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Immunocytochemical examinations of hippocampal nerve cells after experimental administration of dexamethasone

Long-term glucocorticosteroid therapy is associated with numerous serious side-effects such as: Cushing-like look, obesity, diabetes, symptoms of ulcer disease, osteoporosis, children growth disorders (12). Recent reports underlie the prominent influence of glucocorticosteriods (GCs) on the central nervous system. Prolonged exposure to elevated level of glucocorticosterois can cause degenerative changes in the brain (6, 7, 8, 9). These 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 (1, 10, 11, 14, 15, 16). Neuronal damage induced by these hormones occurs mainly in the hippocampus – the structure of the brain containing the highest concentration of glucocorticosteroid receptors (13). The purpose of our experiment was an immunocytochemical examination of hippocampal neurons after experimental administration of synthetic glucocorticosteroid – dexamethasone.

MATERIAL AND METHODS

The experiments were carried out on adult Albino Swiss mouse males (19-21g). 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 two groups (including 20 animals each). The control group – the animals receiving distilled water (i.p. 0.2 ml/24h) for 28 days. The experimental group – the animals receiving dexametha-

sone. Dexamethasone (Dexaven-Jelfa S.A., Poland) was administered intraperitoneally in the single dose 8 mg/kg/24h for 28 days. 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 10% formaldehyde (pH 7.4) 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 immunocytochemical analysis paraffin sections were processed on object glasses covered with Vectabond reagent (Vector Laboratories). Sections were multiple rinsed in phosphate buffered saline (PBS), then pretreated with 1%H_2O_2 to block any possible endogenous peroxidase. After incubation in 5% normal serum sections were put into 0.2% Triton X-100. Sections were rinsed in PBS again and incubated in the solution 1:500 of monoclonal antibody anti-MAP2, in temperature 4^0C for 72 h. Then they were incubated in the solution of biotinylated secondary antibody for 24h in temperature 4^0C and in the solution of avidin-biotin peroxidase complexes for 1h at room temperature. The bound peroxidase was revealed by incubating the sections in a medium containing 0.05% 3,3'-diaminobenzidine (DAB, Sigma) and 0.01%H_2O_2 for 10 min at room temperature. Rinsing the sections in H_2O stopped the reaction. Sections were finally counterstained with cresyl violet, dehydrated in ethyl alcohol, cleared in xylene and cover-slipped with Canadian balm.

RESULTS AND DISCUSSION

IMMUNOCYTOCHEMICAL ANALYSIS OF THE HIPPOCAMPUS

Significant reduction in MAP2 immunoreactivity were revealed in the experimental group in comparison with the control group (Fig. 1, Fig. 2). It was particularly obvious in neurons of the pyramidal cell layer in the CA3 region. Perykarions of nerve cells and their dendrites in this region showed considerable lowering of MAP2 immunoreactivity after administration of dexamethasone. A change of the type of reaction from diffuse to granular was observed in nerve cells showing small degree of morphological damage.

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. Obtained results of immunocytochemical analysis in the experimental group indicate that dexamethasone causes a damage of neuronal cytoskeleton especially in the CA3 region. MAP2 (microtubule associated proteins) constitute a group of proteins associated with microtubules. In physiological conditions they regulate the dynamics of their transformation and form connections that join microtubules with other elements of cytoskeleton and with some cell organelles. Thus they influence the cytoskeleton stability (3).

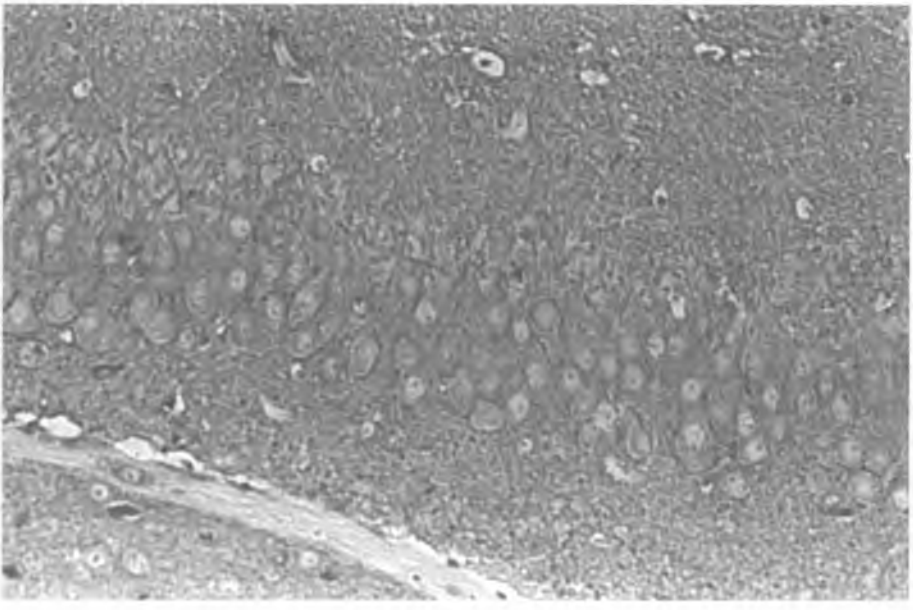

Fig. 1. Control group. MAP2 immunoreactivity in hippocampal neurons of the CA3 region. Magn. 400x

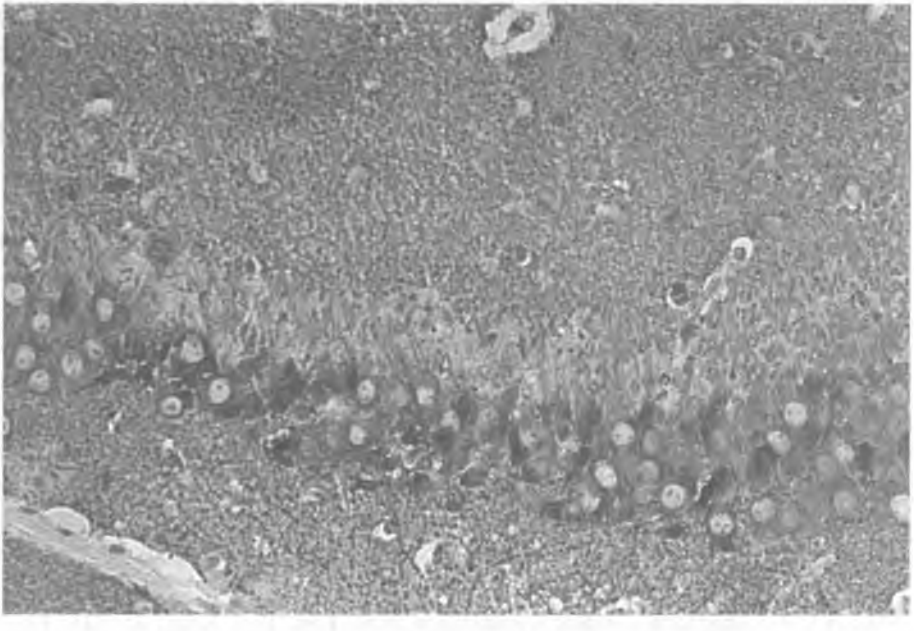

Fig. 2. Experimental group. MAP2 immunoreactivity in hippocampal neurons of the CA3 region after 28-day administration of dexamethasone. Significant decrease of MAP2 immunoreactivity in comparison with the control group. Magn. 400x

Similar changes in MAP2 immunoreactivity were described after administration of glutamate to nerve cell culture from the spinal cord or the brain cortex. So, the changes after toxic doses of dexamethasone are very similar to those observed in the case of stimulation of receptors for glutamate.

The mechanism of neurotoxic effects of dexamethasone 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 (5). 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 in neurons (2). The changes in MAP2 immunoreactivity after administration of dexamethasone observed in our experiment confirm neurodegenerative effect of long-term, high-dose GCs therapy.

CONCLUSIONS

Immunocytochemical examinations of hippocampal neurons after experimental administration of dexamethasone revealed a decrease of MAP2 immunoreactivity in the CA3 hippocampal neurons under the influence of this glucocorticosteroid. The obtained results indicate a damage of cytoskeleton in nerve cells after toxic doses of dexamethasone.

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SUMMARY

The purpose of this research was an assessment of MAP2 immunoreactivity in hippocampal neurons after administration of toxic doses of dexamethasone. Experiments were led on Albino-Swiss mouse males. The obtained results indicate that dexamethasone causes significant decrease of MAP2 immunoreactivity in hippocampal nerve cells of the CA3 region. Our results show damage of neuronal cytoskeleton in this area of the brain.

Badania immunocytochemiczne komórek nerwowych hipokampa po doświadczalnym podaniu deksametazonu

Przeprowadzone badania miały na celu ocenę immunoreaktywności MAP2 neuronów hipokampa po podaniu toksycznych dawek deksametazonu. Doświadczenia przeprowadzono na samcach myszy Albino-Swiss. Otrzymane wyniki wskazują na to, że deksametazon powoduje wyraźne obniżenie immunoreaktywności MAP2 komórek nerwowych hipokampa w polu CA3. Przemawia to za uszkodzeniem cytoszkieletu neuronów w tym obszarze mózgu.