REVIEW PAPER/*ARTYKUŁ POGLĄDOWY*

Neurotoxicity of lead. Hypothetical molecular mechanisms of synaptic function disorders

Neurotoksyczność ołowiu. Hipotetyczny molekularny mechanizm zaburzeń funkcji synaptycznych

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Abstract

Lead (Pb) toxicity is still a major health problem associated with both environmental and occupational exposure. Special attention is given to the neurotoxic effect of lead. Along with the newly emerging data, the Pb concentration in the body that can be considered safe is declining. Numerous studies on the neurotoxicity of Pb have shown multiple cellular 'molecular targets' of this metal at the biochemical and molecular levels, and differences in sensitivity to its toxic action among various neural cells. One possible target of the neurotoxic effect of Pb (at the synapse level) is *N*-methyl-*D*-aspartic acid (NMDA) receptors. This review presents the hypothetical molecular mechanism by which Pb disrupts synapse formation and plasticity in developing hippocampal neurons and the role of the NMDA receptor-dependent signaling pathway and brain-derived neurotrophic factor (BDNF) as a mechanism of Pb neurotoxicity at the synapse level.

Key words: lead (Pb), neurotoxicity, NMDA receptor, BDNF, brain, hippocampus.

Streszczenie

Toksyczność ołowiu (Pb) stanowi problem zdrowotny wynikający z narażenia środowiskowego i zawodowego. W centrum zainteresowania jest neurotoksyczne działanie Pb. W badaniach nad neurotoksycznością Pb obserwuje się tendencję do obniżania progu „bezpiecznego stężenia" Pb. Na poziomie biochemicznym i molekularnym wykazano wiele „punktów uchwytu" Pb w procesach komórkowych oraz niejednakową wrażliwość poszczególnych rodzajów komórek nerwowych na jego toksyczne działanie. Jednym z celów działania Pb na poziomie synapsy są glutaminianergiczne receptory jonotropowe dla kwasu *N*-metylo-*D*-asparaginowego (NMDA). W pracy przedstawiono przypuszczalny mechanizm molekularny, poprzez który Pb zakłóca formowanie i plastyczność synapsy w rozwijających się neuronach hipokampa, oraz omówiono rolę zaburzenia szlaku sygnałowego zależnego od receptora NMDA i czynnika wzrostu pochodzenia mózgowego (BDNF) jako mechanizmu neurotoksycznego działania Pb na poziomie synapsy.

Słowa kluczowe: ołów (Pb), neurotoksyczność, receptor NMDA, BDNF, mózg, hipokamp.

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Introduction

Lead (Pb) is a common toxic element, the concentrations of which in living organisms are closely related to anthropogenic environmental contamination. In developed countries, growing awareness of the effects of Pb on the environment and on human health has resulted in efforts to restrict the use of Pb [1,2]. However, on a global scale, the total level of Pb emissions into the atmosphere is still high [3,4]. In addition, lead compounds are not biodegradable and therefore the current levels of environmental Pb contamination cannot be effectively reduced [5].

Neurotoxicity and the threshold of 'safe' concentrations of Pb

Pb toxicity is still a major health problem, which is associated with both environmental and occupational exposure. Special attention has been given to the neurotoxic effects of lead [6]. In accordance with newly emerging data, the level of Pb concentration in the organism that can be considered safe is declining. Some researchers even argue that there is no safe concentration of Pb, as in fact any concentration of Pb in the organism results in the impairment of biochemical processes in the brain [7]. Moreover, whole blood lead concentrations (PbB), even those below 10 μg/dL, are inversely associated with children's intelligence quotient (IQ) scores at three and five years of age, and associated declines in IQ are greater at these concentrations than at higher concentrations [8]. What is more, Pb accumulates in some tissues/organs and under certain conditions (e.g. pregnancy, osteoporosis, hormonal disorders) it is released into the bloodstream and therefore in effect to the brain. According to recent data, even the bone pool of Pb, previously considered to be a deposition site and therefore a form of detoxification, can also be a mobile pool of Pb [9-12].

Neurotoxicity of Pb exposure in children

Acute Pb contamination in children (PbB – 80 μg/dL), which is currently very rare, can have a dramatic effect on the central nervous system, i.e. brain edema, convulsions, and coma, and can lead to encephalopathy [13]. Exposure to lower doses of Pb can lead to subtle, non-specific disorders of brain functions – reduced perception; impaired cognition, hearing and sight; and even disorders in neurobehavioural functioning, including aggression. It was also shown that Pb may be one of the factors that induce lower IQ in schoolchildren [13,14]. These lead-associated abnormalities have generally been reported in children, but recently they have also been indicated in adults [14]. Immaturity has a great significance in Pb toxicity, as in toxicity in general. Intestinal and blood-brain barriers which have not yet reached full development are less effective in limiting Pb absorption from the gastric tract and its penetration from the blood vessels to the brain's blood vessels and brain parenchyma. In addition, immature cellular defense mechanisms make the brain more susceptible to Pb toxicity. Importantly, the effect of Pb on cognitive and behavioral functions seems to be irreversible [13]. It has been shown that chelation therapy, which is recommended for children with a PbB above 45 μg/dL, may reduce the amount of Pb in the organism but will not compensate cognitive and behavioral problems resulting from Pb exposure earlier in childhood [15]. Additionally, schoolchildren with elevated PbB levels may have problems with concentration during classes and may also exhibit excessive activity. This phenotype is similar to that of attention deficit hyperactivity disorder (ADHD) and indeed a positive correlation has been found between an increase in PbB and ADHD [16,17]. It has also been reported that prenatal exposure to Pb can result in antisocial and illegal behavior among teenagers, and also that people with elevated PbB levels in childhood are arrested more often, both as teenagers and adults [18,19]. For this reason, Pb has been considered to be a 'developmental toxin' and for obvious reasons researchers tend to focus on its effect on children.

The margin between the level of exposure and toxic effects is small. Therefore, for risk assessment and follow-up of time trends, there is a need for adequate information on exposure. The latest study [20] of PbB levels in schoolchildren aged 7-11 in 2007-2008 (433 in total), in urban locations in six European countries, showed only marginal differences, with a range of about 1.5 times between the lowest and the highest PbB geometric mean (Slovenia: 1.34 μg/dL; Sweden: 1.40 μg/dL; Czech Republic: 1.55 μg/dL; Poland: 1.63 μg/dL; Croatia: 1.79 μg/dL; Slovakia 1.94 μg/dL). In the same study, it was also shown that the geometric mean of PbB in children from non-European cities was much higher (Ecuador: 3.17 μg/dL and Morocco 7.10 μg/dL), [20]. In a prospective study Stroh *et al.* (2009) [21] measured PbB level in 3879 Swedish school children during the period 1978-2007. The geographical infor-

mation system (GIS) analysis in this study revealed that although the emissions from the smelter and children's PbB levels had decreased considerably since 1978, proximity to the Pb smelter continued to affect levels of PbB, even in recent years (geometric mean: near smelter 2.29 μg/dL; far from smelter 1.98 μg/dL). The analysis also revealed that proximity to major roads noticeably affected the children's PbB levels during the period 1978-1987 (geometric mean near major roads: 4.43 μg/dL; far from roads: 3.83 μg/dL), due to the considerable amount of Pb in petrol. This effect was not visible after 1987 due to prohibition of Pb in petrol. The results show that proximity to the Pb smelter still has an impact on the children's PbB levels. This is alarming since it could imply that living or working in the vicinity of a former Pb source could pose a threat years after reduction of emissions. The analysis also revealed that urban children exposed to Pb from traffic were only affected during the early period, when there were considerable amounts of Pb in petrol, and that the prohibition of Pb in petrol in later years led to reduced PbB levels of urban children [21]. However, the study of Barton (2011) [22] in about 300 6-year-old preschool children from the Krakow (Poland) urban area, during the period 1997-2004, showed a PbB range of 1.9-32.7 μg/dL, and indicated that 5% of the studied children exceed the 'safe' PbB level. Also a study in the U.S. (1999-2002) estimated that 1.4% of children aged 1-5 years had Pb in blood ≥ 10 μg/dL [23]. The prevalence of elevated PbB levels (≥ 10 μg/dL) among children in the United States decreased from 8.6% in 1988-1991 to 1.4% in 1999-2004, which is an 84% decline. From 1988-1991 and 1999-2004, children's geometric mean PbB levels declined in non-Hispanic black (5.2-2.8 μg/dL), Mexican American (3.9-1.9 μg/dL), and non-Hispanic white children (3.1 μg/dL to 1.7 μg/dL). However, levels continue to be highest among non-Hispanic black children relative to Mexican American and non-Hispanic white children. Blood lead levels were distributed as follows: 14.0% were < 1.0 μg/dL; 55.0% were 1.0 to < 2.5 μg/dL; 23.6% were 2.5 to < 5 μg/dL; 4.5% were 5 to < 7.5 μg/dL; 1.5% were 7.5 to < 10 μg/dL; and 1.4% were ≥ 10 μg/dL [23].

Neurotoxicity of Pb exposure in adults

Until recently, adults were believed to be 'resistant' to the neurotoxic effects of lead thanks to a fully developed blood-brain barrier and defense mechanisms. However, recent studies indicate that various environmental factors, including Pb, may be responsible for neurological deficiencies in adults [24]. For example, it has been suggested that long-term exposure to such environmental factors – the most important of which has been suggested to be Pb – can result in the impairment of cognitive functions [25]. It is also possible that neurodegenerative changes in the brain may be associated with Pb contamination, as a factor that accelerates or enhances the development of neurodegenerative diseases in general [26,27].

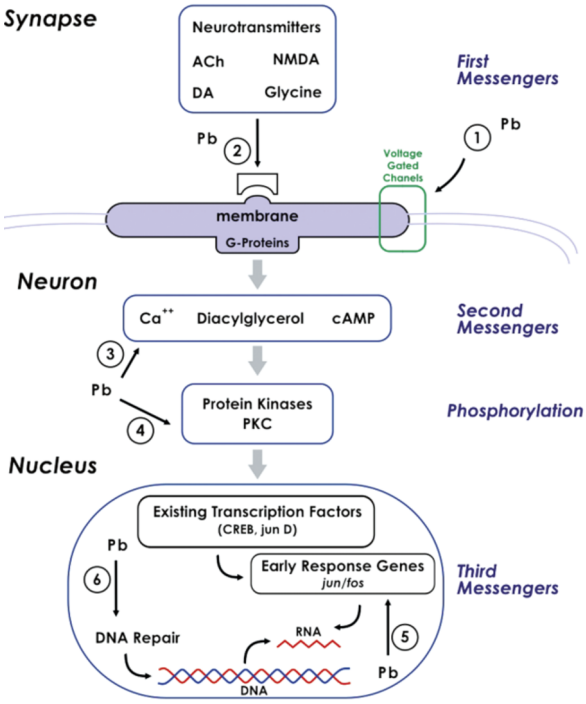
It was shown that Pb participates in the activation of astroglia and inflammatory processes in the brain [28,29], and as is currently known, brains of patients suffering from Alzheimer disease and other neurodegenerative diseases experience chronic inflammatory reactions which involve microglial cells and astrocytes accumulating in the area of amyloid beta plaque [30].

Epidemiological studies have shown a positive correlation between increased levels of Pb in the brain and the incidence of Parkinson disease [31,32]. Exposure to Pb has also been shown to have some influence on the etiology of amyotrophic lateral sclerosis (ALS). Data from the United States National Health Institute have shown an increased risk of ALS among people reporting occupational Pb exposure [33,34]. It was also shown that lead has a negative effect on the peripheral nervous system. Zheng *et al*. [35], in their study on Pb-exposed workers, showed that high PbB was associated with significantly lower sensory and motor conduction velocities in the median, ulnar and peroneal nerves. Also Bilińska *et al*. [36,37], who studied asymptomatic workers chronically exposed to inorganic Pb, recorded electrophysiologically the subclinical damage in the peripheral nervous system and showed a neurotoxic effect of Pb on peripheral nerves, manifested by damage to slow-conducting motor nerves.

In conclusion, there is visible and growing interest among researchers in the effect of Pb on the nervous system throughout human life, not only during the periods of higher susceptibility which occur in the course of the developmental stage.

N-methyl-*D*-aspartic acid (NMDA) receptors

Numerous studies on the neurotoxicity of Pb have shown multiple cellular 'molecular targets' of this metal at both biochemical and molecular levels (Fig. 1), and variations in sensitivity to its toxic activity among the



ACh – acetylcholine, NMDA – N-methyl-D-aspartic acid, DA – dopamine, Pb – lead, Ca^{2+} – calcium, cAMP – cyclic adenosine monophosphate, PKC – Protein kinase C

Fig. 1. Potential cellular 'molecular targets' of Pb in neurons: 1) voltage-gated channels; 2) neurotransmitters (first messenger); 3) second messengers; 4) protein kinases; 5) third messenger; 6) DNA repair. Based on Finkelstein *et al.* [68]

various neural cells [38]. One possible target of the neurotoxic effect of Pb at the synapse level is the NMDA receptors (NMDAR) [39,40]. These receptors play a major role in fast synaptic transmission via their associated ion channel, which is permeable for Ca^{2+}, Na^{+}, K^{+}, which are key factors in the processes connected with neural network formation and synaptic plasticity (and therefore for learning and memory). NMDAR activation requires two co-agonists (glutamic acid and glycine), and the removal of the Mg^{2+} blockade through the depolarization of the neuron's cellular membrane. Glutamic acid is considered to be the main excitatory neurotransmitter in the CNS (central nervous system) of mammals and is responsible for the transmission of the functional signal in many parts of the brain, e.g. the cortex and hippocampus. Most neurons have glutamatergic receptors (either metabotropic receptors associated with G proteins or ionotropic receptors, such as NMDA, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) or KA (kainic acid), which suggests that this system plays a crucial physiological role. Depolarization of the glutamatergic neuron endings results in releases of glutamic acid to the synaptic cleft, where it is bound to the NMDAR. From the synaptic cleft, glutamic acid is reuptaken by Na^{+}-dependent transporters, which are present in the glial cell membrane and to a lesser extent in the neuron membranes. Under physiological conditions, this reuptake contributes to the termination of the glutamic acid's interaction with the receptors in the synapse [40].

NMDAR are distributed widely across the CNS but are predominant in the hippocampus (particularly in the CA1 region), cortex and basal ganglia, where they display postsynaptic expression. Because the NMDA receptor's channel is Ca^{2+}-permeable, its opening leads to increased concentrations of Ca^{2+} in the neuron and thus to the activation of multiple Ca^{2+}-dependent enzymes (kinases, calmodulin, phospholipases and calpain). The biochemical processes thus activated lead to the synthesis of new proteins and functional changes in the synapses.

The involvement of the NMDAR in the signaling pathways in the neuron means that it is a major mediator in synaptic plasticity processes and in memory and learning. However, excessive activation of the NMDAR can cause excitotoxicity, i.e. excessive stimulation of the receptors. This causes an uncontrolled increase in the intracellular concentration of Ca^{2+}, which can even lead to fatal damage to the neurons [41].

The role of NMDA receptors in learning and memory

NMDA receptors play a major role in the processes of learning and memory that occur in the hippocampus, as demonstrated by a simple experiment consisting of the intra-ventricular administration of amino-phosphono-valeric acid (APV), an antagonist of NMDAR. In rats, APV caused memory impairment similar to that observed following damage to the hippocampus [42]. Knock-out mice which lacked NMDAR in the hippocampus also showed impairments in spatial learning, which provides further evidence about the role of the NMDAR in memory pathway formation.

Before discussing the relationship between NMDAR and the capability of acquiring knowledge, a few facts relating to the physiology of memory should be mentioned. At the neuron level, the main mechanism responsible for acquiring new skills and for learning is long-term potentiation (LTP) [43,44].

What is the role of LTP in memory processes? Information coming to the cortex from the sensory systems, once integrated into a whole impression, is forwarded

to the hippocampus, where it is stored (the memory trace persists for several weeks). The mechanism of the memory trace in this area is based on the creation of prolonged activity potential in a number of separate neural networks. The sustaining of this stimulation is associated with the phenomenon of LTP [44]. This effect is obtained in synapses possessing NMDA receptors. When glutamate is released and simultaneously bound to the postsynaptic NMDA receptor, it results in a prolonged influx of Ca^{2+} via the channel associated with the NMDA receptor, causing an increase in the concentration of intracellular Ca^{2+} in the postsynaptic neuron. Consequently, a protein kinase cascade system is triggered, which, through the phosphorylation or dephosphorylation of proteins essential to neurotransmission, leads to modulation of their function. This in turn activates the postsynaptic neuron. This cascade system is further maintained in a state of neuronal excitation by a factor mobilized by kinase activity and released into the area surrounding synapses by the postsynaptic neuron. There are many indications that this substance is nitric oxide [45].

Changes in the intracellular concentration of Ca^{2+}, which are capable of either enhancing or weakening synaptic transmission through the activation of various biochemical cascades, are crucial to the processes of synaptic plasticity. A short-term high concentration of Ca^{2+} in the postsynaptic neuron, which activates protein kinases, is a prerequisite for the induction of LTP. A moderate, but longer lasting (several minutes long) increase in the concentration of Ca^{2+} preferentially stimulates dephosphorylation by activating phosphatases, and leads to long-term depression [46].

It is therefore believed that LTP is one of the basic neuronal phenomena underlying at least some forms of learning and memory, and is used as a model by these processes. The maintenance of the activity of the neuronal circuits is a prerequisite for storing the memory trace. Repeated stimulation of the area of recent memory in the same way, thus strengthening its trace by mobilizing the following neural network, is the basis of the learning process. After some time, a record of recent memory is retransmitted to the gnosis centers. This record is stored there by biochemical processes involving the production of mRNA and proteins, which allows the number of synaptic receptors to increase and new synaptic connections between neurons to be created (synaptic plasticity). This is why impaired LTP has been reported to be one of the causes of learning disability at the molecular level [46].

Toxicity of Pb in relation to NMDA receptors

It has been shown that Pb affects LTP, causing cognitive impairment both in slices of the hippocampus (CA1 and dentate gyrus) in rats exposed to Pb, and also in *in vitro* conditions. Pb inhibits LTP and induces both an increase in the threshold and a decrease in the amplitude of LTP [47]. What is the mechanism of LTP inhibition by Pb?

In order to explain this, we need to look again at the construction of the NMDAR. To date, two main families of NMDA receptor subunits have been cloned. The subunit NR1 is represented by one gene and NR2 by four: NR2A, B, C, and D. The NMDA receptor is a tetramer, a combination of two NR1 and two NR2 subunits. The NR1 subunit proteins are not homogeneous and occur as *splice variants*. The process of alternate cutting and folding of the precursor mRNA (alternative splicing) leads to a number of variants of protein output through an additional/altered amino acid sequence in the amino- or carboxyl terminal (i.e. the presence or absence of the fifth exon at the amino terminus and of exons 21 and 22 at the carboxyl terminus ($2 \times 2 \times 2 = 8$ known variants thus far). Such a structure, including all possible variants of the subunit combinations, creates the theoretical possibility of a huge number of NMDAR subtypes ($8 \times 8 \times 4 \times 4 = 1024$) [9,48]. In nature, however, some forms are predominant, while others are very rare. In the hippocampus, the expression of subunits NR2A and NR2B is most common. The NR2B subunit displays a similar expression in ontogenesis and the early postnatal stage, whereas the expression of NR2A and NR1 increases gradually; this increase is associated with the maturation of the brain and the increased intensity of synaptic plasticity in the early postnatal period. These individual subunits may also be associated with other intracellular proteins and hence with different signal pathways in the cell. NR2 subunits can be associated with a signaling pathway leading to the life or death of the neuron, a pathway which protects neurons against oxidative stress, or with transcription factors for a number of immediate early genes (IEGs) which affect the transmission of signals in the synapse and synaptic plasticity. The three protein domains of subunits NR1 and NR2 pass through the cellular membrane (the M1, M3, and M4 membrane inserted fragments). The M2 fragment forms only a loop in the membrane and constitutes one wall of the ion channel. This section of the ion channel is crucial to its

Ca^{2+} permeability and its blockade by Mg^{2+} and other substances [49,50].

The NMDA receptor can be modulated by many independent endogenous and exogenous factors. These affect its function in different ways. As has been mentioned, L-glutamic acid is its main endogenous agonist, and NMDA acid is its most commonly used exogenous agonist. Mg blocks the receptor's channel under physiological conditions (in a voltage-dependent manner). Under physiological conditions, Zn^{2+} also interacts with the receptor (in a voltage-independent manner). The effect of other inhibitors on the channel of the NMDA receptor has therefore been described as similar to that of Mg or Zn [49].

The inhibiting effect of Pb^{2+} on the current of ions through the channel of the NMDA receptor was discovered by Büsselberg [51] and Guilarte [52], who described it as a highly specific effect and independent of voltage (i.e. similar to Zn^{2+} activity). However, it is probably non-competitive in relation to the Zn^{2+} binding site [53].

Taking these factors into consideration, we carried out studies that would help to clarify this question. In this study we used molecular biology techniques (directed mutagenesis and receptor protein cloning) to carry out a substitution of amino acids in the NMDA receptor Zn^{2+} binding site for each of the receptor subunits [39]. Using the voltage clamp technique, we made electrophysiological measurements and showed that Pb blocked the NMDA receptor channel and that the blockade depends on the structure of its subunits; that in the case of NR2A the binding of Pb occurs only partly in the Zn binding site; and that in the case of NR2B the Pb binding site only partly overlaps with the binding site for Zn. This points to a particular sensitivity on the part of the NMDA receptor subunit NR2A in relation to Pb – which could be of particular importance given the fact that, as has been mentioned, the expression of the NR2A subunit increases gradually with the maturation of the brain and with the increasing intensity of synaptic plasticity in the early postnatal period. The lead concentration (0.1-10 μM of free Pb^{2+}) used in our study was higher than that observed in *in vivo* studies. It has been proposed that the synaptic concentrations of free Pb^{2+} in experimental animals chronically exposed to environmentally relevant levels of the metal are in the nanomolar range [54]. However, the total Pb concentration (measured by atomic absorption spectrometry) in rats gestationally and lactationally exposed to lead caused a Pb concentration in the hippocampus as high as 7.44 μg/g dry mass and 5.06 μg/dL in whole blood [55]. The fact that the Pb^{2+} IC50 (half maximal inhibitory concentration) for access to the NMDAR channel observed in our *in vitro* study was in the low micromolar range (1.3-11.3 μM) may indicate that the effect on the receptor of chronic environmental exposure to this metal is likely mediated via an indirect mechanism.

Zhang *et al*. [56] showed that Pb interferes with normal receptor ontogenesis – that chronic exposure to Pb results in a decrease in protein expression in the NR2A subunit. Expression in the NR2B subunit was not affected, nor was receptor expression increased in the hippocampus or the cerebral cortex of animals exposed to Pb during the prenatal and early postnatal periods. Similar observations were performed in the case of cultures of isolated hippocampal neurons [6]. These data indicate that Pb delays the ontogenetic inclusion of the NMDA receptor NR2A subunit into the maturation of the synapses. Such a delay affects the NMDA-receptor-dependent signaling pathways in the neuron. This is because, as has been quoted above, many of these pathways depend on the composition of the subunits and/or the location of the receptor. The signaling pathway associated with MAP kinase [56], the Ca^{2+}/calmodulin kinase II (CaMKII) pathway [58], and the cAMP response element binding protein (CREB) phosphorylation disorder, as well as the pathway which is associated with the signal induced by brain-derived neurotrophic factor (BDNF) [6], all cause distributions in the transmission of signals in the synapse and synaptic plasticity.

Disorders of the pathway associated with the BDNF signal as an effect of Pb inhibition of the NMDA receptor

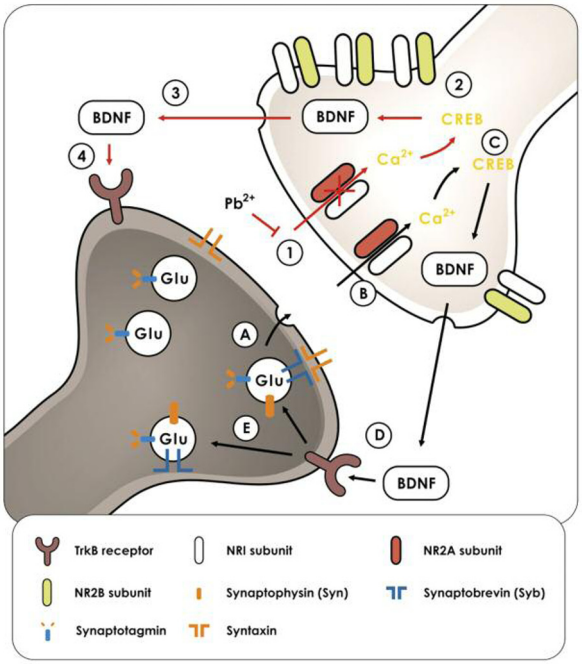
In developing neurons, the functional stabilization of the sites of the presynaptic release of neurotransmitters is controlled by postsynaptic retrograde signals. One of these retrograde signals, BDNF, affects the morphology of the axon, synaptic connections, and also the ultrastructure of the neuron [59]. Neal *et al*. [60] demonstrated that the concentration of BDNF in cultured hippocampal neurons incubated with Pb decreases, as does the expression of the synaptic proteins involved in synaptic vesicle release (syntaxin, synaptophysin and synaptobrevin). Moreover, the incubation of

hippocampal neurons with exogenous BDNF and their simultaneous exposure to Pb almost completely reversed these Pb-induced changes in protein levels and restored the synaptic vesicular release of the neurotransmitters.

The production and release of BDNF is influenced by postsynaptic NMDA receptor activation [61,62]. In vivo studies using confocal microscopy have shown that BDNF can be secreted postsynaptically in cultured hippocampal neurons, which have an active (stimulated) NMDA receptor [63]. This NMDAR-dependent release of BDNF can be critical in the creation of sites of presynaptic release of neurotransmitters [62]. Moreover, it has been observed [64] that this increased expression of BDNF in hippocampal neurons is associated with activation of the NMDA receptor subunit NR2A, and can be inhibited by activation of the NR2B subunit. The signal given by BDNF induces changes in the gene expression of pre- and postsynaptic proteins. Exogenous BDNF increases the expression of synaptotagmin, syntaxin and synaptobrevin in hippocampal slices and increases expression of the NMDA receptor NR2A subunit but not of NR2B. Moreover, BDNF knock-out mice have been found to show decreased expression of both subunits NR2A and NR2B, but no reduction in the expression of either syntaxin or synaptobrevin. Therefore, it seems that the NMDA receptor NR2A subunit may be preferentially associated with the activation pathway through the BDNF and that this subunit may modulate presynaptic plasticity through changes in the expression of genes and proteins [64].

It has been shown that exposure to Pb during neuronal synaptogenesis in the hippocampus reduces the number of synapses in which the NR2A subunit of the NMDA receptor is expressed. This may suggest that the production and release of BDNF is impaired under such circumstances, as shown by Neal *et al*. [60] (quoted above). The BDNF in knock-out mice exhibits a reduction in the release of synaptic vesicles; however, it is possible to reverse this using exogenous BDNF [65]. These data strongly indicate the role played by the NMDAR-dependent signaling pathway and by BDNF as a mechanism of Pb neurotoxicity at the synapse level.

A model of the molecular mechanism by which Pb disrupts synapse formation and plasticity in developing hippocampal neurons might appear as laid out in Fig. 2 [66]. The black arrows in the diagram indicate the unimpaired processes, while the red arrows show processes which are impaired by Pb. Under normal conditions in the glutamatergic synapse, the synaptic vesicles, which contain glutamate, are subject to Ca^{2+}-dependent fusion with the cell membrane in response to a pre-synaptic signal (A). This process is mediated by interaction of the vesicle with the synaptic proteins synaptobrevin (v-SNARE) and syntaxin (t-SNARE) or with the SNAP-25 protein. The result of this is fusion of the vesicles and the presynaptic release of glutamate (Glu), which activates the postsynaptic NMDA receptors (B). Ca^{2+} enters the receptor channel of the postsynaptic neuron, activating (reverse) intraneural signaling pathways. Under normal conditions in the maturing synapse, expression of the NR2A subunit of the NMDA receptor mainly occurs, which determines the initiation of the pathway associated with CREB protein phosphorylation (C). This CREB protein activation causes the transcription of BDNF, which is transported and reversely released. The released BDNF binds to the receptor TrkB (tropomyosin-related kinase receptor B) (D). In the presynaptic neuron, the activation of the TrkB receptor increases the number of docked vesicles and increases the incorporation of synaptophysin and synaptobrevin into the synaptic vesicles and increases vesicular release (E).



BDNF – brain-derived neurotrophic factor, CREB – cAMP response element binding protein, Ca^{2+} – calcium, Pb^{2+} – lead, Glu – glutamate

Fig. 2. A model of the molecular mechanism by which Pb disrupts synapse formation and plasticity in developing hippocampal neurons. Based on Neal and Guilarte [66]

During exposure to Pb, the partially blocked NMDA receptor prevents influx of Ca^{2+} into the postsynaptic neuron (1). In addition, during Pb exposure the expression of the NMDA receptor subunit NR2A is reduced, whilst NR2B subunit expression is increased; this increase takes place mainly outside the synapses (extrasynaptically). Consequently, the NR2B subunit becomes extrasynaptically operational. The activation of the CREB protein is reduced, as is the activation of the signaling pathway associated with its activation, which cause a de - crease in BDNF expression (2). The secretion of BDNF is also impaired (3), resulting in a reduction in the activation of the presynaptic Trk receptors by BDNF (4). The lack of a positive reverse signal results in a decrease in the incorporation of the synaptic vesicle proteins synaptobrevin and syntaxin; vesicular release is also impaired.

The NMDAR receptor itself also provides an interesting paradox – it may either be beneficial for the health of neurons or kill them. Recent studies [67] have shown that these characteristics of the NMDA receptors are dependent on their location: if the receptors are located synaptically, the mobilization of a protective shield for neurons occurs. This takes place through the trigger of pathways associated with calcium signaling through the activation of nuclear signaling pathways. However, the stimulation of extrasynaptic receptors leads to neuronal death. This difference results from the activation of different genes, which trigger opposing signaling pathways in the neuron.

Therefore, those disorders of the higher brain functions which are observed in the case of exposure to Pb during the early stages of brain development may be caused by the following processes: blocking of the activity of NMDA receptors, ontogenetic impairment of the expression of the receptor subunits during brain maturation, disorders in synaptic and extrasynaptic expression, and finally disturbances in the neuronal signaling pathways [66,67].

Disclosure

Authors report no conflict of interest.

References

1. CDC 2004 (Centers For Disease Control And Prevention). National Center For Environmental Health. A review of evidence of health effects of blood lead levels < 10 µg/dl in children. Atlanta; available at: http://www.cdc.gov/nceh/lead/ACCLPP/meetingMinutes/lessThan10MtgMAR04.pdf.
2. CDC 2005 (Centers For Disease Control And Prevention). National Center For Environmental Health. Preventing lead poisoning in young children. Atlanta.
3. EP. 2005 (Environmental Protection). The restriction of the use of certain hazardous substances in electrical and electronic equipment regulations No. 2748; available at: http://www.legislation.gov.uk/uksi/2005/2748/pdfs/uksi_20052748_en.pdf.
4. EP. 2009 (Environmental Protection). The restriction of the use of certain hazardous substances in electrical and electronic equipment (amendment) regulations, No. 581; available at: http://www.legislation.gov.uk/uksi/2009/581/pdfs/uksi_20090581_en.pdf
5. EU. 2008 (European Commission). Institute For Health And Consumer Protection Toxicology And Chemical Substances (& ECB). Opinion of the TC NES on the Environment Part of Industry Voluntary Risk Assessments on Lead and Lead Compounds; available at: http://echa.europa.eu/doc/trd_substances/VRAR/Lead/tcnes_opinion/tcnes_opinion_env.pdf.
6. Neal A.P., Worley P.F., Guilarte T.R. Lead exposure during synaptogenesis alters NMDA receptor targeting via NMDA receptor inhibition. *Neurotoxicology* 2011; 32: 281-289.
7. Koller K., Brown T., Spurgeon A., et al. Recent developments in low-level lead exposure and intellectual impairment in children. *Environ Health Perspect* 2004; 112: 987-994.
8. Lanphear B.P., Hornung R., Khoury J., et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect* 2005; 113: 894-849.
9. Gulson B., Mizon K., Smith H., et al. Skeletal lead release during bone resorption: effect of bisphosphonate treatment in a pilot study. *Environ Health Perspect* 2002; 110: 1017-1023.
10. Garrido Latorre F., Hernández-Avila M., Tamayo Orozco J., et al. Relationship of blood and bone lead to menopause and bone mineral density among middle-age women in Mexico City. *Environ Health Perspect* 2003; 111: 631-636.
11. Nash D., Magder L.S., Sherwin R., et al. Bone density-related predictors of blood lead level among peri- and postmenopausal women in the United States: The Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2004; 160: 901-911.
12. Riedt C.S., Buckley B.T., Brolin R.E., et al. Blood lead levels and bone turnover with weight reduction in women. *J Expo Sci Environ Epidemiol* 2009; 19: 90-96.
13. Canfield R.L., Henderson C.R., Cory-Slechta D.A., et al. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N Engl J Med* 2003; 348: 1517-1526.
14. Jakubowski M. Low-level environmental lead exposure and intellectual impairment in children-the current concepts of risk assessment. *Int J Occup Med Environ Health* 2011; 24: 1-7.
15. Rogan W.J., Dietrich K.N., Ware J.H., et al. The effect of chelation therapy with succimer on neuropsychological development in children exposed to lead. *N Engl J Med* 2001; 344: 1421-1426.
16. Braun J.M., Kahn R.S., Froehlich T., et al. Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children. *Environ Health Perspect* 2006; 14: 1904-1909.
17. Eubig P.A., Aguiar A., Schantz S.L. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ Health Perspect* 2010; 118: 1654-1667.

18. Needleman H.L., McFarland C., Ness R.B., et al. Bone lead levels in adjudicated delinquents. A case control study. *Neurotoxicol Teratol* 2002; 24: 711-717.
19. Wright J.P., Dietrich K.N., Ris M.D., et al. Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood. *PLoS Med* 2008; 27: e101.
20. Hrubá F., Strömberg U., Černá M., et al. Blood cadmium, mercury, and lead in children: an international comparison of cities in six European countries, and China, Ecuador, and Morocco. *Environ Int* 2012; 41: 29-34.
21. Stroh E., Lundh T., Oudin A., et al. Geographical patterns in blood lead in relation to industrial emissions and traffic in Swedish children, 1978-2007. *BMC Public Health* 2009; 9: 225.
22. Barton H.J. Advantages of the use of deciduous teeth, hair, and blood analysis for lead and cadmium bio-monitoring in children. A study of 6-year-old children from Krakow (Poland). *Biol Trace Elem Res* 2011; 143: 637-658.
23. Jones R.L., Homa D.M., Meyer P.A., et al. Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988-2004. *Pediatrics* 2009; 123: 376-385.
24. Costa L.G., Aschner M., Vitalone A., et al. Developmental neuropathology of environmental agents. *Annu Rev Pharmacol Toxicol* 2004; 44: 87-110.
25. Mendola P., Selevan S.G., Gutter S., et al. Environmental factors associated with a spectrum of neurodevelopmental deficits. *Ment Retard Dev Disabil Rev* 2002; 8: 188-197.
26. Haraguchi T., Ishizu H., Kawai K., et al. Diffuse neurofibrillary tangles with calcification (a form of dementia): X-ray spectrometric evidence of lead accumulation in calcified regions. *Neuroreport* 2001; 12: 1257-1260.
27. Basha R., Reddy G.R. Developmental exposure to lead and late life abnormalities of nervous system. *Indian J Exp Biol* 2010; 48: 636-641.
28. Baranowska-Bosiacka I., Dąbrowska-Bouta B., Strużyńska L. Regional changes in purines and selected purinergic receptors in immature rat brain exposed to lead. *Toxicology* 2011; 279: 100-107.
29. Struzynska L., Dabrowska-Bouta B., Koza K., et al. Inflammation-like glial response in lead-exposed immature rat brain. *Toxicol Sci* 2007; 95: 156-162.
30. Cakała M., Strosznajder J.B. The role of cyclooxygenases in neurotoxicity of amyloid beta peptides in Alzheimer's disease. *Neurol Neurochir Pol* 2010; 44: 65-79.
31. Kuhn W., Winkel R., Woitalla D., et al. High prevalence of Parkinsonism after occupational exposure to lead-sulfate batteries. *Neurology* 1998; 50: 1885-1886.
32. Coon S., Stark A., Peterson E., et al. Whole-body lifetime occupational lead exposure and risk of Parkinson's disease. *Environ Health Perspect* 2006; 114: 1872-1876.
33. Kamel F. Lead and other neurotoxins as a risk factors for amyotrophic lateral sclerosis. *Crisp Data Base National Institute of Health.* 2003. http://toxnet.nlm.nih.gov.
34. Fang F., Kwee L.C., Allen K.D., et al. Association between blood lead and the risk of amyotrophic lateral sclerosis. *Am J Epidemiol* 2010; 171: 1126-1133.
35. Zheng G., Tian L., Liang Y., et al. δ-Aminolevulinic acid dehydratase genotype predicts toxic effects of lead on workers' peripheral nervous system. *Neurotoxicology* 2011; 32: 374-382.
36. Bilińska M., Brzezowska D., Koszewicz M., et al. Subclinical lead neuropathy. *Pol Merkur Lek* 2004; 17: 244-247.
37. Bilińska M., Antonowicz-Juchniewicz J., Koszewicz M., et al. Distribution of conduction velocity in the ulnar nerve among lead exposed workers. *Med Pr* 2005; 56: 139-146.
38. Baranowska-Bosiacka I., Chlubek D. Biochemical mechanisms of neurotoxic lead activity. *Postępy Biochem* 2006; 52: 320-329.
39. Gavazzo P., Zanardi I., Baranowska-Bosiacka I., et al. Molecular determinants of Pb2+ interaction with NMDA receptor channels. *Neurochem Int* 2008; 52: 329-337.
40. Marchetti C., Gavazzo P. NMDA receptors as targets of heavy metal interaction and toxicity. *Neurotox Res* 2005; 8: 245-258.
41. Albensi B.C. The NMDA receptor/ion channel complex: a drug target for modulating synaptic plasticity and excitotoxicity. *Cur Pharm Des* 2007; 13: 3185-3194.
42. Niewoehner B., Single F.N., Hvalby Ø., et al. Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. *Eur J Neurosci* 2007; 25: 837-846.
43. Takeda A., Itagaki K., Ando M., et al. Involvement of N-methyl-D-aspartate receptor subunits in zinc-mediated modification of CA1 long-term potentiation in the developing hippocampus. *J Neurosci Res* 2012; 90: 551-558.
44. Howland J.G., Wang Y.T. Synaptic plasticity in learning and memory: stress effects in the hippocampus. *Prog Brain Res* 2008; 169: 145-158.
45. Johnstone V.P., Raymond C.R. A protein synthesis and nitric oxide-dependent presynaptic enhancement in persistent forms of long-term potentiation. *Learn Mem* 2011; 18: 625-633.
46. Miyamoto E. Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. *J Pharmacol Sci* 2006; 100: 433-442.
47. Hori N., Büsselberg D., Matthews M.R., et al. Lead blocks LTP by an action not at NMDA receptors. *Exp Neurol* 1993; 19: 192-197.
48. Lau A., Tymianski M. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch* 2010; 460: 525-542.
49. Van Dongen A.M. Biology of the NMDA Receptor. *CRC Press*, Boca Raton 2009.
50. Monyer H., Sprengel R., Schoepfer R., et al. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* 1992; 256: 1217-1221.
51. Büsselberg D., Michael D., Platt B. Pb2+ reduces voltage- and N-methyl-D-aspartate (NMDA)-activated calcium channel currents. *Cell Mol Neurobiol* 1994; 14: 711-722.
52. Guilarte T.R., Miceli R.C., Jett D.A. Biochemical evidence of an interaction of lead at the zinc allosteric sites of the NMDA receptor complex: effects of neuronal development. *Neurotoxicology* 1995; 16: 63-71.
53. Lasley S.M., Gilbert M.E. Lead inhibits the rat N-methyl-D-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. *Toxicol Appl Pharmacol* 1999; 159: 224-233.
54. Goldstein G.W. Evidence that lead acts as a calcium substitute in second messenger metabolism. *Neurotoxicology* 1993; 14: 97-101.
55. Baranowska-Bosiacka I., Gutowska I., Marchlewicz M., et al. Disrupted pro- and antioxidative balance as a mechanism of neurotoxicity induced by perinatal exposure to lead. *Brain Res* 2012; 1435: 56-71.

56. Zhang X.Y., Liu A.P., Ruan D.Y., et al. Effect of developmental lead exposure on the expression of specific NMDA receptor subunit mRNAs in the hippocampus of neonatal rats by digoxigenin-labeled in situ hybridization histochemistry. *Neurotoxicol Teratol* 2002; 24: 149-160.
57. Cordova F.M., Rodrigues A.L., Giacomelli M.B., et al. Lead stimulates ERK1/2 and p38MAPK phosphorylation in the hippocampus of immature rats. *Brain Res* 2004; 998: 65-72.
58. Zhang G.S., Ye W.F., Tao R.R., et al. Expression profiling of Ca(2+)/calmodulin-dependent signaling molecules in the rat dorsal and ventral hippocampus after acute lead exposure. *Exp Toxicol Pathol* 2012; 64: 619-624.
59. Cohen-Cory S., Kidane A.H., Shirkey N.J., et al. Brain-derived neurotrophic factor and the development of structural neuronal connectivity. *Dev Neurobiol* 2010; 70: 271-288.
60. Neal A.P, Stansfield K.H., Worley P.F., et al. Lead exposure during synaptogenesis alters vesicular proteins and impairs vesicular release: potential role of NMDA receptor-dependent BDNF signaling. *Toxicol Sci* 2010; 116: 249-263.
61. Jiang X., Tian F., Mearow K., et al. The excitoprotective effect of N-methyl-D-aspartate receptors is mediated by a brain-derived neurotrophic factor autocrine loop in cultured hippocampal neurons. *J Neurochem* 2005; 94: 713-722.
62. Walz C., Jüngling K., Lessmann V., et al. Presynaptic plasticity in an immature neocortical network requires NMDA receptor activation and BDNF release. *J Neurophysiol* 2006; 96: 3512-3516.
63. Kolarow R., Brigadski T., Lessmann V. Postsynaptic secretion of BDNF and NT-3 from hippocampal neurons depends on calcium calmodulin kinase II signaling and proceeds via delayed fusion pore opening. *J Neurosci* 2007; 27: 10350-10364.
64. Chen Q., He S., Hu X.L., et al. Differential roles of NR2A- and NR2B-containing NMDA receptors in activity-dependent brain-derived neurotrophic factor gene regulation and limbic epileptogenesis. *J Neurosci* 2007; 27: 542-552.
65. Pozzo-Miller L.D., Gottschalk W., Zhang L., et al. Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *J Neurosci* 1999; 19: 4972-4983.
66. Neal A.P., Guilarte T.R. Molecular neurobiology of lead (Pb(2+)): effects on synaptic function. *Mol Neurobiol* 2010; 42: 151-160.
67. Hardingham G.E., Bading H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci* 2010; 11: 682-696.
68. Finkelstein Y., Markowitz M.E., Rosen J.F. Low-level lead-induced neurotoxicity in children: an update on central nervous system effects. *Brain Res Rev* 1998; 27: 168-176.