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Perinatal exposure to lead (Pb) promotes Tau phosphorylation in the rat brain in a GSK-3β and CDK5 dependent manner: Relevance to neurological disorders

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ABSTRACT

Hyperphosphorylation of Tau is involved in the pathomechanism of neurological disorders such as Alzheimer's, Parkinson's diseases as well as Autism. Epidemiological data suggest the significance of early life exposure to lead (Pb) in etiology of disorders affecting brain function. However, the precise mechanisms by which Pb exerts neurotoxic effects are not fully elucidated. The purpose of this study was to evaluate the effect of perinatal exposure to low dose of Pb on the Tau pathology in the developing rat brain. Furthermore, the involvement of two major Tau-kinases: glycogen synthase kinase-3 beta (GSK-3β) and cyclin-dependent kinase 5 (CDK5) in Pb-induced Tau modification was evaluated. Pregnant female rats were divided into control and Pb-treated group. The control animals were maintained on drinking water while females from the Pb-treated group received 0.1% lead acetate (PbAc) in drinking water, starting from the first day of gestation until weaning of the offspring. During the feeding of pups, mothers from the Pb-treated group were still receiving PbAc. Pups of both groups were weaned at postnatal day 21 and then until postnatal day 28 received only drinking water. 28-day old pups were sacrificed and Tau mRNA and protein level as well as Tau phosphorylation were analyzed in forebrain cortex (FC), cerebellum (C) and hippocampus (H). Concomitantly, we examined the effect of Pb exposure on GSK-3β and CDK5 activation. Our data revealed that pre- and neonatal exposure to Pb (concentration of Pb in whole blood below 10 μg/dL, considered safe for humans) caused significant increase in the phosphorylation of Tau at Ser396 and Ser199/202 with parallel rise in the level of total Tau protein in FC and C. Tau hyperphosphorylation in Pb-treated animals was accompanied by elevated activity of GSK-3β and CDK5. Western blot analysis revealed activation of GSK-3β in FC and C as well as CDK5 in C, via increased phosphorylation of Tyr-216 and calpain-dependent p25 formation, respectively. In conclusion, perinatal exposure to Pb up-regulates Tau protein level and induces Tau hyperphosphorylation in the rat brain cortex and cerebellum. We suggest that neurotoxic effect of Pb might be mediated, at least in part, by GSK-3β and CDK5-dependent Tau hyperphosphorylation, which may lead to the impairment of cytoskeleton stability and neuronal dysfunction.

1. Introduction

Lead (Pb) is widely recognized as a potent environmental toxin of central nervous system (CNS). The developing brain is particularly susceptible to Pb-induced neurotoxicity (Bihaqi et al., 2014a; Liu et al., 2014; Rahman et al., 2011). Epidemiological

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data suggest that in addition to genetic and life-style factors, environmental exposure to Pb during the early phase of life may have a latent and long-term impact on brain function, playing a role in the pathogenesis of neurodegenerative processes as well as in the etiology of CNS disorders (Bihaqi and Zawia, 2013). Acute Pb intoxication during childhood (with lead concentrations in whole blood (Pb-B)—80 μg/dL), which is currently very rare, may contribute to serious brain dysfunction, manifested by brain edema, convulsions, coma, and lead encephalopathy (Zhang et al., 2015). However, the most critical effects of Pb toxicity occur among children exposed to even low levels of this metal during fetal and/or postnatal development (Pb-B in children below 10 μg/dL). Exposure to low doses of Pb may contribute to the impairment of learning and memory processes, intellectual capacity as well as cognitive and behavioural deficiencies (Baranowska-Bosiacka et al., 2013, 2012a; Baranowska-Bosiacka and Chlubek, 2006; Bellinger, 2008; Canfield et al., 2003; Chiodo et al., 2004; Counter et al., 2005; Jusko et al., 2008; Rahman et al., 2011). Neurodevelopmental disorders such as Autism, Schizophrenia and Attention-Deficit Hyperactivity Disorder (ADHD) are likely the result not only of genetic and life-style factors but also early life exposure to environmental risk factors such as Pb (Fuentes-Albero et al., 2015; Lindsky and Shneider, 2005; Rahbar et al., 2014; Stansfield et al., 2012; Yassa, 2014). Furthermore, more recent studies suggest the important role of Pb in the pathogenesis of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD) (Basha et al., 2005a, 2005b; Bihaqi et al., 2014a, 2014b; Bihaqi and Zawia, 2013; Coon et al., 2006; Gu et al., 2012; Liu et al., 2014; Weisskopf et al., 2010; Wu et al., 2008). Early-life exposure to Pb induces overexpression of AD-related genes (APP, BACE1) and their products, as well as their transcriptional regulator—specificity protein 1 (Sp1), enhancing AD pathology in older rodents and primates (Basha et al., 2005b; Bihaqi et al., 2014b; Wu et al., 2008). Thus, Pb toxicity is an important health problem on a global scale resulting from the both environmental and occupational exposure.

Although multiple cellular targets of Pb have been shown at the biochemical and molecular levels, the precise mechanisms by which Pb exerts its neurotoxic effect has not been elucidated.

The processes essential for normal neuronal functioning, synaptic plasticity and transmission, such as neurite growth, synaptogenesis and axonal transport, require an intact microtubule structure (Garcia and Cleveland, 2001; Rahman et al., 2012). One of the microtubule-associated proteins (MAPs), responsible for stabilization and regulation of microtubule network, is Tau protein (Mietelska-Porowska et al., 2014). The function of Tau depends on its phosphorylation state. Hyper-phosphorylated Tau has less affinity for binding with microtubules (Igbal et al., 2010; Kawakami et al., 2011; Lei et al., 2010) and impairs mitochondrial respiration, axonal transport, post-synaptic function as well as compromises cell signalling leading to cognitive impairments (Mietelska-Porowska et al., 2014). In its longest isoform Tau contains approximately 85 potential phosphorylation sites, but phosphorylation at Ser396 seeming to play the pivotal role for its functioning, and in particular destabilizes microtubules (Bramblett et al., 1993; Johnson and Stoothoff, 2004; Lei et al., 2010; Li and Paudel, 2006). Increased levels of p-Tau(Ser396) has been found in both AD and PD brains (Lei et al., 2010; Muntane et al., 2008). The dysfunction of Tau protein evoked by its abnormal phosphorylation leads to intracellular accumulation of this protein, aggregation, and the formation of neurofibrillary tangles (NFTs) (Zhang et al., 2012). Neurofibrillary degeneration is observed in many neurodegenerative disorders like AD, PD and other tauopathies such as, Parkinsonism-dementia complex of Guam, Progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD), Pick's disease (PiD), Huntington disease (HD) or FTDP-17 Tau (frontotemporal dementia with parkinsonism linked to chromosome 17 and Tau mutations) (Bihaqi and Zawia, 2013; Kawakami et al., 2011; Lei et al., 2010; Wang and Mandelkow, 2015).

The effect of Pb on Tau pathology has been reported (Bihaqi et al., 2014a; Bihaqi and Zawia, 2013; Liu et al., 2014; Rahman et al., 2012). However, there is still an open question on how pre- and neonatal exposure to Pb at low doses may affect Tau phosphorylation, which then could be involved in the mechanisms of Pb neurotoxicity. Only a few works have analyzed the neurotoxicity of Pb at blood concentrations considered "safe for humans" (below 10 μg/dL), particularly in the developing brain. Moreover, we chose this model because exposure to environmental toxins during the early phase of life is one of the possible causal factors for abnormal development. The brain in prenatal and early postnatal periods is undergoing rapid growth and is very sensitive to environmental pollutants, including heavy metals (Kim et al., 2010). Hence, the aim of this paper is to examine whether pre- and neonatal exposure to Pb (concentration of Pb in rat offspring blood below the 'safe level') may lead to Tau dysfunction in a developing rat brain. We studied the expression of Tau and its phosphorylation state. Moreover, the involvement of two major Tau-kinases: glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase (CDK5), in Pb-induced Tau modification were evaluated. Attention is focused on the neurodevelopment of the forebrain cortex, hippocampus and cerebellum as these regions have been reported to be sensitive to the toxicity of lead (Collins et al., 1982; Strużyńska et al., 2007). Our data indicated that perinatal Pb administration caused GSK-3β and CDK5-dependent Tau phosphorylation, what in further consequence may lead to the impairment of cytoskeleton stability.

2. Materials and methods

2.1. Reagents

The following antibodies were used in the current study: anti-phospho Tau (Ser396), anti-phospho GSK-3β (Ser9), anti-GSK-3β (Cell Signaling Technology, Beverly, MA, USA), anti-phospho GSK-3β (Ty216) (BD Biosciences Pharmingen, NJ, Franklin Lakes,USA), anti-Tau, anti-CDK5, anti-p25/p35, anti-α-spectrin (Santa Cruz Biotechnology, CA, USA), anti-phospho Tau (Ser199/202), anti-GAPDH, anti-rabbit IgG (Sigma-Aldrich, St. Louis, MO, USA), anti-mouse IgG (GE Health Care UK, Little Chalfont, Buckinghamshire, UK). RNeasy Lipid Tissue Mini Kit was obtained from Qiagen (Poland). Reagents for reverse transcription (FirstStrand cDNA synthesis kit with oligo-dT primers) and PCR (Power SYBR Green PCR Master Mix) were obtained from Fermentas and Applied Biosystems (Foster City, CA, USA). 3,3′-Diaminobenzidine (DAB) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Procedures involving animals were carried out in strict accordance with international standards of animal care guidelines, and every effort was made to minimize suffering and the number of animals used. Experiments were approved by the Local Ethical Committee on Animal Testing at the Pomeranian Medical University in Szczecin, Poland (approval No 30/2008).

Three-month old female (250 ± 20 g) Wistar rats (n = 6) were kept for a week in a cage with sexually mature males (2:1). All animals were allowed free access to food and water and were kept in a room with a controlled temperature under a LD 12/12 regime. After a week, they were separated from the males, and each female was placed in an individual cage. Pregnant females were divided into two groups: control and Pb-treated. Females from the Pb-treated group (n = 3) received 0.1% lead acetate (PbAc) in drinking