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EFFECT OF BUTYROXAN ON ULTRASTRUCTURAL CHANGES IN THE PITUITARY PRODUCED BY TETANUS TOXIN

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UDC 616-076.4:616.814.3-616.981.551.001.6

KEY WORDS: ultrastructure of the posterior lobe of the pituitary; tetanus toxin; butyroxan.

A previous investigation [3] showed that a unique response of the hypothalamo-hypophyseal neurosecretory system (HHNS) arises to injection of tetanus toxin (TT). During the first 5 h the output of neurosecretion was greatly activated and increased, and after 3 days the accumulation of neurosecretory material was associated with degenerative changes in the pituicytes and structural changes in the endothelium.

In the present investigation ultrastructural changes were studied in the caudal section of the HHNS at different times after injection of TT and administration of pharmacologic agents with predominantly central action. The α-adrenoblocker butyroxan used for this purpose, a Soviet drug which blocks mainly central adrenergic structures [1], statistically significantly increased the length of survival of experimental rats by almost 2 days after injection of 0.67 or 1.0 lethal dose of TT [2]. It can be tentatively suggested that the HHNS plays an important role in the mechanism of this effect.

EXPERIMENTAL METHOD

Experiments were carried out on 17 male albino rats weighing 260-280 g. A lethal dose of TT in 0.25 ml of 0.85% NaCl solution was injected intramuscularly into the left leg. An aqueous solution of butyroxan was injected intramuscularly into the right leg in a dose of 10 mg/kg body weight immediately after the injection of TT, and this was repeated daily until the animal died. Signs of local tetanus appeared in the rats after 36 h, followed by generalized ascending tetanus with spontaneous convulsions after 96-108 h and death of the animals on the 5th-6th day. Control animals received injections of butyroxan only, in the same dose.

Material for electron microscopy was taken 5 h and 3 days after injection of the toxin and butyroxan. Pieces of the posterior lobe of the pituitary were fixed in glutaraldehyde in phosphate buffer, postfixed in osmium tetroxide solution, dehydrated in acetone, and embedded in a mixture of Epon and Araldite and in Epon-812. Ultrathin sections cut on the LKB-8800 ultramicrotome were stained with lead citrate and uranyl acetate and studied in the JEM-100 electron microscope.

EXPERIMENTAL RESULTS

The ultrastructure of the posterior lobe of the pituitary in rats receiving physiological saline did not differ from that of the corresponding region of the brain in intact animals [3].

Only slight activation in the caudal section of HHNS was observed 5 h after injection of butyroxan. The number of fibers filled with elementary granules and empty vesicles, i.e.,

Central Research Laboratory, Rostov-on-Don Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 63-65, February, 1982. Original article submitted April 28, 1981.

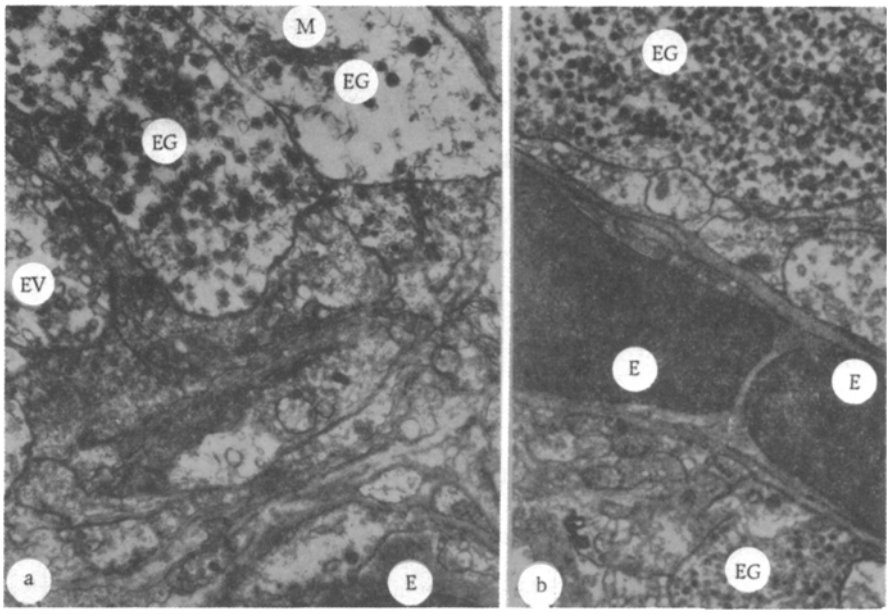

Fig. 1. Ultrastructure of posterior lobe of pituitary 5 h (a) and 3 days (b) after injection of butyroxan: a) axons containing elementary granules and empty vesicles. 7200 ×; b) capillary surrounded by neurosecretory fibers and filled with elementary granules. 4200 ×. EG) Elementary granules; EV) empty vesicles; M) mitochondria; E) erythrocytes.

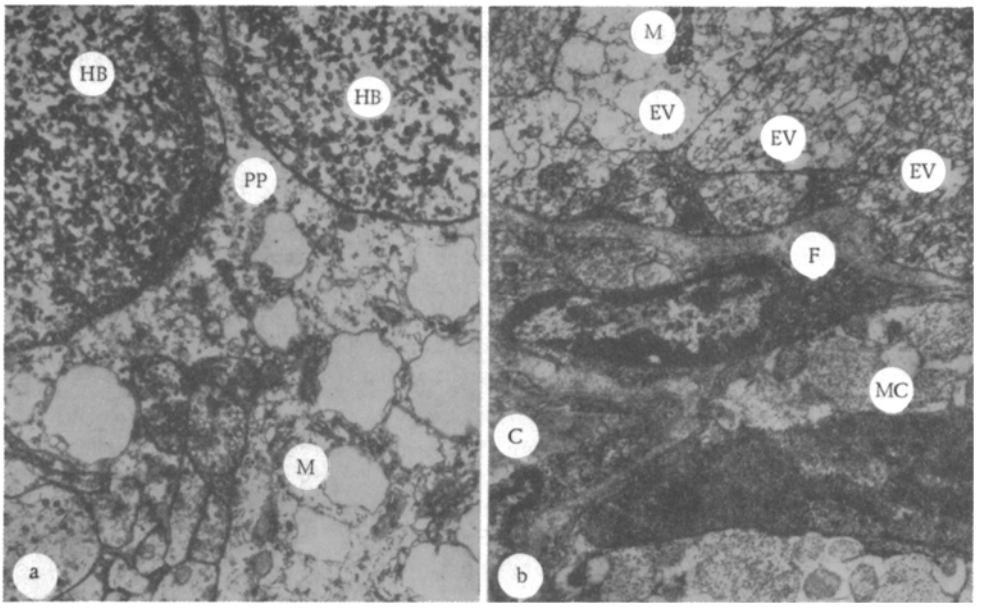

Fig. 2. Ultrastructure of posterior lobe of pituitary 5 h (a) and 3 days (b) after injection of TT and butyroxan: a) Herring's bodies with multiple elementary granules. 4200 ×; b) perivascular location of mast cell and neurosecretory fibers containing empty vesicles. 4200 ×. HB) Herring's bodies; PP) process of pituicyte; MC) mast cell; F) fibrocyte; C) capillary; EV) empty vesicles; M) mitochondria.

with the outer membranes of granules left after discharge of the neurosecretion, became roughly the same. There was no change in the number or location of the synaptic vesicles. Mitochondria only rarely appeared swollen, and more often they consisted of single, slightly elongated bodies with indistinctly outlined cristae (Fig. 1a). The Herring's bodies contained

a fair amount of neurosecretion, but aggregated and laminated bodies could be seen in cases when octapeptides had been discharged into the blood stream. The glial cells and capillaries were unchanged.

Activation of the terminal portion of the neurosecretory system had not yet appeared 3 days after injection of butyroxan. Most fibers and endings contained numerous elementary granules. Meanwhile the number of axons with empty vesicles and synaptic vesicles was reduced; the latter, moreover, were found more often among granules in large neurosecretory endings rather than in the zone of axovasal contacts which is traditional for them. The mitochondria in them were small, their membranes were stable, and the electron density of their matrix was high. Various osmiophilic inclusions were found in the Herring's bodies, reflecting degenerative changes in considerable areas of axoplasm. When local injury to the fibers was present, single multilamellar bodies or myelin figures were observed.

Capillaries of the posterior lobe of the pituitary showed structural changes corresponding to those observed during gradual accumulation of neurosecretory material and its reduced discharge into the blood stream. There was correspondingly a sharp decrease in width of the periendothelial space, pinocytotic vesicles in the endothelium became few in number, microvilli disappeared, and often fibers and terminals with a considerable quantity of neurosecretion could be seen around the capillaries (Fig. 1b).

Dilated cisterns of the endoplasmic reticulum and lamellar complex were seen in the astropituicytes, and numerous lipid drops and swollen mitochondria were present. The trend of the changes in the oligopituicytes was the same, but in addition secondary lysomes appeared in them.

Injection of butyroxan, with its strong central α-adrenoblocking activity, thus has a moderate activating action on the caudal portion of HHNS within a few hours of its injection. On the 3rd day accumulation of neurosecretory elementary granules was observed and the liberation of neurohormones was inhibited. Distinct activation of intracellular organelles was observed in both types of pituicytes.

After intramuscular injection of TT followed by butyroxan not only was the survival of the animals prolonged [2], but the function of the posterior lobe of the pituitary was modified. The first fact to which attention was drawn was that after 5 h the toxin virtually did not stimulate the discharge of neurosecretion. Moreover, under low power of the electron microscope fibers containing mainly granules could be seen. Synaptic vesicles were few in number and difficult to detect, and only solitary empty vesicles were present; the large terminals also were filled with neurosecretion (Fig. 2a). The mitochondria in the axons were elongated and had the same appearance as in rats receiving physiological saline. The ultrastructure of the angioarchitectonics had no special features. Changes were found in the glial cells in the astropituicytes only and were connected with accumulation of lipid drops.

Later, after 3 days, TT followed by butyroxan had an activating effect on the discharge of neurosecretion, which coincided in time with increased vascular permeability. The periendothelial space was widened, microvilli were formed on the luminal surface of the endothelial cells, and pinocytosis was intensified. The appearance of mast cells with a perivascular distribution deserves special attention (Fig. 2b). The granularity of their cytoplasm was a very characteristic feature. Sometimes instead of granules, large vacuoles of the corresponding size could be seen, whereas other granules contained a small amount of electron-dense material, on account of the utilization of biologically active substances (heparin, histamine, various enzymes, mucopolysaccharides, etc.). Neurosecretory fibers close to these vessels were filled only with empty synaptic vesicles (Fig. 2b). The mechanism of liberation of neurohormones evidently includes not only the usual method of their discharge into the blood stream with the participation of acetylcholine of the synaptic vesicles [5], but also another method — with the participation of histamine of the mast cells [4].

It is a noteworthy fact that under the influence of tetanus toxin, especially during the first few hours after its injection, pituicytes were activated, as shown by the increase in number and size of the lipid drops. Similar structural changes in the course of immunization with tetanus toxoid also were observed in Porton mice on the 28th and 100th days of the experiment, and at these times, moreover, glial cells were more than 4 times more numerous than in the control [8]. This phenomenon evidently reflects more intensive detoxication processes and is linked with increasing endocytosis by pituicytes during activation of the caudal section of HHNS. There are grounds for such a suggestion because in experiments with

horseradish peroxidase the enzyme was phagocytosed in large quantities by the glial cells of the posterior lobe of the pituitary during intensification of neurohypophyseal secretion in rats [9]. Similar changes arise in pituicytes after injection of certain psychotropic drugs [7]. As regards the liberation of neurosecretion, administration of an α-adrenoblocker followed by tetanus toxin evokes a two-wave reaction in the caudal portion of the HHNS in rats. The first wave is characterized by inhibition of neurosecretory activity, the second by mobilization of the contents of the granules into the capillaries.

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