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ULTRASTRUCTURAL CHANGES IN EXPERIMENTAL TETANUS

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UDC 611.814.34:616-076.4:576.851.551

In experiments on rats an electron-microscopic study was undertaken of the caudal part of the hypothalamic-hypophyseal neurosecretory system in experimental tetanus. Activation of the system was found in the early stages after injection of tetanus toxin, evidently on account of the injection of heterologous protein, for a similar effect was given by injection of the inactivated toxin. Parallel changes were observed in vascular permeability, lipid metabolism, and the clotting system of the blood.

KEY WORDS: hypothalamic-hypophyseal neurosecretory system; tetanus toxin.

Light-optical studies of the hypothalamic-hypophyseal neurosecretory system (HHNS) in experimental tetanus have demonstrated the successive stages of the secretory disturbances: activation, preceding the appearance of the clinical features, and followed by inhibition of synthesis and liberation of neurohormones after injection of tetanus toxin [3]. However, many of the finer details of the response of the components of the posterior lobe of the pituitary still await discovery, for which electron-microscopy is the essential method.

This paper describes a study of the ultrastructural changes in the caudal part of the HHNS at different periods after injection of tetanus toxin.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male albino rats weighing about 250 g. A lethal dose of tetanus toxin in 0.24 ml 0.85% sodium chloride solution was injected intramuscularly into the left calf. After 24 h the rats developed signs of local tetanus, and after 3 days general ascending tetanus with spontaneous convulsions, followed by death on the fourth day. Another group of animals received the same dose of inactivated toxin (heated to 56°C for 2 h). The control animals received physiological saline but the general conditions of the basic experiments remained the same.

Material was taken for electron microscopy 5 h and on the third day after injection of the toxin. Pieces of the posterior lobe of the pituitary were fixed in glutaraldehyde in phosphate buffer and postfixed in osmium tetroxide solution, dehydrated in acetone, and embedded in a mixture of Epon and Araldite. Ultrathin sections, stained with lead citrate and uranyl acetate, were examined in the UÉMV-100 and JEM-100B electron microscopes.

EXPERIMENTAL RESULTS

The ultrastructure of the posterior lobe of the pituitary of the rats receiving physiological saline was virtually indistinguishable from that in intact animals. Nerve fibers and terminals contained many neurosecretory elementary granules, a few empty and synaptic vesicles, and also small mitochondria. The endings of the axons were in contact with capillaries, a special feature of which was their fenestrated endothelium. Glial cells, represented by pituicytes, corresponded to astrocytes and oligodendrocytes of the neuroglia. Mast cells, characteristic of the neurohypothesis of dogs and opossums [1, 4], are not found in rats.

Central Research Laboratory, Rostov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 4, pp. 489-493, April, 1978. Original article submitted October 12, 1977.

 0007-4888/78/8504-0536 $07.50

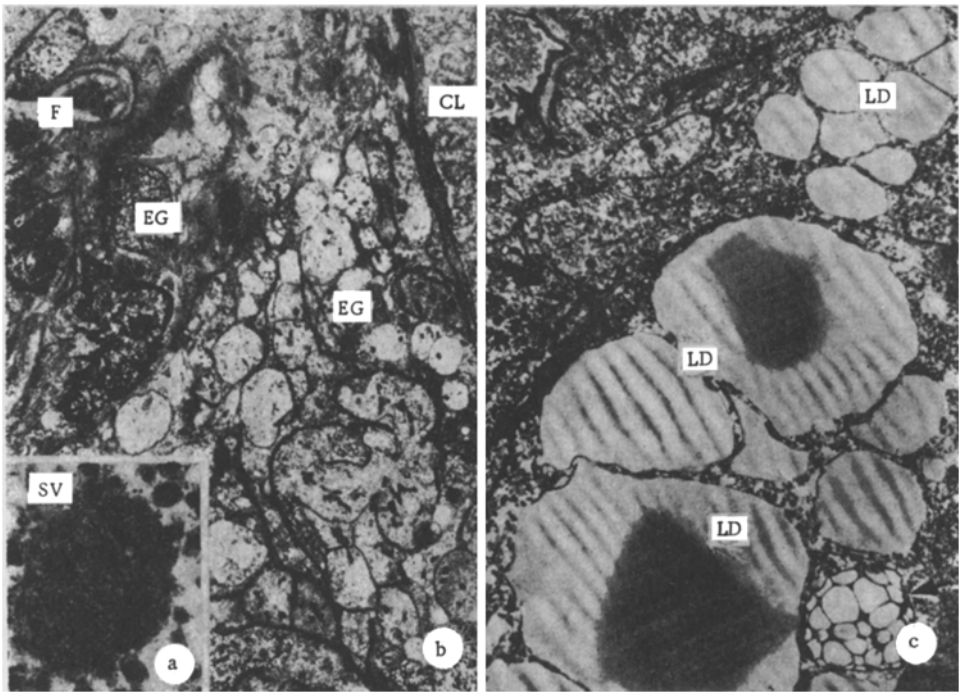

Fig. 1. Ultrastructure of posterior lobe of pituitary 5 h after injection of tetanus toxin: a) axons contain elementary granules and many empty vesicles. Precipitates of fibrin, blocking the capillary lumen, can be seen in the top left-hand corner of the figure (6000×); b) collection of synaptic vesicles resembling "caviar" (19,000×); c) cytoplasm of pituicytes filled with lipid material, bottom left – lipochondrion (marked by arrow) (12,500×). SV) Synaptic vesicles; EG) elementary granules; F) fibrin; CL) capillary lumen; LD) lipid drops.

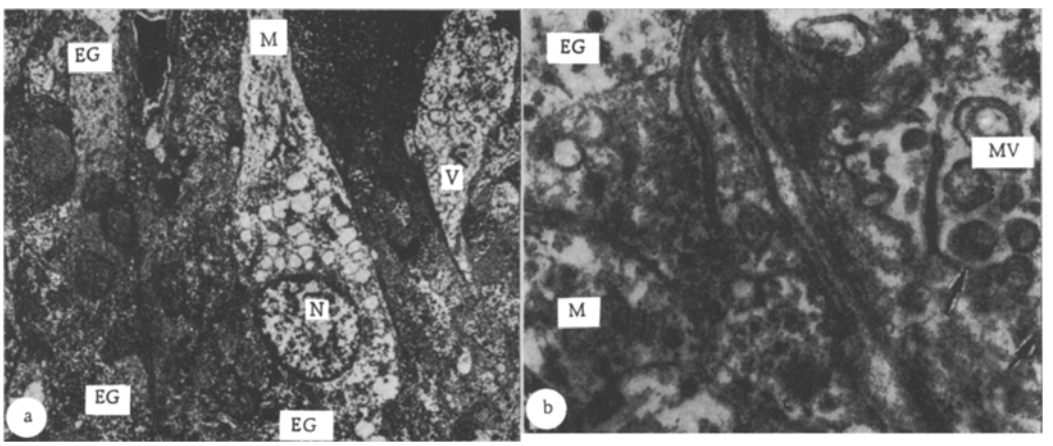

Fig. 2. Ultrastructure of posterior lobe of pituitary 3 days after injection of tetanus toxin: a) appearance of numerous elementary granules, restoration of ultrastructure of mitochondria; large vacuoles are visible in cytoplasm of pituicytes at site of lipid drops (14,000×); b) injury to plasmalemma of endothelial cell (arrows), destruction of microvilli (clasmatosis) (22,500×). V) Vacuoles; M) mitochondria; N) nucleus; EG) elementary granules; MV) microvilli.

Many terminals were discharging neurosecretion 5 h after injection of the toxin, and they appeared less osmiophilic in panoramic films. However, some fibers contained a fair number of elementary granules (Fig. 1a). Collections of synaptic vesicles also were observed in this region, sometimes grouped so closely together that they resembled crystalline structures. Such structures have been described previously in the

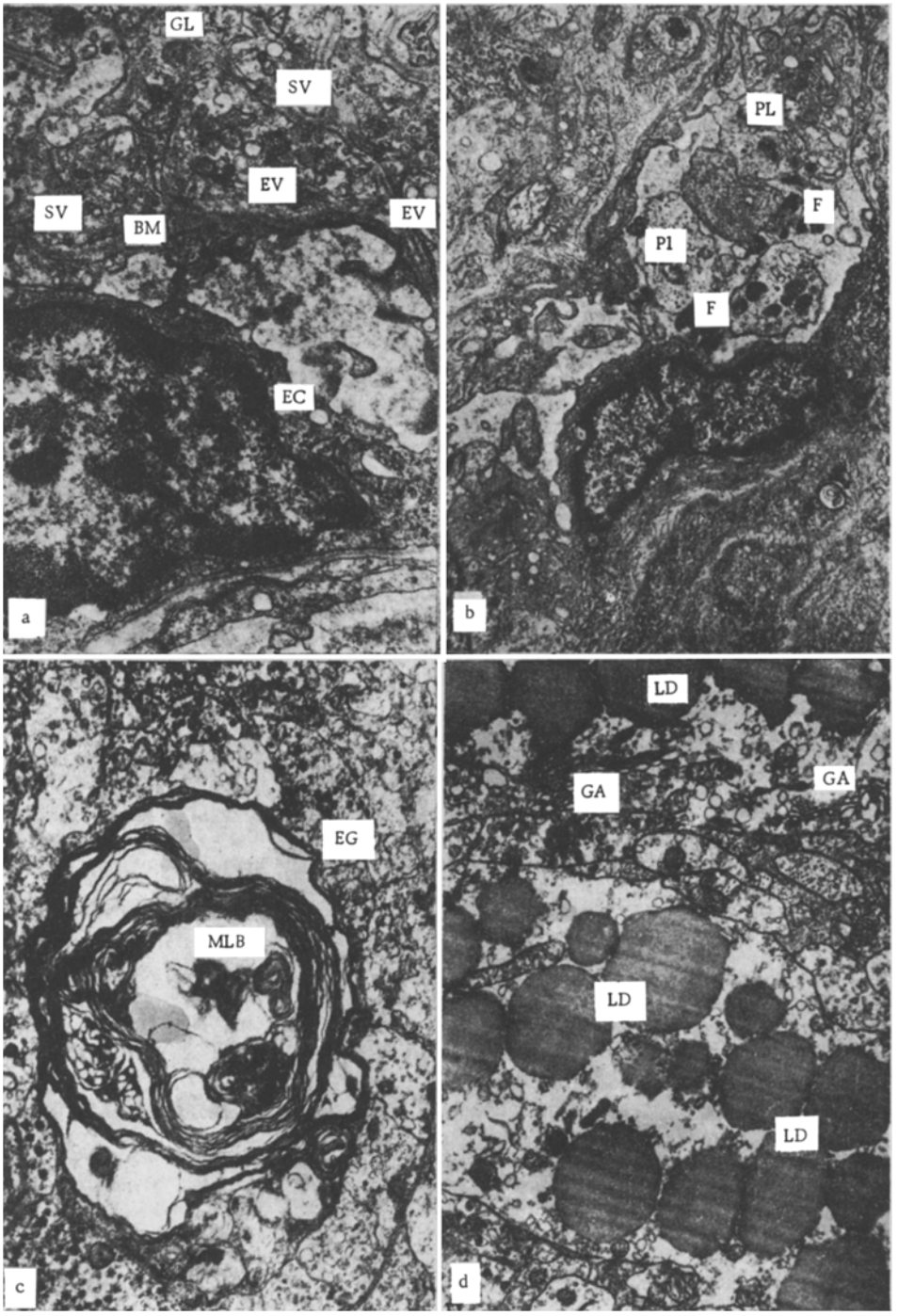

Fig. 3. Ultrastructure of posterior lobe of pituitary after injection of inactivated tetanus toxin (control): a) expulsion of contents of elementary granules and their conversion into empty vesicles; in some axons glycogen granules can be seen (5 h; 16,600×); b) intravascular coagulation and phenomenon of viscous metamorphosis (5 h, 11,000×); c) large multilamellar body (3 days, 15,000×); d) appearance of multiple lipid drops in cytoplasm of pituicytes (3 days, 14,000×). GL) Glycogen; EG) elementary granules; EV) empty vesicles; SV) synaptic vesicles; BM) basement membrane; EC) endothelial cell; PL) platelets; F) fibrin; MLB) multilamellar bodies; LD) lipid drops; GA) Golgi apparatus.

capillary endothelium of the posterior lobe of the pituitary in dogs receiving endotoxin [2]. Mitochondria in the nerve cells were small, and those in the endings were moderately swollen.

Ultrastructural changes in the capillaries were distinguished by their polymorphism: Together with dark endotheliocytes with a large osmiophilic nucleus and similar cytoplasm, edematous cells were seen. The luminal surface of some of them had well-developed microvilli. Signs of intravascular coagulation were observed, in the form of fibrin precipitates blocking the lumen (Fig. 1a). The cytoplasm of both types of pituicytes contains large lipid drops and mitochondria (Fig. 1c).

Differences in the osmiophilia of the lipid material were discovered and could indicate its possible liberation from the cell. Polyvesicular structures described previously in the cytoplasm of astrocytes [5] and in human sweat glands [6], and known as lipochrondria, also were found. Because of their phagocytic powers, one of us (E.A.B.) [1] has postulated that they may be concerned in the fate of the empty vesicles, i.e., membranes of the elementary granules. These membranes, which are lipoprotein in nature, are evidently an object of phagocytosis and subsequently are taken into the general cytoplasmic pool of the cell, where they are utilized for its needs (energy production, biosynthesis of macromolecular compounds, and so on). This hypothesis is confirmed by the definite correlation found between the degree of liberation of neurosecretion and the lipid content in the pituicytes of rats exposed to various forms of stress (dehydration, trauma, injection of formalin) [7].

On the third day marked accumulation of neurosecretion was observed and the mitochondria appeared as elongated formations with moderately dense matrix and ordinary cristae (Fig. 2a). There were fewer synaptic vesicles than at the previous period of investigation and the vesicles were uniformly distributed in the axoplasm, virtually none being concentrated in groups. The mitochondria reacted differently in the pituicytes and their processes. Besides unchanged organelles, some grossly swollen mitochondria were seen, together with others which had degenerated and were converted into myelin figures. Another constant feature was the appearance of numerous lysosomes, lipid drops, and mitochondria.

In the lumen of the blood vessels of the posterior lobe of the pituitary damaged endothelial cells were observed, with microvilli desquamated as a result of clasmatosis (Fig. 2b). In the zone of axo-vasal contacts, despite the presence of the synaptic vesicles and elementary granules, no empty vesicles were seen, evidence of the deposition of neurosecretory material.

The pattern of changes in the caudal region of HHNS in experimental tetanus described above is thus evidence of activation of the components of this system as early as during the first 5 h, and also of changes in vascular permeability, lipid metabolism, and the blood clotting system. After 3 days accumulation of neurosecretory material was combined with degenerative changes in the pituicytes and injury to the endothelium.

Moderate liberation of the contents of the elementary granules was observed 5 h after injection of the inactivated toxin; independently of the degree of liberation of neurosecretion, glycogen granules were observed in the axoplasm of the fibers (Fig. 3a). Wherever forced mobilization of neurosecretion had occurred, the Herring's bodies appeared optically empty. Some of them contained thick-walled vacuoles and honeycombed bodies, while a few endings were filled with large osmiophilic inclusions, aggregates, and formed lamellar bodies.

In the capillaries the periendothelial spaces were considerably widened, the number of pores and pinocytotic vesicles in the endothelium was increased, and signs of intravascular coagulation and the phenomenon of viscous metamorphosis of the platelets could be seen (Fig. 3b). The spur to intravascular clotting was evidently the initial disturbances in the first phase. In addition, vacuolation of the cytoplasm of the platelets and swelling of the mitochondria took place. Damage to the platelets was accompanied by their standard response: liberation of a special plastin component, the so-called thrombocytoplastin factor. In addition, a process of adhesion and aggregation of the platelets, combined with morphological and functional changes in them, amounting altogether to the phenomenon of viscous metamorphosis, was not infrequently observed (Fig. 3b). Bordering on such platelets, precipitates of fibrin could be identified, although these could be formed quite separately from the platelets also.

A special feature of the ultrastructural changes in the pituicytes of the first and second types was a disturbance of lipid metabolism, as a result of which numerous lipid droplets accumulated in their cytoplasm.

After 3 days the general trend of the changes observed previously still continued. Individual neurosecretory endings were converted into multilamellar structures (Fig. 3c). The quantity of lipid material in the pituicytes was increased many times over and it occupied the whole of the cytoplasm and displaced the organelles to the periphery (Fig. 3d). Dystrophic and degenerative changes in the axons and glia were sometimes irreversible in character.

In response to injection of tetanus toxin a unique response of the HHNS thus develops; activation of the system is evidently explained by injection of the heterologous protein, for a similar effect is given by injection of the inactivated toxin. Changes in the clotting system of the blood, lipid metabolism, and vascular permeability are noteworthy.

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EFFECT OF CYTOTOXIC IMMUNE SERA ON FORMATION OF FOCI OF HEMATOPOIESIS (MICROCOLONIES) IN THE MOUSE SPLEEN

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UDC 615.365.015.4:612.411

Injection of antierythrocytic or antithrombocytic immune serum into unirradiated mice stimulates the formation of foci of hematopoiesis (microcolonies) in the spleen. It is suggested that the formation of microcolonies by stem cells is not specific for the irradiated organism.

KEY WORDS: hematopoiesis; cytotoxic sera.

A few days after irradiation of mice foci of hematopoiesis, or microcolonies, are formed in certain parts of the spleen [3, 4]. Whether this phenomenon is characteristic of irradiated animals only or not is an interesting question. There are data in the literature [1, 2] to show that foci of myelopoiesis can be formed in the lymph nodes after injection of sarcolysin (phenylalanine mustard) and foci of myelo- and erythropoiesis in the liver after injection of cyclophosphamide. Microcolony formation in hematopoietic organs, including the spleen, is evidently connected with an acute deficiency of blood cells in the body, with the consequent activation of hematopoiesis.

To verify this hypothesis, a deficiency of erythrocytes or platelets was induced in mice and hematopoiesis was subsequently activated by injection of the corresponding cytotoxic immune sera.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred albino mice and inbred CBA mice weighing 20-24 g. In series I the animals were given three or four intraperitoneal injections of cytotoxic immune serum, agglutinating erythrocytes (AES) or platelets (ATS) of mice in a titer of 1:8, in a dose of 0.15 ml (diluted 1:20) at intervals of 24 h. The serum was obtained after three injections of $5 \cdot 10^8$ mouse erythrocytes or platelets into rabbits. The first injection was given intradermally (with Freund's complete adjuvant) and subsequent injections intravenously at intervals of 4 weeks. The sera obtained on the 7th day were exhausted with serum,

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 4, pp. 493-495, April, 1978. Original article submitted May 30, 1977.

0007-4888/78/8504-0540$07.50