ELSEVIER

Physiology & Behavior 86 (2005) 399 – 406

PHYSIOLOGY & BEHAVIOR

Trace metal regulation of neuronal apoptosis: From genes to behavior

Cathy W. Levenson *

Program in Neuroscience and Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL 32306-4340, United States

Abstract

The genetically programmed form of neuronal death known as apoptosis plays a role in many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Huntington's disease. Apoptosis is also responsible for neuronal death after traumatic brain and spinal cord injury, stroke, and seizures. The cognitive and behavioral consequences of all of these disorders can be devastating. Unfortunately the mechanisms that regulate neuronal apoptosis are complex. However, it is this very complexity that provides us with a wide array of potential targets for the development of anti-apoptotic strategies. Thus, our lab is currently exploring the molecular and cellular mechanisms responsible for neuronal apoptosis, with a particular focus on the role of the metals copper, zinc, and iron. Each of these metals is essential for normal central nervous system (CNS) development and function. However, imbalances, either excess or deficiency, can result in neuronal apoptosis. In this review, we show the relationship between these metals in neurodegenerative disorders and CNS injury, and the mechanisms that govern neuronal survival and apoptosis.

Keywords: Zinc; Copper; Iron; Parkinson's disease; Wilson's disease; Motor behavior; Trauma; Anorexia

1. Introduction

Neuronal apoptosis, a genetically programmed form of neuronal death, plays a role in a wide variety of neurodegenerative disorders including Alzheimer's disease [1], Parkinson's disease [2], Wilson's disease [3], amyotrophic lateral sclerosis [4], and Huntington's disease [5]. All of these disorders have serious behavioral consequences. For example, Alzheimer's disease (AD), the most common cause of dementia, is characterized by memory loss, mood alterations, depression, anxiety, irritability, sleep disturbances, and psychosis. By 2047 over 8 million people in the United States alone will likely suffer from this debilitating disease [6]. Parkinson's disease, which affects about 1% of adults over 60 years of age [7], results from the death of dopaminergic neurons in the substantia nigra and is characterized by disruptions in motor behavior including rigidity, postural abnormalities, and tremor at rest, as well as cognitive decline. Wilson's disease is a genetic disorder that results in the copper-mediated apoptosis of dopaminergic neurons leading to motor and cognitive symptoms similar to classical Parkinson's disease. Neuronal apoptosis is also involved in the neuronal death that occurs after traumatic brain injury, spinal cord injury, seizure, and hypoxic–ischemic events such as stroke [8,9].

Understanding the mechanisms responsible for apoptosis in chronic neurodegenerative disorders and injury is complicated by the fact that there are hundreds, if not thousands, of players in the possible cascade of events leading to neuronal apoptosis. The mechanisms of apoptosis are not only cell specific, but also specific to the apoptotic trigger that initiates the cascade. Recently, however, it has been recognized that this complexity provides a wealth of possible targets for the design for new therapeutic and preventative strategies [10,11]. For example, we have evidence that the trace elements zinc, copper, and iron are involved in the cellular and molecular processes that regulate neuronal apoptosis in a number of neurodegenerative disorders. They also regulate apoptosis,

* 237 Biomedical Research Facility, Tallahassee, FL 32306-4340, United States. Tel.: +1 850 644 4122; fax: +1 850 644 0989.
E-mail address: levenson@neuro.fsu.edu.

0031-9384/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2005.08.010

neuronal survival and repair mechanisms after injury. Thus, this review will explore our recent data on the apoptotic mechanisms at work in copper toxicity associated with Wilson's disease, abnormalities in iron metabolism that can lead to Parkinson's disease, and the role of zinc in traumatic brain injury, as well as behaviors such as anorexia.

2. Copper-mediated neuronal apoptosis

The trace element copper is essential for normal central nervous system (CNS) development and function. It is needed at the catalytic site of many critical enzymes including the free radical scavenger superoxide dismutase (Cu,Zn-SOD) [12], dopamine β-monooxygenase, that converts dopamine into norepinephrine [13], and peptidylglycine α-amidating monooxygenase (PAM) that is responsible for the post-translational modification of dozens of neurohormones and neuropeptides including oxytocin, thyrotropin releasing hormone (TRH), neuropeptide Y (NPY), substance P, gonadotropin-releasing hormone (GnRH), cholecystokinin (CCK), and α-MSH [14,15].

Although these functions of copper are essential, copper is also a highly toxic, redox-active metal that has been associated with several neurodegenerative disorders. For example, copper-mediated aggregation of amyloid β (Aβ) has been linked to the development of Alzheimer's disease [16]. Our work has focused on the fact that excess cellular copper results in DNA damage and apoptosis. Copper transport is in large part regulated by copper-transporting P-type ATPases. Mutations in the Wilson's ATPase, ATP7B, result in Wilson's disease (WD) that is characterized by organ and cellular copper accumulation [17]. While this disorder most frequently presents with liver disease including jaundice, hepatitis, cirrhosis, edema, and ascites, it is interesting to note that approximately 20% of patients present first with neurological symptoms including spasticity, rigidity, dysarthria, and muscle spasms. Patients frequently experience neuropsychiatric symptoms including depression, memory loss, anxiety, mania, and a variety of schizo-affective type disorders. Thus, our work has explored the mechanisms responsible for copper-mediated apoptosis in WD.

Much of our work as focused on the possible role of the tumor suppressor p53 in copper-mediated apoptosis. p53-mediated neuronal death plays a role in a variety of neurodegenerative disorders including Parkinson's, Alzheimer's, and Huntington's diseases [18] and is a key mechanism in the elimination cells with genomic damage that might otherwise become cancerous [19]. Thus, mutations that result in the deletion of the p53 gene, or the formation of a mutant conformation that cannot function as a transcription factor, result in abnormal cellular proliferation. In fact, approximately 50% of all human malignant cells carry mutations in the p53 gene, making it the single most frequently mutated gene in human cancer cells [20,21].

A variety of cellular stressors such as oxidation [22], and hypoxia [23], have been shown to result in DNA damage, p53 induction, and apoptosis. As illustrated in Fig. 1, these and other cellular stressors trigger the transcription and activation of p53. Following p53 oligomerization and phosphorylation it is translocated to the nucleus where it acts as a DNA binding transcription factor to induce a host of downstream genes, such as bax, that induce apoptosis. p53 target genes include, not only genes that induce apoptosis, but also genes involved in cell cycle arrest, DNA repair, and cell survival (Table 1).

We have shown that treatment of human liver cells (human hepatoma, Hep G2) with copper to produce cellular copper levels similar advanced WD results in a significant elevation in p53 [24]. While there were small increases in p53 mRNA abundance after copper-treatment, most of the increases in p53 were post-transcriptional. Both immunocytochemistry and Western analysis of cytosolic and nuclear fractions showed that within a few hours of copper exposure p53 was synthesized and translocated to the nucleus. These events were accompanied by clear evidence of apoptosis [24].

We have also studied copper-mediated apoptosis in cultured human neurons. Ntera-2 cells (NT2) are neuronal precursors that undergo retinoic-acid dependent differentiation into post-mitotic neurons in culture (NT2-N). As expected, levels of copper similar to those found in the basal ganglia of WD patients significantly reduced neuronal survival. Curiously, the basal ganglia is a region of the brain particularly susceptible to copper-mediated damage. This is likely due to high concentrations of dopamine. This catecholamine is highly susceptible to oxidative damage, and the resulting oxidative products appear to be neurotoxic. Furthermore, we also have unpublished data showing that copper-mediated oxidation converts the dopamine metabolite salsolinal into a neurotoxic compound that in part mediates neuronal death.

While much of the copper-mediated neuronal death that we have observed was the result of apoptosis (determined both biochemically and morphologically), there was also evidence of copper mediated necrosis [25]. This was accompanied by a striking elevation in transcription of the gene that codes for the heat shock protein, Hsp 70. This chaperone protein is responsible for maintaining protein folding under conditions of cellular stress and likely plays a role in neuronal survival. While it has mostly been associated with mitochondria, we have also detected Hsp 70 in the nucleus of copper-treated neurons, suggesting a role for this chaperone protein in the folding and translocation of nuclear proteins.

As seen in hepatocytes, apoptosis was accompanied by nuclear accumulation of p53 that was largely the result of post-transcriptional mechanisms. However, this work extended the previous findings by showing that in neurons copper-mediated apoptosis is dependent on p53. Trans-