ULTRASTRUCTURAL MECHANISMS OF THE EFFECT OF MYELOPEPTIDES ON SOME BRAIN FORMATIONS IN RATS RECEIVING ENDOTOXIN, AND THE ROLE OF RECEPTOR-MEDIATED ENDOCYTOSIS

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Involvement of structures of the CNS in the response to intravenous injection of endotoxin was demonstrated previously [2, 3, 10]. Evidently an important role in this process is played by lipopolysaccharide (LPS) molecules, which interact with various receptor formations, including receptors of nerve cells [1]. It can therefore be postulated that blockade of neuronal receptors will abolish the structural changes produced by LPC. Myelopeptides (MP) are known to be regulatory mediators of the bone marrow [9]. Besides their immunocorrective action they also possess endorphin-like properties, binding with opiate receptors of neurons and lymphocytes [8].

The aim of the present investigation was to undertake an ultrastructural study of the effect of MP on some brain formations in animals receiving endotoxin.

EXPERIMENTAL METHODS

Experiments were carried out on 20 male albino rats weighing 150-180 g, divided into two groups. The animals of group 1 were given an injection of 2 mg/100 g body weight of LPS of E. coli, equivalent to LD_{90} [12]. Animals of group 2 received an intramuscular injection of MP (2 mg/100 g) 24 h before the injection of LPS. For each main group, there was a corresponding control. Material for electron-microscopic investigation (fragments of sensomotor cortex and of the choroid plexuses of the lateral ventricles) taken 5 h after the injection of LPS, was fixed with glutaraldehyde–osmic acid fixative, made up in the usual way, and embedded in Epon 812. Ultrathin sections, cut on an LKB 8800 ultramicrotome, were stained with uranyl acetate and lead citrate and examined in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

Multivesicular bodies, secondary lysosomes, and numerous coated vesicles (CV) were found 5 h after intravenous injection of LPS in the cytoplasm of the sensomotor cortical neurons (Fig. 1a). CV are known to be not characteristic of the ultrastructure of the neuronal cytoplasm [7], and their appearance is associated with specific uptake of various ligands due to receptor-mediated endocytosis (RME) [13]. As a rule, subsurface cisterns are discovered. A greatly widened rough endoplasmic reticulum, swollen mitochondria, and hyperplastic membrane of the lamellar complex also were visible (Fig. 1a). Changes in the nucleus and ultrastructures of the cytoplasm fit into the pattern characteristic of severe forms of ischemia and known as ischemic-homogenizing disease [5]. Ultrastructural analysis of the choroid plexuses at these same times revealed thrombosis of the small vessels and universal sludge in the large vessels (Fig. 1b).

Changes in the epithelial cells consisted essentially of the appearance of signs of cloudy-swelling or hydropic degeneration (Fig. 1c). Structural changes in the intracellular organelles, evidence of intensification of the secretory function of the epitheliocytes, leading to the formation of numerous vesicular structures, containing a substance resembling

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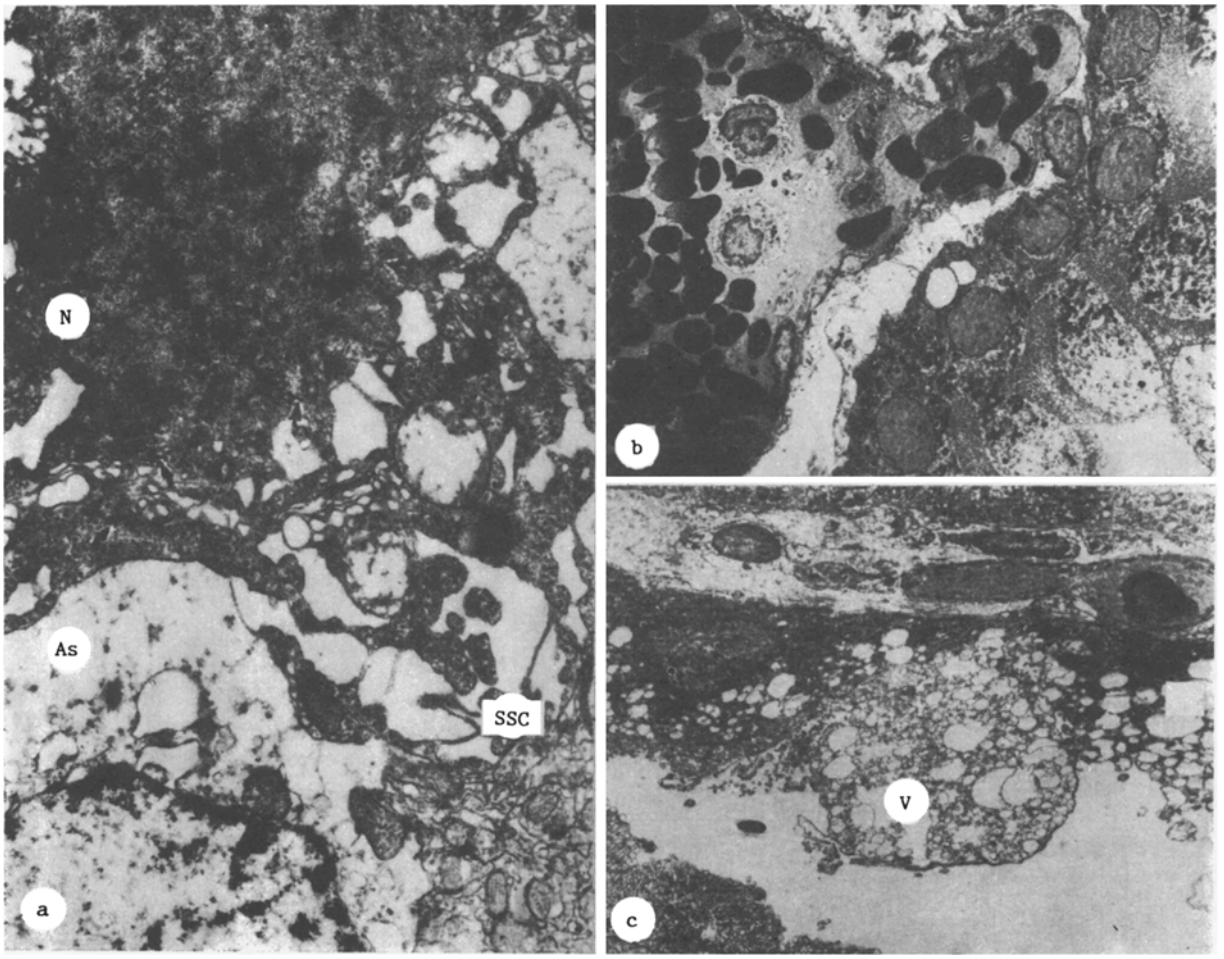

Fig. 1. Intravenous injection of LPS. a) Sensomotor cortical neuron with signs of ischemic-homogeneizing disease (arrows indicate coated vesicles). 11,000×; b) Stasis in vessels of choroid plexus, marked edema of stromal cells, formation of vesicular structures in epitheliocytes. 1400×; c) Vacuolar degeneration of epitheliocytes in choroid plexus. 2500×. As) astrocyte, N) neuron, SSC) subsurface cistern, V) vacuole.

plasma, was noted. Single necrotic epitheliocytes were desquamated into the lumen of the ventricle.

Injuries common to the blood–brain and blood–CSF barriers are increased vascular permeability, destruction of the endothelium, diapedetic hemorrhages, and thrombosis of the vessels. In other words, signs of a thrombohemorrhagic syndrome were recorded in the brain, just as in other organs [6, 11], i.e., hemorrhages and thrombosis coexist simultaneously.

In control experiments with intravenous injection of physiological saline, no ultrastructural changes were present in the functional elements of the sensomotor cortex and choroid plexuses. In the cytoplasm of the neurons there were only single CV or none at all.

The state of the ultrastructures of the sensomotor cortex and choroid plexuses 5 h after intravenous injection of LPS indicates that interaction of its molecules with membrane receptors leads to toxic damage to the cells, giving rise to irreversible changes in neurons and epitheliocytes [2, 10]. In addition, injuries to the vascular barriers of the brain took place [2]. In the light of the known ability of MP to interact with neuronal receptors [8, 9], it is interesting to study its effect on the ultrastructure of the brain in conjunction with LPS.

Intramuscular injection of MP alone gave rise to massive formation of CV in nerve cells in the region of the lamellar complex after 24 h, together with single CV, lying freely in the cytoplasm (Fig. 2). Otherwise the ultrastructure of the sensomotor cortex was normal. An ultrastructural study of the choroid plexuses likewise revealed no changes in response to injection of MP.

Fig. 2. Coated vesicles in sensomotor cortical neurons following intramuscular injection of MP (arrows). 56,000×.

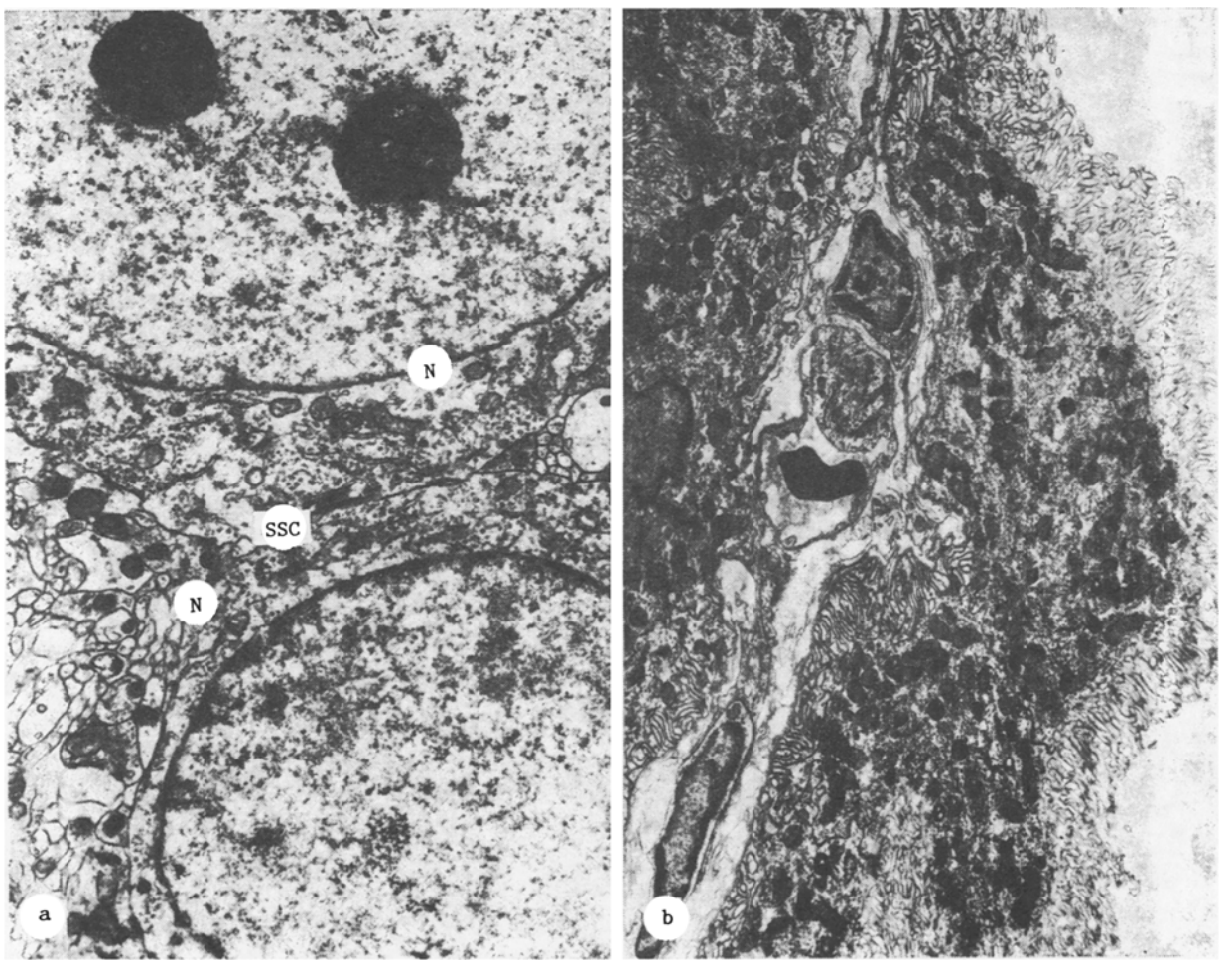

Fig. 3. Intravenous injection of LPS against the background of MP. a) Sensomotor cortical nerve cells, 9000×; b) villus of choroid plexus, 3000×. N) Neuron, SSC) subsurface cistern.

Thus 24 h after intramuscular injection of MP its molecules passed through the blood–brain barrier and interacted with receptors on the surface of the nerve cells, evidently making contact for other ligands with them impossible.

In the experiments with LPS and MP, special attention must be paid to preservation of the neurons (Fig. 3a), in which no damage was observed to the protein-synthesizing and energy-producing systems, or activation of proteolysis.

It has to be pointed out that CV, as before, were present in large numbers in the cytoplasm of the cells. Gliocytes and synapses also appeared intact, by contrast with the experiments with LPS, where degeneration of the dark type, which is irreversible, was observed in the synapses [4].

A study of the choroid plexuses showed that MP prevents thrombus formation induced by LPS in vessels of varied caliber (Fig. 3b), probably on account of a marked decrease in permeability of the blood–CSF barrier. Edema of the stroma was less marked and the decrease in the number of vesicular spheres was evidence of delay of CSF production against the background of MP. No damaged leukocytes were found, such as are frequently encountered in endotoxemia. At the same time, it must be pointed out that MP evidently has no special effect on fibrin formation or on sludging of the blood cells.

The absence of signs of vacuolar degeneration of the epitheliocytes, as well as their desquamation and destruction, are convincing evidence of the protective action of MP on these structures.

MP thus blocks neuronal receptors and enters into competitive relations with LPS molecules, displacing them from binding sites, preventing their internalization into the cytoplasm, and abolishing the toxic effect of LPS. To judge from the state of the blood–brain and blood–CSF barriers, their permeability was greatly reduced. MP also abolishes many ultrastructural disturbances arising in endotoxemia, but has no action on the clotting system of the blood.

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