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Kierownik: prof. dr hab. Krystyna Czerny

206. ERRATA

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Influence of hypoxia–induced convulsions on morphological structure of hippocampal cells

Wpływ drgawek wywołanych niedotlenieniem
na morfologię komórek hipokampa

Clinical observations indicate that seizures induced by hypoxia are a common kind of convulsive activity in infants and elderly patients (6, 11). These seizures are an important risk factor of further epileptogenesis (5, 7, 11) and are associated with memory disturbances.

The hippocampus plays an important role in many complex functions such as memory and emotional behavior (2). Convulsions independently of their reason cause hypoxia, which may lead to permanent damage of the brain (11). Observations on human material have revealed that the hippocampus is the area which shows the most constant and pronounced abnormalities in cases with severe epilepsia (10).

The purpose of our experiment was to examine the influence of hypoxic seizures induced by exposure of animals to spontaneous breathing in the gas mixture containing 5% O_2 and 95% N_2 on morphological structure of mouse hippocampal cells.

MATERIAL AND METHODS

The experiment was carried out on adult Albino Swiss mice. The animals were divided into three groups: the control one and two experimental groups (including 5 animals each). Animals from experimental groups were subjected to experimental hypoxia. Experimental hypoxia was obtained by exposure of mice to spontaneous breathing in the gas mixture containing 5% O_2 and 95% N_2. After 24 hours from single 10 second episode of seizures in the case of experimental group I and 72 hours in experimental group II, animals from both experimental groups were decapitated and their brains were collected. For examination on the level of the light microscope the obtained tissue material was fixed in the Baker's fluid (1% $CaCl_2$ in 10% solution of neutral formalin). The procedure in the case of the

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control group was the same. 6 μm thick paraffin sections cut in the frontal plane were stained with hematoxylin and eosin and with cresyl violet and were assessed by the light microscopy.

RESULTS

CONTROL GROUP

The H + E and cresyl violet stainings revealed the regular structure 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 CA1 region they were arranged in 2 to 4 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 from experimental groups I and II the morphology of the hippocampus on the level of the light–microscope was similar to the control group. We did not observe any morphogical changes in the pyramidal neurons after 24 and 72 hours from hypoxia–induced seizures. In animals from experimental group I the CA1 region consisted of several (2 to 4) layers of pyramidal nerve cell bodies. They possessed round or oval in shape, clear nuclei with distinct nucleoli (Fig. 3). Animals from experimental group II also did not show morphological changes in the CA1 pyramidal neurons (Fig. 4).

DISCUSSION

It is a well–known fact that neurons in the various regions of the hippocampal formation vary with respect to their reaction toward pathological influences, i.e., they display a form of selective vulnerability. Sommer was the first to report that CA1 region of the hippocampus is the most sensitive to damage during anoxia (12). Clinical observations indicate that seizures induced by hypoxia are important risk factor of further epileptogenesis and are associated with memory disturbances (5, 7, 11). These data indicate that hypoxia–induced seizures can cause permanent damage of neurons in the central nervous system. Previous studies on animals showed that hypoxic–ischemic destruction of hippocampal neurons were not morphologically apparent 4 hours after the insults but matured 24–72 hours later (13). This delayed necrosis may facilitate further epileptic discharges from the hippocampus (1).

In our experiment histological examinations of hippocampal cells did not show morphological features characteristic of neural damage in both experimental groups (24 hrs and 72 hrs after hypoxia–induced seizures). On the level of the light microscope we did not observe the swelling or shrinkage of pyramidal neurons in the most vulnerable to hypoxia region of the hippocampus such as the CA1 region. These results lead to conclusion that in our model of hypoxia–induced seizures hippocampal cells are not permanently damaged. Similar results were observed in the cat model used by Brown et al., no hippocampal damage was detected by light or electron microscopy in spite of the fact that seizures were induced for several hours (3). In other previous studies examination of hippocampal cells on the level of the light microscope after experimental anoxia showed condensation of the nucleus and cytoplasm in the most neurons of the CA1 and CA2 regions (4, 8). These changes were typical of apoptotic mechanism of cell death. Cells undergoing apoptosis are shrunken whereas necrosis leads to cell swelling (9). In these studies anoxia was obtained by a twenty–minute complete ischemia. In the case of anoxia–induced damage in CNS the time of anoxia seems to play the most important role in development of nerve cell destruction. In our experiment seizures induced by hypoxia lasted about 10 seconds. Hypoxia of CNS obtained in our model seems too slight for the induction of permanent morphological damage in the hippocampus.

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STRESZCZENIE

Obserwacje kliniczne wskazują, że niedotlenienie jest częstą przyczyną napadów drgawek u noworodków oraz że w przyszłości drgawki te są ważnym czynnikiem ryzyka rozwoju padaczki.

Celem pracy była analiza histologiczna komórek hipokampa po drgawkach wywołanych niedotlenieniem. Wyniki przeprowadzonych badań wskazują, że w modelu zastosowanym w naszym doświadczeniu nie dochodzi do trwałego uszkodzenia struktur hipokampa. Komórki hipokampa badane po 24 i 48 godzinach od drgawek nie wykazują jakichkolwiek zmian morfologicznych na poziomie mikroskopu świetlnego.

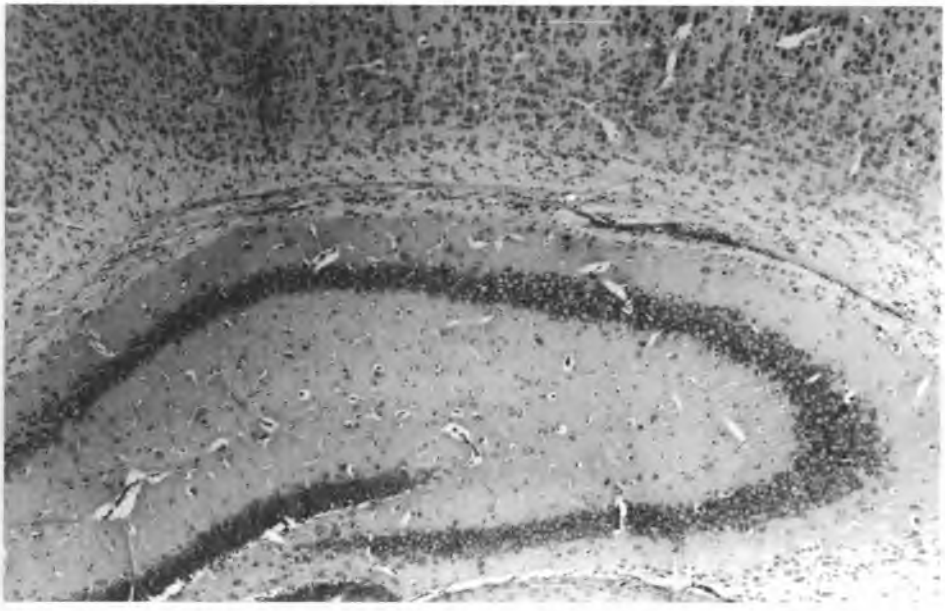

Fig. 1. Control group. A low–power photomicrograph of a frontal section through the hippocampus. Stained with cresyl violet

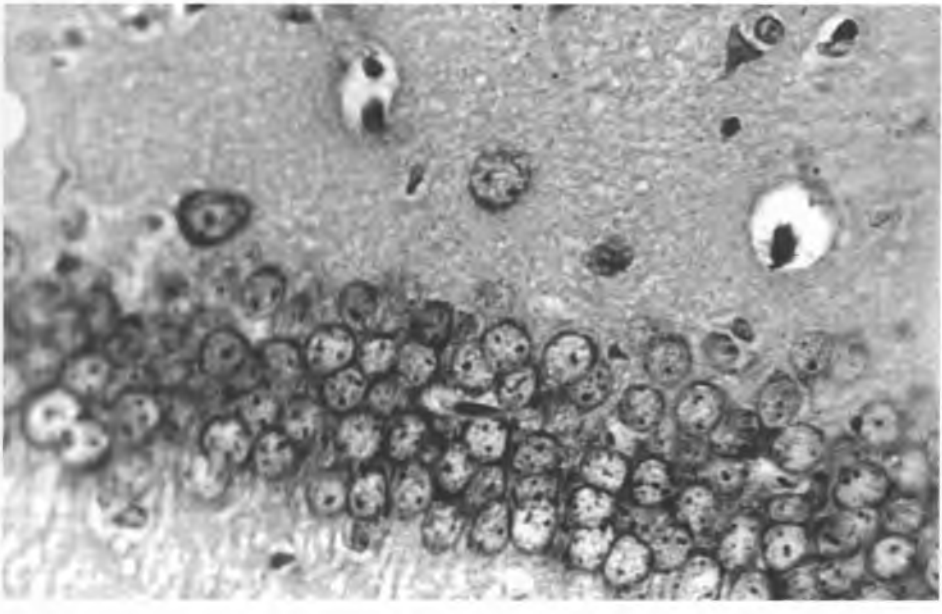

Fig. 2. Control group. The CA1 region of the hippocampus. Piramidal neurons are arranged in 2 to 4 layers. They possess clear, round nuclei with distinct nucleoli. Stained with cresyl violet. Magn. 720 x.

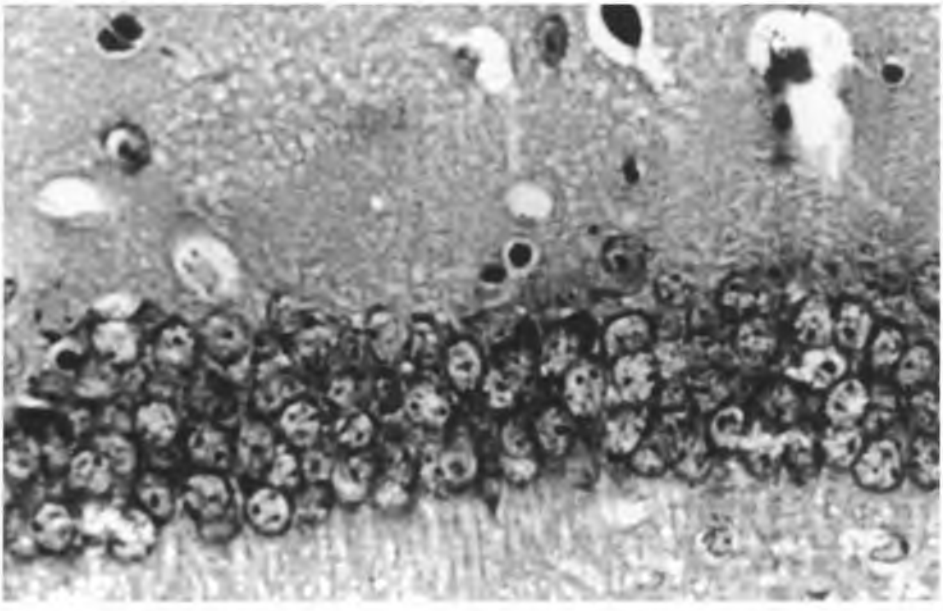

Fig. 3. Experimental group I (24 hrs after hypoxia–induced seizures). The CA1 region of the hippocampus. Neurons do not show morphological changes in comparison with the control group. Stained with cresyl violet. Magn. 720 x.

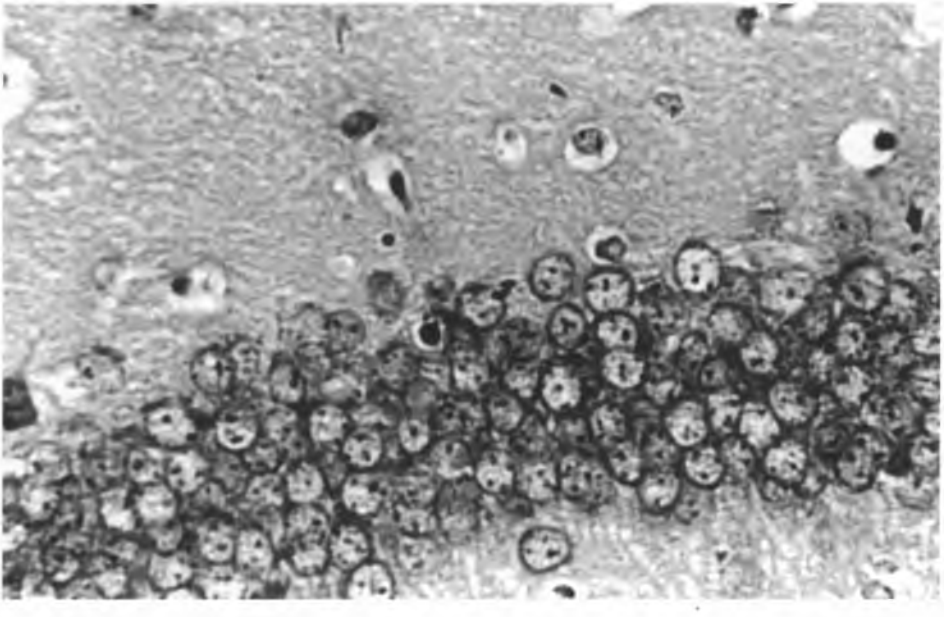

Fig. 4. Experimental group II (72 hrs after hypoxia–induced seizures). The CA1 region of the hippocampus. Neurons do not show morphological changes in comparison with the control group. Stained with cresyl violet. Magn. 720 x.