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Retinal degeneration following lead exposure – functional aspects

Degeneracja siatkówki oka w narażeniu na ołów – aspekty funkcjonalne

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Summary

Due to the prevalence of lead (Pb) in the environment, the neurotoxic effects on the human body have become an important clinical problem. Despite that Pb concentration in the environment decreased after banning its use in petrol, it is still a significant issue which can affect child development and vision. This paper focuses on the degeneration of the retina under exposure to lead. We present the most frequent sources of exposure to lead in the environment and the influence on vision, mechanisms leading to the apoptosis of photoreceptor cells, as well as strategies for blocking rod apoptosis. We also present Pb-induced disorders in the calcium metabolism of photoreceptor cells and Ca^{2+}-dependent enzymes.

Key words: **lead (Pb) • retinal degeneration • neurotoxicity • photoreceptor apoptosis**

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List of abbreviations: **cGMP** – cyclic guanosine monophosphate; **ERG** – electroretinography; **GAP** – GTPase-activating protein; **GCAP** – guanylate cyclase-activating protein; **LRAT** – lecithin retinol acyltransferase; **NMDA** – N-methyl-D-aspartic acid; **RDH** – retinol dehydrogenase, **β-PDE, Pde6b** – rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta.

Introduction

The eye is a key human sense organ; degenerative diseases of the retina and loss of vision are therefore an important clinical challenge and social problems. It is important to identify the causes and mechanisms leading to degenerative changes so that appropriate preventative or therapeutic methods can be applied. The factors leading to the apoptosis of photoreceptors include changes in the expression, structure and/or function of phototransduction cascade proteins, photoreceptor outer segment proteins, transcription factors and mitochondrial proteins [23].

Essential metals performing physiological functions in the body, such as copper, zinc and iron, are involved in many metabolic pathways in cells and in the control of essential metabolic and signaling functions, including those associated with vision. Exposure to heavy metals, such as lead, cadmium, mercury and thallium, results in toxic effects, for example competitive replacement of the aforementioned essential metals in the cells. The toxicity of heavy metals may also result from an interaction between the metal ion and the specific protein which alters the protein structure and function. Transport proteins are particularly vulnerable to the toxic effects of heavy metals. It has been shown that the retinal pigment epithelium chelates ions and is capable of binding both essential and heavy metals due to their high affinity for melanin present in the pigment epithelium melanosomes [15,63]. Erie et al. provides data on the concentration and distribution of the various heavy metals in the eye structures of humans or animals, and their potential impact on the eye and vision [15].

Pb in the environment

Lead remains one of the most widely used and universal materials known to man. It is a common metal in the environment, present in three forms - metallic lead, and organic and inorganic lead salts [60]. Toxicity results in many acute and chronic cardiovascular, neurological, haematological and immunological pathologies [45]. Before its harmfulness was discovered, Pb was used in the manufacture of paints, as an additive to gasoline and in many other products and technological processes [13,47]. Many European countries, under Directive 98/70/EC of the EU [11], as well as the United States [14], introduced a ban on the use of tetraethyl lead as an anti-knock agent in fuels [38]. This decision helped to decrease the concentration of Pb in the blood in the European population [34].

In some countries however, Pb is still widely used, e.g., in the production of Pb-acid batteries and the synthesis of sulfuric acid (approximately 33% of total use) [38]. It is also used in the construction industry and in the production of bullets [64]. The high density of Pb makes it useful for radiation protection in the nuclear industry and hospitals. For the same reason, Pb is also a good insulator of acoustic waves, used to reduce noise at factories and engine rooms in ships [39]. Its properties are often indispensable, and therefore the risk of exposure to lead in the future may be as high as nowadays. Approximately 4,000,000 tonnes of lead are excavated per year. More than 50% of the production comes from Australia, China and the United States [40]. Another 3,000,000 tonnes come from recycling, mainly in the Western world [39].

In 1991 the United States Secretary of the Department of Health and Social Welfare described Pb as a major environmental threat to the health of children in the United States [13]. Exposure to lead contributes to the creation of about 600,000 new cases of intellectual disability among children annually [65]. Today, old lead paint is the largest source of environmental Pb in the United States. Exposure to the harmful effects of this element may occur due to improper removal of lead-based paints, for example during abrasion or sanding. It has been shown that nearly two million children between 1 and 5 years of age residing in the United States have blood lead levels of 10 μg/dL or more, which puts them at risk of abovementioned adverse health effects. In less developed countries the percentage of children with blood Pb levels exceeding 10 μg/dL is even higher [59]. The currently accepted standard for lead in the blood (threshold dose) is 5 μg/dL [9]. In 2009 Prokopowicz et al. [55] conducted a study on a group of 80 women (50-59 years old). They were recruited in one of the major cities in Poland – Wrocław. The results showed that the mean concentration of Pb in the blood of examined women was 2.15μg/dL. The Swedish study revealed that the median value for southern Swedish women equal 2.20 μg/dL (reviewed in [63]).

The European Food Safety Authority (EFSA) examined food for the concentration of lead. Over nine years 144,206 samples were collected and Pb levels in more than half of the food tested proved to be lower than the level enabling quantitative or qualitative detection. On average, the concentrations ranged from 0.3 μg/kg in milk for infants to 4,300 μg/kg in dietary products. The median of all categories was 21.4 μg/kg. Between 2003 and 2010 concentrations of Pb in food dropped by about 23%. The average exposure to Pb in the diet was estimated to be 0.68 μg/kg body weight per day in the European population throughout the entire life. The highest exposure was reported in small and older children, 1.32 and 1.03 μg/kg body weight per day, respectively. Among infants Pb concentrations ranged between 0.83 and 0.91 μg/kg body weight per day [17]. Hrubá et al. [29] demonstrated that the concentrations of Pb in the blood of children aged 7-14 years old from urban areas in Europe (in 2010-2012) are as follows: Sweden: 1.4 μg/dL; Czech Republic: 1.55 μg/dL; Poland: 1.63 μg/dL; Croatia: 1.79 μg/dL. The highest concentrations were detected in children in Slovakia (mean: 1.94 μg/dL) and the lowest in Slovenia - 1.34 μg/dL.

Neurotoxic effects of Pb

Lead has a particularly toxic effect on the nervous system. Long-term side effects of low concentrations of lead on cognitive function during the development of humans have been documented over the past two decades [59]. The

neurotoxicity of lead has special repercussions in children; a direct relationship has been shown between exposure to low concentrations of lead and a decrease in behavioral performance and cognitive function in children during adolescence [18]. Pre- and neonatal exposure to lead compounds increases the risk of disorders with symptoms depending on the concentration and duration of exposure and genetic susceptibility [26]. Symptoms can occur immediately after exposure to Pb or with a delay. These include memory impairment, impaired vision and hearing, and mental retardation [60].

Concentrations of lead in the blood, even those amounting to less than 10 µg/dL, are inversely proportional to the intelligence quotient (IQ) in children aged 3 and 5 years [16]. Tests show a 1-3 point decrease in IQ points at blood lead concentrations of 10 to 20 µg/dL and by 5-10 points for children in which the concentrations were greater, up to 30 µg/dL [41]. Exposure to low Pb levels can lead to subtle non-specific abnormalities of the nervous system and behavioral disorders, including aggression. In addition, children of school age who have an increased concentration of lead in the blood can experience problems with concentration and hyperactivity. This phenotype is similar to attention deficit hyperactivity disorder – ADHD (reviewed in [4]). A positive correlation was found between blood Pb concentrations and the incidence of ADHD [16]. It was also shown that prenatal exposure to lead could lead to antisocial and illegal behavior among adolescents. People who have experienced elevated levels of Pb in the blood in childhood are more likely to be arrested both as adolescents and adults [44,66]. Descriptions of neurological changes due to Pb toxicity in children often include cases of learning disorders, especially visual-perceptual learning, e.g., a reduced ability to distinguish shape, direction and spatial orientation. Children are more susceptible to the effects of lead probably because they absorb higher doses of lead via the gastrointestinal tract (50% of the dose), as well as their developing nervous system is more vulnerable to damage than in adults [22].

The mechanism of neurotoxicity of Pb is not fully understood. It is known that lead disrupts the structural components of the blood-brain barrier through primary damage to astrocytes and indirectly by violating the small vessel endothelium. In the brain, damage is mainly found in the prefrontal cortex, hippocampus and cerebellum. Some characteristic features of lead poisoning can be associated with this specific anatomical pattern of damage. The effects of poisoning are revealed by anatomical symptoms and by impairment of neurotransmitter systems that are critical in the formation of emotional reactions, memory and learning [18].

A low-level long-term Pb exposure leading to even low concentration of lead in the blood inhibits the activity of enzymes and causes the formation of reactive oxygen species, thereby exacerbating oxidative stress, which disturbs the homeostasis of cellular mechanisms that play an important role in the pathogenesis of lead toxicity. Other cellular and molecular mechanisms responsible for the toxicity of Pb involve the intensification of neuronal apoptosis and the effect on Ca^{2+}-dependent enzymes [18,45].

Lead is a highly neurotoxic element, causing disturbances in the functioning of neurons. Studies have shown that lead ions penetrate nerve cells and astroglial primarily by voltage-dependent calcium channels (VDCC) [62]. The negative effect of Pb on the nervous system results from its influence on three main neurotransmission systems - glutamatergic, dopaminergic and cholinergic (reviewed in [2]). NMDA receptors appear to be the main target of lead in the central nervous system. Also, their density increased in the brains of adult rats chronically exposed to lead. Excessive activation of NMDA receptors can cause excitotoxicity, which leads to cell death [2,10]. It competes with calcium for binding sites on proteins in the EF hand, a helix-loop-helix structural domain. These proteins belong to the family of binding proteins. Lead substitutes calcium in the activation of calmodulin, whereas at higher concentrations it inhibits its activity. Nanomolar Pb levels stimulate phosphorylation activated by calmodulin and calmodulin-dependent activation of phosphodiesterase [35,62]. This may lead to an increased release of neurotransmitters by the synaptic vesicles, thus increasing the amplitude of spontaneous synaptic currents [2].

Physiology of vision, the role of electroretinography in the diagnosis of retinal degeneration.

Phototransduction is the key element in the process of vision, allowing the conversion of the light stimuli into electrical signals, which can then be interpreted by the brain. The process is schematically presented on Figure 1.

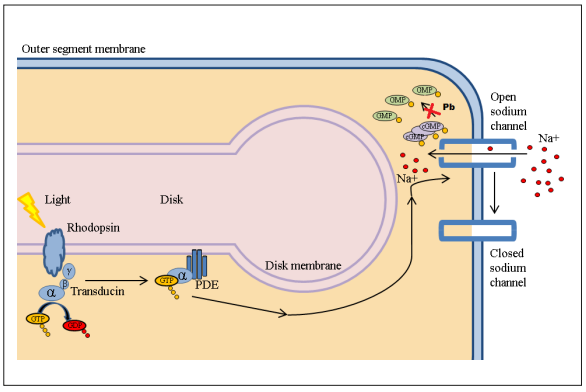

Fig. 1. Phototransduction cascade [36,37,43,52,56,58]

The bioelectrical activity in the retina can be assessed by electroretinography (ERG). This test is used to detect a number of anomalies in the functioning of the retina [50,51]. An electroretinogram records bioelectrical response of the retina to light stimulus. Eindhoven and Jolly in 1908 suggested that light triggers a chain reaction leading to the formation of specific waveforms: a, b and c. An additional waveform which is less frequently recorded (after switching off the impulse) is called wave d. Wave a is a

component of the ERG which reflects the activity of photoreceptors of the retina in response to a flash of light. Wave b is initiated in retinal cells postsynaptic to the photoreceptors. Wave b is attenuated by each procedure causing blockage of synaptic transmission from photoreceptors, such as flushing with cobalt ions or solutions with a high concentration of magnesium and low calcium concentration [53]. Thus, the b wave amplitude may serve as criterion of sensitivity of the retina [42]. Schematic ERG of human eye is presented on Figure 2.

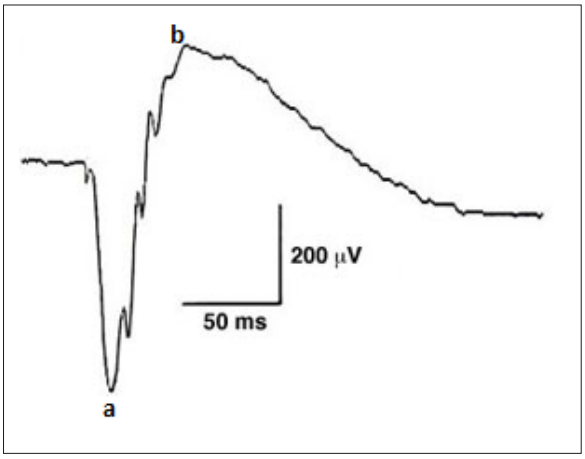

Fig. 2. ERG of human eye [36]

Effect of Pb on the eye and vision

It has been shown that lead can accumulate in the tissues of the human eye. Erie J. et al. [15] used mass spectrometry to determine the concentration of Pb and other toxic heavy metals in the fluids and tissues of human eyes. The results are shown in Table 1. The lowest average Pb concentration was found in the vitreous body, while the highest occurred in the pigment epithelium and choroid. Choroid and pigment epithelium showed the widest range of measured values [15]. The other studies showed that Pb and Cd accumulated in the tissues of the human eye, especially those containing pigment, such as the pigment epithelium, choroid, iris or ciliary body [5,54]. Melanin contained in pigment grains binds metal ions in melanosomes because of their high affinity for sulfhydryl groups. Melanin may play a protective role by acting as a detoxifying agent through the uptake of metals from the adjacent retina and photoreceptors. On the other hand, binding of heavy metals by melanin raises their concentration in pigment epithelium and retina which is harmful to these structures of the eye. This process is similar to continuous exposure to drugs with melanin affinity, such as chloroquine and phenothiazine derivatives. Heavy metals can cause degeneration of the retinal pigment epithelial cells or photoreceptors [15]. They compete for the same binding sites with metals such as zinc, copper, calcium, magnesium, molybdenum and iron which are physiologically present in melanosomes, especially the pigment epithelium [54]. Heavy metals are capable of replacing the previously attached ion, thereby causing disturbances in the metabolism of the cells [12,15].

In respect of the eye, it has been shown that one of the negative effects caused by Pb is a selective loss of retinal photoreceptors and bipolar cells. This effect has also been observed in humans [21,27]. Elevated concentrations of Pb may also result in cataracts in men [61] and a low pressure glaucoma in women [67]. Intoxication with Pb compounds in animal models results in progressive degeneration of the rods of the retina, which accurately reflects the degenerative changes observed in humans [27]. This model allows the development of pharmacological strategies to allow the inhibition of apoptosis of retinal cells improving the quality of life of patients suffering from degenerative diseases of the retina.

Mechanisms of action of Pb on the retina

Exposure to Pb in vitro and in vivo induces electrophysiological and biochemical changes in scotopic (rod-dependent), but not photopic (cone-dependent) ERG [24]. The phenotype of changes in the retina is clearly dependent on the duration of exposure to Pb [26]. Long-term and rod-dependent changes, resulting from exposure to low, medium and high concentrations of lead, have been shown in mice and rats (highest blood concentrations were 20, 30-60 and >60 μg/dL, respectively). Functional studies using ERG in this model showed a decreased b wave amplitude, and a prolonged latent phase and time needed to adapt to darkness. Similar changes occurred in isolated retinas treated

Table 1. The concentrations of Pb in the human eye [15]

	Lead			
	Dry weight (ng/g)		Wet weight (ng/g)	
	Mean ± SD	Range	Mean ± SD	Range
Aqueous humor	-	-	0	0
Vitreous humor	-	-	0.5 ± 1.0	0.0-3.5
Lens	13 ± 18	0- 97	4 ± 4	0-13
Ciliary body	321 ± 127	127-464	73 ± 70	27-175
Pigment epithelium/ choroid	432 ± 485	29-2165	82 ± 109	6-378
Retina	53 ± 54	0-172	5 ± 7	0-27

with micromolar concentrations of lead chloride [49]. Biochemical studies of the retina revealed that exposure to Pb resulted in a concentration-dependent inhibition of the hydrolysis of cGMP, causing an increase in cGMP levels in both dark adaptation and in light adaptation [20,24].

Fox et al. [21] showed that the loss of photoreceptor cells (rods) and bipolar cells is caused by apoptotic cell death during development and adulthood. Exposure of rat mothers to Pb was obtained by introducing 0.02% or 0.2 % lead acetate in drinking water. On day 21 of age (weening) the concentration of Pb in the blood of their baby rats was 1 µg/dL (control), 19 µg/dL (0.02%) and 59 µg/dL (0.20%). From 45 to 90 days of age, the concentration of Pb was 5-7 µg/dL in all groups. Quantitative studies by scanning electron microscopy and field inversion electrophoresis (FIGE) showed that the daily rate of apoptosis was almost linear among rods, and sigmoidal for bipolar cells. RNA analysis showed the delay in expression of the gene encoding the β subunit of cGMP phosphodiesterase (β-PDE) by about 1-2 days. A significant decrease in the expression was observed throughout the entire period of development of the baby rats. In addition, retinal cGMP phosphodiesterase activity was also delayed by 1-2 days and significantly reduced. There was also a significant increase in intra-rod and retinal Ca^{2+} concentration. Incubation of mitochondria isolated from the retina of the eyes of rats exposed to lead with glutamate/malate or succinate/rotenone as substrates showed a significant and substrate-independent decrease in mitochondrial ATP. These results strongly suggest that apoptosis of rods (and possibly bipolar cells) induced by exposure to Pb is caused by an overload of Ca^{2+} and Pb. This is due to a lower expression and activity of cGMP phosphodiesterase, both during development of the retina as well as in adults. Furthermore, the obtained results indicate that changes in mitochondrial function due to the presence of increased concentrations of Ca^{2+} and Pb may play a key role in the pathogenesis of retinal degeneration, specific to these cells. These results are confirmed in preliminary studies which showed the release of mitochondrial cytochrome c and increased caspase activity in the retinas of rats exposed to the lead. In vitro studies on isolated mitochondria incubated in the presence of retinal Ca^{2+} or Pb demonstrated a similar dose-dependent reduction in the production of ATP. These effects are blocked by cyclosporin A, suggesting that mitochondrial ion channels remain open [21].

The role of Pb in the rod apoptosis pathway

Apoptosis, active cell death, is a process which may be induced by many physiological and pathological signals. Mitochondria and caspases play an important role in the effector and executive phases of apoptosis. As mentioned earlier, exposure to Pb, both in vivo and in vitro, increases intracellular Ca^{2+} concentration in rods and whole retina. Under physiological conditions, calcium ions are responsible for many processes in the cell, such as growth, differentiation and synaptic activity. Although the physiological increase in intracellular Ca^{2+} concentration is necessary for correct functioning of the cells, an excessive influx of Ca^{2+} ions together with the release of calcium from the intercellular space can lead to an overload of regulatory mechanisms and consequently to cell death. Pb is accumulated in the body, damaging mitochondria which play an important role in the regulation of intracellular calcium concentrations. The increased influx of calcium into the mitochondria can exacerbate mitochondrial electron transport thereby increasing the production of reactive oxygen species (ROS). The decrease in mitochondrial function may change the usually mild synaptic transmission by glutamate into pathological excitotoxicity - leading to the death of the cell [60]. Studies suggest that a long-term elevated intracellular concentration of Ca^{2+} plays a fundamental role in rod apoptosis [28,46]. Elevated calcium levels and activation of hydrolytic enzymes leads to excessive energy expenditure and decreased energy production [44]. The apoptosis effector phase is accompanied by an irreversible opening of mitochondrial ion channels induced by a variety of apoptosis inducers, such as persistent elevated levels of Ca^{2+} in the mitochondrial matrix, prooxidants and substances reacting with thiol groups [23,28]. This leads to depolarization of the mitochondrial membrane, the release of cytochrome c, caspase activation, and fragmentation of chromatin. Pb is also an inhibitor of rod-specific cGMP phosphodiesterase [23]. In retinal rods, the cGMP level is relatively high in the dark and the continuous supply of Na^+ and Ca^{2+} through ion channels maintains the cells in a partly depolarized state. When the visual pigment (rhodopsin) absorbs a photon, it becomes enzymatically active and catalyzes the conversion of GTP to GDP on the G protein - transducin. GTP-bound transducin activates cGMP phosphodiesterase, which in turn catalyzes the hydrolysis of cGMP. As a result, ion channels in the cell membrane close, resulting in hyperpolarization of the membrane. This is followed by the decreased release of the transmitter to the secondary cells of the retina. A return to equilibrium requires both disabling of the metabolic pathway and synthesis of cGMP to open the channels. Ca^{2+} is transported through the light-independent sodium/calcium exchanger Na^+/Ca^{2+}-K^+, which leads to a decrease in intracellular Ca^{2+} concentration. This stimulates the activation of guanylate cyclase to re-synthesize cGMP and the deactivation of rhodopsin by rhodopsin kinase. A similar metabolic pathway can be observed in cones, but each component is different from its rod counterpart. Cones are much less sensitive to light [6]. It has been shown that during and after exposure to lead, the expression of rod cGMP phosphodiesterase decreases [21]. There is also a decrease in its activity leading to increased permeability of ion channels, cell overload with Ca^{2+} ions and a reduction in mitochondrial ATP synthesis. This results in the release of cytochrome c and increased caspase activity and, consequently, cell death [21,23]. It has been shown that the opening of mitochondrial ion channels and apoptosis can be inhibited using for example proteins such as Bcl-2, Bcl-x (L), bongkrekic acid, and cyclosporin A [28].

Nucleotide receptors and retinal degeneration

The family of membrane receptors for extracellular nucleotides includes adenosine receptors (P1) and the receptors of ATP or other nucleotides (P2). The latter are

a diverse family of receptors, with two subtypes: P2X – functioning as non-selective ion channels, and P2Y – G protein-coupled [8]. Both P1 and P2 receptors are referred to as nucleotide receptors, and their presence has been demonstrated in cells of the central and peripheral nervous system [7]. It has been proved that interactions between neurons and glial cells are based, among others, on purinergic signalling, the disruption of which may play a role in neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease [7].

P2X receptors are ATP-gated ion channels, permeable to K^+, Na^+ and Ca^{2+}. These high-speed receptors (about 10 ms) [57] play an important role in fast neurotransmission in the nervous system (central and peripheral), and also in the processes of neuroprotection and neurodegeneration [25,32]. Their subtypes are diverse in terms of structure, location, sensitivity to ATP and permeability of ion channels [31,48]. P2X7 receptor exhibits specific properties as it is activated by high ATP concentrations (100 to 1000 μM), while other homomeric forms of P2X receptor are activated by ATP already at a concentration of 1-10 μM [1]. It has been demonstrated that P2X7 receptor may be responsible for the apoptosis of many cell types, including the cells of the retina. *In vitro* studies have shown that activation of P2X7 receptors in isolated retinal ganglion cells increases intracellular calcium concentration, which may result in cell death. It has also been found that the mechanical and chemical isolation of nerve cells causes an increase in their sensitivity compared to natural conditions and reduces retinal ganglion cell survival [30]. It is so because *in vivo*, retinal ganglion cells are located near the Müller and astrocytic cells which may release compounds to protect against damage (e.g. adenosine, an agonist of P1 receptors). It is therefore possible that the ganglion cells may be more resistant to high concentrations of ATP *in vivo* than *in vitro*. However, *in vivo* research conducted in rats with BzATP (P2X7 receptor agonist) introduced into their vitreous body showed a reduced number of ganglion cells and enhanced apoptosis of neurons in both the center and at the edge of the retina. The results of these studies are important for patients with glaucoma since ATP is a P2X7 agonist, the concentration of which is increased in response to increased intraocular pressure. It has also been shown that patients with glaucoma have a 9- fold increased ATP concentration in the aqueous humor of the eye [30]. This mechanism can therefore be responsible for the degeneration of retinal ganglion cells in glaucoma.

P2Y receptors are stimulated by pyrimidine and purine nucleotides. Their action is slower (about 100 ms) than the P2X receptors because their mechanism of action is related to the interaction with G proteins and second messenger induction [57]. Additionally, molecular structure of P2Y receptors, their sensitivity to nucleotides and mechanisms of action are significantly different from P2X receptors [33]. They are involved in physiological processes of the nervous system, including the regulation of neurotransmitter secretion and initiation of the calcium wave in astrocytes [19].

In the literature there are no data on changes in the expression of P2X7 receptors in the retina of the eye during exposure to Pb. The results of our own study indicate that significant increase in expression of this receptor has been shown in the hippocampus of young rats exposed to lead [3]. The same paper also reported increased expression of connexin proteins Cx43 (responsible for ATP release from astrocytes to the intercellular space), accompanied by neurodegenerative changes in the hippocampus [3].

Strategies for blocking rod apoptosis

Understanding the mechanism of rod apoptosis allowed the development of potential neuroprotective strategies aimed mainly at easing the effect of Ca^{2+} overload. There are several pharmacological approaches to stop apoptosis; including an increase in the expression of anti-apoptotic proteins Bcl-2 and administration of growth factors. Other strategies consist of an attempt to block or reduce overloading of cells with Ca^{2+} [23]. In the case of rods and neurons, a long-term increase in Ca^{2+} concentration stabilizes mitochondrial ion channels in a high conductivity state (open). As a result, the inner membrane of the mitochondria becomes permeable, resulting in an irreversible decrease in membrane potential, loss of ATP, swelling and release of pro-apoptotic factors into the cytosol, and the activation of the caspase cascade. Physiologically, rod mitochondria contain low concentrations of Ca^{2+} in both dark and light adaptations. Therefore, a therapeutically useful substance should reduce the influx of Ca^{2+} into the cell, the penetration of Ca^{2+} into the mitochondria, or should block the cytochrome c dependent apoptotic cascade. Currently, one can distinguish five main neuroprotective strategies: 1) blocking the entry of Ca^{2+} ions to the rods through regulation of the ion channels activated by cGMP [23]. L-cis diltiazem was used to block the rod cGMP-activated conductance [66]; 2) blocking the passage of Ca^{2+} to the rods through L-type channels [23,67]; 3) inhibition of the mitochondrial sodium-calcium exchanger; 4) inhibition of the release of cytochrome c by mitochondria; and 5) inhibition of caspase 3 and the subsequent caspase cascade [23]. Increasing the mitochondrial buffering capacity of Ca^{2+} might also protect cells from low- to moderate Ca^{2+} and/or Pb overload. It can be achieved by the overexpression of Bcl-2 or Bcl-x_L [23].

Conclusion

The degeneration of the retina is a complex clinical problem with different etiologies; one of the causes is exposure to environmental hazards, including Pb. The prevalence of Pb in the environment makes it impossible to completely eliminate the risk of exposure, because it is still widely used in industry. In addition, long-term exposure to relatively low concentrations of Pb, generally asymptomatic and subclinical (i.e. microintoxication) is currently the dominant form of environmental contamination, especially in children.

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