Toxicology 390 (2017) 146–158

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Full length article

Glycogen metabolism in brain and neurons – astrocytes metabolic cooperation can be altered by pre- and neonatal lead (Pb) exposure

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ARTICLE INFO

Keywords:
Lead (Pb) neurotoxicity
Brain glycogen metabolism
Glycogen synthase (Gys1)
Glycogen phosphorylase kinase (PHKB)
Glycogen phosphorylase brain isoform (PYGB)
Glycogen phosphorylase muscle isoform (PYGM)

ABSTRACT

Lead (Pb) is an environmental neurotoxin which particularly affects the developing brain but the molecular mechanism of its neurotoxicity still needs clarification. The aim of this paper was to examine whether pre- and neonatal exposure to Pb (concentration of Pb in rat offspring blood below the "threshold level") may affect the brain's energy metabolism in neurons and astrocytes via the amount of available glycogen. We investigated the glycogen concentration in the brain, as well as the expression of the key enzymes involved in glycogen metabolism in brain: glycogen synthase 1 (Gys1), glycogen phosphorylase (PYGM, an isoform active in astrocytes; and PYGB, an isoform active in neurons) and phosphorylase kinase β (PHKB). Moreover, the expression of connexin 43 (Cx43) was evaluated to analyze whether Pb poisoning during the early phase of life may affect the neuron-astrocytes' metabolic cooperation. This work shows for the first time that exposure to Pb in early life can impair brain energy metabolism by reducing the amount of glycogen and decreasing the rate of its metabolism. This reduction in brain glycogen level was accompanied by a decrease in Gys1 expression. We noted a reduction in the immunoreactivity and the gene expression of both PYGB and PYGM isoform, as well as an increase in the expression of PHKB in Pb-treated rats. Moreover, exposure to Pb induced decrease in connexin 43 immunoexpression in all the brain structures analyzed, both in astrocytes as well as in neurons. Our data suggests that exposure to Pb in the pre- and neonatal periods results in a decrease in the level of brain glycogen and a reduction in the rate of its metabolism, thereby reducing glucose availability, which as a further consequence may lead to the impairment of brain energy metabolism and the metabolic cooperation between neurons and astrocytes.

1. Introduction

Lead (Pb) toxicity is an important global health problem resulting from environmental and occupational exposure (CDC, 2012; ATSDR, 2013). Various papers show a correlation between elevated blood Pb levels in children and impaired memory, concentration, learning and reduced IQ levels (Canfield et al., 2003; Bihaqi et al., 2014; Lidsky and Shneider, 2005; Rahman et al., 2012). More recent studies also suggest the important role of Pb in the pathogenesis of neurodevelopmental disorders such as autism, schizophrenia and attention deficit hyperactivity disorders (ADHD) (Fuentes-Albero et al., 2015; Lindsky et al., 2005; Rahbar et al., 2014, 2012; Stansfield et al., 2012; Yassa, 2014; Kim et al., 2013; Nicolescu et al., 2010). Furthermore, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases are likely to be the result not only of genetic and lifestyle factors but also of early life exposure to environmental risk factors such as Pb (Bihagi et al., 2014; Bihagi and Zawia, 2013; Coon et al., 2006; Gu et al., 2012; Liu et al., 2014; Weisskopf et al., 2010). However, the precise mechanism of Pb neurotoxicity has not been fully elucidated. Some point to electrophysiological, neurochemical and molecular changes as the basis of the disorders observed (for a review see Baranowska-Bosiacka et al., 2012a).

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http://dx.doi.org/10.1016/j.tox.2017.09.007
Received 30 June 2017; Received in revised form 28 August 2017; Accepted 7 September 2017
Available online 12 September 2017
0300-483X/

It has been shown that even acceptable Pb levels in children's blood result in neurocognitive impairment (Canfield et al., 2003; Chiodo et al., 2004; Counter et al., 2005; Jusko et al., 2008). The direct neurotoxic effects of Pb can be seen in structural abnormalities in synapses associated with alterations in the level of key synaptic proteins (Gąsowska et al., 2016), impaired processes of storing and releasing neurotransmitters (Basha and Reddy, 2015; Mansouri et al., 2013; Fortune and Lurie, 2009), and the impairment of signaling pathways and the energy metabolism of neurons (Baranowska-Bosiacka et al., 2011a), leading to serious neurological disorders. The known causes of brain energy metabolism disorders include the impairment of glycolysis enzyme activity, reduced mitochondrial membrane potential, the impairment of ATP synthesis, as well as the decreased expression and activity of Na^+/K^+ ATPase activity (Baranowska-Bosiacka et al., 2012b, 2013a,b).

Glycogen plays a crucial role in brain energy metabolism and its metabolism determines correct metabolic co-operation between the neuron and the astrocyte. This, in turn, is crucial for normal neurotransmission and brain plasticity. According to many studies, interruptions in the delivery of glycogen to astrocytes, e.g. during hypoglycemia, contribute to the generation of lactate, which is then transported to the neighboring neurons and is metabolized there. Hence, astrocyte glycogen plays a specific protective function against hypoglycemia, while maintaining the function of the neurons (see e.g. Falkowska et al., 2015). The regulation of glycogen metabolism is a perfectly coordinated, multi-stage system in which glycogen synthase (Gys1) and glycogen synthase kinase (GSK) play a crucial role, as do enzymes catalyzing its degradation, i.e. glycogen phosphorylase (PYG) and glycogen phosphorylase kinase (PHK).

In our previous study (Gąssowska et al., 2016) pre- and neonatal exposure to Pb (concentration of Pb in whole blood below 10 μg/dL) caused a significant increase in the phosphorylation status of glycogen synthase kinase 3β (GSK-3β) in the brains of rats. GSK-3β is identified as an enzyme down-regulating the activity of glycogen synthase. The excessive phosphorylation of GSK-3β, observed under conditions of Pb toxicity as well as in many neurological disorders, may contribute in this way to the deregulation of glycogen metabolism. Moreover, GSK-3β can also act as one of the major Tau protein kinases. Our previous study revealed a significant increase in the phosphorylation of Tau with a parallel rise in the level of Tau protein in Pb-treated rat brain. The dysfunction of the microtubule-associated protein Tau (MAP Tau) induced by its abnormal phosphorylation leads to the 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, such as Alzheimer's (AD) and Parkinson's diseases (PD) and other tauopathies.

In addition to Tau, one of the cytoskeleton proteins is also connexin 43, which is involved not only in the formation of the blood-brain barrier, but also in the formation of astrocyte-neuron connections and the coordination of metabolites such as glucose and lactate, which are also glycogen metabolites. It has been shown that disorders of expression of this protein may also play a role in the etiology of neurodegenerative diseases (see also Freitas-Andrade and Naus, 2016). The effect of Pb on brain energy metabolism has been reported in a variety of models of acute and chronic toxicity (Baranowska-Bosiacka et al., 2011a,b; Marchlewicz et al., 2009; Strużyńska et al., 1997). However, it is still an open question as to how pre- and neonatal exposure to Pb at low doses may affect glycogen metabolism and neuron-astrocyte metabolic cooperation, which could then be involved in the mechanisms of Pb neurotoxicity. In our study, we analyzed the neurotoxicity of Pb at blood concentrations considered "threshold for humans" in the developing brain. We chose this model because exposure to environmental toxins during the early phase of life is a possible causal factor for abnormal development. The brain in prenatal and early postnatal periods undergoes rapid growth and is very sensitive to environmental pollutants, including heavy metals (Kim et al., 2010).

Hence, the aim of this paper was to examine whether pre- and neonatal exposure to Pb (concentration of Pb in rat offspring blood below the "threshold level") may affect the brain's energy metabolism in neurons and astrocytes via the amount of available glycogen. We investigated the glycogen concentration in the brain, as well as the expression of the key enzymes involved in glycogen metabolism in brain: glycogen synthase 1 (Gys1), glycogen phosphorylase (PYGM, an isoform active in astrocytes; and PYGB, an isoform active in neurons) and phosphorylase kinase β (PHKB). Moreover, the expression of connexin 43 (Cx43) was evaluated to analyze whether Pb poisoning during the early phase of life may affect the neuron-astrocytes' metabolic co-operation. We focused on the forebrain cortex (FC), cerebellum (C) and hippocampus (H), as these regions have been reported to be sensitive to Pb toxicity (Baranowska-Bosiacka et al., 2011a,b; Collins et al., 1982; Strużyńska et al., 2007).

2. Material and methods

2.1. Reagents

The following antibodies were used in the current study: Anti-Glycogen synthase 1 (Gys1) antibody (Abcam, Cambridge, UK), glycogen phosphorylase muscle isoform PYGM and glycogen phosphorylase brain isoform PYGB (Santa Cruz Biotechnology, USA), glycogen phosphorylase kinase (PHKB) (Abcam, Cambridge, UK). The Glycogen Assay Kit II (Colorimetric) was purchased from Abcam, Cambridge, UK. The 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).

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. The 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 experimental. Females from the experimental group (n = 3) received 0.1% lead acetate (PbAc) in drinking water *ad libitum*, starting from the first day of gestation. The solution of PbAc was prepared daily in disposable plastic bags (hydropac, Anilab, Poland) from solid reagent directly at the desired concentration, and was not acidified. Pregnant females from the control group (n = 3) received distilled water until the offspring were weaned. The volume of liquids taken in did not differ significantly between the experimental and control groups. Offspring (males and females) stayed with their mothers and were fed by them. During the feeding of pups, mothers from the experimental group were still receiving PbAc in drinking water *ad libitum*. The pups were weaned at postnatal day 21 (PND 21) and placed in separate cages. From that moment, the young rats of the study and control groups received only distilled water *ad libitum* until PND 28.

We chose an oral route of exposure to 0.1% lead acetate as this mimics environmental exposure and is used as a common model of lead poisoning for rodents (Kang et al., 2009; Xu et al., 2005). In addition, our previous study (Baranowska-Bosiacka et al., 2012b), revealed that this treatment protocol results in a concentration of Pb in whole blood (Pb-B) of rat offspring below the "threshold for humans" (10 ug/dL)