DIFFERENTIATION OF NEUROSECRETORY CELLS OF THE HYPOTHALAMUS AFTER WHOLE-BODY γ-RAY IRRADIATION

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Data on differentiation of the neurosecretory cells of the supraoptic and paraventricular nuclei of the anterior hypothalamus during the period of postnatal development in normal and irradiated animals are described. Ionizing radiation reduces the volume of the nuclei of the secretory neurocytes and leads to forced elimination of neurosecretion from the perikaryon of the neurocytes along the axons into the posterior lobe of the neurohypophysis.

The neuroendocrine complex of the hypothalamus, pituitary, and peripheral endocrine glands constitutes the principal channel effecting protective and adaptive responses of the animal to extremal factors, notably to ionizing radiation. Voitkevich et al. [1] have described neuro-endocrine disintegration induced by irradiation.

It has therefore become necessary to study differentiation of the secretory neurocytes of the hypothalamus in the period of early postnatal development of animals and the effect of ionizing radiation on this process.

EXPERIMENTAL METHOD

Experiments were carried out on 65 young Wistar rats. The newborn animals were irradiated on a "Luch" apparatus in a dose of 200 R at a dose rate of 32.8 R/min. Acute radiation sickness, terminating in total death on the 16th-18th day after irradiation, was diagnosed in the experimental animals. The test objects consisted of neurocytes of the supraoptic (SON) and paraventricular (PVN) nuclei of the anterior hypothalamus, and the posterior principal lobe of the neurohypophysis. The animals were sacrificed 5, 10, and 15 days after irradiation. The region of the hypothalamus and pituitary was fixed in Bouin's fluid and in 10% formalin solution. Serial paraffin sections through the hypothalamus and pituitary were stained with paraldehyde-fuchsin and pseudoisocyanin [8]. The diameter of the secretory neurons was determined by means of an ocular micrometer with moving scale, and the volume was calculated from this figure. The numerical results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The volume of cell nuclei is known to be a true criterion of their functional state, the character of their differentiation, and their possible degeneration. Results of karyometry and cytometry were used to study the kinetics of ontogenetic differentiation of the SON and PVN neurocytes.

In intact young rats there is a progressive increase in volume of the nuclei of the SON neurons. In rats aged 30 days the nucleoplasm has reached its maximum size characteristic of the sexually mature

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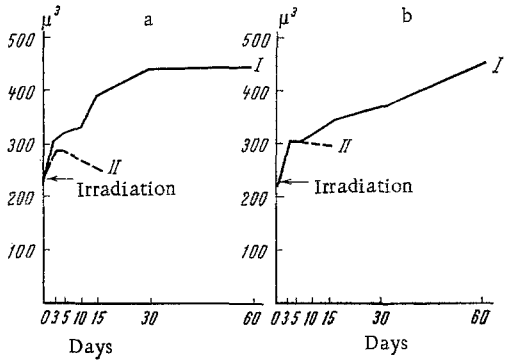

Fig. 1

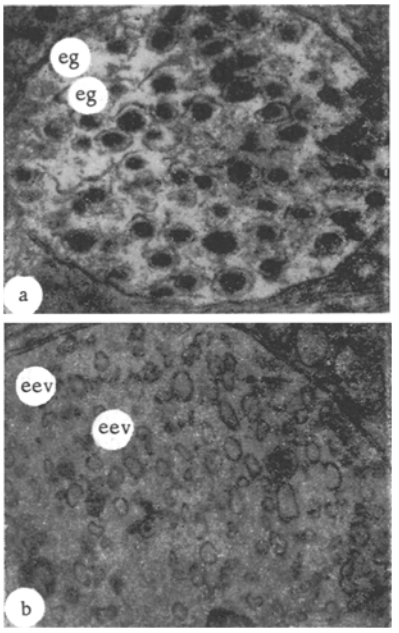

Fig. 2

Fig. 1. Changes in nuclear volume of neurons in normal and irradiated animals: a) supraoptic nucleus; b) paraventricular nucleus; I) control; II) irradiation.

Fig. 2. Fragments of neurosecretory endings in the posterior lobe of the neurohypophysis: a) 15-day-old rat (control): eg – elementary granules (24,000×); b) 15 days after irradiation: eev – electron-empty vesicles (21,000×).

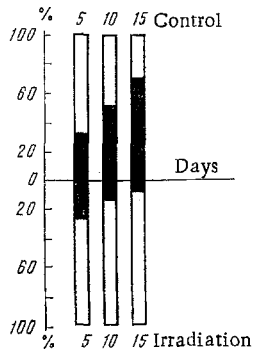

Fig. 3. Relative numbers of elementary granules and empty vesicles in secretory endings of the neurohypophysis. Shaded area denotes granules, unshaded area – vesicles.

animals (Fig. 1a). The karyometric results showed that irradiation of the newborn rats inhibited this increase in nuclear volume of the neurons. In experimental rats aged 5 days, a statistically significant decrease in the nuclear volume of the SON cells was observed (Fig. 1a). On the 15th day after irradiation the difference in nuclear volume of the SON neurocytes between the control and experimental animals was increased. For instance, in the intact rats at this time the mean nuclear volume of the SON cells was $394 \pm 8\ \mu^3$, compared with $268 \pm 12\ \mu^3$ in the irradiated animals. The results of karyometry of the PVN neurocytes at different periods in the control and experimental rats are given in Fig. 1b. In the experimental animals a significant decrease in the nuclear volume in the PVN neurons was observed on the 15th day after irradiation.

Examination of serial sections through the hypothalamus stained with pseudoisocyanin showed that in normal conditions, with an increase in the time after birth, the quantity of fluorescent material in the cytoplasm of the SON neurons steadily increased. Irradiation, however, led to the rapid elimination of the fluorescent granules of secretion from the perikaryon of the SON neurocytes. For instance, five days after irradiation of the newborn rats, solitary fluorescent granules of secretion were present in the perinuclear zone of most neurons. Nevertheless, the cytoplasm of individual SON neurocytes showed strong fluorescence. At subsequent times (10 and 15 days after irradiation) the differences between the SON neurons of the irradiated and control animals became more conspicuous. The cytoplasm of the neurons of the irradiated rats was pale and contained numerous vacuoles, sometimes confluent. Staining with pseudoisocyanin revealed solitary fluorescent granules of secretion in the cytoplasm of the neurocytes, whereas in the neurons of the control animals large agglomerations of fluorescent material were observed.

Differentiation of the PVN neurocytes in the control animals was delayed by comparison with the SON neurocytes. Neurosecretory material was first discovered in the cytoplasm of the PVN neurocytes on the

3rd day after birth. The quantity of neurosecretion in the cytoplasm increased with age. At the age of 45 days, neurosecretion was first found along the course of the axons. Morphological changes in the PVN neurocytes were found in irradiated rats ten days after irradiation, and consisted of a decrease in the quantity of paraldehyde-fuchsinophilic material in the cytoplasm of the neurocytes. By the 15th day after irradiation, the cytoplasm of most PVN neurons contained only a few granules of neurosecretion. The same picture was also seen in the axons. It was therefore important to study the changes induced by irradiation in the nerve fibers whose endings form contacts on the blood capillaries in the posterior lobe of the neurohypophysis.

A study of ultrathin sections through the posterior lobe of the neurohypophysis showed that under the influence of ionizing radiation the elementary granules of the neurosecretion lose their electron-dense contents and are converted into electron-empty vesicles (Fig. 2). Counting the numbers of dense granules and empty vesicles in the secretory endings of the neurohypophysis showed that irradiation in a dose of 200 R induced a significant decrease in the number of granules and an increase in the number of empty vesicles (Fig. 3).

The results of this investigation showed that ionizing irradiation causes the forced elimination of neurosecretion from the perikaryon of the secretory neurocytes and its entry into the capillary blood stream of the posterior lobe of the neurohypophysis. This is because, as a carrier of antidiuretic hormone, the neurosecretion participates in the regulation of water and mineral metabolism and can thus exert some influence on the development of radiation sickness [4-6]. After whole-body irradiation of the young rats, not only the rate of elimination of neurosecretion was changed, but its synthesis also was disturbed. It can be postulated on the basis of the experimentally observed morphological pictures that in the early stages after irradiation the biosynthesis of octapeptides is intensified, and in the late stages this is replaced by inhibition. Disturbance of secretion formation in the SON and PVN neurocytes was also connected, evidently, with changes in the state of the nucleus. These changes were expressed morphologically as a sharp decrease in its volume. By the 15th day after irradiation the nuclear volume of the SON neurocytes was almost halved by comparison with the control. Previous work has shown that irradiation of embryos, as well as of young rats, delays the growth and differentiation of cortical, cerebellar, and hippocampal neurons, the epithelium of the mucous membrane of the mouse forestomach, and of various other structures [3, 7]. This demonstrates a substantial inhibitory effect of ionizing radiation also on differentiation of the neurosecretory cells of the SON and PVN in the hypothalamus.

The present observations revealed a difference in the responses of the developing neurons of the PVN and SON to irradiation. It may be that the slightly higher resistance of the PVN neurocytes to irradiation is due to their low functional activity in the period of irradiation, because it is known that intensively functioning neurons undergo more severe radiation changes [2]. It is demonstrative that differentiation and, consequently, the formation of the specific function of the PVN neurocytes are delayed by comparison with the function of the SON neurons by 2-3 days. Consequently, at the time of irradiation of the newborn rats, the level of function of the PVN neurocytes was relatively low.

Ionizing radiation thus induces forced elimination of neurosecretion from the perikaryon of the developing neurocytes and inhibits the increase in their nuclear volume. This last fact suggests an inhibitory action of ionizing radiation on differentiation of the SON and PVN neurons in the postnatal period of development.

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