ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LIV, 25 SECTIO D 1999

Katedra i Zakład Histologii i Embriologii Akademii Medycznej w Lublinie
Kierownik: prof. dr hab. Krystyna Czerny

Katedra i Zakład Farmakologii Klinicznej Akademii Medycznej w Lublinie
Kierownik: prof. dr hab. Iwona Żebrowska-Łupina

JOANNA SEKITA-KRZAK, KRYSTYNA CZERNY,
IWONA ŻEBROWSKA-ŁUPINA, ANDRZEJ WRÓBEL,
MIROSŁAW ŁAŃCUT

Ultrastructural examination of hippocampal cells after experimental administration of dexamethasone

Badania ultrastrukturalne komórek hipokampa
po doświadczalnym podaniu dexametazonu

Glucocorticosteroids (GCs) are one of the most commonly prescribed classes of medications in medicine, with numerous applications ranging from physiologic replacement therapy to immunosuppression. Due to the presence of GC receptors in almost all cells, both the desired and undesired effects of GC therapy are manifold. Increasing evidence indicates that GCs are toxic to neurons and have an important role in neurodegenerative process. This is particularly so in the hippocampus, a principal neural target tissue for GCs with the highest concentration of GCs receptors in the central nervous system (CNS). The purpose of our experiment was the ultrastructural examination of hippocampal neurons after experimental administration of synthetic GC – dexamethasone.

MATERIAL AND METHODS

The experiment was carried out on white mouse males. The animals were divided into two groups: the control one and the experimental one (including 10 animals each) regarding the age of animals and stress influence. Control group – the animals receiving distilled water intraperitoneally in the dose of 0.2 ml/24h. Experimental group – the animals receiving Dexaven for 28 days.

Dexaven was administered intraperitoneally in the single dose 8 mg/kg/24h. After 24 hrs from the last Dexaven dose animals from experimental group were decapitated and their brains were collected for histological examination. The procedure in the case of animals from the control group was the same. After 24 hrs from the last dose of distilled water animals were decapitated and their brains were collected. For ultrastructural examinations the obtained tissue material was fixed in 4%

glutaraldehyde. Next, fragments of brains after being dehydrated were embedded in Epon 812 (4). Half-thin sections, 1 μm thick stained with methylene blue were examined in light microscope in order to select the areas for ultrastructural studies. The preparations were observed through 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

The amount of pyramidal neurons in the CA3 and CA4 regions significantly decreased. Pyramidal neurons in the CA3 region showed pathological changes leading to the condensation of nucleus and cytoplasm. Neurons were dark and irregular in shape (Fig. 2, 3). They possessed dark, irregular nuclei. The surface of nucleus was covered with numerous convolutions and protuberances of various size. The nucleus was electrondense and contained compact homogenous chromatin. The perinuclear cisterna was dilated. The nucleolus was enlarged (Fig. 2, 3). In the most severely shrunken neurons the nucleolus was invisible (phot. 4). The cytoplasm became more condensed. It consisted of areas of clumped free ribosomes separated by clear spaces representing the dilated cisternae of rough endoplasmic reticulum and damaged mitochondria. Mitochondria were slightly swollen or had torn membranes, but in many neurons undamaged mitochondria were visible (Fig. 5). Shrunken, neurons were surrounded with swollen processes of glial cells (Fig. 3, 5). Between such neurons we observed other type of cells. They possessed nucleoli regular in shape: round or oval. In these cells chromatin formed large, electrondense masses beneath the nuclear envelope and similar single agglomerations inside (Fig. 6). The nucleolus was not visible. The nuclear envelope showed the lack of ribosomes on the surface of external nuclear membrane or the lack of the whole external nuclear membrane in many places. The perinuclear cisterna was dilated. The cytoplasm of such cells was electron lucent. Cell organelles such as free ribosomes, cisternae of rough endoplasmic reticulum, mitochondria were few. The cisternae of rough endoplasmic reticulum were dilated. We observed microglial cells. They were not numerous and were arranged singly between other cells. Electron microscopy examination also revealed the swelling of endothelial cells in blood vessels and the swelling of glial processes surrounding these vessels (Fig. 7). We also observed the damage of myelin sheaths.

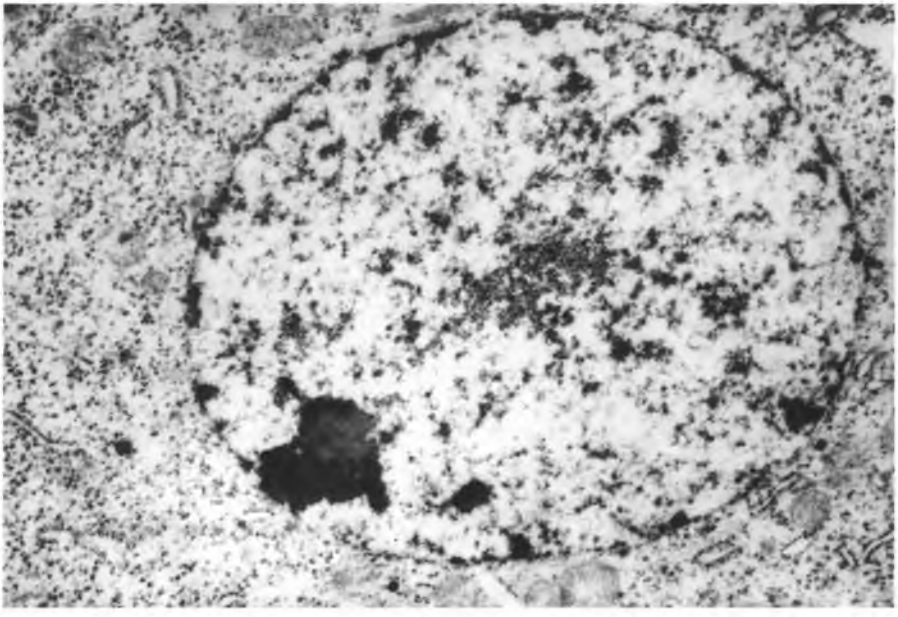

Fig. 1. Control group. Pyramidal neuron in the CA3 region. Magn. 4200 x.

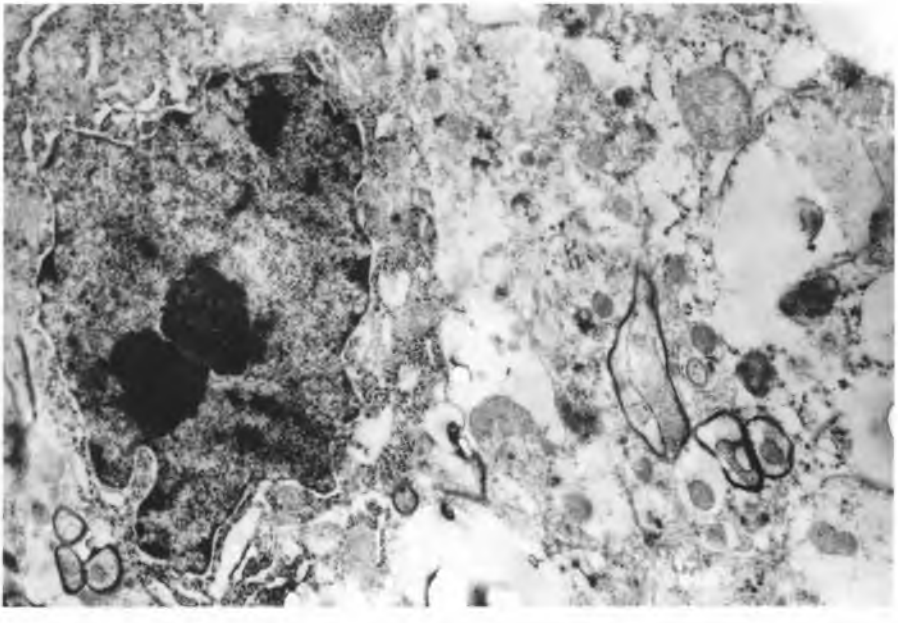

Fig. 2. Experimental group. Dark, shrunken pyramidal neuron with enlarged nucleolus in the CA3 region. Magn. 4200 x.

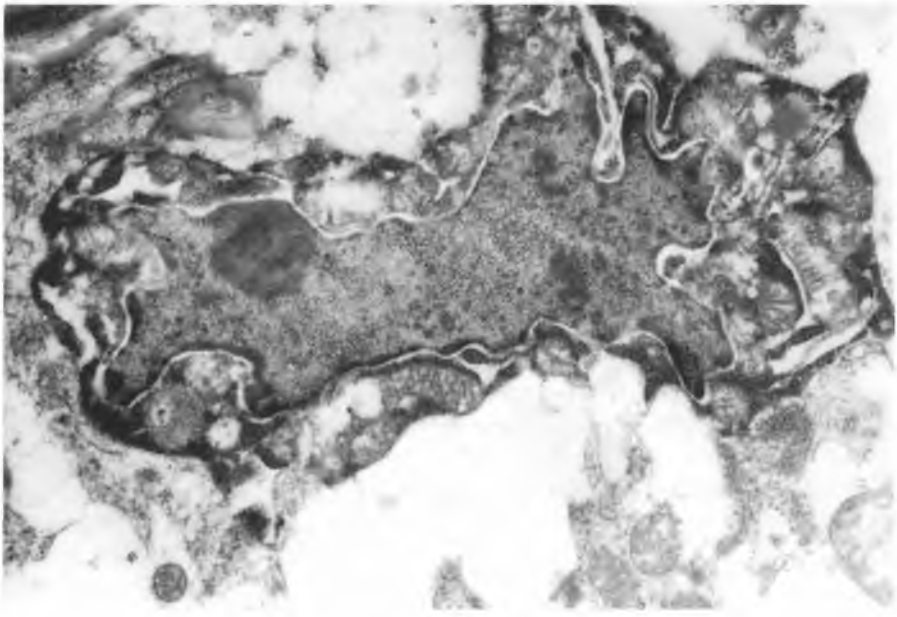

Fig. 3. Experimental group. Damaged neuron in the CA3 region. Swollen processes of glial cells surrounding this neuron. Magn. 4200 x.

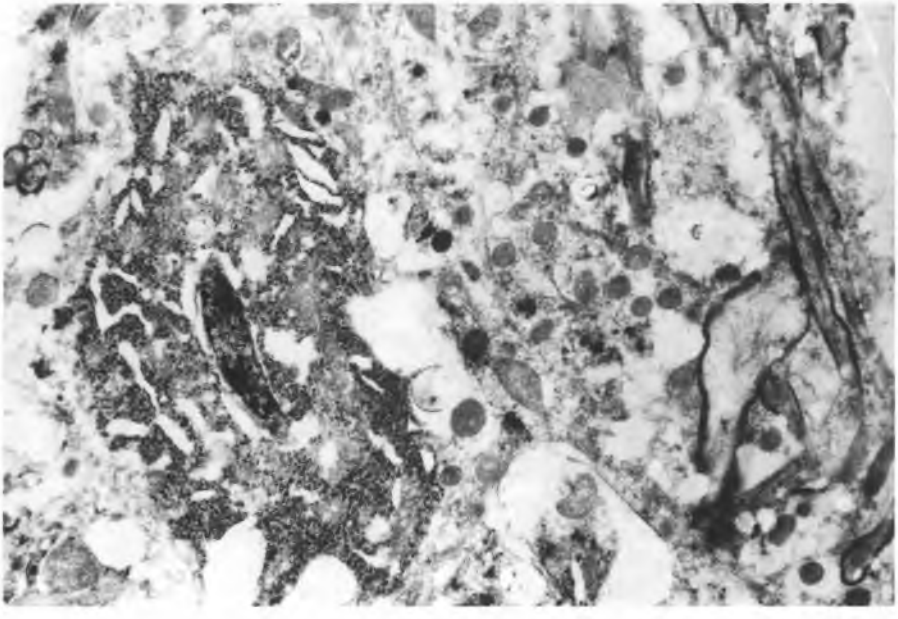

Fig. 4. Experimental group. One of the most severely shrunken neurons in the CA3 region. A swelling of glial processes around it. Magn. 4200 x.

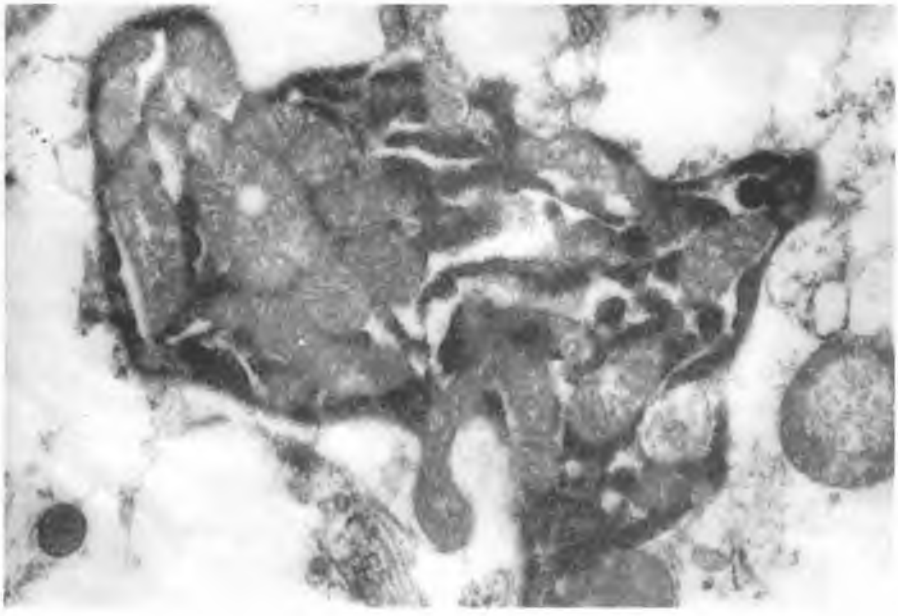

Fig. 5. Experimental group. A large amount of undamaged mitochondria in the shrunken neuron. Magn. 9800 x.

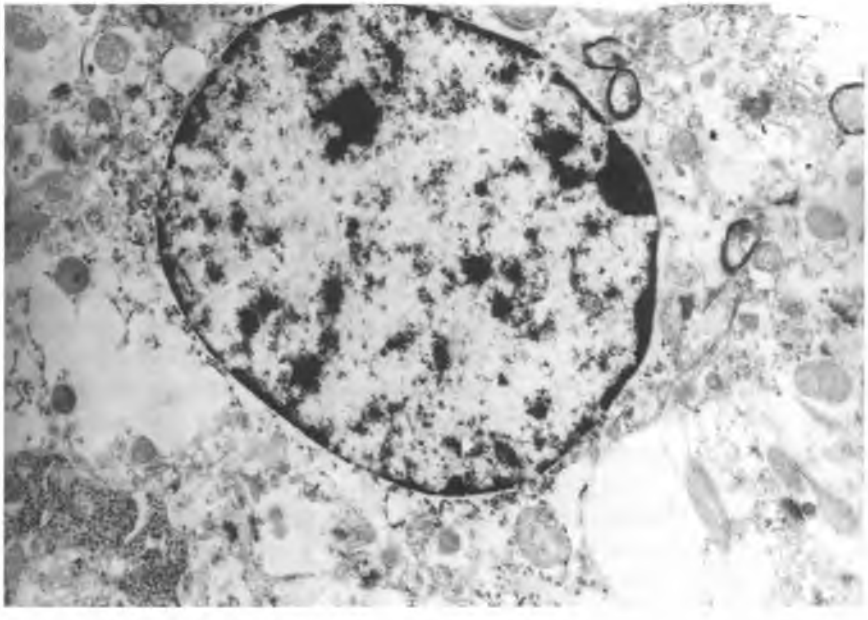

Fig. 6. Experimental group. The cell with chromatin clumped beneath the nuclear envelope, devoid of nucleolus. Magn. 4200 x.

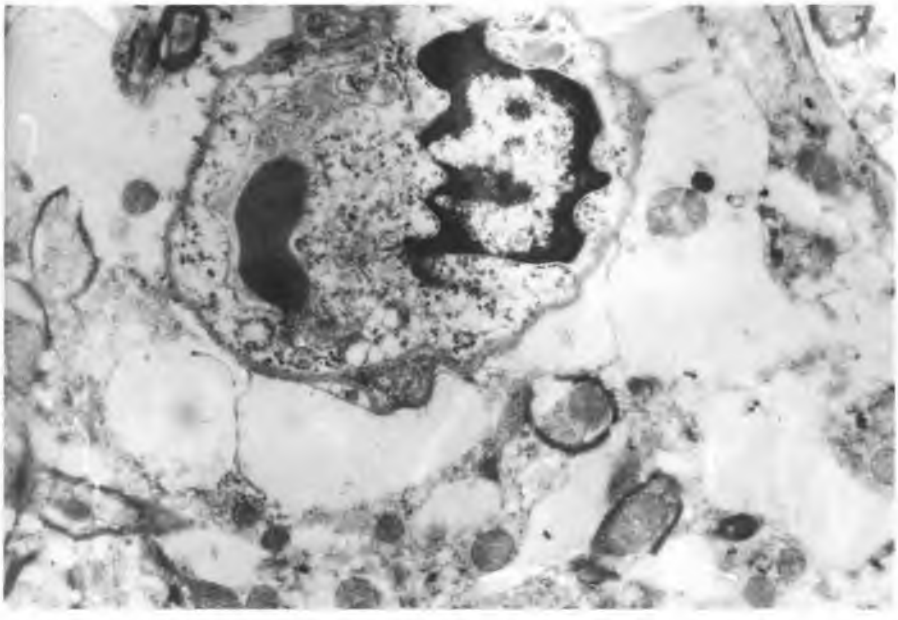

Fig. 7. Experimental group. The swelling of endothelial cell in blood vessel and of glial processes in its vicinity. Magn. 5600 x.

DISCUSSION

There is no doubt that GCs are toxic to CNS. This is particularly so in the hippocampus, a principal neural target tissue for GCs with the highest concentration of GCs receptors in CNS (7).The results of the present study revealed pathological changes in the ultrastructure of hippocampal cells caused by exogenous GC, dexamethasone. The primary and most severaly 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 nucleus and cytoplasm. Finally these degenerating cells died. In any tissue, cell death may follow only two distinct morphological and biochemical patterns: necrosis or apoptosis (l, 5, 6, 10). Necrosis is a pathological event that occurs in a large area of tissue under severe stress (3). Necrosis is a passive process, typified by cell and organelle swelling, less of synthetic functions, disruption of cellular membrane with spillage of the intracellular contents into the extracellular space (9). The result of this process is usually an inflammatory reaction that leads to local cellular infiltration. In contrast, apoptotic cells undergo a physiological suicide program, which requires an active metabolism and protein synthesis (6, 10). Apoptosis typically affects single cells scattered throughout the tissue and is not associated with inflammation (9). Apoptosis is also known to be involved in pathophysiological events (8). Apoptosis consists of three interconnected phenomena: 1) early morphological changes leading to dramatic plasma membrane blebbing, cell volume contraction and nuclear pyknosis, 2) late morphological changes characterised by the break up of the nucleus into the discrete fragments and formation of apoptotic bodies, 3) ingestion (phagocytosis) of apoptotic bodies and cell debries by adjacent cells. Following dexamethasone administration we observed these phenomena in the hippocampus. Shrunken, dark neurons observed in our experiment presented typical early morphological changes characteristic of apoptosis: shrinkage of cell, condensation of nucleus and cytoplasm, preservation of cell membrane. Although apoptosis involves a stereotyped sequence, minor modifications of the process occur in certain cells, perhaps because of their particular structural features. For example, relatively restricted fragmentation of both nucleus and cytoplasm, was a pattern of apoptosis of cortical thymocytes (10). Similar situation may be characteristic also of post–mitotic cells of CNS parenchyma. It can explain why the fragmentation of the nucleus was not observed in our experiment. Under the influence of dexamethasone neurons put in motion the suicide program. This program requires an active protein synthesis. Enlarged nucleoli and a large amount of free ribosomes observed in neurons in our experiment indicate the active protein synthesis in these cells. A lack of inflammatory infiltration at the place of damage also confirms this mechanism of cell death. GCs are classic inductors of apoptosis (8). But the mechanism by which GCs induce apoptosis in neurons is not completely understood. Cells with round or oval nuclei in which chromatin was clumped beneath the nuclear envelope and with clear cytoplasm observed in our experiment may correspond to swollen astroglial cells. The swollen glial processes surrounded shrunken neurons and vessels located in the vicinity of degenerating neurons. Redistribution of the fluid from cell undergoing shrinkage degeneration to the vicinity undergoing swelling is characteristic of apoptosis. The swelling of endothelial cells in blood vessels and of glial processes surrounding these vessels may be additional features for the drug (dexamethasone). They are the signs of cytotoxic oedema.

CONCLUSIONS

All our results led to a conclusion that dexamethasone damages the pyramidal neurons especially in the CA3 and CA4 regions of the hippocampus and degenerating neurons in this area have characteristic features of apoptosis.

REFERENCES

1. Bursch W. et al.: The biochemistry of cell death by apoptosis. Biochem. Cell Biol., 68, 1071, 1990.
2. Distelhorst C.W. et al.: Role of calcium in glucocorticosteroid–induced apoptosis of thymocytes and lymphoma cells. Blood, 91, 3, 731, 1998.
3. Franceschi C. et al.: Cell proliferation, cell death and aging. Aging, 1, 3, 1989.
4. Glauert A.M.: Practical methods in electron microscopy. North – Holland Publish. Comp. Amsterdam, 1, 3, 1974.
5. Kamińska B., Stańczuk M.: Molekularne mechanizmy neurodegeneracji. Post. Biol. Kom., 25, suppl. 11, 15, 1998.
6. Kerr J.F.R. et al.: In C.S. Potten (Ed.), Perspective on mammalian cell death. Oxford Science Publ., Oxford, p3, 1987.
7. McEwen B. et al.: Adrenal steroid receptors and actions in the nervous system. Phys. Rev., 66, 1121, 1986.
8. Motyl T.: Apoptoza – śmierć warunkująca życie. Post. Biol. Kom., 25, 3, 315, 1998.
9. Orrenius S. et al.: Ca–activated mechanism in toxicity and programmed cell death. ISI Atlas of Science, 890 319, 1988.
10. Wylline A.H. et al.: Cell death: the significance of apoptosis. [In:] G.H. Bourne, J.F. Danielli (Ads.). International Review of Cytology. Vol. 68, 251, Academic Press, New York–London–Toronto–Sydney–San Francisco, 251, 1980.

Otrz.: 1999.10.13

STRESZCZENIE

Do niepożądanych działań glikokortykosterydów należy ich toksyczny wpływ na ośrodkowy układ nerwowy. Celem pracy była ocena na poziomie mikroskopu elektronowego zmian morfologicznych w neuronach hipokampa po doświadczalnym podaniu dexametazonu. Wyniki przeprowadzonych badań wskazują, że dexametazon uszkadza komórki nerwowe zwłaszcza w okolicy CA3 i CA4 hipokampa, wywołując w nich zmiany o charakterze apoptozy.