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Histological changes in neurons of the globus pallidus after experimental administration of dexamethasone

In recent years it has been reported that prolonged exposure to elevated level of glucocorticosteroids (GCs) causes degenerative changes in the central nervous system (CNS). These changes occur both as the result of hypersecretion of endogenous GCs or exogenous GCs administered in high doses for therapeutic purposes (7,8,9,11). It is thought that neurotoxic action of GCs is responsible for senile dementia, 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 (6,12).

Neuronal damage induced by GCs occurs mainly in hippocampal formation, a principal neural target site for GCs with the highest concentration of GCs-receptors in the CNS. Because high concentration of GCs-receptors also occurs in the paleostriatum we decided to investigate whether synthetic GC-dexamethasone causes degenerative changes in neurons of the globus pallidus, which is paleostriatal structure.

MATERIAL AND METHODS

The experiments were carried out on adult Albino Swiss mice males weighting 19–22g at the beginning of the experiment. 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. Mice were housed 20 per cage under standard laboratory conditions, with free access to granular standard diet and tap water. Their weight was monitored daily. The animals were divided into two groups (10 animals each). Control group –the animals receiving distilled water (ip. 0.2ml/24h) for 28 days and experimental group – the animals receiving dexamethasone. Dexamethasone (Dexaven-Jelfa S.A., Poland) was administered ip. in a single dose 8 mg/kg/24h for 28 days. 24 hrs after the last distilled water or last dexamethasone injection all animals were perfused with 0.9% NaCl with heparin, followed by 10% formaldehyde (pH 7.4). Following decapitation, brains were removed from the skulls 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 slices were serially cut in the frontal plane. For histological analysis selected paraffin-embedded tissue slices were stained with cresyl violet and assessed by the light microscope. We examined morphology of neurons in the globus pallidus of both hemispheres.

RESULTS

BODY WEIGHT CHANGES AND MORTALITY

Mice from the experimental group showed statistically significant decrease of the body weight in comparison with the control group. The mortality in experimental group was about 25%.

HISTOLOGICAL EXAMINATION

Light microscopy examination of cresyl violet stained slides from the control group revealed the regular structure of the globus pallidus (Fig. 1, 1A). Nerve cells were large, triangular or polygonal in shape. Their nuclei were clear, round or oval in shape with distinct nucleoli. The cytoplasm contained small, irregular granules of tigroid (rough endoplasmic reticulum).

Significant morphological changes were observed in the experimental group (Fig. 2, 2A). After 28-day administration of dexamethasone the amount of neurons decreased. In this region nerve cells were sparse. Numerous neurons showed far-reaching morphological changes including perykarion shrinkage and an increased intensity of cytoplasm staining. Nuclei of damaged nerve cells were dark, irregular in shape and shrunken in comparison with clear round nuclei of the control group. We also observed an increased amount of glial cells which were dispersed between neurons. Neuronal damage was often asymmetrical. In these cases morphological changes concerned cells from one hemisphere of the brain only.

The intensification of changes varied in particular animals but the general pattern of damage was similar in all cases.

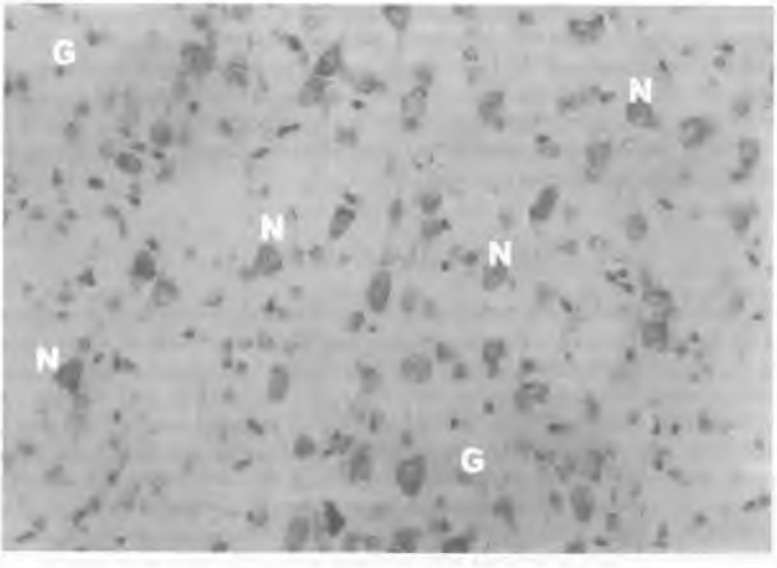

Fig. 1. Control group. Frontal section of the globus pallidus. Large neurons (N) with clear, rounded nuclei and glial cells (G) are visible. Staining with cresyl violet. Magn. 400x

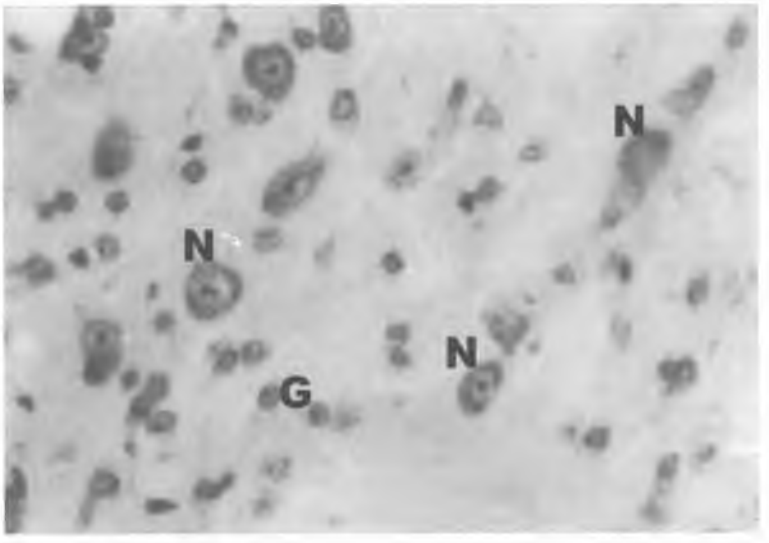

Fig. 1A. Control group. Large neurons (N) with clear, rounded nuclei and glial cells (G) are visible. Staining with cresyl violet. Magn. 800x

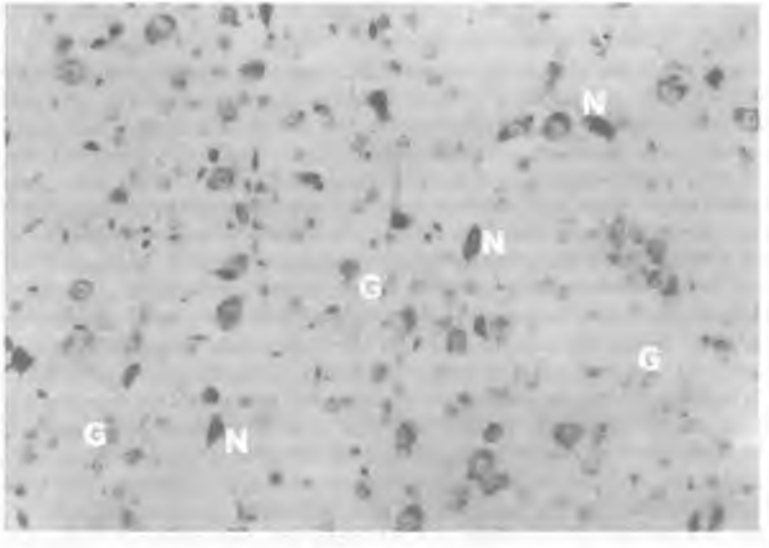

Fig. 2. Experimental group (dexamethasone – 8mg/kg for 24h). Shrunken, dark neurons (N) with pycnotic nuclei are visible. Increased amount of glial cells (G). Staining with cresyl violet. Magn. 400x

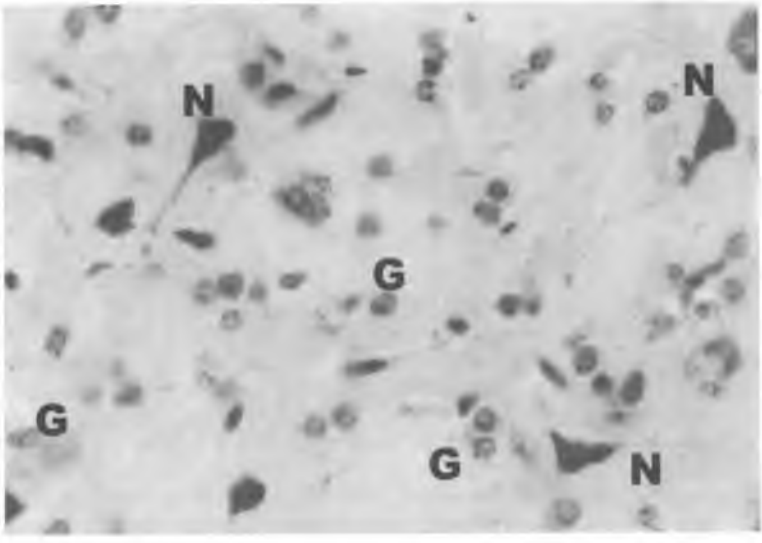

Fig. 2A. Experimental group (dexamethasone – 8mg/kg for 24h). Shrunken, dark neurons (N) with pycnotic nuclei are visible. Increased amount of glial cells (G). Staining with cresyl violet. Magn. 800x

DISCUSSION

There is no doubt that glucocorticosteroids (GCs) are toxic to the central nervous system (4, 6, 9). The results of the present study revealed pathological changes in nerve cells of the globus pallidus caused by exogenous GC-dexamethasone. The globus pallidus is a philogenetically old structure among nuclei basales. It represents paleostriatum in oposition to nucleus caudatus and putamen which represent neostriatum. It is characterized by abundant nerve fibers myelinated and nonmyelinated with one nerve cell type. Morphological changes observed in neurons of the globus pallidus after long-term administration of dexamethasone on the level of the light microscope (shrinkage of perykarions, more intensely cytoplasm staining, pyknotic changes of the nucleus, lack of morphological features characteristic for inflammation) are typical of apoptosis (3). In any tissue, cell death may follow two distinct morphological and biochemical patterns: necrosis or apoptosis. GCs are classic inductors of apoptosis (3). They induce apoptosis in thymocytes and lymphoma cells (1). The mechanism by which GCs induce apoptosis in neurons is not completely explained. It is supposed that the mechanism of neurotoxic effects of GCs is connected with the impairment of glucose uptake in neurons and thus induced the state of increased metabolic vulnerability. This effect is similar to classic GC 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. GCs increase the concentration of glutamate in the extracellular space (5, 10). Activation of NMDA receptors by high concentration of glutamate may be decisive for the induction of degenerative changes typical of apoptosis in nerve cells under the influence of GCs (2).

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SUMMARY

The aim of our research was morphological assessment of neurons in the globus pallidus after 28-day administration of dexamethasone. The experiments were carried out on adult Albino Swiss mice males. It was found that prolonged administration of this glucocorticosteroid causes degenerative changes in neurons of the globus pallidus (shrinkage of nerve cells, increased stainability of nucleus and cytoplasm). We also observed increased numbers of glial cells in this area of the brain.

Zmiany morfologiczne w komórkach nerwowych gałki bladej po doświadczalnym podaniu dexametazonu

Celem pracy była ocena morfologiczna neuronów gałki bladej po 28-dniowym podawaniu dexametazonu. Badania wykonano na samcach myszy Albino-Swiss. Stwierdzono, że przewlekłe podawanie tego glikokortykosteroidu powoduje zmiany degeneracyjne komórek nerwowych gałki bladej, polegające na ich obkurczeniu oraz zwiększonej barwliwości jądra i cytoplazmy. Obserwowaliśmy też zwiększenie liczby komórek glejowych w tym obszarze mózgu.