In response to a sharp intensification of cardiac activity, such as takes place at the beginning of physical exertion, activation of metabolism is observed in the subepicardial layers of the myocardium, which evidently play the leading role in the maintenance of cardiac activity in this situation.

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CHANGES IN ULTRASTRUCTURAL SYNAPTOARCHITECTONICS CAUSED BY ENDOTOXIN

É. A. Bardakhch'yan and B. A. Saakov

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The ultrastructure of the synapses of the sensomotor cortex in dogs was studied in response to intravenous injection of typhoid endotoxin. The most marked changes were observed in the dendritic apparatus of the neurons. Activation in cortical structures was associated with an increase in the number of functioning synapses and reorganization of interneuronal connections. The extent of the contacting membranes and the number of synaptic vesicles were increased, and the contents of the dense-core vesicles were liberated. Depression was associated with degenerative changes in the synapses.

KEY WORDS: *endotoxin; neurons; synapses.*

Intravenous injection of filtrates of intestinal microorganisms and of typhoid endotoxin lead within the space of 1 h to the appearance of a powerful discharge of impulses in some nerve fibers with an increase in the amplitude and frequency of the spikes [2]. In the sensomotor cortex a considerable increase of bioelectrical activity is observed and precedes the development of characteristic morphological changes [3, 4]. However, a closer electron-microscopic analysis revealed irreversible changes in some internal organs (lungs, liver, kidney) as early as 30-60 min after injection of endotoxin [5, 6]. It was therefore interesting to study the ultrastructural changes in the CNS responsible for the development of electrophysiological phenomena observed previously.

This paper describes the results of electron-microscopic observations on the state of nerve cells and synapses in the sensomotor cortex of dogs after injection of endotoxin.

EXPERIMENTAL METHOD

Experiments were carried out on 10 adult mongrel dogs weighing 8-17 kg into which *Salmonella typhi* endotoxin (lipopolysaccharide) was injected intravenously in a dose of 5 mg/kg. Physiological saline was injected into three control animals. The animals were decapitated 1 h after the injection and pieces of the sensomotor cortex were fixed in glutaraldehyde, made up in phosphate buffer, and postfixed in OsO_4 solution. The material was

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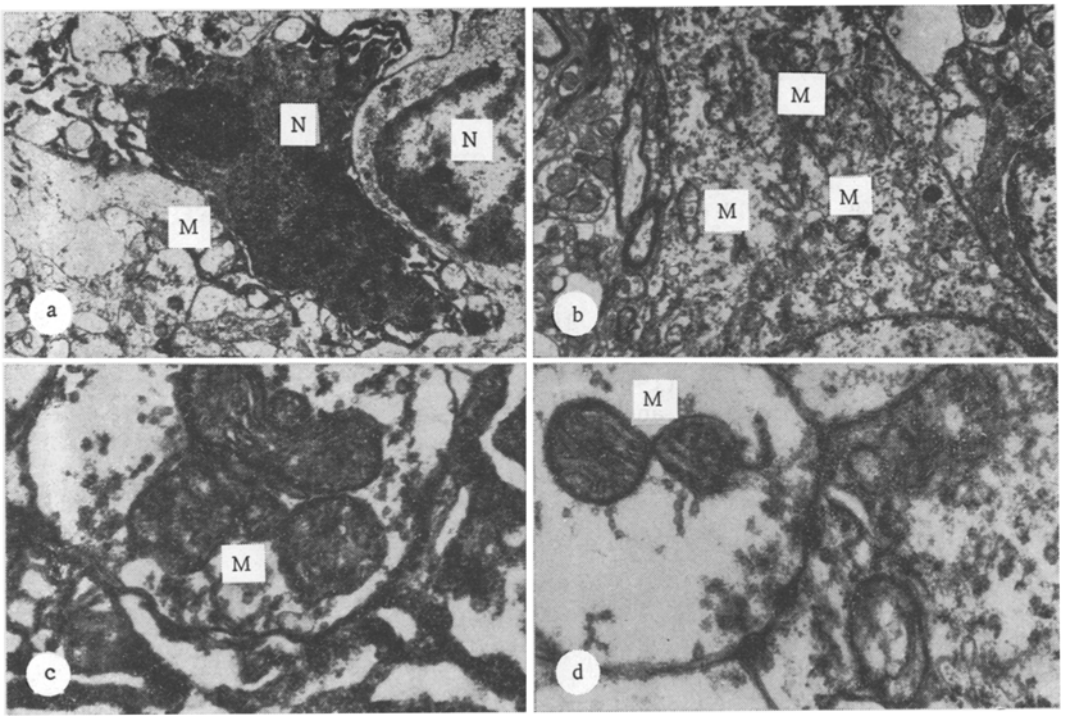

Fig. 1. Ultrastructure of sensomotor cortex after injection of endotoxin: a) pycnomorphic neuron (15,000×); b) pale neuron with swollen mitochondria (17,000×); c) diffuse arrangement of numerous synaptic vesicles in axoplasm (40,000×); d) sharp increase in number of synaptic vesicles (40,000×). N) Nucleus; M) mitochondrion.

dehydrated in acetone and embedded in Epon. Ultrathin sections stained with uranyl acetate and lead citrate were examined in the UEMV-100K electron microscope.

EXPERIMENTAL RESULTS

After intravenous injection of endotoxin the pale and dark nerve cells were found to be highly sensitive, but the former were more resistant to the harmful action of the lipopolysaccharides. The mitochondria in the dark neurons were swollen, their matrix was translucent, and the cristae were partly or totally destroyed. The karyoplasm had high electron density and the nucleoli were usually displaced toward one pole of the nucleus (Fig. 1a). The pale cells appeared relatively unchanged, although swollen mitochondria could also be found in their cytoplasm (Fig. 1b). Axosomatic contacts on the dark neurons contained many synaptic vesicles, but the zones of the synapses were palely stained and the synaptic space could not always be distinguished (Fig. 1c). Similar synaptic boutons on pale cells were characterized by a sharp increase in the mumber of vesicles, although other features of membrane specialization were fairly clearly defined (Fig. 1d). In both cases the absence of typical accumulations of synaptic vesicles in the zone of liberation of the mediator was conspicuous. An increase in the synaptic vesicles in the presynaptic zone were observed in axodendritic synapses (Fig. 2a). Axon terminals often contained vesicles with an electron-dense core (Fig. 2b), evidence of the different character of mediation in the nerve fiber. Meanwhile the number of synapses located on dendritic spines and the number of active sites on contacting membranes were increased (Fig. 2c). Evidence of increased "informativeness" (to use Bogolepov's terminology [8]) in this region was the presence of contacts between one spine and two axons simultaneously (Fig. 2d). Such convergence of nervous impulses must certainly indicate increased functional activity of these synapses.

Meanwhile degenerative changes could be observed, in the form of a marked increase in density of the presynaptic membrane, increased osmiophilia of the axoplasm, and the appearance of structures in it consisting of small vesicles surrounded by a membrane (Fig. 2e). Sometimes destruction of the submicroscopic components of the synapses could lead to the appearance of myelin figures (Fig. 2f). Larger vesicles, which had lost their osmiophilic contents, could be seen in the same place. These are known to be carriers of catecholamines and to empty in response to the action of various stimuli [15].

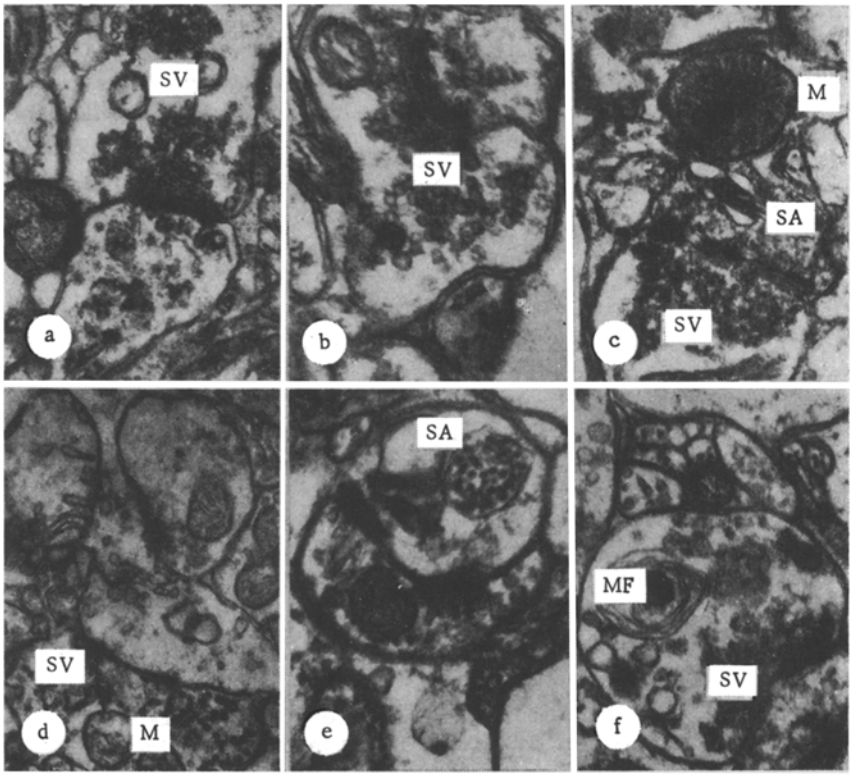

Fig. 2. Ultrastructure of synapses after injection of endotoxin: a) increase in extent of synaptic membranes and concentration of synaptic vesicles in presynaptic zone (55,000×); b) axodendritic synapse; axoplasm contains vesicle with dense core (arrow; 68,000×); c) axospinous synapse; increase in number of active sites (49,000×); d) one spine makes simultaneous contact with two axons (33,000×); e) absence of synaptic space, dilatation of cisterns of spinous apparatus (42,000×); f) appearance of myelin figure and liberation of contents of vesicles with dense core (arrows; 49,000×). SV) Synaptic vesicle; SA) spinous apparatus; M) mitochondrion; MF) myelin figure.

Changes in the synaptic boutons in response to injection of endotoxin are thus characterized most frequently by the development of degenerative changes of pale type, although in some of them more profound disturbances characteristic of degeneration of the dark type also developed.

In control experiments the ultrastructure of the sensomotor cortex was unchanged and, for practical purposes, appeared to be the same as in other mammals in the absence of any intervention [8].

As was stated previously [4], bioelectrical activity after injection of endotoxin changed in two directions: Either increased activity persisted for a long time or in the course of a few hours periods of activation alternated with depression. How can these electrophysiological phenomena be explained? Disturbances of both types of electrogenesis were probably connected with structural and functional changes in the nerve cells and with the state of the synaptic apparatuses. Axodendritic forms of connection are known to play a leading role in the transmission of nervous impulses in the cortex. During an increase in the functional capacity of the somatosensory cortex and intensification of sensory afferentation the number of spines has been shown to increase [11-13].

It is a noteworthy fact that the increase in the number of axodendritic connections correlates with changes in the intracellular organization of the nerve cells. This is evidently connected with increased functional activity of neuronal ensembles in the period of maximal manifestation of the stimulating action of the endotoxin. Under these circumstances an increase in the number of axon terminals with active zones was found electron-microscopically and synapses with two or three enlargements of their synaptic membranes were found more frequently. Another feature reflecting increased functional activity of the synapses during

excitation by endotoxin was a tendency toward concentration of synaptic vesicles near the presynaptic membrane. This redistribution of vesicles is evidence of a sharp increase in presynaptic afferentation [7]. Similar changes have been found in synapses of the frog tectum mesencephali after injection of picrotoxin, which gives rise to the appearance of prolonged paroxysmal discharges on the EEG [10].

The large vesicles observed in these experiments were evidently membranes of larger vesicles with a hard core left behind after discharging their biologically active substances (catecholamines). Similar structures have been found after injection of reserpine, which liberates monoamines [14], in alcohol intoxication [1], and after administration of amphetamine [9].

Whereas activation in the sensomotor cortex can be explained by an increase in the number of functioning synapses, and by the recruiting of additional and the reorganization of pre-existing interneuronal connections, the periods of depression are evidently associated with the development of degenerative changes in the synapses. Functional overstrain, caused by prolonged stimulation by endotoxin circulating in the blood, leads to structural disturbances of the nerve cells and, what is particularly important, to damage of the dendritic apparatus of the neurons. An increase in the afferent flow affects the dendritic spines — the specific postsynaptic receptive structures. A marked expansion of the components of the spinous apparatus takes place in the spines, or multivesicular bodies and myelin figures appear.

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