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The neurotoxic effects of manganese on the dopaminergic innervation of the gill of the bivalve mollusc, *Crassostrea virginica*

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ABSTRACT

We examined effects of manganese on the nervous system and innervation of lateral cilia of *Crassostrea virginica*. While essential in trace amounts, tissue manganese accumulation is neurotoxic, inducing Manganism, a Parkinson's-like disease in humans. Lateral cilia of the gill of *C. virginica* are controlled by a reciprocal serotonergic–dopaminergic innervation from their ganglia. Oysters were incubated 3 days in the presence of up to 1 mM manganese, followed by superfusion of the cerebral ganglia, visceral ganglia or gill with dopamine or serotonin. Beating rates of cilia were measured by stroboscopic microscopy of isolated gill preparations or gill preparations with the ipsilateral cerebral and/or visceral ganglia attached. Acute manganese treatments impaired the dopaminergic, cilio-inhibitory system, while having no effect on the serotonergic, cilio-excitatory system, which is in agreement with the proposed mechanism of manganese toxicity in humans. Manganese treatments also decreased endogenous dopamine levels in the cerebral and visceral ganglia, and gills, but not serotonin levels. We demonstrated that manganese disrupts the animal's dopaminergic system, and also that this preparation can be used to investigate mechanisms that underlie manganese neurotoxicity. It also may serve as a model in pharmacological studies of drugs to treat or prevent Manganism and other dopaminergic cell disorders.

1. Introduction

Manganese is an element present in all animal tissues and required as an enzyme cofactor or activator for numerous reactions of metabolism (Cotzias, 1958; Wedler, 1993). While essential in trace amounts, excessive manganese exposure can result in toxic accumulations in human brain tissue and resulting extrapyramidal symptoms similar to those seen in patients with idiopathic Parkinson's disease (Calne et al., 1994; Aschner, 2000; Levy and Nassetta, 2003; Dobson et al., 2004). This Parkinson's-like neurological condition first described in 1837 in two manganese ore-crushing mill workers (Couper, 1837) has been referred to as Manganism (Mena et al., 1967; Barbeau, 1984; Donaldson, 1987; Gorell et al., 1999). Inhalation of manganese from the atmosphere is believed to be the primary cause of manganese toxicity (Andersen et al., 1999). In addition to mining and manganese ore processing, high levels of airborne manganese are possible in a number of other occupational settings, including welding, dry battery manufacture, and use of certain organochemical fungicides like Maneb. (NAS, 1973; Meco et al., 1994; Reidy et al., 1992; Iregren, 1999; Olanow, 2004). Most recently, questions are being asked about the safety of ambient manganese in the general population and there is a growing concern that chronic, low-level occupational or increased environmental exposure to manganese may be a contributing factor in a variety of neurological conditions including the high numbers of people diagnosed with Parkinson's disease in the United States and elsewhere (Mergler, 1996, 1999; Lucchini et al., 1999; Davis, 1999; Kaiser, 2003; Levy and Nassetta, 2003; Sadek, et al., 2003; Jankovic, 2005; Racette, et al., 2005; Ostiguy et al., 2006; Dorman et al., 2006).

Although manganese toxicity has been recognized for some time, the primary mechanism underlying its neurotoxic effects remains elusive. Clinically, Manganism resembles idiopathic Parkinson's disease, a dopaminergic cell disorder. Symptoms common to both disorders include gait imbalance, rigidity, tremors and bradykinesia (Mena et al., 1967, 1970; Rosenstock et al., 1971; Huang et al., 1989), suggesting a similar etiology of neuronal damage in the substantia nigra with a resulting deficiency of the neurotransmitter dopamine for the striatum. However, compared to Parkinson's, there are some differentiating features seen with Manganism including symmetry of effects, more prominent dystonia, a characteristic “cock walk,” an intention rather than resting tremor, earlier behavioral and cognitive dysfunction, difficulty turning, and a poor response to Levodopa (Barbeau et al., 1976; Huang, et al., 1993, 1998; Calne et al., 1994; Lu et al., 1994; Koller et al., 2004; Olanow, 2004; Jankovic, 2005; Cersosimo and Koller, 2006) suggesting different or more extensive damage in the basal ganglia or to the dopaminergic system. Human and animal studies have shown that toxic exposure to manganese results in metal accumulations in various areas of the basal ganglia

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and dysfunction of cells of both the striatum and the globus pallidus (Eriksson et al., 1992; Calne et al., 1994; Brenneman et al., 1999; Nagatomo et al., 1999; Newland, 1999; Pal et al., 1999; Baek et al., 2003). Other studies have shown that manganese selectively targets dopaminergic neurons in the human basal ganglia (Pal et al., 1999; Olanow, 2004) and decreases dopamine levels in the striatum (Mena et al., 1970; Parenti et al., 1986; Eriksson et al., 1987; Vescovi et al., 1991; Sistrunk et al., 2007). Considering the clinical similarities between Manganism and Parkinson's disease, and the fact that manganese accumulates in brain regions rich in dopaminergic neurons, it has long been suggested that manganese neurotoxicity involves a disruption in dopaminergic neurotransmission (Neff et al., 1969; Hornykiewicz, 1972; Graham, 1984).

Bivalves and other marine invertebrates are often used in metal environmental toxicology studies because their tissues readily accumulate trace metals to concentrations that are usually much higher on a wet weight basis than what is present in the surrounding seawater (Rainbow, 1993; Phillips and Rainbow, 1993; Boening, 1999). Numerous reports have been made on the bioaccumulation of various heavy metals in the eastern oyster, *Crassostrea virginica*, and other oyster species (Capar and Yess, 1996; Bu-Olayan and Subrahmanyam, 1997; Scanes and Roach, 1999; Abbe et al., 2000; Fang et al., 2001; Spooner et al., 2003; Rodney et al., 2007). While concentrations of dissolved manganese in freshwaters, even that which is free of anthropogenic sources, can range from 10 to >10 000 μg/L (Reimer, 1999), manganese concentrations in open seawater tend to range from 0.4 to 10 μg/L (US EPA, 1984; Zeri et al., 2000) and rarely rise to over 200 μg/L except in areas of severe hypoxia or coastal regions with high river flows (Eaton, 1979). As an essential nutrient, manganese is actively assimilated and utilized by both plants and animals and studies show significant bioaccumulation of manganese by aquatic biota at lower trophic levels. (Folsom et al., 1963; Thompson et al., 1972; Bryan and Hummerstone, 1973; Pentreath, 1973; Rai and Chandra, 1992). Our lab reported that *C. virginica*, incubated in the presence of $MnCl_2$, readily accumulated manganese into its tissues (Murray et al., 2007). Despite its potential for bioaccumulation compared to other aquatic metals, manganese is believed to be one of the least toxic and only a few published reports exist on manganese toxicity in marine organisms. A 2004 IPCA report based upon available toxicity data, suggest that effects of total manganese on marine species of phytoplankton, invertebrates and fish have been observed in laboratory tests that range from a low of 1.5 mg/L based upon a 5-d EC 50 for the marine diatom *Ditylum brightwellii* (Canterford and Canterford, 1980) to a high of 300 mg/L based up a 7-d LC 50 for the adult clam, *Mya arenaria* (Eisler, 1977). The only published study involving *C. virginica* reported manganese toxicity at 16 mg/L based upon a 48 h LC 50 for oyster embryos (Calabrese et al., 1973). Although there is no current marine quality guideline for manganese, the IPCS (2004) suggested a guidance value of 0.3 mg/L for the protection of 95% marine species with 50% confidence.

In this study we sought to use *C. virginica* as a model to study the physiological effects of manganese on a known dopaminergically innervated system. Dopamine, serotonin and other biogenic amines are present in the nervous tissue and gill of *C. virginica* (Downer, et al., 2006). The innervation of the lateral ciliated cells in the gill by the nervous system of *C. virginica* is by a cilio-excitatory serotonergic system and a cilio-inhibitory dopaminergic system, schematically shown in Fig. 1 (Carroll and Catapane, 2007). The anatomy of the oyster showing the gill, cerebral ganglia and visceral ganglia is shown in Fig. 2. Application of serotonin to the cerebral ganglia, visceral ganglia or gill activates quiescent cilia and increases their beating rates. Similar treatments with dopamine decrease the beating rates of the cilia of the lateral cells of the gill and causes cilio-stasis. It is postulated that the animal's cerebral ganglia contain dopaminergic and serotonergic neurons, which synapse in the visceral ganglia with a second set of dopaminergic and serotonergic neurons, which peripherally innervate the gill via the branchial nerve. At each ganglion serotonin acts as an exciter of cilio-excitatory circuits while dopamine acts as an exciter of cilio-inhibitory circuits. Within the gill, the epithelial cells containing the lateral cilia have serotonin and dopamine receptors that when activated increase or decrease the beating rates of the cilia, respectively.

The oyster, *C. virginica* provides a relatively simple nervous system with a serotonergic–dopaminergic innervation component that directs an observable and measurable physiological response, and may be useful in investigating the mechanisms that underlie both manganese neurotoxicity and other dopaminergic cell disorders.

2. Materials and methods

Oysters (*C. virginica*) were incubated in Instant Ocean® artificial seawater (ASW) obtained from Aquarium Systems Inc. (Mentor, OH, USA). Dopamine, serotonin, 1-octanesulfonic acid (sodium salt, SigmaUltra) and HPLC standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents including manganese chloride ($MnCl_2 \cdot 4H_2O$, ASC grade) were obtained from Fisher Scientific (Pittsburgh, PA, USA).

Adult *C. virginica* of approximately 80 mm shell length were obtained from Frank M. Flower and Sons Oyster Farm in Oyster Bay, NY, USA. They were maintained in the lab for up to two weeks in temperature-regulated aquaria in ASW at 16–18 °C, specific gravity of 1.024 ± 0.001, salinity of 31.9 ppt, and pH of 7.2 ± 0.2. Each animal was tested for health prior to experimentation by the resistance it offered to being opened. Animals that fully closed in response to tactile stimulation and required at least moderate hand pressure to being opened were used for the experiments. In order to ensure that each oyster would receive equal manganese exposure during the experiment and not just close up, healthy specimens were shucked by removing their right shell before being placed in individual temperature-controlled aerated containers of ASW for 3 days in the presence

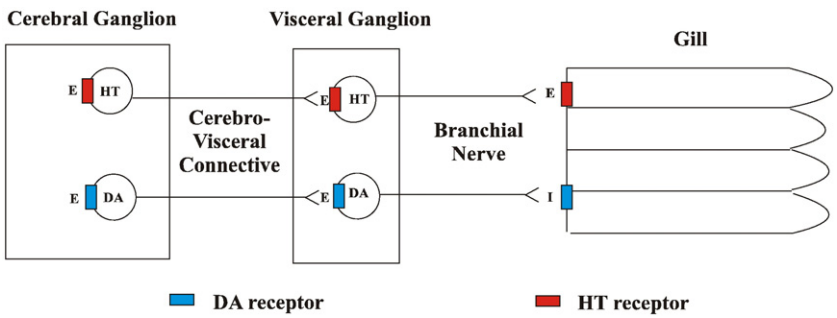

Fig. 1. Schematic representation of the innervation of the lateral ciliated cells of the gill of *Crassostrea virginica*. Serotonin (HT), Dopamine (DA), E = excitatory neurotransmitter, I = inhibitory neurotransmitter.