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Functional Morphology of the Rat Choroid Plexus in Experimental Models

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In the present study were carried out ultrastructural and morphometrical investigations of the rat choroid during development and after hypokinesia and low doses of ionizing irradiation. Investigations of the rat choroid plexus during development provide evidence that light and dark epithelial cells finish their differentiation on 30 days postnatum. Changes of the epithelial cells during development suggest that dark and light cells are modulations of the same basic cells with possible functional differentiation starting from 17 days postconception and continue to 22 months. The observed ultrastructural and morphometrical data during hypokinesia may be related to the increased functional activity of the dark epithelial cells and earlier adult changes, for which epithelial ultrastructure gives evidence in the investigated period. Investigations after total-body irradiation of rats with low doses have shown that changes of the epithelial and endothelial cells of the plexus choroideus are more marked after irradiation with oxygen ions and neutrons in comparison with gamma rays.

Key words: rat choroid plexus epithelial cells and blood vessels, ultrastructure and morphometry, development, hypokinesia and low doses irradiation.

Introduction

The choroid plexuses are specialized highly vascular anatomycal structure which protrude into the lateral ventricle, as well as in the third ventricle and fourth ventricle. The surface of the choroid plexus consist of numerous villi each covered with single layer of epithelial cells surrounded by vascular connective tissue cells [8]. As a secretory source of vitamins, peptides and hormones for neurons, the choroid plexus provides substances for brain homeostasis [4]. Most blood vessels in the plexus choroideus are wide-calibre (approximately 15 μm) fenestrated capillaries [7]. Investigation of the influence of hypokinetic conditions indicates that immobilization of pregnant rats for 5 days considerably influences the morphology of the corpus luteum and luteal cells [6]. X-rays, neutrons, alpha- and beta-particles come from environment or are produced by human activities. The real long-term effects of this background radiation are nevertheless a mystery, which is why they are currently being investigated by the scientific projects.

The *aim* of the present study is to investigate the ultrastructural and morphometrical changes of the rat choroid plexus during development and after hypokinesia and exposure to low doses of ionizing irradiation.

Materials and Methods

Development. Wistar rats (n=60) aged 17 and 20 days postconception, 5, 15, 30, 45 and 60 days postnatum and 4, 7, 10, 13 and 22 months were used. The animals were fixed by immersion [14] and by intracardial perfusion [5].

Hypokinesia. One-month aged Wistar rats were divided into two groups: I group (n=20) rats subjected to 3, 6, 9 and 12 months of hypokinesia in specially constructed individual cages for physiological immobilization [15] and II group – control rats (n=20) under normal vivarium conditions.

Irradiation. Three-months aged female Wistar rats were divided into four group: I group –irradiated with single dose of 10^4 particles /cm^2 of oxygen ions (n=3), II group – irradiated with fast neutrons (n=3) to 1.5 MeV at the dose of 1.0 Gy, III group – irradiated with gamma rays Co^{60} (n=3) at the dose of 1.0 Gy and IV group – control six months aged rats (n=4). Three months after irradiation the animals were intracardially perfused [7]. The choroid plexuses were embedded in Durcupan and examined with JEOL JEM 1200EX transmission electron microscope.

We obtained morphometric data from the light microscope at 1000× magnification using a square grid system [13] calibrated for linear measurement in μm and area measurement in μm². All values were expressed as mean ± SEM, and statistically analyzed by Student t-test.

Results and Discussions

In our investigations of the rat choroid plexus from aged 17 days postconception to 22 months we established the three periods of the ***development***. The **first period** may be divided into three phases of the differentiation. During the *first phase* (17-20 days postconception) the epithelial cells are pseudostratified and they have electron-light cytoplasm with many glycogen granules and scanty cell organelles, concentrated at the apical part (Fig. 1 A, B). During the *second phase* (20 days postconception – 15 days postnatum) the epithelial cells come to low columnar, in the apical part of the epithelial cells there are many cytoplasmic protrusions with glycogen, short and fine microvilli (Fig. 2 A). The most marked ultrastructural changes of the epithelial cells on the 15 days postnatum are many cytoplasmic protrusions, filled with glycogen, many vacuoles, granular endoplasmic reticulum and mitochondria with unformed cristae (Fig. 2 B). The

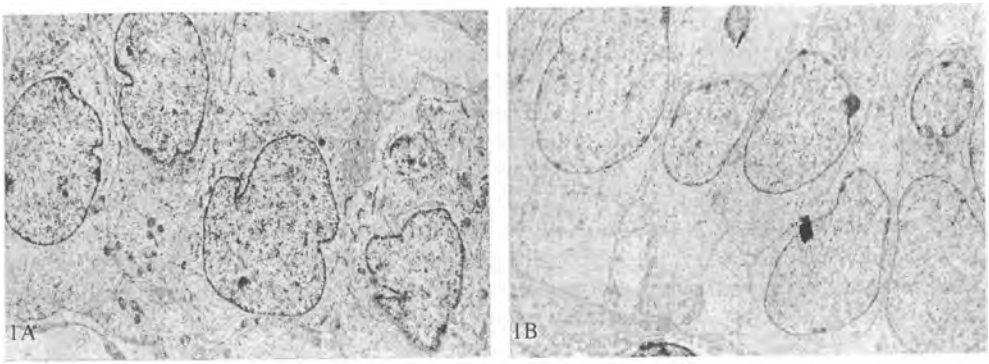

Fig. 1A – Pseudostratified epithelial cells of the rat choroid plexus aged 17 days postconception. × 3 000; 1B – High columnar epithelial cells of the rat choroid plexus aged 20 days postconception. × 3 000

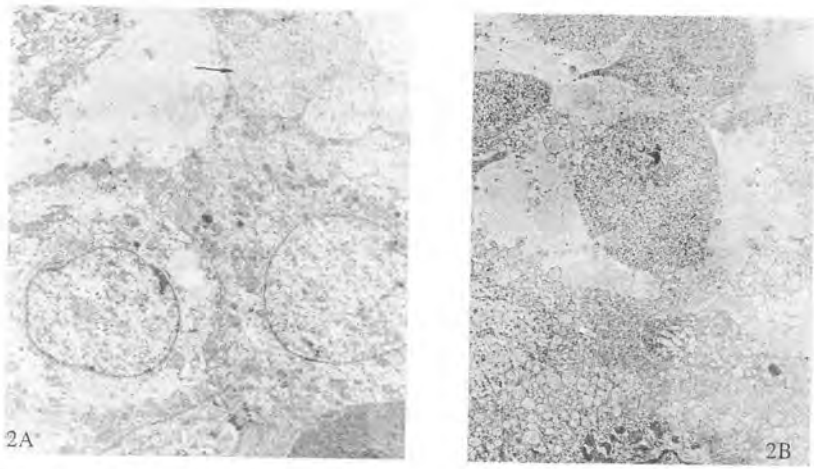

Fig. 2A – Rat choroid plexus aged 5 days postnatum. ×2 500; 2B – Rat choroid plexus aged 15 days postnatum. ×3 000

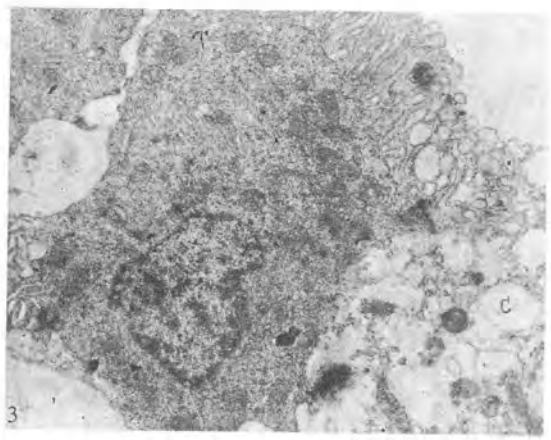

Fig. 3. Light and dark epithelial cells of the rat choroid plexus aged 30 days postnatum. ×6 000

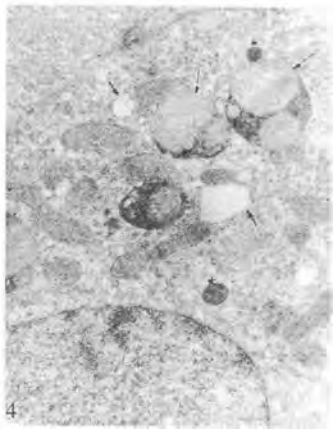

Fig. 4. Rat choroid plexus aged 22 months. × 12 000

concentration of glycogen in the rat choroid plexus epithelial cells increased to 5 days postnatum and decreased at the 15 days postnatum. S t u r r o c k made similar observations in the mouse choroid plexus [12]. During the *third phase* of the rat choroid plexus development (15-30 days postnatum) the epithelial cells come to cuboidal, the electron density of the epithelial cytoplasm is increased, the microvilli are well shaped and tight packed, and the connective tissue and the blood vessels are well differentiated (Fig. 3). From morphometrical analysis of the rat choroid plexus during development it was established that the nuclear, cytoplasmic and cell area of the dark epithelial cells is smaller than the same parameters of the light epithelial cells during the whole investigated period. The nuclear, cytoplasmic and cell area of the light and dark epithelial cells increased from 17 days postconception to 5 days postnatum and decreased from 5 days postnatum to 15 days postnatum. Changes of the nuclear, cytoplasmic and cell area of the light and dark epithelial cells from 17 days postconception to 15 days postnatum are proceeded simultaneously with the ultrastructural data for glycogen accumulation. On

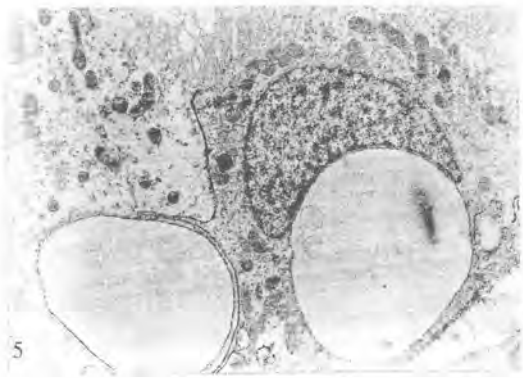

Fig. 5. Rat choroid plexus after 3 months hypokinesia. × 5 000

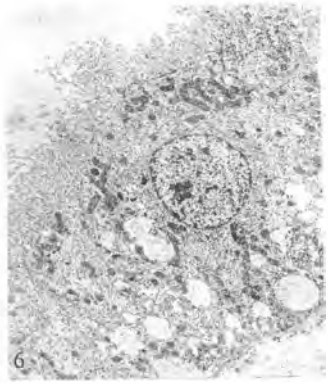

Fig. 6. Rat choroid plexus after 6 months hypokinesia. × 3 000

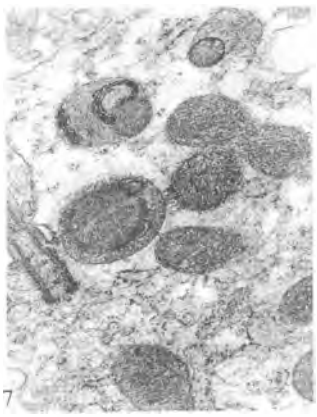

Fig. 7. Apical part of the epithelial cell of the rat choroid plexus after 9 months hypokinesia. × 30 000

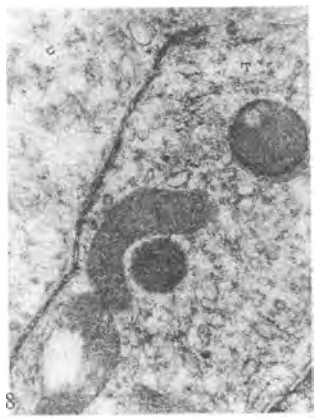

Fig. 8. Rat choroid plexus after 12 months hypokinesia. × 25 000

the basis of the ultrastructural and morphometrical investigations of the rat choroid plexus during the **second period** of development (45 and 60 days postnatum, 4, 7, 10 and 13 months) it was established that the epithelial cells are cuboidal, as the average area of the light cells is 204.5 μm^2, and of the dark cells – 137.2 μm^2. The nuclei of the epithelial cells are rounded, located basally and have homogeneous chromatin. The large numbers of mitohondria are present, concentrated at the apical ends of the cells. The most marked ultrastructural changes of the epithelial cells during the **third period** of development (13-22 months) are the presence of many lipid droplets, second lysosomes, imbibing mitochondria and dense bodies (Fig. 4). The main morphological changes noted with age suggest a decrease in efficiency of choroid plexus cells in old age [4].

The prolonged ***hypokinesia*** provoked significant ultrastructural and morphometric changes of the rat choroid plexus. The most marked ultrastructural changes of the rat choroid plexus during 3 and 6 months of hypokinesia are elongated mitochondria, giant electron-light vacuoles, dense bodies, multivesicular bodies and coated vesicles in the epithelial cytoplasm (Figs. 5, 6). These changes pointed out the increased absorptive ac-

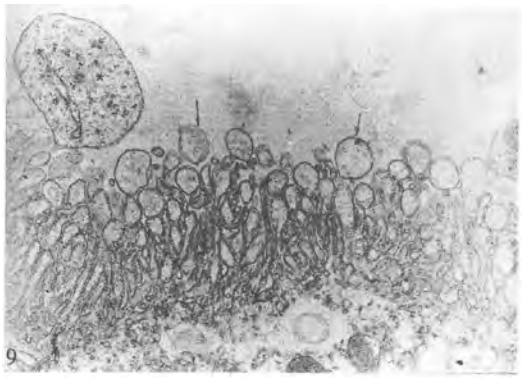

Fig. 9. Rat choroid plexus 3 months after irradiation with high energy oxygen ions. × 10 000

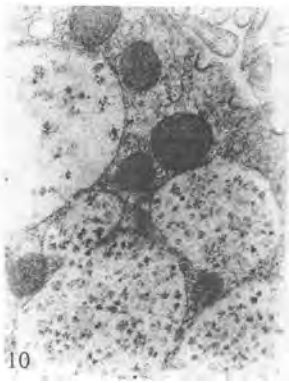

Fig. 10. Rat choroid plexus 3 months after irradiation with high energy oxygen ions. × 25 000

tivity of the choroid plexus epithelium and probably depend on the increased vasopressin [11]. The epithelial cytoplasm of the rat choroid plexus cells during 9 months of hypokinesia, in contrast to control, contains a great number of dense bodies, multivesicular bodies, coated vesicles and electron-dense lysosome-like bodies with lamellar structures (Fig. 7). These changes may be associated with increased transcellular transport by fenestrated capillaries and earlier adult changes. Destructive changes of the choroid plexus epithelium (numerous swollen mitochondria, lipid droplets, second lysosomes and electron-dense bodies with lamellar structures) observed during 12 months of hypokinesia are an evidence for earlier adult changes (Fig. 8). The obtained morphometrical data point out that the increased relative part of the dark cells, the large value of the nuclear, cytoplasmic and cell area of the dark cells, and decreased nuclear, cytoplasmic and cell area of the light cells more significantly during 12 months of hypokinesia, in comparison with dark cells, may be related to the increased functional activity of the dark epithelial cells and earlier adult changes [9].

Choroid plexus of the brain is an ideal model for studying the development of ***radiation*** damage due to a close contact between vascular and epithelial cells, which normally have very slow turnover [1]. On the apical surface of the epithelial cells of the rat choroid plexus 3 months after irradiation with *high energy oxygen ions* were seen cytoplasmic protrusions and elongated and dilated microvilli (Fig. 9). In the epithelial cytoplasm there are seen dense bodies, vesicles, multivesicular bodies and vacuoles, containing glycogen granules (Fig. 10). In the epithelial cytoplasm of the rat choroid plexus 3 months after irradiation with *fast neutrons* were seen well defined Golgi apparatus, coated vesicles, pinocitotic vesicles and multivesicular bodies (Fig. 11). Most characteristic ultrastructural changes of the rat choroid plexus 3 months after irradiation with *gamma rays* were elongated mitochondria, many pinocytotic vesicles on the basolateral intercellular junctions as well as in the endothelial cells of the capillaries. Many epithelial cells possessed two nuclei after exposure with oxygen ions and gamma rays. The obtained morphometrical data after irradiation of the rat choroid plexus have shown that the nuclear and cytoplasmic area of the light and dark epithelial cells is changed statistically significant after irradiation with oxygen ions and gamma rays. The relative part of the capillaries, i.e. vessel <16μm in diameter were 70.66% in control rats, and 34.86% in fast neutrons, 56.34% in oxygen ions and 70.52% in gamma rays irradiated rats. The initial loss of capillaries and the increase in number of large vessels in plexus choroideus 3 months after irradiation were consistent with the effects attributed to re-

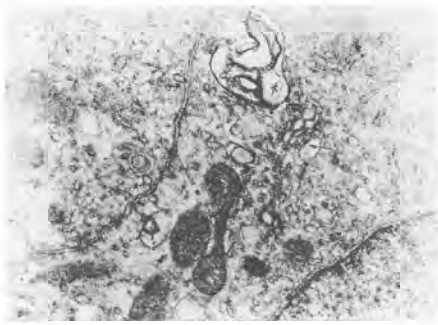

Fig. 11. Rat choroid plexus 3 months after irradiation with fast neutrons. × 15 000

generation in the plexus choroideus [10]. These changes may be indicative of compensatory reactions in the organism following radiation exposure. Experimental study have shown a significant reduction in the number of blood vessels > 16 μm in diameter and atrophy of the choroid plexus epithelial cells only after 25 Gy of X-rays [2, 3]. These results clearly demonstrate that the effect on blood vessels after irradiation can be induced in the choroid plexus by single dose of 1.0 Gy fast neutron, oxygen ions and gamma rays. We suggest a hypothesis that the vascular damage is predominant factor leading to development of late effects in irradiated normal tissues.

Conclusion

Plexus choroideus performs a multiplicity of functions for the central nervous system. From fetal period of development through adult, and extending into terminal physiological stages, plexus choroideus actively engages with building, maintaining and repairing of the brain homeostatic mechanisms which are vital to neuronal system.

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