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PHYSIOLOGY

Cortexin and Combination of Nitrite with Cortexin Decrease Swelling and Destruction of Cerebellar Neurons in Hemorrhagic Stroke

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It is known that signal transmission from neuron to neuron is mediated by neurotransmitters, which are released from synaptic vesicles, interact with the corresponding postsynaptic receptors, and transmit impulses in chemical synapses. There is another type of transmission, where neurotransmitters diffuse into intercellular space and interact with extrasynaptic receptors. Endogenic peptides are recognized as signal molecules in interneuronal and neuroeffector transmission, playing the roles of neuromodulators, neurotransmitters, or physiologically active substances with cerebroprotective and antiseizure activity [1]. Nitric oxide (NO) has similar properties when its concentration does not exceed physiological levels [1, 2].

Multiple studies demonstrated that peptides, such as the peptide-based drug cortexin, positively influence the body adaptation to extreme stress conditions [3–5]. In the cerebral cortex, the action of cortexin is tissue-specific with cerebroprotective, antiseizure, and nootropic effects [3, 5]. The NO-generating compound sodium nitrite also exerted a protective effects when it was injected at relatively low doses, such as 0.5 mg/100 g body weight, which did not resulted in the formation of additional methemoglobin [6–8]. Cortexin is often used for treatment of patients who are simultaneously taking nitro-containing vasodilators. We studied the effects of cortexin and its combination with nitrites on the ultrastructure of cerebellar neurons in rats with hemorrhagic stroke induced by acoustic stress.

Male 4.5-month-old rats of the Krushinskii–Molodkina (KM) strain were used for the study. The animals were selected for the experiments in accordance with their age, body weight, and sex from a population of KM rats. Twenty-six male rats weighing 260 ± 40 g were used for the study of the cortexin effects on the progress of hemorrhagic stroke. The animals were divided into two groups. The experimental animals were intraperitoneally injected with the neuropeptide cortexin dissolved in physiological solution at a dose of 0.2 mg/kg body weight for ten days. The rats were housed six or seven per cage under the vivarium conditions, a natural light/dark cycle, at room temperature (+20°C) and were allowed free access to water and food. Thirteen rats were preliminary treated with cortexin. Thirteen control rats were intraperitoneally injected with the same volume of physiological saline for ten days.

Hemorrhagic stroke was modeled in accordance with the standard scheme [9]. Rats of KM strain were put into a chamber and underwent acoustic stress. Acoustic stimulation was started with continuous 1.5-min presentation of a loud (110–115 dB) electric bell ring, which was followed by a 15-min series of alternating 10-s strong and weak (80–90 dB) acoustic stimuli with 10-s intervals between them. After a 3-min pause, the rats were again subjected to a 1-min continuous strong acoustic stimulus (110–115 dB).

After the end of the experiments, the animals were decapitated, and the cerebella were dissected and fixed with 2.5% glutardialdehyde dissolved in 0.1 M sodium cacodylate buffer, pH 7.2, which contained 0.5% tannin acid and 3% sucrose. The specimens were postfixed with 1% OsO_4 dissolved in the same buffer solution,

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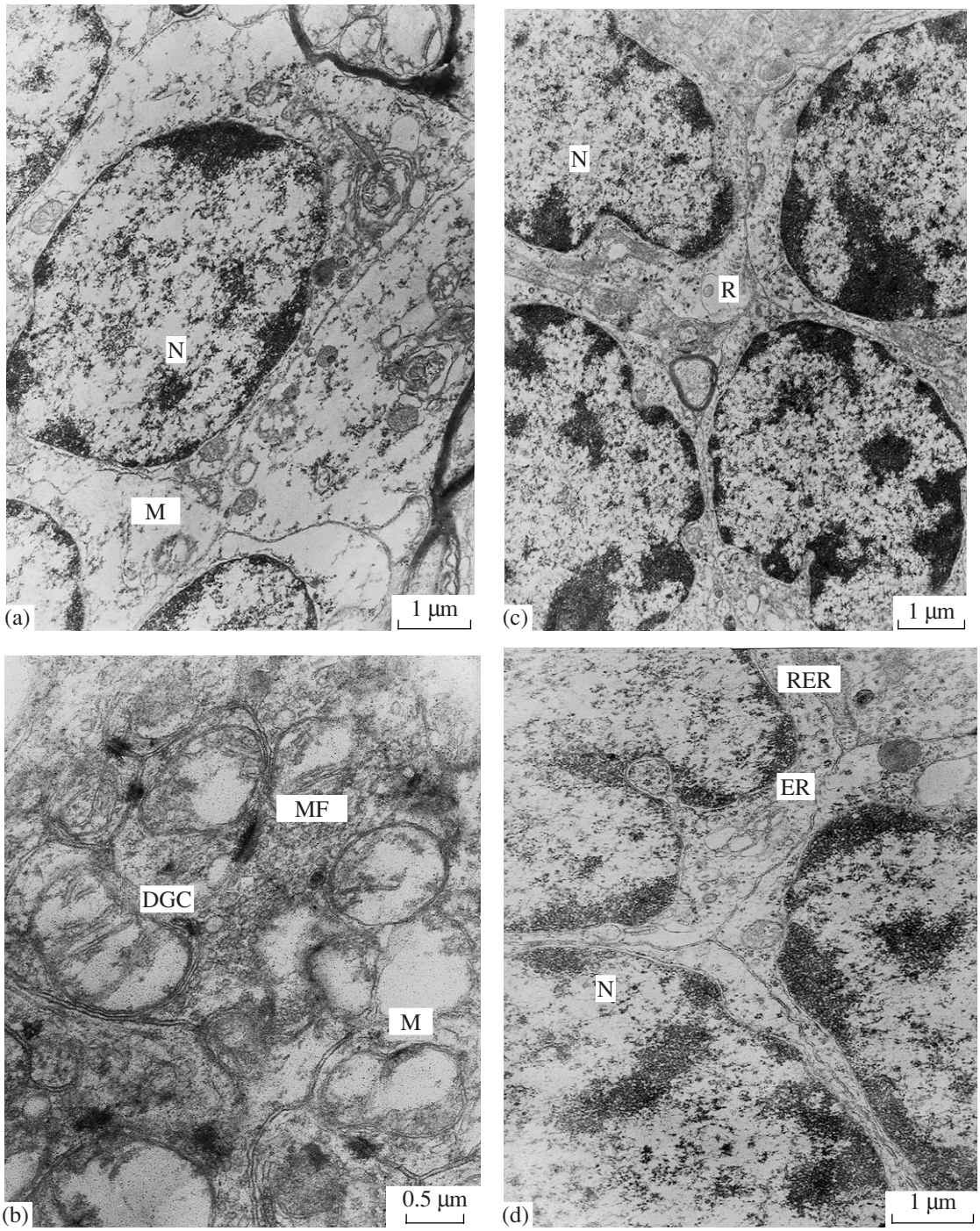

Fig. 1. Effects of treatment with (a, b) physiological saline, (c) cortexin, or (d) combination of cortexin with nitrite on ultrastructure of cerebellar granule cells in KM rats after hemorrhagic stroke induced by acoustic stress. DGC, dendrite of granule cell; M, mitochondria; MF, mossy fibers; R, ribosome; RER, rough endoplasmic reticulum; ER, endoplasmic reticulum; N, nucleus. (a) cerebellar granule cell with swelled cytoplasm and damaged M, ER, myelin sheath, and MF neurofilaments from the control animals treated with physiological solution; (b) synaptic contact of mossy fiber with DGC with substantially damaged mitochondria in the cerebellum of the control animals treated with physiological solution; (c) cerebellar granule cells from the rats preliminary treated with cortexin. Good preserved N, cytoplasm, cytoplasmic organells, and ribosomes chains are observed; (d) cerebellar granule cells from the rats preliminary treated with combination of cortexin and nitrite. The cells are good preserved; ER cisterns, and RER are observed.

pH 7.2, for 1 h at 4°C. Then, they were dehydrated in ethanol solutions with increasing concentrations, absolute alcohol, and acetone, and, after that, they were embedded in a mixture of Epon-812 and araldite according to the method [10]. The sections were stained with uranyl acetate and lead citrate and studied using a JEM-1200EX-11 electron microscope (Japan) under an accelerating voltage of 90 kV.

We studied granular cells (GCs) of the boundary layer between granular cells and Purkinje cells in three groups of animals. Cerebellar GCs from the brain of the animals that were injected with physiological saline after acoustic stress are presented in the Fig. 1a. The nuclei of these cells contained dense clots of membrane-bound and central chromatin. In the cytoplasm, undamaged mitochondria, endoplasmic reticulum (ER) cisterns, ribosomes, and rough endoplasmic reticulum (RER) were absent. This cellular destruction is a result of swelling, which is typical of hemorrhagic stroke. Other cell structures, such as GC dendritic terminals, also contained swelled mitochondria (MCH), and some of them had local membrane destructions (Fig. 1b).

In the cerebella of the experimental group of rats treated with cortexin, we observed substantial changes that showed a positive effect of this peptide on cerebellar neurons in KM rats. Cerebellar GCs of rats injected with cortexin are presented in the Fig. 1c. These cells did not display manifest swelling, contained intact mitochondria and ER chains of ribosomes.

In the cerebellum of the experimental animals treated with cortexin and nitrite, we observed changes that indicated positive influence of this combination on the structure of cerebellar GCs. Cerebellar GCs of the rats of the third experimental series are presented in Fig. 1d. The nuclei of GCs were not substantially changed; chromatin bodies were equally distributed over the nuclei. Mitochondria, ER, ribosomes chains, and RER were observed in the cytoplasm of GCs. The dendrites of GCs, as well as their mitochondria and structure of mossy fibers (MF) terminals, were also good preserved. Specifically, in the first series of experiments, we observed damages of myelin sheaths (“garneting”) and neurofilaments in MF cross sections (Fig. 1a). The occurrence of these damages was lower in the second series of experiments and was very low in the third series of experiments. Thus, we suppose that cortexin and combination of cortexin with nitrite prevented GCs swelling and destruction of neuron structure in hemorrhagic stroke.

What are the mechanisms of protective cortexin effects in KM rats that are genetically prone to hemorrhagic stroke after audiogenic stress stimulation? It is known that interaction of neuropeptides with peptidergic receptors affects NO generation in the mammalian brain and myocardium [2–4]. In turn, NO activates wound healing and repair of other damages in different tissues [11]. This effect is mediated by Ca^{2+} ions, which activate constitutive NO-synthases and increase the levels of NO and its metabolites [2, 3].

The data on the effects of different NO-generating compounds allow us to make some conclusions on the beneficial action of NO: (1) a decrease in the blood pressure due to the vasodilator action of NO-generating compounds [6, 12]; (2) an improvement of blood oxygen supply and attenuation of hypoxia/ischemia [6, 13]; (3) redistribution of proteins from the cytoplasm to the membrane-bound state and a decrease in oxidative damage of the most vulnerable components of cell membranes, such as unsaturated fatty acids [13]; (4) the ability of NO_2^- ions formed from NO or released from drugs, food, and water to accept electrons of the mitochondrial respiratory chain and increase the content of ATP in neurons [14]. In conclusion, we have to emphasize that activation of peptidergic receptors is coupled, as a rule, with Ca^{2+} ion mobilization, activations of constitutive NO-synthases, and changes in the contents of NO and its metabolites [2–4]. Recently, the phenomenon of distant ischemic preconditioning was described [15]. Essentially, the brain and the myocardium become more resistant to long-term ischemia/reperfusion after preliminary treatment with one to three sessions of short-term ischemia/reperfusion. The sequences of events and the mechanisms of this phenomenon in cells are not completely understood; however, we may safely assert that peptides, the nuclear factor NF-κB, and NO play an important role in distant ischemic preconditioning [15]. Detailed study on the effects of combined and separate treatments with peptide-based drugs, including cortexin in combination with NO-generating compounds or inhibitors of NO-synthases, is very important.

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