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The effects of 17β-estradiol and ethanol on zinc- or manganese-induced toxicity in SK-N-SH cells

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Abstract

Serious neurodegenerative disorders are increasingly prevalent in our society and excessive oxidative stress may be a key mediator of neuronal cell death in many of these conditions. A variety of metals, such as manganese and zinc, are essential trace elements but can reach localized toxic concentrations through various disease processes or environmental exposures and have been implicated as having a role in neurodegeneration. Both manganese and zinc exist as bivalent cations and are essential cofactors/activators for numerous enzymes. Evidence suggests one action of these metals, when concentrated beyond physiological levels, may be to inhibit cellular energy production, ultimately leading to increased radical formation. Our studies were undertaken to directly investigate the toxic effects of manganese and zinc in an immortalized neuronal-like cell line (SK-N-SH) by testing interactions with the antioxidant, 17β-estradiol, and the neurotoxin, ethanol. Employing undifferentiated SK-N-SH cells, we found that these metals caused biphasic effects, enhancing cell proliferation at low doses and inducing cell death at higher doses. Zinc was both more efficacious and more potent than manganese in enhancing growth and in causing cell death. 17β-Estradiol and ethanol enhanced the proliferative actions of zinc and manganese across a wide concentration range. Furthermore, co-treatment with either 17β-estradiol or ethanol afforded protection against manganese-, but not zinc-induced toxicity. Finally, combined administration of 17β-estradiol and ethanol to SK-N-SH cells resulted in both a loss of growth enhancement and protective properties that were observed when these substances were administered individually. We also noted that the toxic effects occurred more rapidly from zinc than manganese exposure. Taken together, these data suggest that oxidative stress likely has a role in cell death resulting from toxic exposure to either zinc or manganese, but there is a difference in the precise mechanism of their effects.
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1. Introduction

We are living in a time of increasing incidence of serious, neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases (Brookmeyer et al., 1998; Lilienfeld and Perl, 1993). Many of the essential transition metals, such as zinc, manganese, copper and iron are implicated in some neurodegenerative diseases (Bush, 2003; Carrie et al., 2003; Cuajungco and Lees, 1997a,b; Gorell et al., 2004; HaMai et al., 2001; Powers et al., 2003; Todorich and Conner, 2004). These metals exhibit bimodal properties: at low levels, they are essential for proper functioning of many enzymes, signaling proteins and transcription factors, and may even possess antioxidant properties.

Excessive levels of zinc have been implicated in both acute and chronic insults resulting in neurodegeneration. During acute pathological states, such as ischemia or excitotoxic events, zinc accumulates in dead or dying neurons (see Koh, 2001; Cuajungco and Lees, 1997a,b for review). This is in marked contrast to normal conditions, where histochemically visible zinc is observed mainly in presynaptic vesicles or boutons of glutamatergic neurons. Upon stimulation, zinc is released along with glutamate, where it modulates glutamatergic transmission (Frederickson and Bush, 2001). In acute neurodegenerative conditions,

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loss of zinc from presynaptic neurons and its concurrent appearance in postsynaptic neurons has led to the hypothesis that neurodegeneration arising from ischemic or excitotoxic events is associated with excessive stimulus and, therefore, exaggerated release of zinc with subsequent uptake into postsynaptic neurons. It has also been proposed that high levels of zinc may originate from other depots within the cell after stressful events (Frederickson and Bush, 2001; Lee et al., 2000). Collectively, these data demonstrate that zinc appears to accumulate in dead or dying neurons, possibly contributing to neurodegeneration. Zinc has also been shown to accumulate within the β-amyloid plaques found in Alzheimer disease and may have complex interactions with β-amyloid and other heavy metals, such as iron and copper (Bush, 2003; Cuajungco and Lees, 1997a,b). The precise mechanism of neuronal toxicity incurred by extended exposure to supra-physiological levels of zinc is unknown. Recently, Daniels et al. (2004) reported an association between down regulation of Erk and p38 stimulation with zinc toxicity and radical production in Nα2 neuroblastoma cells. Evidence also suggests a role for zinc in the inhibition of cellular energy production, another mechanisms for increased oxidative stress (Dineley et al., 2003).

Similar to zinc, excessive manganese exposure can also induce neurodegeneration, resulting in Parkinson's-like symptoms (Aschner et al., 1999; Aschner, 2000; Lai et al., 1984). Although the mechanisms of manganese toxicity have also not been fully elucidated, it can induce uncoupling of oxidative phosphorylation and cause a lowering in the levels of glycolytic and TCA cycle enzymes. These actions can account for, at least in part, the manganese-induced neural cell death (Gavin et al., 1992; Malthankar et al., 2004).

Within the last decade, an explosion of research has implicated a role for 17β-estradiol (the most potent and physiologically significant of the estrogens) in the growth, maintenance and protection of neurons in the adult brain (see Wise, 2003 for review). The beneficial effects of estrogens have been demonstrated in studies comparing the incidence of neurodegenerative disorders in women (Norbury et al., 2003). Recently, estrogen replacement has been shown to be beneficial in delaying the onset of some neurodegenerative disorders, such as Alzheimer's and Parkinson's disease (Cho et al., 2003). Moreover, 17β-estradiol promotes neuronal viability in both immortalized cell lines and primary cell cultures (Behl, 2002). It is a protective agent against a variety of stressors and toxic insults, including ischemia and excitotoxicity (Zaulyanov et al., 1999; Wise, 2003). 17β-Estradiol is believed to exert its neuroprotective effects through both receptor- and non-receptor-mediated mechanisms. It can activate the MAP kinase pathway, attenuate NMDA receptor activation and/or activate the cAMP/PKA/CREB pathway to modulate intracellular calcium concentrations, in addition to its classical genomic actions (Behl and Holsboer, 1999; Kajta et al., 2001; Wise et al., 2001). The potential of estradiol for direct antioxidant action is also suggested by its structure (Moosman et al., 1997; Prokai et al., 2003). Furthermore, Lee et al. (2004) recently uncovered an important interaction between zinc and estradiol, with the observation of reduced synaptic zinc levels and zinc transporter expression in mice treated with estradiol. It has also been shown to be effective against metal-induced oxidation (Keller et al., 1997). This highlights the need to better understand interactions between estradiol and heavy metals, such as zinc and manganese.

Ethanol is another very common chemical with the potential for harmful interactions with a number of heavy metals, such as zinc and manganese. High levels of intake (associated with the development of alcohol abuse or alcoholism) leads to brain damage, including neuronal loss and gliosis (see Alcohol Health and Research, 2000, for review). Moreover, ethanol can enhance the lead-induced inhibition of brain antioxidant mechanisms (Jindal and Gill, 1999), suggesting it may promulgate toxic effects of heavy metals. In light of the potential for harmful actions of high zinc and manganese, the neuroprotective potential of 17β-estradiol and the neurotoxic potential of ethanol, we wanted to directly test interactions between the metals, estradiol and ethanol in a neuronal-like cell line.

2. Experimental procedures

2.1. Culture of SK-N-SH cells

SK-N-SH cells are characterized as neuronal-like, derived from a human female osteocarcinoma (ATCC). Cells were grown in minimal essential media, supplemented with 1 mM pyruvate, 2 mM glutamine, 25 mM sodium bicarbonate and 10% fetal bovine serum. This cell line was maintained and experiments run with cells in the undifferentiated state. When grown to near confluence (80%), cultures were gently trypsinized then aliquoted to 96-well plates at a plating density of ~3000 cells/well. After allowing for adherence, cells were concurrently exposed to various experimental treatments for 48 h (a time previously established as optimal for measurement of manganese-induced cell death). Manganese chloride was studied across a concentration range of 10–3000 μM. Zinc chloride was studied across a concentration range of 10–1000 μM. Ethanol effects were assessed across a concentration range of 10–400 mM, whereas 17β-estradiol was studied across a range of 0.1–100 μM. Ethanol or 17β-estradiol and heavy metal interaction experiments employed concentrations of ethanol and estradiol showing maximal stimulation of SK-N-SH cell proliferation. Metal levels were studied at growth enhancing and toxic doses. Plates containing ethanol were incubated in closed plastic bags with ethanol in beakers to maintain the desired ethanol concentration. At 48 h, cells were washed free of the treatment media, and assessed for cell viability using the MTT. To verify the MTT assay as an accurate measure of cell

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