Effect of Mesodiencephalic Modulation on Small Intestinal Mucosa after Massive Blood Loss in Rats

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Effect of mesodiencephalic modulation on small intestinal mucosa was studied in rats subjected to massive blood loss. Massive blood loss induced structural changes in intestinal epitheliocytes typical of tissue hypoxia: intraintestinal edema, mitochondrial alterations, and microcirculation disturbances. A single session of mesodiencephalic stimulation carried out 60 min after massive blood loss produced a pronounced adaptive effect, restored disturbed structure of the intestinal villi, and induced compensatory hypertrophy of the mitochondria. This suggests that mesodiencephalic modulation promotes normalization of intracellular energy metabolism, improves the absorption capacity of bordered enterocytes, and restores the disturbed intestinal barrier.

Key Words: *small intestine; mucosa; intestinal barrier; blood loss; mesodiencephalic modulation*

Acute disadaptation is accompanied by a pronounced activation of regulatory mechanisms in humans and animals. In many cases, the difficulties in the treatment and functional recovery during acute disadaptation are related to the fact that the general nonspecific background does not prevent the development of the disease.

Supposedly, the leading role in the development of adaptive reactions is played by diencephalic structures [1-3]. Mesodiencephalic modulation (MDM), a method directly affecting these cerebral subdivisions, is aimed at activation of the adaptive mechanisms by specific electric stimulation. As an electrical method of activation of the adaptive mechanisms, MDM has some advances in the clinical and rehabilitation practice [8]. The parameters of electrical stimulation (amplitude and duration of electrical pulses, repetition rate and duration of the pulse train, electrode positioning, and other factors) are so chosen that the effect of MDM is focused on subcortical structures, while its effect on the cortex is minimized [4-7,11].

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According to P. K. Anokhin theory of sensory and biological convergence in neurons [1], an important role in the adaptation to extreme conditions in humans is played by the central humoral systems. Bioactive substances (neuropeptides) are synthesized in the diencephalic structures of the brain. Their synthetic analogs (dalargin) are used to treat gastrointestinal diseases [10]. MDM activates synthesis of neuropeptides, while repeated MDM sessions increased their basal plasma level.

Not only overt pathological states, but also their urgent manifestations can be considered from the viewpoint of adaptation and self-regulation concept developed by I. M. Sechenov and I. P. Pavlov. Experiments on animals with urgent pathology characterized by most pronounced disadaptation make it possible to reveal the mechanisms of action and improve the rehabilitation methods used to treat the therapeutic and surgery patients.

Massive blood loss is accompanied by rapid ischemic damage to the intestine and disturbance in the intestinal barrier, which dictates the usage of cytoprotectors in the early posthemorrhagic period [12,13].

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However, even timely administration of pharmacological actoprotectors, the substances characterized by a potent stimulating effect on the metabolism of enterocytes, is inefficient in many cases [14]. Our aim was to study the effect of MDM on the small intestinal mucosa under conditions of acute disadaptation provoked by massive blood loss.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (250-300 g) anesthetized with 5% hexenal (0.3 ml/100 g, intraperitoneally). In all animals of the experimental and control groups (n=14), two plate electrodes were subcutaneously implanted in the frontal area (anode) and occipital fossa (cathode). A catheter was inserted into the vena cava inferior. The wires from head electrodes were passed through a subcutaneous tunnel and fixed to a connector attached to the interscapular skin. During the experiments, this connector was connected to wires from an MDM-101 transcranial electrical stimulator. The vascular catheter was passed through the soft tissues in the pelvic area and brought out under the tail skin at a distance 5 cm from the tail tip.

The rats were tied in individual cages [9]. The tie did not restrict motion within the cage and made it possible to manipulate with electrodes and angiocatheter and to perform MDM without provoking stress reactions.

The experiments were started on postoperation day 10. Acute blood loss was modeled by draining venous blood (3.5% body weight during 25 min on the average) through a syringe connected to the intravenous catheter. In special experiments, blood loss corresponding to 3.5% body weight was critical, and even a slight increase of this threshold value resulted in a rapid fatal outcome.

In the experimental group, a 30-min MDM session (0.5 mA) was carried out 1 h after blood loss. Four types of direct and alternating currents were applied. The frequency of alternating current varied from 60 to 90 Hz. The control animals were not subjected to MDM after blood loss, although the manipulation modeling the connection of the wires to MDM stimulator were performed.

All rats were sacrificed 4 h after blood loss by air embolism (via the implanted angiocatheter).

For histological examination and electron microscopy specimens of small intestinal mucosa were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Araldite. Semithin sections were stained with methylene blue and azure, ultrathin sections were contrasted with lead nitrate and examined under an EVM-100 B electron microscope.

RESULTS

In control rats, histological examination of the small intestinal mucosa revealed some flattened intestinal villi, smoothed contours of the lining epithelium, and intact clear-cut contours of the brush border. The bordered epitheliocytes had high cylindrical shape, centrally located oval nucleus with diffuse chromatin and slightly loosened cytoplasm predominantly in basal enterocytes. The lamina propria of the intestinal villi was characterized by moderate edema and disintegration of the cellular elements. The subepithelial capillaries and venules were dilated and contained large erythrocyte aggregates with decreased tinctorial properties, which attested to a low hemoglobin content. Some capillaries contained no blood cells (Fig. 1, *a*, *b*).

Electron microscopy of bordered enterocytes (BE) revealed edema of the basal cytoplasm, organelle disintegration, and accumulation of mitochondria in the perinuclear zones (Fig. 1, *c*). The mitochondria of BE localized near the Golgi apparatus and apical cytoplasm was swollen. They had round shape and partially vacuolated or lysed crystae. Light mitochondrial matrix contained no dense osmiophilic inclusions (calcium granules). The microvilli of the brush border retained the standard elongated shape, and there were no disturbances in the structure of the terminal reticulum (Fig. 1, *d*, *e*).

The structural changes in BE of the small intestinal mucosa after blood loss attest to tissue hypoxia manifested by intracellular edema, changes in the mitochondria supplying the energy for enzyme synthesis and intracellular transport of nutrients. The tissue hypoxia was caused by microcirculatory disturbances in the intestinal villi. During hemorrhagic shock, the tissue hypoxia was caused and maintained by blood accumulation in the intestine, decelerated blood flow, and aggregated bloodstream.

The specimens of the experimental group demonstrated structural recovery of the absorptive epithelium and blood capillaries. The intestinal villi retained their specific elongated shape, folded epithelium with high cylindrical BE and homogeneous distribution of the organelles in BE cytoplasm. There were no signs of stromal edema and dilation of blood vessels. Subepithelial capillaries and venules were characterized by moderate plethora and contained small erythrocyte aggregates or individual erythrocytes with normal hemoglobin content (Fig. 2, a).

Electron microscopy revealed no signs of intracellular edema. Round and oval mitochondria with numerous clear-cut crystae and dense osmiophilic inclusions were uniformly distributed in the BE cytoplasm up to the basal membrane (Fig. 2, *b*). Smooth collapsed and moderately widened cisterns of the Golgi

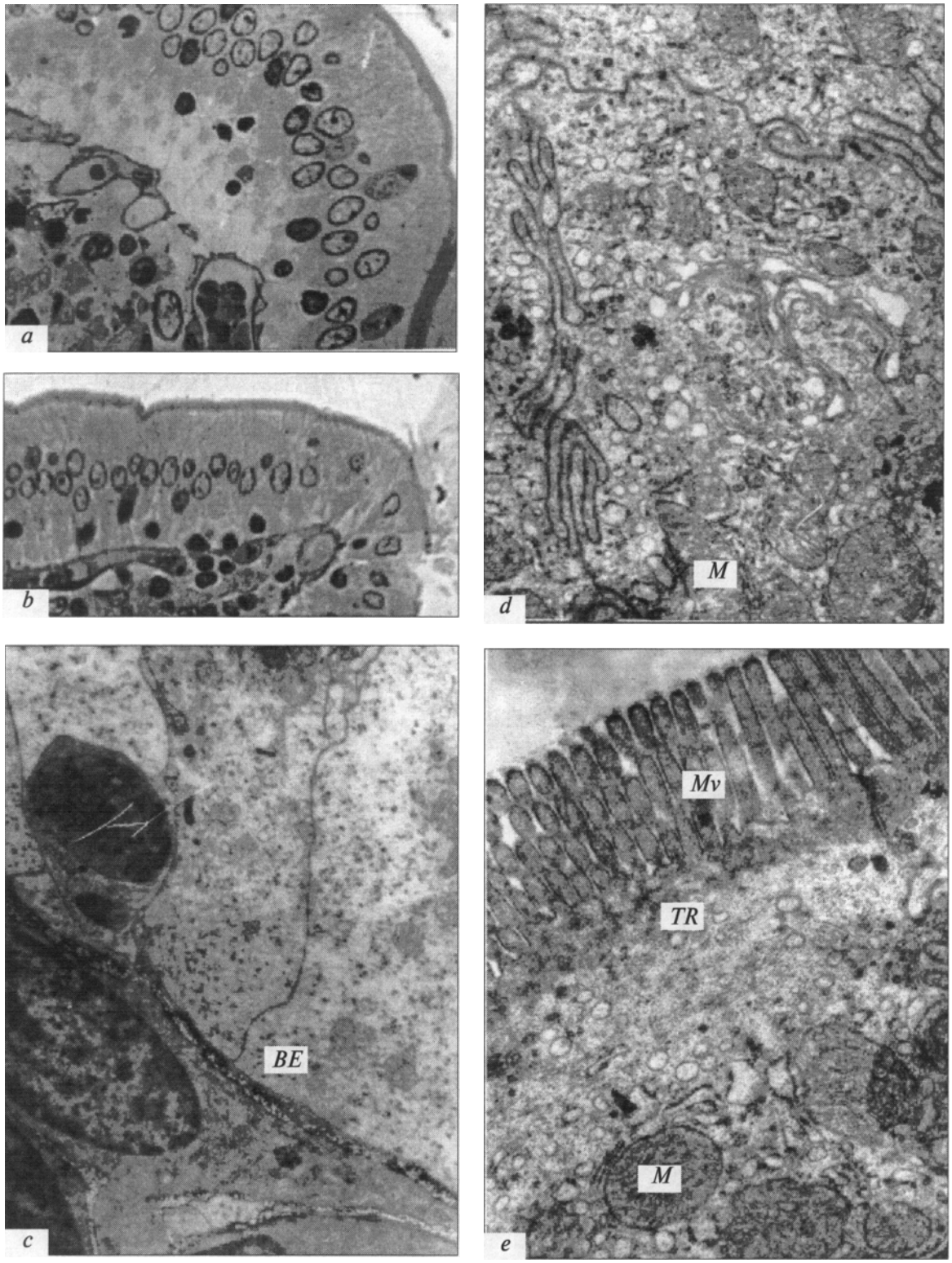

Fig. 1. Alterations in small intestinal mucosa after blood loss (control group). *a, b*) loosened cytoplasm in bordered enterocytes (*BE*) and erythrocyte aggregates in subepithelial capillaries. Methylene blue and azure staining, ×900; *c*) edema of basal BE cytoplasm, ×12,000; *d*) light mitochondrial matrix (*M*) and lysis of the crystae, ×26,000; *e*) loosened terminal reticulum (*TR*) and microvilli (*Mv*) of the brush border in BE, ×27,000.

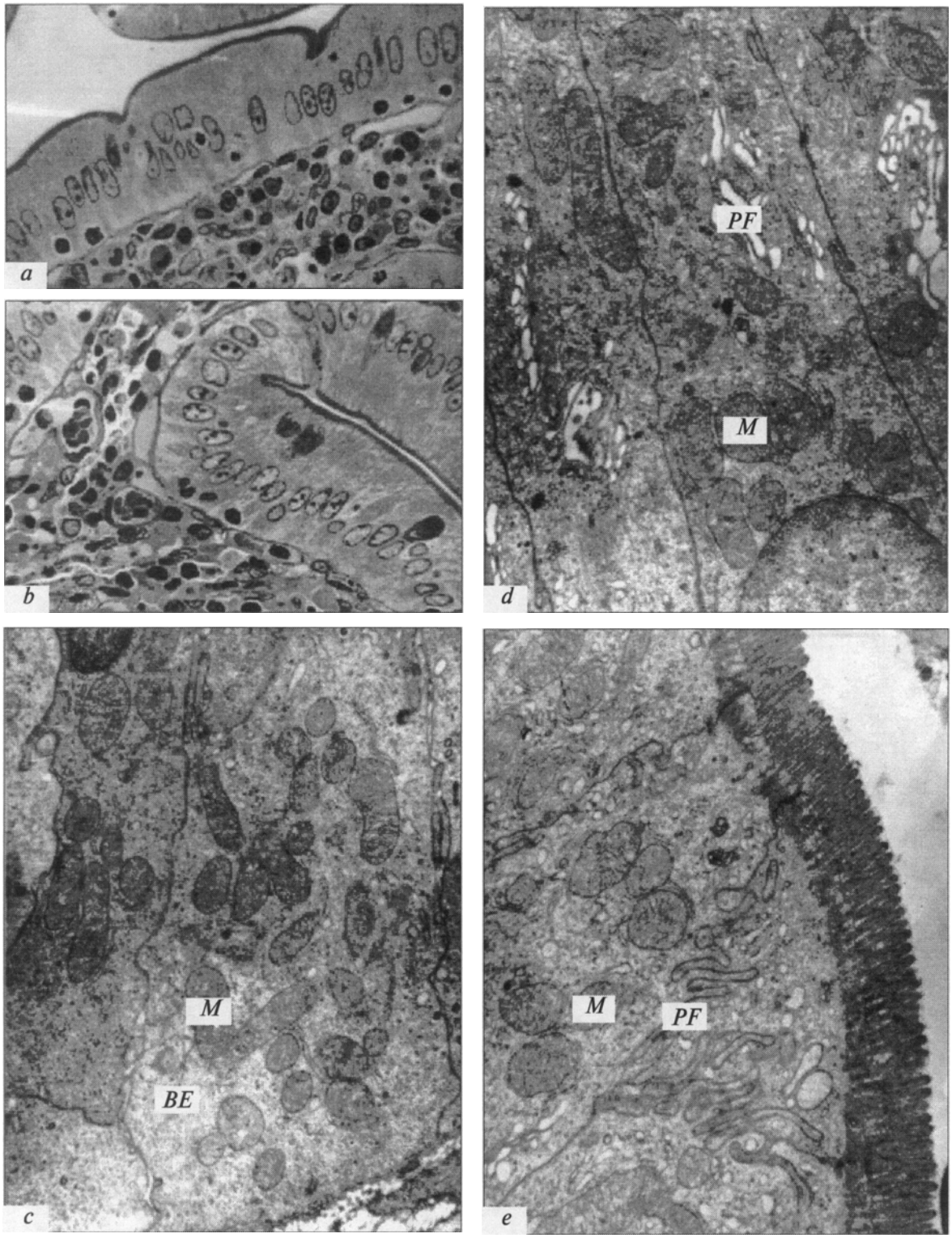

Fig. 2. Alterations in small intestinal mucosa after blood loss and mesodiencephalic modulation. *a, b*) high cylindrical bordered enterocytes (*BE*) with uniformly dense cytoplasm and moderate plethora in subepithelial capillaries. Methylene blue and azure staining, ×900; *c*) large mitochondria (*M*) in basal cytoplasm of BE, ×18,000; *d*) hyperplasied mitochondria near Golgi apparatus of BE, ×8,000; *e*) microvilli (*Mv*) of a standard height, pronounced plasmalemma folds (*PF*), and large mitochondria in the apical cytoplasm of BE, ×24,000.

apparatus encompassed with large mitochondria with hyperplasic crystae were seen in the perinuclear and apical cytoplasm. The hyaloplasm had narrow cisterns of the granular endoplasmic reticulum and polyribosomes (Fig. 2, *c*). The apical plasmalemma formed numerous villi of a standard height with preserved terminal network in their basement (Fig. 2, *d*, *e*).

Thus, MDM applied 60 min after blood loss not only promoted structural recovery of the intestinal villi, but also induced compensatory hypertrophy of the mitochondria. This suggests that MDM activates adaptive mechanisms: it rapidly restores intracellular energy metabolism, improves the absorption function of BE, and restores disturbed intestinal barrier after acute blood loss.

The beneficial effect of MDM can be explained by activated synthesis and release of neuropeptides participating in normalization of disturbed reparative processes in the small intestinal mucosa.

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