ULTRASTRUCTURAL CHANGES IN THE ADRENAL MEDULLA AND CORTEX IN ENDOTOXIN SHOCK

É. A. Bardakhch'yan and Yu. G. Kirichenko

UDC 616.94-06:616-001.36]-092.9-07:616.45-091.8-076.4

KEY WORDS: endotoxin shock; chromaffin cells; adrenocorticocytes.

The need to study the pathogenesis of septic shock calls for the development of an experimental model of this condition, namely endotoxin shock. In such a model special attention is paid to the adrenals, activation of which in endotoxemia is accompanied by increased release of catecholamines and steroid hormones into the bloodstream [12]. In a series of investigations into the initial stage of endotoxic shock, the writers previously reported ultrastructural changes in cells of the adrenal medulla and cortex, and the characteristics of disturbance of vascular permeability of the blood-adrenal medulla and blood-adrenal cortex barriers [5, 6]. Meanwhile, the fact that endotoxin shock occurs in successive periods [8] suggest that it is possible to distinguish an intermediate stage, from 1.5-2 to 9 h in duration, when the structural disturbances become most marked.

This paper describes an electron-microscopic study of adrenal injuries 5 h after the development of endotoxemia.

EXPERIMENTAL METHOD

Endotoxin shock was produced in experiments on 20 mongrel dogs weighing 6-10 kg and five chinchilla rabbits weighing 1.8-3.2 kg by intravenous injection of typhoid endotoxin or *Escherichia coli* endotoxin in a dose of 5 mg/kg body weight, equivalent to LD_{80} [8]. The adrenal medulla and cortex were studied 5 h after injection of endotoxin (intermediate stage of shock). In control experiments (four dogs and two rabbits) the animals received an intravenous injection of the equivalent volume of physiological saline. The blood pressure was recorded with an ultrasonic pressure transducer by the direct method. All experimental animals were killed by intravenous injection of a lethal dose of pentobarbital. Material for electron microscopy (pieces of the adrenal medulla and cortex) was obtained immediately after sacrifice and was processed by the usual method. Ultrathin sections obtained on the LKB-8800 Ultrotome were stained with uranyl acetate and lead citrate and studied in the IEM-100S electron microscope.

EXPERIMENTAL RESULTS

At the height of the manifestations of the intermediate stage of endotoxin shock, i.e., after 5 h, the animals of the experimental group exhibited adynamia, vomiting, diarrhea and, sometimes, convulsions. Breathing was fast and irregular and the blood pressure fell to 20 mm Hg. Injection of physiological saline into the dogs and rabbits did not cause any such changes in them. On electron-microscopic analysis of the adrenal medulla and cortex of the control animals, the ultrastructure of the gland cells was found not to differ from that described in intact animals [3, 13].

Ultrastructural study of the chromaffin cells in the intermediate stage of endotoxin shock showed almost total disappearance of catecholamine granules, which were replaced by empty profiles. Considerable changes were observed in the nuclei. Their membranes were undulating and formed many invaginations, with organelles sunk into them, and the perinuclear space was often dilated. Marked intracellular edema was observed with disorganization of the organelles and reduced electron density. Cisterns of the endoplasmic reticulum were strongly dilated, and the lamellar complex was hypertrophied, with elements of it located not only peri-

Central Research Laboratory, Rostov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 97-100, July, 1986. Original article submitted July 18, 1985.

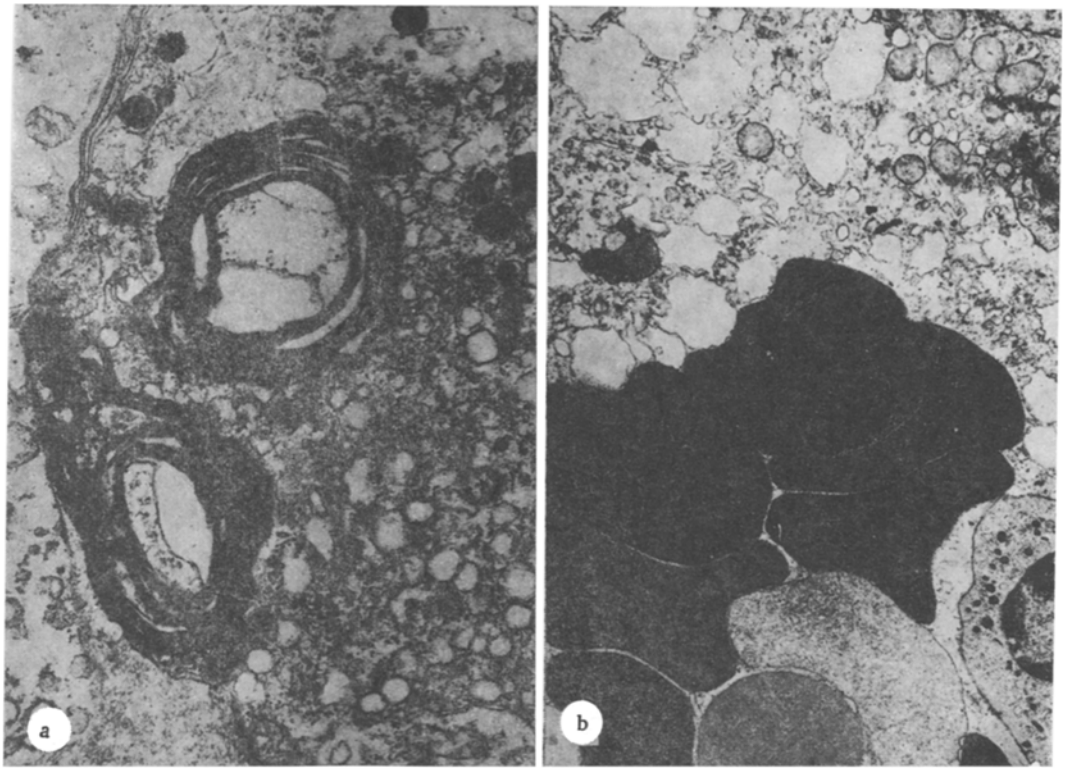

Fig. 1. Ultrastructural changes in adrenal medulla and cortex in intermediate stage of endotoxin shock: a) Myelin-like figures in cytoplasm of chromaffin cell (2100 ×); b) extravasated blood cells among adrenocorticocytes of zona fasciculata of adrenal cortex (7800 ×).

nuclearly, but also in other parts of the cell. The cristae in the mitochondria were reduced, their matrix translucent, and often only the membrane remained at the site of the organelle. Destruction of the mitochondrial membrane often ended with the formation of myelin-like structures (Fig. 1a). A considerable increase was noted in the number of primary and secondary lysosomes.

Ultrastructural changes in the chromaffin cells in the intermediate stage of endotoxin shock correlate with severe microcirculatory disturbances leading to intravascular coagulation and hemorrhages, and with changes in the innervation system, where signs of irreversible degeneration are found. These lesions, which lie at the basis of disturbance of the synthetic, secretory, and trophic functions of the medulla, indicate that stress enhances the powers of adaptation of the cells, and most of them are in the stage of exhaustion and death.

In the intermediate stage of endotoxic shock parallel structural disturbances were found in cells of the zona fasciculata and zona reticularis of the adrenal cortex, so that their description can be combined. During the electron-microscopic investigation of the adrenocorticocytes attention was drawn primarily to massive hemorrhages into the parenchyma, which were particularly numerous in the zona fasciculata (Fig. 1b). Extravasation mainly affected erythrocytes and leukocytes, although platelets also were found.

Changes in the structure of the organelles of the adrenocorticocytes 5 h after injection of endotoxin were more marked and more widespread than in the initial stage of shock. The number of mitochondria was increased and most of them were in close contact with lipid drops. Tears were observed in the mitochondrial membranes and their matrix communicated freely with the cell cytoplasm. The inner membrane often formed invaginations and vacuoles, and organelles were transformed into myelin figures. Circular inclusions were recorded in submitochondria (Fig. 2a), and were distinguished by high electron density. Their functional significance was not clear, although it is considered that accumulation of osmiophilic material of this kind, evidently lipid in nature, is a sign of destruction of mitochondria and of death of the cell as a whole [7].

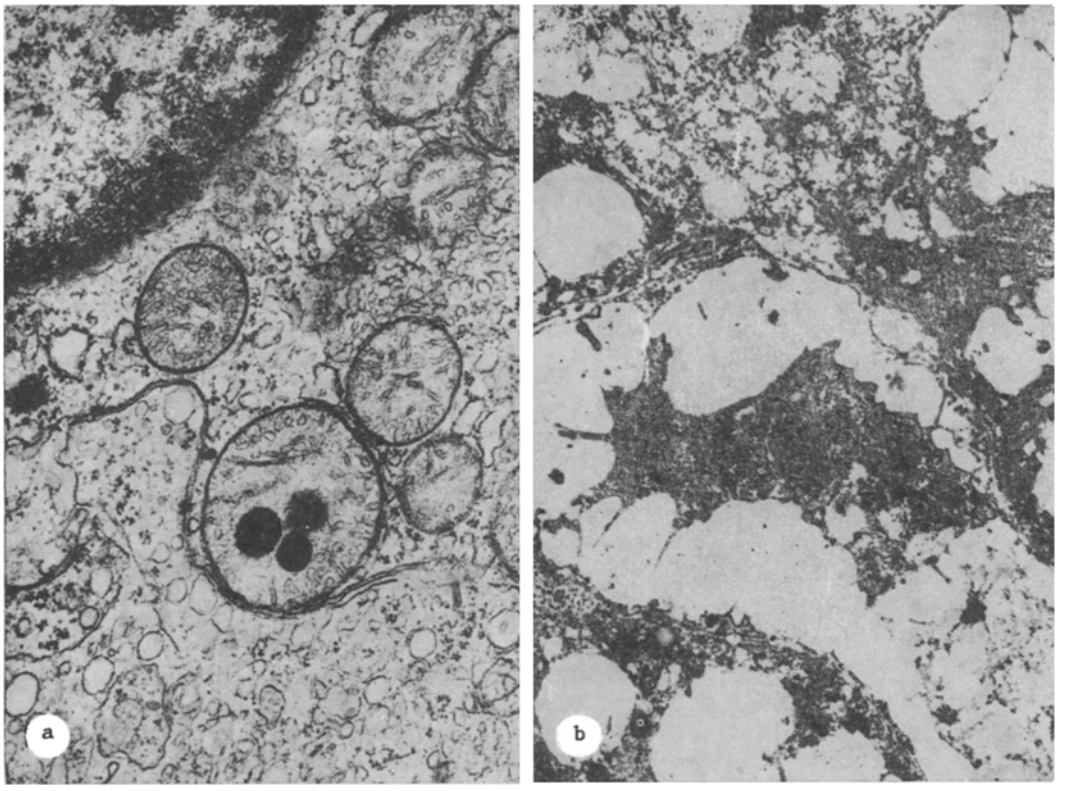

Fig. 2. Changes in adrenocorticocytes in intermediate stage of endotoxin shock: a) Electron-dense inclusions in mitochondria of adrenocorticocyte in zona reticularis (49,000 ×); b) necrotically changed adrenocorticocytes (8500 ×).

However, in most adrenocorticocytes concentration of lipids, pycnosis of nuclei, con-ensed (de-energized) forms of mitochondria, fragmented membranes of the endoplasmic reticulum, and few ribosomes, lying freely in the cytoplasm, were recorded. In other words, what was taking place was disadaptation of the cells followed by their death (Fig. 2b).

Decompensation of the adrenal cortical cells in endotoxemia can evidently be explained not only by hyperfunction in the gland [9]. As was shown previously, an important pathogenetic role in endotoxemia is played by the formation of a thrombohemorrhagic syndrome in the cortex, based on a sharp increase in permeability of the blood-adrenal cortex barrier [4]. These ultrastructural changes, on the one hand, are due to a change in the type of secretion of steroid hormones, the switch to the so-called "emergency holocrinia," and on the other hand, they are linked with the modifying action of the endotoxin and of biologically active substances on the vessel wall [1, 2, 10, 11]. As a result, extensive perforations arise in the endothelial lining of the cortical capillaries, resulting in hemorrhages and thrombosis. Consequently, microcirculatory disturbances are a key factor in the dystrophic changes taking place in adrenocortical cells, terminating in total necrosis of the parenchyma. The vascular phenomena of endotoxin are probably potentiated in target organs that are in a hypermetabolic state, and this explains the selective damage to the adrenal cortex in endotoxemia.

LITERATURE CITED

1. É. A. Bardakhch'yan and Yu. G. Kirichenko, Patol. Fiziol., No. 1, 79 (1983).
2. É. A. Bardakhch'yan and E. I. Pal'chikova, Byull. Éksp. Biol. Med., No. 2, 185 (1984).
3. V. M. Gordienko and V. G. Kozyritskii, Ultrastructure of Glands of the Endocrine System [in Russian], Kiev (1978).
4. N. F. Kan'shina, Arkh. Patol., No. 5, 71 (1980).
5. Yu. G. Kirichenko, B. A. Saakov, and É. A. Bardakhch'yan, Byull. Éksp. Biol. Med., No. 10, 500 (1980).
6. B. A. Saakov, Yu. G. Kirichenko, and É. A. Bardakhch'yan, Folia Morphol., 31, 225 (1983).
7. V. N. Shvalev, A. A. Sosunov, K. L. Mar'yan, and A. Yu. Anikin, Arkh. Anat., No. 1, 50 (1984).

8. J. J. Coalson, H. K. Woodruff, L. I. Greenfield, et al., Surg. Gynec. Obstet., 135, 908 (1972).
9. B. J. Levin and L. E. Cluff, J. Exp. Med., 121, 247 (1965).
10. F. L. Mandache, D. Mateescu, V. Ginroiu, et al., Rev. Roum. Méd. Ser. Endocrinol., 17, 79 (1979).
11. C. Hittermayer, U. N. Riede, and W. Sandritter, Pathol. Res. Pract., 165, 287 (1979).
12. P. M. Naik, V. M. Dadkar, and U. K. Sheth, Indian J. Med., 68, 353 (1978).
13. I. Rhodin, J. Ultrastruct. Res., 34, 23 (1971).

PARAMEMBRANOUS NEUROFILAMENTOUS STRUCTURES OF CEREBRAL CORTICAL SYNAPSES DURING ISCHEMIA AND THE EARLY POSTISCHEMIC PERIOD

V. V. Semchenko and S. S. Stepanov

UDC 616.831-005.4-092.9-06:616.831.3-018.82

KEY WORDS: cerebral cortex, synapses, ischemia, postischemic period.

Dense projections (DP) of the presynaptic grid (PG), the substance of the synaptic space, and the postsynaptic condensation (PC) constitute the paramembranous neurofilamentous system of subsynaptic units (SSU) of interneuronal junctions and maintain the integrative function of the brain [7, 9, 11]. Being highly labile formations, the structural components of SSU undergo marked changes during embryonic and postnatal development and during exposure to various factors [3, 8, 10]. It has been shown that in total ischemia and in the period of recirculation, interneuronal integration is disturbed [2]. A definite role in the development of this process may perhaps be played by changes in SSU of interneuronal connections.

The aim of this investigation was to study structural changes in SSU of interneuronal junctions in the cerebral cortex during short-term total ischemia and in the early postischemic period.

EXPERIMENTAL PERIOD

Experiments were carried out on 16 male albino rats weighing 190-210 g under ether anesthesia. Total ischemia and the postischemic period were simulated by inducing clinical death for 5 min from blood loss, followed by resuscitation [6]. The brain was fixed by perfusion with a mixture of 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.4) with sucrose at the end of ischemia, and after 5, 30, and 90 min of the postischemic period. Oriented pieces of sensomotor cortex were stained while being taken through the 100% ethanol stage in a 5% solution of phosphotungstic acid (PTA) and embedded in plane-parallel order in Araldite. Tangential ultrathin sections were cut through the molecular layer of the neocortex and 15 random fields of neuropil were photographed from one animal under magnification of 15,000 of the ÉVM-100LM microscope. The total number of PTA-positive junctions and the number of indefinite and definite synapses were counted per 100 μ^2 of neuropil from negative under a final magnification of 30,000×. Among the definite synapses, asymmetrical junctions of the A, B, and C type and symmetrical junctions of the D type were distinguished by the degree of definiteness of shape of the PG and the height of DP [8]. The data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The total number of PTA-positive junctions and the number of indefinite and definite symmetrical synapses during ischemia and in the early postischemic period did not differ from their number in the cerebral cortex of the control animals (Table 1). The total number of definite junctions was reduced only after recirculation for 90 min, due to reduction of the

Department of Histology and Central Research Laboratory, Omsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 100-102, July, 1986. Original article submitted June 12, 1985.

0007-4888/86/0007-0991$12.50