Ultrastructure of the Pineal Gland after γ-Irradiation under Conditions of Inhibition of Adrenocortical Function

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Electron microscopy showed that whole-body γ-irradiation in sublethal doses led to the appearance of injuries in pinealocytes, glial cells, and vessels of the pineal gland in rats. Limitation of the nonspecific effect of γ-irradiation via inhibition of adrenocortical function with metopirone in physiological doses reduced the radiation-induced ultrastructural damage to the pineal gland.

Key Words: *pineal gland; γ-irradiation; ultrastructure; metopirone*

The pineal gland (PG) receives information on the external light fluctuations and transforms it into the endocrine response of the organism [1,12,16]. Along with indolylamines (serotonin, 5-methoxytryptamine, melatonin, *etc.*), peptides synthesized in PG are involved in the realization of functional relationships of this gland with other organs [1]. Indolylamines participate in the formation of basal endogenous radioresistance of the body and are distinguished as a class of radioprotectors. The radioprotective effects of serotonin are best studied [5,6]. Recent studies demonstrated high antioxidant and radioprotective activity of the main PG hormone melatonin [4,14]. Exogenous melatonin protects cells and tissues from free-radical injuries [12]. The radiomodifying properties of peptides synthesized in the PG were demonstrated [10]. Hence, PG hormones and peptides contribute to the formation of basal endogenous radioresistance of the body. At the same time, ultrastructural damages to PG caused by whole-body γ-irradiation and the possibility their modification are little studied, particularly in view of intricate relationship between the manifestations of the effects of various endocrine changes and the status of the neuroendocrine system. The adrenals produce hormones responsible for the realization of some common reactions of the body in response to damage. We investigated the ultrastructure of PG after whole-body γ-irradiation under conditions of inhibited adrenocortical function.

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats (180-200 g). The animals were divided into 3 groups: 1) intact animals; 2) exposed animals; 3) exposed animals injected with metopirone, a specific inhibitor of glucocorticoid synthesis. The exposure was carried out on a Luch-1 device (^{60}Co source, dose power 17.2×10^4 Gy/sec). The animals of groups 2 and 3 were irradiated for 5 days in a daily dose of 1.25 Gy (total dose 6.25 Gy). Metopirone (Ciba) was injected in a dose of 11 mg/100 g for 2 weeks after irradiation [3,7-9,13]. The animals were decapitated (at 10.00-12.00) under Nembutal narcosis (50 mg/kg) on days 14 and 21 of the experiment. PG was fixed in Karnovskii fluid, dehydrated, and embedded in epon mixture. Ultrathin sections (100 nm) were made on LKB-7A ultramicrotome (LKB), contrast-stained with uranyl acetate and lead citrate, and examined under a JEM-100S electron microscope (JEOL).

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RESULTS

PG ultrastructure in controls was normal and corresponded to published data and our own findings [10,13].

Electron microscopy of PG from group 2 animals showed that the most pronounced ultrastructural changes in the vascular bed occurred on day 14 after the start of irradiation. We observed swelling, edema, and vacuolation of the endothelium, disappearance of cytoplasmatic organelles and pinocytous vesicles, extension and loosening of perivascular spaces (Fig. 1, *a*). In some pinealocytes the outer karyolemma leaflet was detached, cristae in the central parts of the mitochondria were swollen and destroyed, and large vacuoles (presumably, a result of dilation of the endoplasmatic reticulum) and myelin figures appeared in the cytoplasm. This was paralleled by accumulation and enlargement of lipid droplets (Fig. 1, *b*) and appearance of lysosomes. In these animals pinealocytes usually contained one nucleolus with homogenous structure (Fig. 1, *c*).

These changes were still present on day 21 after the start of γ-irradiation. At this term slightly expressed invaginations of the karyolemma and detachment of its outer leaflet were seen in pinealocytes. The cytoplasm volume decreased, and some pinealocytes had just a narrow cytoplasmic rim (Fig. 1, *d*). It is noteworthy that the most pronounced changes were still observed in the cytoplasm: vacuolation due to sharp dilatation of the endoplasmatic reticulum. In some pinealocytes and glial cells swollen mitochondria contained clarified matrix and completely lyzed cristae (Fig. 1, *e*). Detachment of the outer karyolemma leaflet was seen in glial cells; dilated cisterns of the rough endoplasmatic reticulum and hypertrophic Golgi complex were seen in the cytoplasm. The cytoplasm of glial cell contained lipid droplets (often in the Golgi complex zone) and myelin figures. Endotheliocytes of many blood capillaries were edematous, virtually without cytoplasmic organelles. Their nuclei contained loosened chromatin, the external nuclear membrane was often detached along the perimeter. Perivascular spaces were loosened and edematous (Fig. 1, *f*).

Hence, ultrastructural signs of injuries in pinealocytes, glial cells, and particularly PG vessels appear after fractionated whole-body γ-irradiation in sublethal doses. Evaluation of the biological effect of radiation should include both assessment of its direct (immediate) damaging effect and mediated effects on the body. The direct effect of radiation injury is determined by simultaneous damage of cells caused by their direct exposure to ionizing radiation. Mediated (nonspecific) effect is regarded as a manifestation of the general adaptation syndrome and includes damage to membrane structures, uncompensated activation of lipid peroxidation, and reactive changes in the regulatory systems, primarily, endocrine system [2]. In order to discriminate between the direct effects of radiation and changes associated with the reaction of the pituitary-adrenal system to stress (irradiation), we carried out experiments on a special group of animals injected with metopirone in a dose maintaining normal blood level of glucocorticoid hormones [3,7-9,11].

Electron microscopy of PG of group 3 animals 14 days after the start of the experiment showed the appearance of very large nucleoli in pinealocytes; these nucleoli usually consisted of two well discernible components with homogeneous and fibrillar structure. Metopirone treatment promoted recovery of mitochondrion ultrastructure. Enlarged mitochondria (in comparison with the control) with clearly seen cristae appeared in some cells (Fig. 2, *a*). The nuclear membrane formed numerous deep invaginations. The number of polyribosomes and granular endoplasmatic reticulum membranes in the cytoplasm increased. The number of lysosomes and lipid droplets notably decreased; solitary myelin figures were seen in some pinealocytes. Glial cells retained high electron density of the cytoplasm and nucleus, but the detachment of the outer nuclear membrane was less expressed. Like in pinealocytes, invagination of the outer nuclear membrane was more pronounced than in glial cells of control animals; large nucleolus, usually one, was as a rule well discernible. The cytoplasm still contained lipid droplets, mitochondria with clarified matrix and partially lyzed cristae (only near the limiting mitochondrial membrane the cristae were preserved, Fig. 2, *b*). Basal membrane of blood capillaries became dense, edemas in the perivascular spaces decreased. The number of organelles (mitochondria, endoplasmatic reticulum membranes, ribosomes, pinocytotic vesicles) in the endotheliocyte cytoplasm increased (Fig. 2, *c*). Schwann cells had more axons containing vesicles with regularly arranged microtubules, mitochondria, and secretory granules.

These morphological changes in the pinealocytes and glial cells did not disappear on day 21 of the experiment. Like at the previous term, pinealocyte cytoplasm contained numerous membranes of granular endoplasmatic reticulum, short cisterns of smooth endoplasmatic reticulum, numerous polyribosomes and mitochondria with well discernible cristae (Fig. 2, *d*). Pinealocyte nuclei contained large nucleoli with granular and fibrillar components (Fig. 2, *e*). Morphological features of glial cells observed previously remained unchanged. Ultrastructure of blood capillaries did not differ from that in control animals and was characterized by the presence of numerous pinocytotic vesicles in the endothelium. Nerve fiber processes with regularly arranged microtubules, electron-transparent

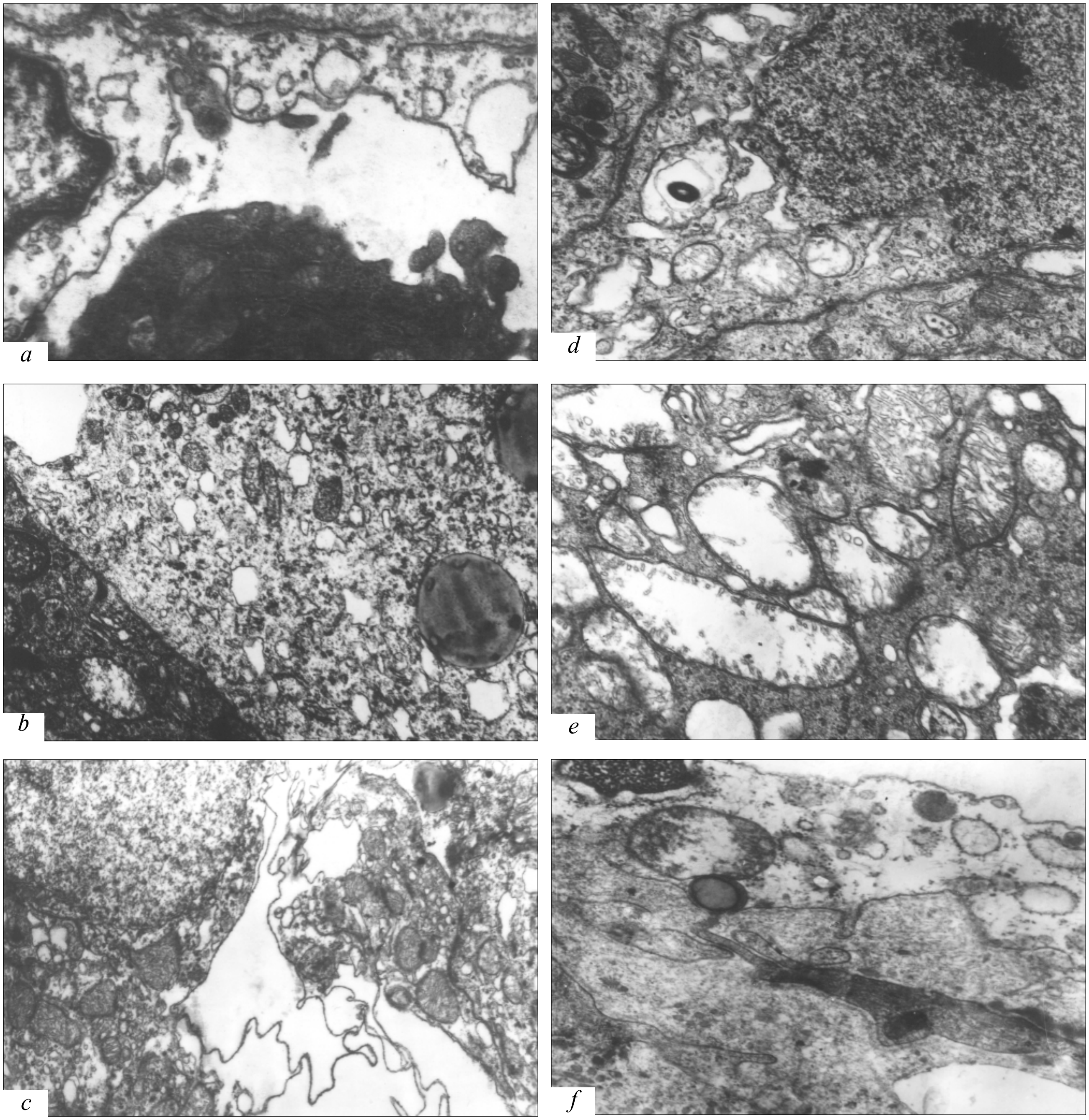

Fig. 1. Ultrastructure of pineal gland on day 14 (*a-c*) and 21 (*d-f*) after irradiation. *a*) capillary with swollen endothelium on one side and dark, presumably dying endothelial cell on the other, ×14,000. *b*) glial cells and fragment of pinealocyte cytoplasm with a large lipid droplet, ×10,500. *c*) pinealocyte with sites of destroyed cytoplasm, ×7000. *d*) pinealocyte; cytoplasm contains mitochondria with swollen cristae, myelin figure, and numerous cisterns of the endoplasmatic reticulum, ×10,500. *e*) fragment of glial cell cytoplasm with mitochondria with completely or partially lyzed cristae, ×14,000. *f*) edematous vascular endothelium, ×14,000.

compact vesicles, and mitochondria were seen in the perivascular spaces (Fig. 2, *f*). Hence, the damaging effect of ionizing radiation on PG ultrastructure was reduced in animals treated with metopirone, which could be due to reduced indirect effect of glucocorticoid hormones and other bioactive substances, whose synthesis and secretion changed under conditions of radiation-induced stress. It is known that irradiation in sublethal doses leads to activation of oxidative stress in various tissues, including traditionally considered radioresistant tissues[2].

Hence, electron microscopy of PG after fractionated γ-irradiation showed that limitation of adrenocortical function within the range of physiological norm with metopirone prevented radiation-induced damage to PG ultrastructure. The structure of the vas-

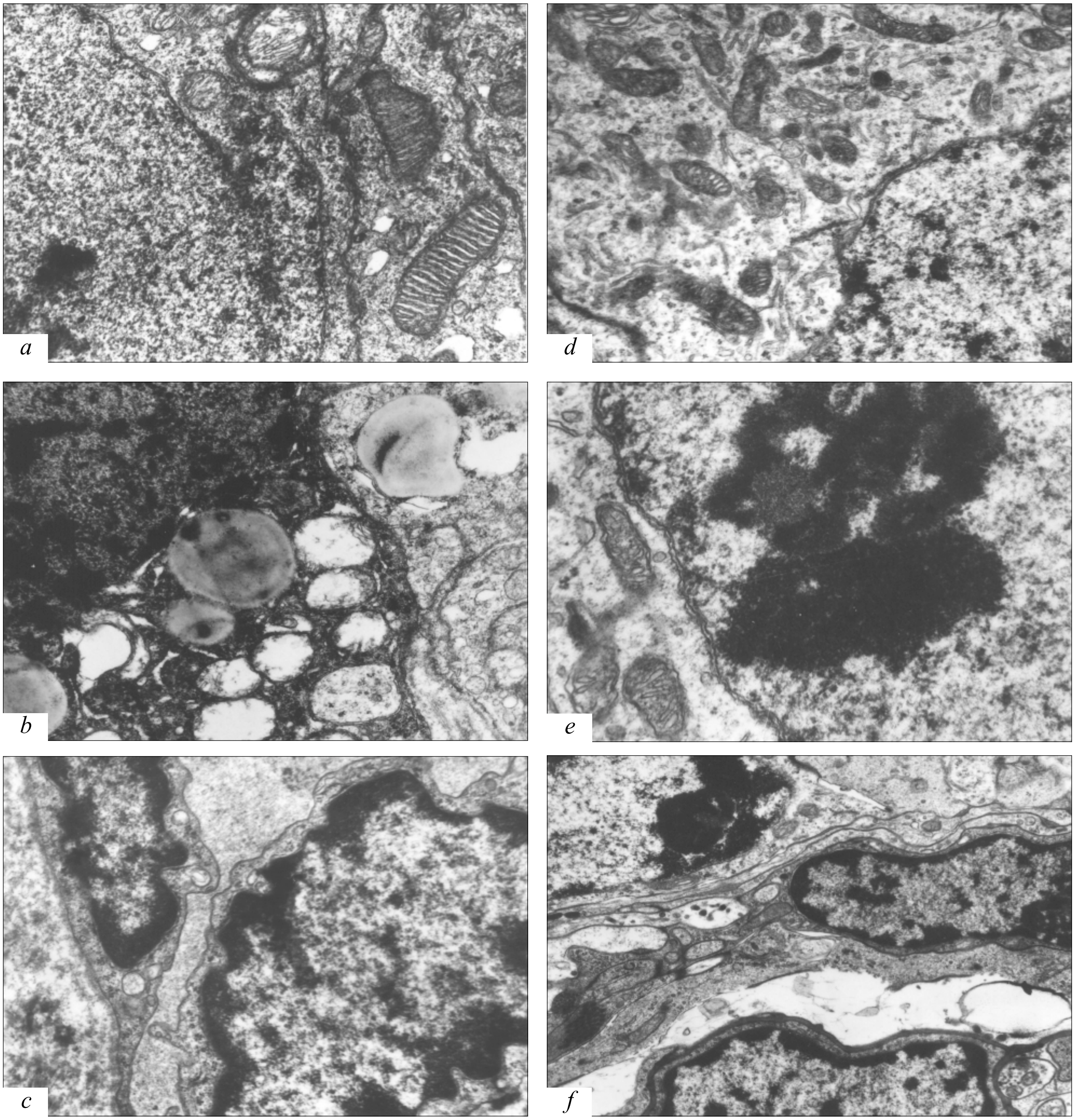

Fig. 2. Ultrastructure of pineal gland of animals treated with metopirone on days 14 (*a-c*) and 21 (*d-f*) after the start of irradiation. *a*) pinealocyte with large mitochondria with well preserved cristae in the cytoplasm, ×10,500; *b*) glial cell with compact karyolemma and cytoplasm containing lipid droplets and swollen mitochondria, ×10,500. *c*) vascular wall differs little from the control, ×10,500. *d*) pinealocyte fragment; normal structure of the nucleus; cytoplasm contains Golgi complex, numerous polyribosomes, rather large mitochondria, ×10,500. *e*) pinealocyte with well preserved karyolemma and large nucleolus, ×17,500. *f*) blood capillary with pinealocytes in the perivascular space, ×7000.

cular wall and, hence, the transporting function of the endothelium were preserved, which protected the integrity of pinealocytes and glial cells. These results are in line with the results of electron-microscopic analysis of the thymus in the same animals [8]. A similar positive effect was observed in studies of other stress factors: toxins [7] and hypokinesia [3,11]. Limitation of functional activity of the adrenal cortex can be used for correction of radiation-induced hormonal disorders.

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