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Protective effects of ACTH (4-9) in degeneration of hippocampal nerve cells caused by dexamethasone: ultrastructural studies

Searching drugs preventing neurotoxic effects of glucocorticosteroids (GCs) has a prominent practical value. In the recent years there have been reports showing that GCs are toxic to neurons and have an important role in neurodegenerative processes (1, 3, 16). This is particularly so in the hippocampus, a principal neural target tissue for GCs with the highest concentration of GCs receptors in central nervous system (15). Degenerative changes occur both as the result of action of endogenous glucocorticosteroids secreted by adrenal cortex and exogenous glucocorticosteroids administered in high doses for therapeutic purposes (18, 19, 13, 14). It is thought that neurotoxic action of glucocorticosteroids is responsible for senile dementia, some cases of dementia in posttraumatic shock, cognitive impairments in patients with depression and Cushing's syndrome, difficulties in memory and concentration in patients treated with exogenous corticosteroids (19, 12).

The purpose of this research is an assessment of neuroprotective action of ACTH (4-9) in degeneration of nerve cells caused by prolonged administration of dexamethasone on the base of ultrastructural examination of hippocampal neurons. We used ACTH (4-9) fragment as a neuroprotective agent because several earlier studies showed its neuroprotective effect (4, 5, 7). Mechanisms that mediate this action of ACTH (4-9) are still not explained.

MATERIAL AND METHODS

The experiments were carried out on adult Albino Swiss mouse males (19–22g). Care and treatment of the animals were in accordance with the guidelines for laboratory animals established by the National Institutes of Health as well as by the Local Ethical Committee of the Medical University of Lublin. The animals were divided into three groups (including 20 animals each). Control group-the animals receiving distilled water (i.p. 0.2 ml/24h) for 28 days. Experimental group I – the animals receiving dexamethasone. Experimental group II – the animals receiving dexamethasone and ACTH (4-9). Dexamethasone (Dexaven-Jelfa S.A., Poland) was administered intraperitoneally in the single dose of 8 mg/kg/24 h for 28 days. ACTH (4-9) (Bachem, Switzerland) was administered subcutaneously in the dose of 50 μg/kg twice a week 30 min prior to dexamethasone. 24 hrs after the last distilled water or last dexamethasone injections all animals were decapitated. Their brains were removed from the skull and fixed in 4% glutaraldehyde. Next, tissue material after being dehydrated was embedded in Epon 812. Half-thin sections, 1 μm thick stained with methylene blue were examined in light microscope in order to select the CA3 hippocampal areas for ultrastructural studies. The preparations were observed in the transmission electron microscope TESLA BS 500.

RESULTS

Control group: Pyramidal neurons in the CA3 region were arranged in several (3–5) layers of cells. They possessed round or oval nuclei. Chromatin formed small electron-dense granules equally dispersed throughout the nucleus. The nucleolus was well visible. The nuclear envelope consisted of two unit membranes separated by a narrow space (perinuclear cisterna). The outer membrane of nuclear envelope was covered with ribosomes. Around the nuclear envelope at sites where the inner and outer membranes fuse, the nuclear pores were visible. In the cytoplasm cell organelles such as rough endoplasmic reticulum, free ribosomes, mitochondria, lysosomes were visible. Free ribosomes were equally dispersed within the cytoplasm (Fig. 1).

Experimental group I: The amount of pyramidal neurons in the CA3 region was significantly decreased. Pyramidal neurons in the CA3 region showed pathological changes leading to a condensation of nucleus and cytoplasm. Neurons were dark and irregular in shape (Fig. 2). They possessed dark, irregular nuclei. The surface of nucleus was covered with numerous convolutions and protuberances of various size. The nucleus was electron dense and contained compact homogenous chromatin. Nucleoli were enlarged. The cytoplasm became more condensed. It consisted of areas of clumped free ribosomes separated by clear spaces representing the dilated cisternae of endoplasmic reticulum. Shrunken neurons were surrounded with swollen processes of glial cells (Fig. 3). Electron

microscopy examination also revealed the swelling of endothelial cells in blood vessels and the swelling of glial processes surrounding these vessels.

Experimental group II: Ultrastructural examinations revealed that the character of morphological changes in hippocampal neurons after administration of ACTH (4-9) is similar to changes observed in the experimental group I, but the degree of damage in the case of majority of animals was significantly smaller. 40% of animals from the experimental group II did not show any morphological changes in hippocampal neurons (Fig. 4). In other animals, we observed pathological changes leading to condensation of neuronal nucleus and cytoplasm, but the amount of damaged nerve cells and intensity of changes were significantly reduced.

DISCUSSION

There is no doubt that glucocorticosteroids are toxic to CNS. This is particularly so in the hippocampus, a principal neural target tissue for glucocorticosteroids with the highest concentration of GCs receptors in CNS (8). The results of the present study revealed pathological changes in the ultrastructure of hippocampal cells caused by exogenous glucocorticosteroid-dexamethasone. The primarily and most severely affected cells in our experiment were pyramidal neurons in the CA3 region. Most of neurons in this region showed pathological changes leading to increasing condensation of the cell nucleus and cytoplasm. Finally these degenerating cells died. In any tissue, cell death may follow two distinct morphological and biochemical patterns: necrosis or apoptosis (2, 6, 20). Shrunken, dark neurons observed in our experiment presented typical morphological changes characteristic of apoptosis: shrinkage of cell, condensation of nucleus and cytoplasm, preservation of cell membrane (8, 9). Apoptosis requires an active protein synthesis. Enlarged nucleoli and a large amount of ribosomes observed in cytoplasm indicate the active protein synthesis in these cells. The lack of inflammatory infiltration in the place of damage also confirms this mechanism of cell death. GCs are classic inductors of apoptosis (9). The mechanism by which glucocorticosteroids induce apoptosis in neurons is not completely explained. In the 1970s, Sibley and Tompkins determined that the initial step in GCs-induced apoptosis is mediated through the GCs receptor and requires translocation of the receptor from the cytoplasm into the nucleus. In the nucleus the GCs receptor functions as a transcription factor, enhancing or repressing the expression of a selected repertoire of genes. Glucocorticosteroids may repress expression of genes necessary for cell survival by attenuating AP – 1 (c - Fos/c - Jun) transcription factor activity, or may induce the transcription of genes involved in carrying out the death programme. On the other hand, it is known that the impairment of glucose uptake in neurons plays an important role in the mechanism of neurotoxic effects of glucocorticosteroids (11). This effect is similar to classic glucocorticosteroid inhibition of glucose transport in numerous peripheral tissues. Energetic depletion enables damaging action of glutamate.

That is so, because the control of glutamate releasing and what is more important glutamate uptake are processes which require a large amount of energy. Glucocorticosteroids increase the concentration of glutamate in the extracellular space. An activation of NMDA receptors by high concentration of glutamate may be deciding for induction of degenerative changes typical of apoptosis in nerve cells under the influence of glucocorticosteroids (6).

On the base of results achieved after administration of ACTH (4-9) we can assume that this fragment of adrenocorticotropic hormone shows a protective effect against neurotoxic influence of dexamethasone. Earlier experiments show that ACTH (4-9) prevents vincristine-and taxol-induced neuropathy (7, 4). This fact seems interesting regarding the mechanism of damaging action of vincristine and taxol. Experimental data indicate that vincristine and taxol induce in neurons changes typical for apoptosis (10, 17). The results of our investigations show that protective action of ACTH (4-9) against neurotoxic effect of dexamethasone is connected with its ability to inhibit apoptotic processes in neurons.

CONCLUSIONS

All our results led to a conclusion that ACTH (4-9) prevents dexamethasone-induced degenerative changes in hippocampal pyramidal neurons. Protective action of ACTH (4-9) against neurotoxic effect of dexamethasone is connected with the inhibition of apoptotic processes in neurons.

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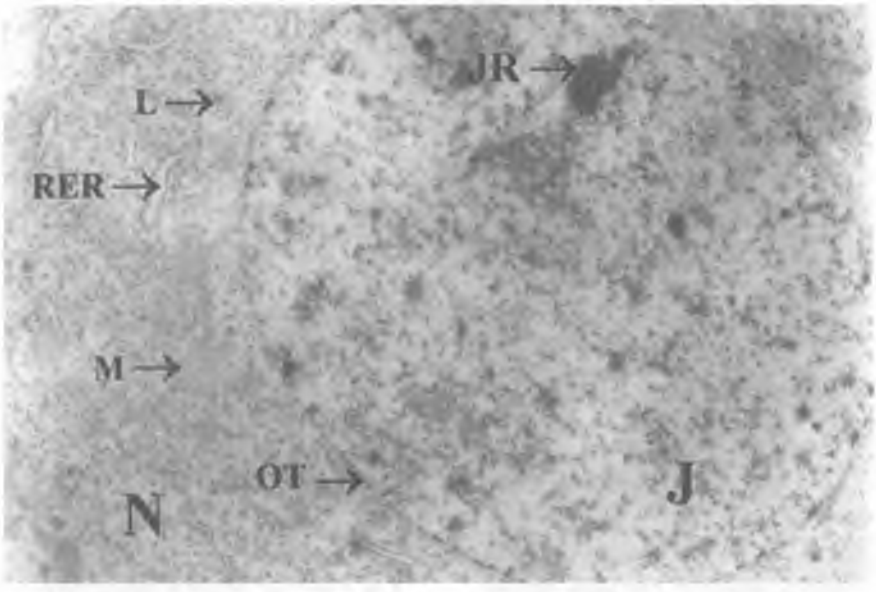
SUMMARY

The purpose of this research was an assessment of protective action of ACTH (4-9) in dexamethasone-induced neurodegeneneration on the base of ultrastructural examinations of hippocampal neurons in the CA3 region. The experiments were carried out on

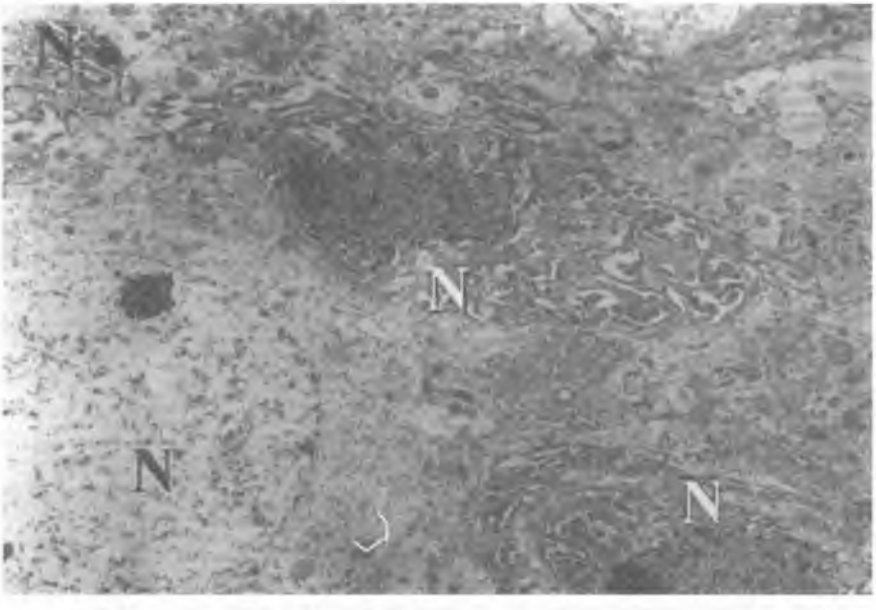
adult Albino Swiss mouse males. The animals were divided into three groups: control group, experimental group 1-dexamethasone 8 mg/kg/24h for 28 days, experimental group 2-dexamethasone and ACTH (4-9) 50 μg/kg twice a week. Results of our investigations show that ACTH (4-9) prevents neurotoxic influence of dexamethasone and its protective action is connected with the ability to inhibit degenerative processes in neurons having a character of apoptosis.

Działanie ochronne ACTH (4-9) w degeneracji komórek nerwowych hipokampa wywołanej deksametazonem: badania ultrastrukturalne

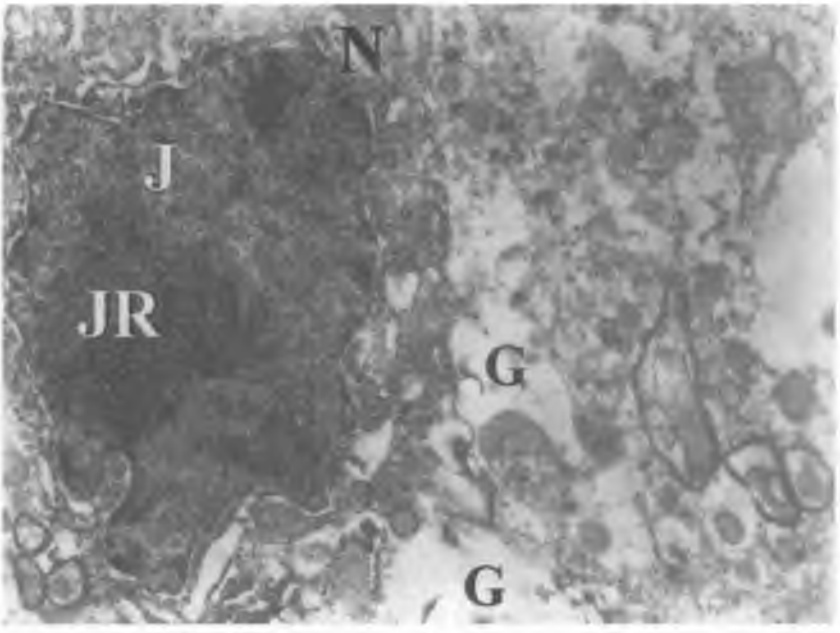
Celem pracy była ocena protekcyjnego działania ACTH (4-9) w neurodegeneracji indukowanej deksametazonem na podstawie badań ultrastrukturalnych neuronów hipokampa w polu CA3. Doświadczenia przeprowadzono na dorosłych samcach myszy białych. Zwierzęta podzielono na trzy grupy: grupę kontrolną, grupę doświadczalną 1-deksametazon 8 mg/kg przez 28 dni, grupę doświadczalną 2-deksametazon i ACTH (4-9) 50 mg/kg dwa razy w tygodniu. Wyniki naszych badań wskazują na to, że ACTH (4-9) zapobiega neurotoksycznemu działaniu deksametazonu, a jego działanie ochronne wiąże się ze zdolnością hamowania procesów degeneracyjnych w komórkach nerwowych o charakterze apoptozy.



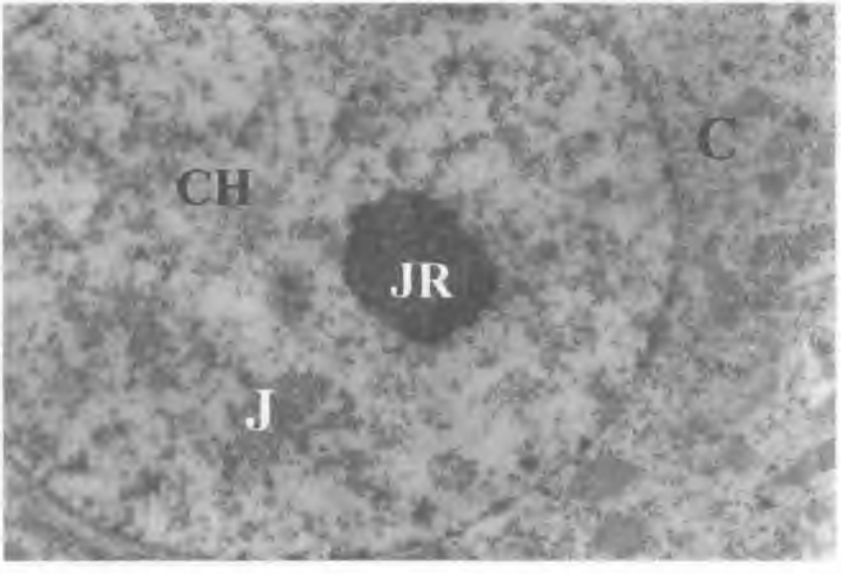
Phot. 1. Control group. Neuron (N) in the CA3 region of the hippocampus. Nucleus (J), nucleolus (JR), mitochondrium (M), rough endoplasmic reticulum (RER), lysosomes (L). Magn. 6000x



Phot. 2. Experimental group I. Neurons (N) in the CA3 region of the hippocampus. Magn. 3000x



Phot. 3. Experimental group I. Shrunken and dark neuron (N) in the CA3 region. Nucleus of neuron (J), nucleolus (JR), swollen processes of glial cells (G). Magn. 6000x



Phot. 4. Experimental group II. Neuron in the CA3 region of the hippocampus. Nucleus (J), nucleolus (JR), chromatin (CH), cytoplasm (C). Magn. 6000x