucas et al., 1982; Singer et al., 1988). For this test, the bedding from a mouse cage housing pregnant females was introduced in the cage of the male mice used in this study. The amount and location of bedding were kept constant each time. The total time spent sniffing the female bedding material was measured using a stop watch during a 5 min testing session. Both vanadium-treated and control mice were subjected to the test at the same day. This test is an easy and effective measure of an animal's olfactory capacity to detect a novel odor.

2.4. HPLC detection of dopamine and its metabolites in olfactory bulb

Following the completion of treatment, mice were sacrificed and the olfactory bulbs were dissected out at the junction between the olfactory bulbs and the rest of the brain for each mouse. The dissected olfactory bulbs were weighed. The differences in weight of olfactory bulb between control and vanadium exposed animals were determined. Levels of dopamine (DA) and its metabolite DOPAC in olfactory lobe tissues were determined by high-performance liquid chromatography (HPLC) with electrochemical detection. The samples were prepared as described previously (Zhang et al., 2007; Ghosh et al., 2013). Briefly, neurotransmitters