ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, N 2, 119 SECTIO D 2002

Department of Histology and Embryology with the Lab of Experimental Cytology
Medical University of Lublin
Department of Clinical Pharmacology, Medical University of Lublin

JOANNA SEKITA-KRZAK, KRYSTYNA CZERNY,
IWONA ŻEBROWSKA-ŁUPINA, MARIA STĘPNIEWSKA,
JÓZEF VISCONTI, JACEK KOZIEJ

Neuroprotective effect of ACTH (4-9) in degeneration of hippocampal nerve cells caused by dexamethasone: morphological studies

In the recent years there have been reports showing that prolonged exposure to elevated level of glucocorticosterois causes degenerative changes in the central nervous system. These changes occur both as a result of action of endogenous glucocorticosteroids secreted by adrenal cortex and exogenous glucocorticosteroids administered in high doses for therapeutic purposes (16, 17, 11, 12). Neuronal damage induced by these hormones occurs mainly in hippocampal formation – the structure of the brain containing the highest concentration of glucocorticosteroid receptors (13). It is thought that neurotoxic action of glucocorticosteroids is responsible for senile dementia (age related neuron depletion due to elevated levels of endogenous glucocorticosteroids secreted during stress), some cases of posttraumatic dementia, cognitive impairments in patients with depression and Cushing's syndrome, difficulties in memory and concentration in patients treated with exogenous corticosteroids (17, 10). Clinical observations of neurotoxic effects of glucocorticosteroids were confirmed in numerous experiments on animals (1, 2, 14).

Searching drugs preventing neurotoxic effects of glucocorticosteroids has a prominent practical value. 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 histological examination of hippocampal neurons. We used ACTH (4-9) fragment as a neuroprotective agent because there are contradictory evidences referring to its neuroprotective effects in literature. Mechanisms that mediate this action 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 SA, Poland) was administered intraperitoneally in the single dose of 8 mg/kg/24h 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 h after the last distilled water or last dexamethasone injections all animals were perfused with 0.9% NaCl with heparin, followed by 10% formaldehyde (pH 7.4). Following decapitation, brains were removed from the skull and postfixed in the same fixative solution at 4^0C for at least 24 h. Specimens were then dehydrated in graded ethanol solutions and embedded in paraffin. 6 μm thick paraffin sections were cut in the frontal plane. For histological analysis they were stained with cresyl violet and assessed by the light microscope. We examined morphology of neurons in the dorsal hippocampus of both hemispheres.

The quantitative analysis of morphological brain changes was carried out by counting the number of damaged neurons in the CA3 region (in slides stained with cresyl violet) using a computer analyser of histological pictures (Lobophot 2, Nicon). Cells with rounded nuclei and visible nucleoli were considered undamaged, while cells with dark shrunken nucleus in which the nucleolus was not discernable were considered damaged. Cell counts were made within 2 adjacent 40x microscopic fields in the pyramidal cell layer (from the point directly ventral to the most lateral extension of the upper limb of the dentate granule cell layer). The percentage of damaged neurons in the CA3 region was counted in all groups. Histological data were subjected to statistical analysis using Chi-square test.

RESULTS

Weight changes: changes in the body weight of all animals are presented in Figure 1. Mouse males from the experimental groups I and II showed significant decrease of the body weight in comparison with the control group after administration of the toxic doses of dexamethasone. The mortality in both experimental groups was similar (25%).

Histological analysis of the hippocampus: light microscopy examination of cresyl violet stained slides from the control group revealed the regular structure of the hippocam-

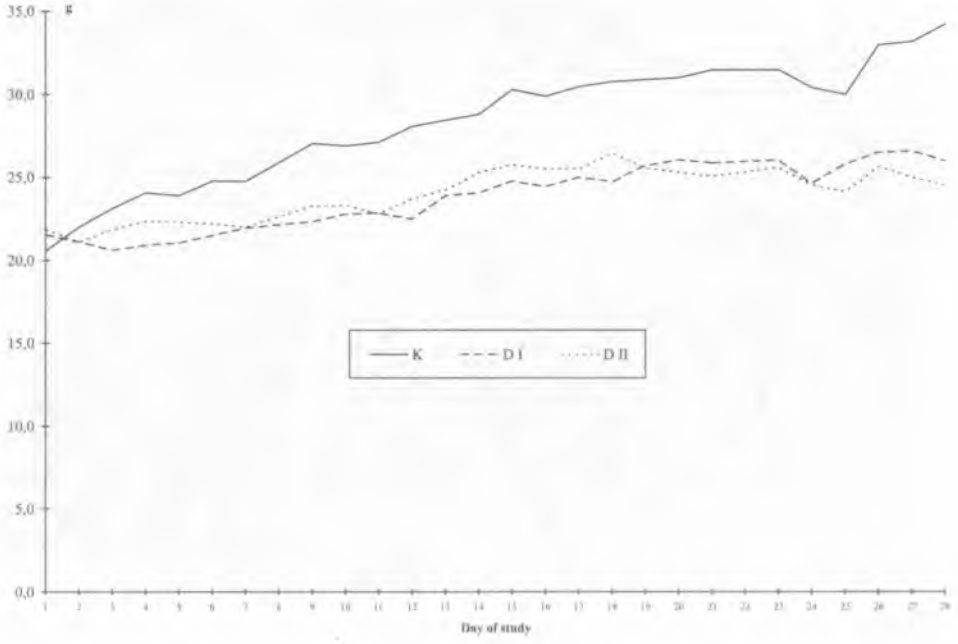

Fig. 1. Body weight changes of animals during experiment. K – control group, D I – experimental group I, D II – experimental group II

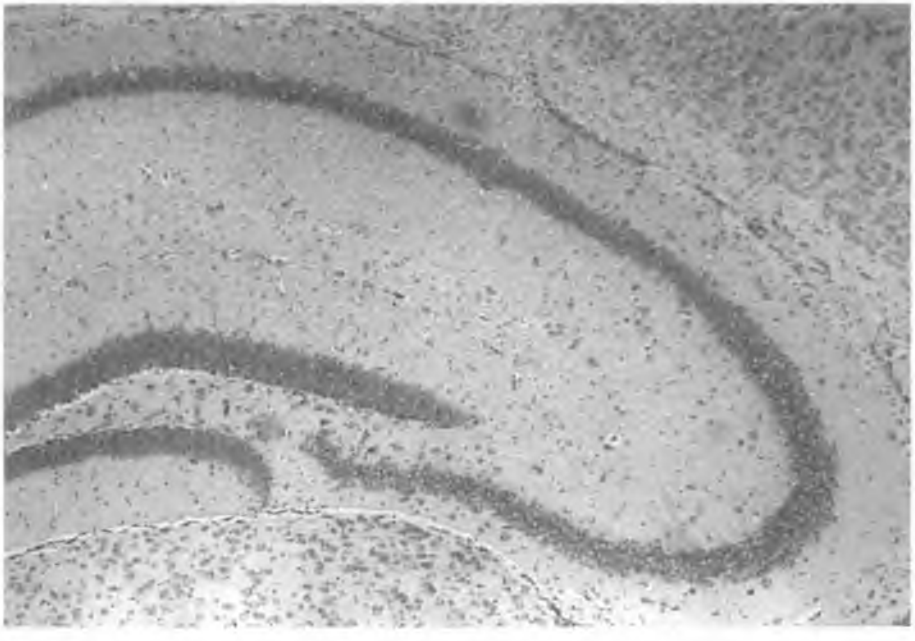

Fig. 2. Control group. Regular structure of the hippocampus. The frontal section through the hippocampus. Staining with cresyl violet. Magn. 200x

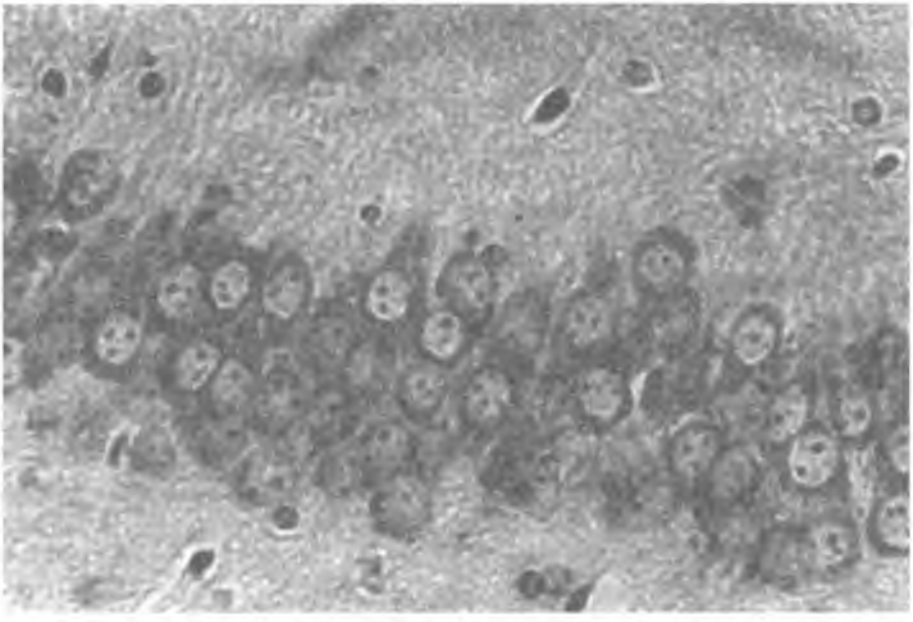

Fig. 3. Control group. The CA3 region of the hippocampus. Staining with cresyl violet. Magn. 800x

Fig. 4. Experimental group I – after administration of dexamethasone. The CA3 region of the hippocampus. Decreased amount of nerve cells. Shrunken and dark neurons. Swelling around damaged neurons. Staining with cresyl violet. Magn. 800x

pus (Fig. 2). The nuclei of pyramidal neurons in CA1–CA4 regions were clear, round or oval in shape with distinct nucleoli. In the CA3 region they were arranged in 3 to 4 layers (Fig. 3).

Significant morphological changes of hippocampal neurons were observed in the experimental group I. After 28-day administration of dexamethasone the amount of pyramidal neurons, especially in the CA3 region was decreased. In this region nerve cells were arranged in 2 to 3 layers in comparison with the bigger amount in the control group. Numerous neurons in the CA3 region showed far-reaching morphological changes including a shrinkage of perykarions and an increased intensity in their staining. Nuclei of damaged nerve cells were dark, irregular in shape and shrunken in comparison with clear round nuclei of the control group (Fig. 4).

In the group of animals receiving ACTH (4-9) together with dexamethasone performed examinations revealed significantly a smaller degree of morphological damage of neurons. The total number of neurons in the CA3 region was similar to the control group and the amount of nerve cells showing morphological changes was significantly reduced in comparison with animals receiving only dexamethasone (Fig. 5).

Statistical analysis of the results received during the quantitative analysis of damaged neurons revealed statistical significance in the CA3 region, when the percentage of dexametasone-induced damaged neurons was compared to either the control group or the experimental group II ($P<0.0001$ for the control group against the experimental

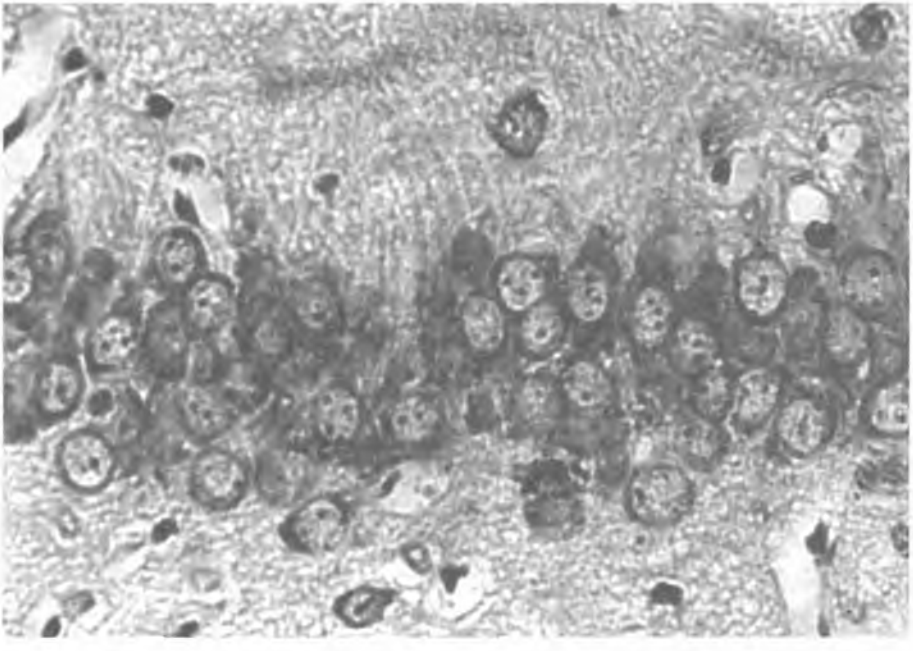

Fig. 5. Experimental group II – after administration of dexamethasone and ACTH (4-9). The CA3 region of the hippocampus. Staining with cresyl violet. Magn. 800x

group I; $P<0.05$ for the control group against the experimental group II; $P<0.01$ for the experimental group I against II). Statistically significant differences from the control were designated by $P<0.05$.

The mean values of percentage numbers of damaged neurons in all groups are given in Figure 6.

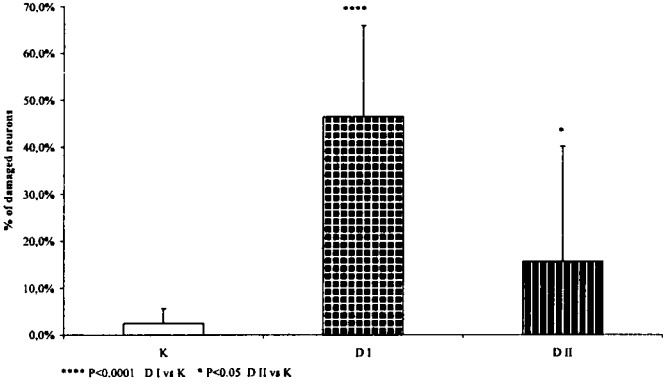

Fig. 6. Percentage of damaged neurons in the CA3 region of the hippocampus. K – control group, D I – experimental group I, D II – experimental group II

DISCUSSION

Our results show that the prolonged exposure to toxic doses of dexamethasone causes degenerative changes in nerve cells of the hippocampus. The most vulnerable to neurotoxic action of this glucocorticosteroid are neurons of the pyramidal cell layer in the CA3 region. Morphological changes observed in nerve cells after long-term administration of dexamethasone on the level of light microscope (shrinkage of perykarions, more intense cytoplasm staining, pyknotic changes of the nucleus, lack of morphological features characteristic of inflammation) are typical of apoptosis (7). The mechanism of neurotoxic effects of glucocorticosteroids is not completely explained. It is supposed that the impairment of glucose uptake in neurons and connected with it the state of increased metabolic vulnerability play an important role in this action (9). 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 (5).

On the base of the achieved results one can assume that the fragment of adrenocorticotropic hormone used in our research shows a protective effect against neurotoxic influence of dexamethasone. Earlier experiments showed that ACTH (4-9) prevents Vincristine-and Taxol-induced neuropathy (6, 3). 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 of apoptosis (8, 15). Mechanism of neuroprotective action of ACTH (4-9) may be connected with its antagonistic action on NMDA receptors (4). The results of our investigations indicate that protective action of ACTH (4-9) against neurotoxic effect of dexamethasone is connected with its ability to inhibit apoptotic processes in neurons. Thus performed investigations confirm other authors' suggestions that ACTH (4-9) affinity for NMDA receptors can be deciding for the protective influence of this chemical in neurodegenerative processes.

CONCLUSIONS

ACTH (4-9) shows a protective effect against degeneration of hippocampal nerve cells. It significantly reduces dexamethasone-induced morphological changes in neurons typical of apoptosis.

REFERENCES

1. Arbel I. et al.: The effects of long-term corticosterone administration on hippocampal morphology and cognitive performance of middle-aged rats. Brain Res., 657, 227, 1994.
2. Elliot E. et al.: Corticosterone exacerbates kainate-induced alterations in hippocampal tau immunoreactivity and spectrin proteolysis *in vivo*. J. Neurochem., 61, 57, 1993.
3. Hamers F. et al.: The ACTH (4-9) analog, ORG 2766, prevents Taxol-induced neuropathy in rats. Eur. J. Pharmacol., 233, 177, 1993.
4. Horvath K. M. et al.: Postnatal treatment with ACTH (4-9) analog ORG 2766 attenuates N-methyl-D-aspartate-induced excitotoxicity in rat nucleus vasalis in adulthood. Eur. J. Pharmacol., 405, 33, 2000.
5. Kamińska B., Stańczyk M.: Molekularne mechanizmy neurodegeneracji. Post. Biol. Kom., 25, 15, 1998.
6. Kiburg B. et al.: *In vivo* modulation of Vincristine-induced neurotoxicity in *Limnaea stagnalis*, by the ACTH(4-9) analogue Org 2766. J. Neurooncol., 30, 173, 1996.
7. Motyl T.: Apoptoza – śmierć warunkująca życie. Post. Biol. Kom., 25, 315, 1998.

8. Muzylak M., Maślińska D.: Neurotoxic effect of Vincristine on ultrastructure of hypothalamus in rabbits. Folia Histochem. Cytobiol., 30, 113, 1992.
9. Philips P. et al.: High-dose dexamethasone decreases regional cerebral glucose metabolism in the rat. Neurology, 37 Suppl 1, 248, 1987.
10. Seckl J. R., Olson T.: Glucocorticoid hypersecretion and the age impaired hippocampus: cause or effect? J. Endocrinol., 145, 201, 1995.
11. Starkman M. N., Schteingart D. E.: Neuropsychiatric manifestations of patients with Cushing syndrome: relationship to cortisol and adrenocorticotropic hormone levels. Arch. Intern. Med., 141, 215, 1981.
12. Stein-Behrens B. et al.: Stress exacerbates neuron loss and cytoskeletal pathology in the hippocamus. J. Neurosci., 14, 5373, 1994.
13. Twardowska K., Rybakowski J.: Oś limbiczno-podwzgórzowo-przysadkowo-nadnerczowa w depresji. Psychiatr. Pol., 30, 741, 1996.
14. Uno H. et al.: Hippocampal damage associated with prolonged and fatal stress in primates. J. Neurosci., 9, 1705, 1989.
15. Wakabayashi T., Spodnik J.: Structural changes of mitochondria during free radical-induced apoptosis. Folia Morphol., 59, 61, 2000.
16. Watanable Y. et al.: Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res., 588, 341, 1992.
17. Wolkowitz O. M. et al.: Cognitive effects of corticosteroids. Am. J. Psychiatry, 147, 1297, 1990.

2002.02.25

SUMMARY

The purpose of this research was an assessment of neuroprotective effect of ACTH (4-9) in degenerative changes of nerve cells induced by dexamethasone. Experiments were led on Albino-Swiss mouse males. We examine morphological changes of neurons in the dorsal hippocampus in slides stained with cresyl violet and we performed quantitative analysis of neurodegenerative changes using a computer analyser of histological pictures. Achieved results indicate that ACTH (4-9) shows neuroprotective effect against neurotoxic influence of dexamethasone. This chemical inhibits dexamethasone induced degeneration of hippocampal nerve cells having morphological features characteristic of apoptosis.

Neuroprotekcyjne działanie ACTH (4-9) w degeneracji komórek nerwowych hipokampa wywołanej deksametazonem: badania morfologiczne

Badania miały na celu ocenę neuroprotekcyjnego działania ACTH (4-9) w degeneracji komórek nerwowych wywołanej deksametazonem. Doświadczenia przeprowadzono na samcach myszy Albino-Swiss. Oceniono morfologię komórek nerwowych hipokampa grzbietowego w preparatach barwionych fioletem krezylu oraz dokonano analizy ilościowej zmian neurodegeneracyjnych przy pomocy komputerowego analizatora obrazów. Otrzymane wyniki wskazują na to, że ACTH (4-9) wykazuje działanie protekcyjne w stosunku do neurotoksycznego wpływu dexametazonu. Związek ten hamuje indukowaną deksametazonem degenerację komórek nerwowych hipokampa o charakterze apoptozy.