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Mechanism of cRNA-Induced Synaptic Vesicles Release from Lead-Exposed Hippocampal Neurons

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Abstract: Lead mainly accumulates in hippocampus. A large number of research results show that the toxic effect of lead accumulation in hippocampus can cause changes in the structure and function of hippocampus itself, which in turn leads to the decline of learning and memory ability and cognitive abnormality. The time interval between action potential reaching nerve endings and subsequent vesicle fusion is very short, which can make protein phosphorylation dephosphorylation play a direct and acute role in single-round vesicle exocytosis. Synapse formation is a dynamic process, which involves the stability of neural network and the recruitment of pre-synaptic and post-synaptic specific proteins. After fusion with vesicles, it is released into synaptic cleft, in which glutamate is transported from presynaptic to synaptic endings as a glutamate transporter, and then combined with postsynaptic membrane receptors to exert synaptic effect. In this paper, the mechanism of synaptic vesicles release from lead-exposed hippocampal neurons was studied by cRNA, and the possible mechanism of synaptic vesicles release from lead-exposed hippocampal neurons was discussed by observing the changes of ultrastructure of neurons, organelles and morphological parameters of synapses in hippocampus.

1. Introduction

Lead is a systemic toxic substance that affects almost every living organ. It widely exists in various production fields and pollutes the environment. If people are in this environment, it may cause chronic damage to the body. The impact of lead exposure on human body and environment is also a global concern. Lead is mainly accumulated in a large amount in the hippocampus. A large number of research results show that the toxic effect of lead accumulation in the hippocampus can cause changes in the structure and function of the hippocampus itself, leading to decreased learning and memory ability, cognitive abnormalities, etc. [1]. The time interval between the arrival of the action potential to the nerve endings and the subsequent vesicular fusion is very short, which can make the protein phosphorylation and dephosphorylation play a direct and acute role in the

single-round vesicular exocytosis, so the protein kinase and phosphatase may play an important role in the subsequent neurotransmitter release event [2]. Synaptic formation is a dynamic process, which involves the stable pre-synaptic and postsynaptic recruitment of specific proteins in the neural network. The formation of the central nervous system requires the cooperation of different types of synapses. The most representative excitatory and inhibitory neurotransmitter in the central nervous system is glutamate, which is located in the prominent anterior vesicle and is released into the synaptic space after fusion with the vesicle [3]. As a glutamate transporter, glutamate is transported from presynaptic to synaptic terminals, and then combined with postsynaptic membrane receptors to play a synaptic effect.

The direct neurotoxicity of lead can lead to cell apoptosis, and excitatory toxicity can affect the storage and release of neurotransmitters and the changes of neurotransmitter receptors, mitochondria, second messengers, cerebral vascular endothelial cells, astrocytes and oligodendrocytes. In the past two decades, considerable achievements have been made in understanding the impact of postsynaptic structures on various forms of synaptic plasticity [4]. On the contrary, due to technical barriers and the complexity of synaptic vesicle release mechanism, it is difficult to clarify the molecular and cellular mechanism of the influence of presynaptic structure on synaptic plasticity. Learning and memory itself is a very complex process, and its cellular and molecular mechanisms are still unclear, but several points are certain, that is, the hippocampus is the key part of learning and memory: postsynaptic long-term potentiation and postsynaptic long-term depression are the possible mechanisms in the formation of hippocampal memory, and are the two main characteristics of synaptic plasticity of nerve cells [5-6]. In this paper, we studied the mechanism of the release of synaptic vesicles of hippocampal neurons induced by lead exposure by cRNA, and explored the possible mechanism of lead exposure on the release of synaptic vesicles of hippocampal neurons by observing the changes of ultrastructure of neurons, organelles and morphological parameters of synapses in the hippocampus.

2. Effect of Lead on Synaptic Plasticity in Hippocampus

In this paper, the effect of low lead exposure on synaptic plasticity in hippocampus and the antagonism of zinc during development were studied. The results show that lead can reduce the amplitude of synaptic plasticity EPSP and PS-induced LTP in hippocampus, and the damage of PS-induced LTP is more serious, while zinc has obvious protective effect on lead-induced LTP damage. The amplitude of LTP induced by EPSP only increased by 26%, but it increased by 94% in lead treatment group. Obviously, lead reduces the LTP amplitude of CA1 and increases the LTP amplitude of CA3. The contents of blood lead and brain lead in lead exposure group were significantly higher than those in control group at each time point ($P < 0.01$). The contents of brain lead and blood lead in lead exposed group decreased gradually, but they were still higher than those in control group. See Table 1.

Table 1 Comparison of Blood Lead and Brain Lead Contents at Different Observation Time Points

Group	Blood lead		Brain lead	
	P14	P21	P14	P21
Control group	14.18±1.31	18.45±3.45	0.74±0.45	4.25±0.23
Lead exposure group	76.45±8.25*	128.74±19.5*	17.45±2.45*	20.14±3.58*

The difference in the effect of lead on LTP of CA1 and CA3 is due to the different mechanisms of LTP production between CA1 and CA3. MF germination was not found in the lead-exposed group and the control group on 14th day after birth, but appeared in the control group on 21st day

after birth, which was higher than that in the lead-exposed P21 group, and the staining intensity of hippocampus in the lead-exposed P21 group was lower than that in the control group. With the gradual increase of the expression level of Znt-1 in hippocampal neurons, there was no significant difference in the average gray values between the lead exposed P14 and P21 groups and the corresponding control groups. See Table 2.

Table 2 Comparison of Gray Values of Znt-1 Expression in Ca3 Area of Hippocampus

Group	Grayscale value	
	P14	P21
Control group	94.25±5.14	64.58±6.25
Lead exposure group	91.23±7.15	66.15±7.23

The process will produce changes in the strength of synaptic connection, and the basis of this change depends on the formation of synaptic connection during the development process. During the culture process of lead exposure to hippocampal neurons, it was found that lead inhibited the occurrence of hippocampal neuron axons and increased the number of dendrites, but increased the length and branches of axons. In the synaptic formation of PC12 cell line induced by nerve growth factor, low lead exposure can make the extension and number of synapses longer and more, Lead can make the shape of protuberance bigger and flat, and lead can also regulate the occurrence of protuberance through calcium signal transduction system.

3. Mechanism of Lead Neurotoxicity

3.1 Excitatory Toxicity

Excitatory toxicity denatures neurons in many acute central nervous system diseases, including ischemia, trauma and epilepsy. The main medium of excitotoxicity injury is calcium ion. In excitotoxicity, excessive release of synaptic glutamate can lead to imbalance of calcium homeostasis. Lead, as a widespread neurotoxic agent in the environment, has significant damage to children's neurodevelopment. When lead enters the blood-brain barrier, it accumulates in the hippocampus, which is the key part of learning and memory, and synaptic plasticity is the important foundation of learning and memory. Histone modification is one of the most important epigenetic modifications that control gene transcription regulation in eukaryotes, and methylation and demethylation of histone are the most important and well-studied modification types of histone [7]. The regulation of gene expression by histone lysine methylation guided by cRNA not only depends on the site of lysine residues and the degree of methylation, but also triggers the transmission of fast excitatory transmitters in the central nervous system. However, AMPA receptors allow Ca2+ to flow in a part of hippocampus, cortex and retinal neurons, and are related to the region of histone lysine in the gene [8].

The blockage of AMPA receptor will affect the communication and communication of neurons, which is an indispensable part of learning and memory. The mechanism of hippocampal nerve injury caused by lead may be that when lead enters hippocampus, it induces the up-regulation of calcyon expression, competitively activates the second messenger IP3, triggers the IP3 receptor, increases downstream CaM expression, and increases intracellular Ca2+ outflow, thus interfering with cell signal transduction, causing cell calcium homeostasis imbalance, and even causing cell injury and apoptosis.

3.2 Oxidative Stress

Oxidative stress is considered to be a redox reaction involving reactive oxygen species and

reactive nitrogen. The key role of oxidative stress is to regulate the function of cells, mainly on microglia and astrocytes, such as the activation of apoptosis process and excitotoxicity, which are the two main causes of neuronal death. When the hippocampal slices were perfused with different concentrations of lead acetate solution, the group peak potential (PS) and excitatory postsynaptic potential (EPSP) recorded in CA1 decreased when stimulated in the clockwise direction, and the decrease of PS was more obvious, and the decrease was proportional to the concentration of Pb [9]. However, lead perfusion had no effect on the extracellular evoked potential produced by retrograde stimulation. Therefore, he speculated that Pb might act on presynaptic sites.

If there is only one peak on the dissolution curve of a single gene, and the peak is about 80 ℃, it indicates that the primer specificity is strong enough to meet the experimental requirements. With cRNA as the internal reference gene, all samples were homogenized, and all genes in all samples were subjected to three repeated experiments. The expression level of all genes was analyzed using the mean value of Ct value. CRNA-guided lead can cause neurotoxicity by directly or indirectly producing lipid peroxidation and oxidative stress reaction, and lead neurotoxicity leads to free radical damage mainly in two ways [10]. There are many mechanisms that cause neuronal death. In addition to mitochondrial dysfunction, excitotoxicity, oxidative stress, from gene to disease level, such as aggregation caused by protein misfolding and ubiquitination caused by abnormal proteasome function, all participate in cell damage.

3.3 Affect Gene Expression

Lead can change the mRNA expression of NMDA receptor subunits in hippocampus, thus affecting the modification of their receptor levels or subtypes, and the tight junction of neurons mediated by NMDA receptors has also changed. Lead exposure can change the axonal structure of neurons, so it is speculated that lead exposure may probably cause nervous system damage during development by affecting the Zn2+balance of hippocampal neurons. Lead can cause neurotoxicity, which is related to Brn-3aPOU transcription factor, which is related to the survival and differentiation of sensory neurons during their development. Therefore, the reason for the low learning ability of lead-exposed offspring rats may be that lead exposure leads to the decrease of zinc in hippocampal neurons and the decrease of free available zinc, which in turn affects the development of hippocampus and reduces the synaptic plasticity of hippocampus.

The total amount of cRNA protein has not changed in lead exposure environment. In this paper, the mRNA expression of downstream genes NRG1 and Meis2 related to neural development regulated by cRNA was detected. Lead guided by cRNA can inhibit DNA repair. In the process of DNA repair, lead plays an interference role such as polymerization and ligation, thus inhibiting DNA repair. Lead can also indirectly affect the combination of cAMP and PKA regulatory subunit by inhibiting adenylate cyclase, thus affecting the phosphorylation of CREB and damaging long-term memory. The expression of DZNT-1 decreased in P7 group after birth, suggesting that the intracellular Zn2+level of hippocampal neurons decreased, and there may be an imbalance of Zn2+in hippocampal neurons, which may be one of the important mechanisms leading to lead-exposed nerve injury in the early development stage.

4. Conclusions

According to whether the cRNA-guided lead exposure hippocampus LTD can be blocked by NMDA receptor blockers, it can be divided into NMDA receptor-dependent LTD and non-NMDA receptor-dependent LTD. The production of LTD is mainly dependent on the long-term decrease of the postsynaptic Ca2+influx and glutamate receptor sensitivity. The effect of lead on the amplitude of LTP in CA1 region is mainly the result of inhibiting the channel current of NMDA receptor by

affecting it. The reason why lead increases the amplitude of CA3 may be that lead reduces the release of synaptic GABA, and lead increases the excitability of CA3 pyramidal cells by reducing GABA-mediated inhibition. Glutamate, the most representative excitatory and inhibitory neurotransmitter in the central nervous system, is located in the prominent anterior vesicle and is released into the synaptic space after fusion with the vesicle. As a glutamate transporter, glutamate is transported from presynaptic to synaptic terminals, and then combined with postsynaptic membrane receptors to play a synaptic effect. To investigate whether chronic lead exposure affects the release of neurotransmitters from the presynaptic membrane of hippocampal neurons. In this paper, cRNA was introduced to obtain that the release of synaptic vesicles of hippocampal neurons exposed to lead has a protective effect, which provides a theoretical basis for traditional Chinese medicine to prevent hippocampal damage caused by lead exposure. A new concept of the range of synaptic plasticity, which is used to characterize the size of synaptic plasticity and is defined as the sum of LTP and LTD amplitudes. In the CA1 region of the hippocampus, lead reduced the amplitude of LTP and LTD, thus narrowing the range of synaptic plasticity.

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