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Neurotoxic effects of glucocorticosteroids – morphological examinations of hippocampal cells after experimental administration of dexamethasone

Neurotoksyczne działanie glikokortykosterydów
– badania morfologiczne komórek hipokampa
po doświadczalnym podaniu dexametazonu

Glucocorticosteroids (GCs) are widely used in anti-inflammatory and immunosuppressive therapy. Many disorders require long-term GCs therapy. Such therapy is associated with numerous serious side effects such as: cushing-like look, obesity, diabetes, symptoms of ulcer disease, osteoporosis, children growth disorders (14). Recent reports underlie the prominent influence of GCs on the central nervous system (CNS). In patients long-termly treated with GCs psychiatric disorders such as depression or euphoria were described (2). Short-term high-dose GC-therapy may cause acute psychosis (13). Increasing evidence indicates that also endogenous GCs, secreted from adrenal cortex during stress are toxic to neurons and have an important role in neurodegenerative processes (14). Such toxicity appears to play a role in neuron loss during aging and compromises the capacity of neurons to survive a variety of metabolic insults like hypoglicemia, anoxia, ischemia (14, 10, 3).

The purpose of our experiment was to find out whether exogenous GCs cause the morphological changes of neurons in CNS and whether these changes depend on the term of GC therapy.

Because the highest concentration of GCs-receptors in CNS occurs in the limbic system, especially in the hippocampus (5), we supposed that the biggest morphological changes would occur in this brain structure. So, we performed the histological examination of mouse hippocampal cells after experimental administration of synthetic GC-dexamethasone (Dexaven).

MATERIAL AND METHODS

The experiment was carried out on white mouse males. The animals were divided into one control group and three experimental groups (including 10 animals each) according to 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 I – the animals receiving Dexaven for 7 days. Experimental group II – the animals receiving Dexaven for 14 days. Experimental group III – 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 groups 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 examination on the level of the light microscope the obtained tissue material was fixed in Baker's fluid (1% $CaCl_2$ in 10% solution of neutral formalin). 6 μm thick paraffin sections cut in the frontal plane were stained with hematoxylin and eosin, with cresyl violet and with Feulgen's method and were assessed by the light microscopy. Furthermore, in the CA3 region the amount of damaged cells were counted using a computer analyser of histological pictures (Lobophot 2, Nicon). Cells with rounded nuclei and visible nucleoli were considered undamaged, while cells with dark shrunked nucleus in which the nucleolus was not discernable were considered damaged. Cell counts were made within two 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). Histological data were subjected to statistical analysis using Chi-square test, in order to determine statistical significance of differences between groups.

RESULTS

CONTROL GROUP

The E + H and cresyl violet stainings revealed the regular strcture of the hippocampus (Fig. 1). All strata of *cornu Amonis* and cells of gyrus dentatus did not show any morphological disturbances. The most characteristic stratum pyramidale of *cornu Amonis* was composed of several layers of pyramidal neurons. 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 5 layers (Fig. 2). *Gyrus dentatus* was composed of the granule cell layer (several layers of medium sized neurons) and of molecular cell layer with small neurons.

EXPERIMENTAL GROUPS

In the case of animals receiving Dexaven for 7 and 24 days (Experimental groups I and II) the morphology of hippocampus on the level of the light microscope was similar to the control group.

Significant morphological changes of hippocampal cells in comparison with the control group were observed after 28-day administration of Dexaven (Experimental group III). After 28-day administration of Dexaven distinct changes appear within the stratum pyramidale *cornu Amonis* (Fig.

3). The amount of pyramidal neurons, especially in the CA3 region decreased. In this region cells were arranged in 2 to 3 layers in comparison with 3 to 5 in the control group. Numerous pyramidal neurons in the CA3 region revealed far–reaching morphological changes: a shrinkage of perykarions, different intensity of staining and changes in morphology of nucleus (Fig. 4). Nuclei of damaged cells were dark, irregular in shape and shrunken in comparison with clear round nuclei of the control group. Nucleoli in these cells were hardly visible or invisible. In some cells apparently dead, disintegration of nucleus was observed. Perykarions of damaged cells were smaller in comparison with control group and shrunken. The cytoplasm in these neurons was more intensely stained. Other neurons with smaller changes in nucleus possessed nucleoli and cytoplasm less intensly stained than in the control group (Fig. 4). Similar destructive alterations were observed in the CA4 region and in three animals in some cells of *gyrus dentatus*. The intensity of changes varied in particular animals but the general pattern of damage was similar in all cases. In five animals changes were asymmetrical. The amount of damaged neurons in the CA3 and CA4 regions was significantly greater in one hemisphere of the brain.

Statistical analysis of the results revealed statistical significance in the CA4 region when the percentage of dexametasone–induced damaged cells was compared to the control group. In all cases statistical significant differences were found {K – D1 $P < 0.01$; K – D2 $P < 0.0001$; K – D3 $P < 0.001$; K – D4 $P < 0.001$; K – D5 $P < 0.0001$; K – D6 $P < 0.0001$). Not all animals were affected to the same extent by dexamethasone.

DISCUSSION

Changes of hippocampal cells observed in our experiment after 28–day administration of dexamethasone show the morphological damage of the hippocampus. The localization of damage indicates that in hippocampus pyramidal neurons are the most sensitive to dexamethasone especially in the CA3 and CA4 regions. The lack of morphological changes on the level of a light–microscope after 7– and 14–day administration of dexamethasone in comparison with damage observed after 28 days indicates the important role of the term of dexamethasone treatment. The risk of neuronal damage increases with the prolongation of GC therapy.

The reasons of asymmetrical damage observed in some animals are not clear. Asymmetrical pattern of damage in the hippocampus was described after experimental ischemia but its reason was not completely explained (11, 7).

Relatively small changes in *gyrus dentatus* observed only in three animals indicate a bigger resistance of its cells to the toxic action of GCs.

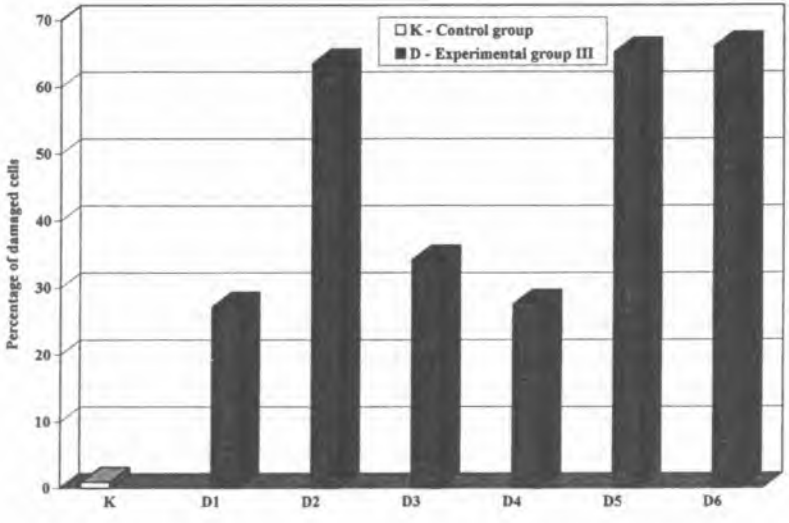
The pattern of morphological damage observed in our experiment is analogous to that described in the hippocampus due to the high level of endogenous GCs secreted during prolonged stress (14). Probably neurotoxicity of endogenous GCs plays an important role in neuron loss during aging (10).

Dark, shrunken pyramidal neurons in our experiment do not possess the features of necrosis. We did not observe cellular swelling or inflamatory infiltration in these cells characteristic of necrosis (8). It can be explained through antiinflammatory action of GCs and their capacity for the induction of apoptosis. Decreased intensity in staining observed in less changed pyramidal neurons can be caused by the swelling but the real cause of this phenomenon is difficult to explain on the level of the light microscope.

GCs are classic inductors of apoptosis (2, 8, 9). Damaged pyramidal neurons in our experiment show several morphological criteria of apoptosis. Shrinkage of cell, dark dense nucleus and nucleus

breaking up although the neurons are still held by a membrane indicate that cell death is carried out in the way of apoptosis (1). The mechanism by which GCs induce apoptosis in neurons is not completely understood. 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 (12). In the nucleus the GCs receptor functions as a transcription factor, enhancing or repressing the expression of a selected repertoire of genes. GCs 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 program.

CONCLUSIONS



Percentage of damaged cells in the CA3 regions
of the hippocampus following 28–day administration of dexamethasone

1. Experimental administration of dexamethasone causes the morphological damage of neurons with features characteristic of apoptosis especially in pyramidal neurons of the CA3 and CA4 hippocampal regions.

2. Neurons of gyrus dentatus are more resistant to GCs than CA3 and CA4 pyramidal neurons of *cornu Amonis*.

3. The term of GCs administration plays an important role in development of morphological changes in neurons. The risk of neuronal damage increases with the prolongation of GC–therapy.

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STRESZCZENIE

Wzrastająca liczba doniesień wskazuje na neurotoksyczne działanie glikokortykosterydów. U pacjentów przyjmujących te leki opisywano zaburzenia psychiczne pod postacią ostrych psychoz, depresji i euforii. W ośrodkowym układzie nerwowym największa liczba receptorów dla glikokortykosterydów znajduje się w układzie limbicznym, zwłaszcza w okolicy hipokampa.

Celem pracy była analiza morfologiczna komórek hipokampa po doświadczalnym podaniu dexametazonu. Otrzymane przez nas wyniki wskazują na to, że w obrębie struktur hipokampa komórkami najbardziej wrażliwymi na działanie dexametazonu są neurony piramidalne w obszarze CA3 i CA4. 28–dniowe podawanie leku wywołuje w nich zmiany degeneracyjne typowe dla apoptozy. Komórki

zakrętu zębatego są bardziej odporne na uszkodzenie. Długość okresu kortykoterapii ma istotne znaczenie dla rozwoju zmian morfologicznych. Ryzyko uszkodzenia komórek nerwowych wzrasta wraz z przedłużaniem czasu podawania glikokortykosterydów.

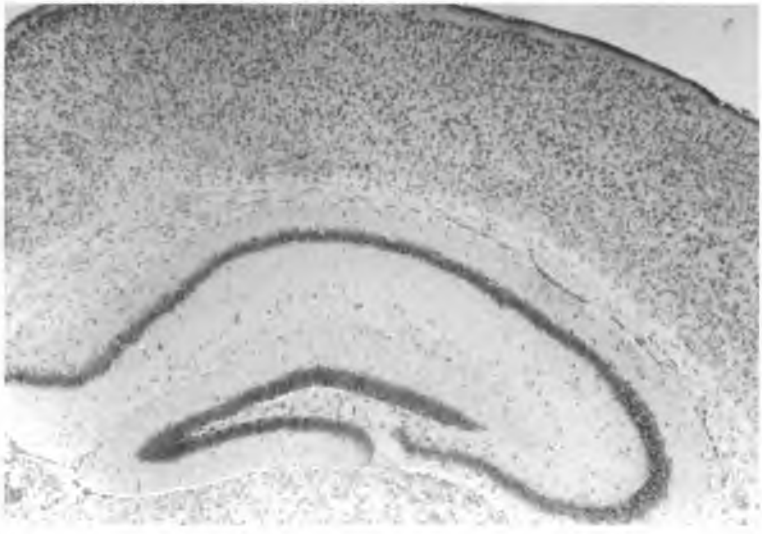

Fig. 1. Control group. A low–power photomicrograph of a frontal section through the hippocampus

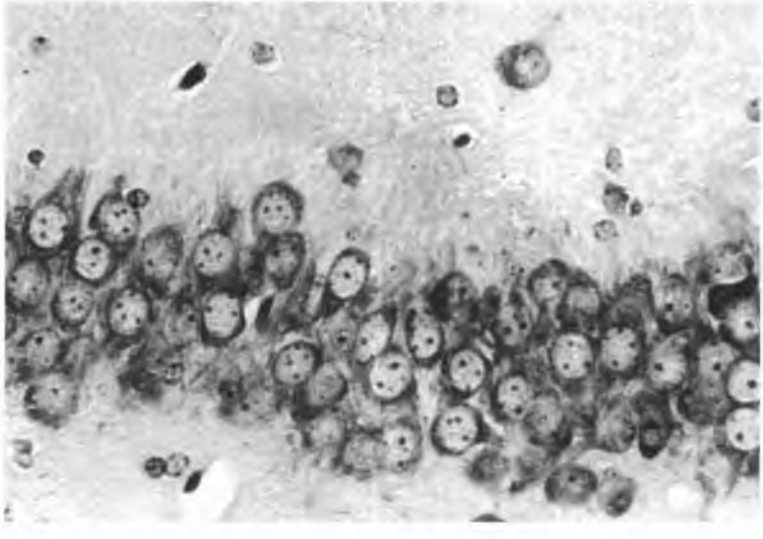

Fig. 2. Control group. The CA3 region of the hippocampus. Pyramidal neurons are arranged in 3 to 5 layers. They possess clear, round nuclei with distinct nucleoli. Magn. 640 x

Fig. 3. Experimental group III (after 28–day administration of Dexaven). A low–power photomicrograph of a frontal section through the hippocampus. In the CA3 and CA4 regions damaged dark piramidal neurons are visible. Small changes in *gyrus dentatus*

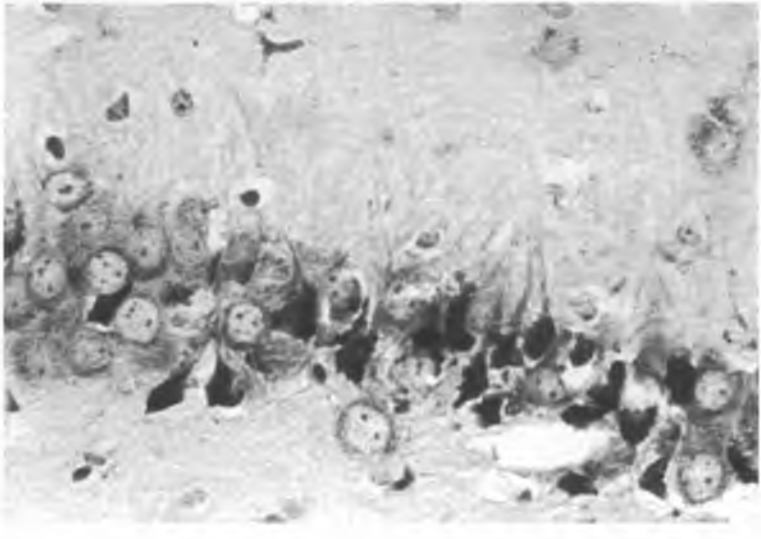

Fig. 4. Experimental group III. The CA3 region of the hippocampus. A decreased amount of pyramidal neurons. Numerous damaged neurons are visible. They are shrunken and dark with dark, irregular in shape nucleus and invisible nucleoli. In neurons with relatively small changes nucleoli and cytoplasm stain less intensely. Magn. 640 x