ISSN 1990-519X, Cell and Tissue Biology, 2020, Vol. 14, No. 1, pp. 57–64. © Pleiades Publishing, Ltd., 2020.
Russian Text © The Author(s), 2019, published in Tsitologiya, 2019, Vol. 61, No. 10, pp. 797–805.

Reactive Changes of Endocrine Cells of Intestinal Mucosal Epithelium with Administration of Melatonin or Doxylamine Succinate (Electron Microscopic Study)

M. L. Churkova*

Mechnikov Northwestern State Medical University, St. Petersburg, 195067 Russia

**e-mail: mariya.churkova@szgmu.ru*

Received June 3, 2019; revised June 11, 2019; accepted June 20, 2019

Abstract—Using an electron microscopy method, the structure of endocrinocytes of mucosal epithelium of duodenum, colon, and rectum in Wistar line rats was studied after daily administration of melatonin or doxylamine succinate for 1 month. It was demonstrated that morphological traits of increased functional activity (which is often accompanied by intracellular regeneration phenomena) are observed 1 day after the last administration of the studied substances. Mitochondria of endocrinocytes are primarily subject to changes. In addition, a large number of poorly differentiated endocrine cells were registered in the intestinal epithelium of rats subjected to experimental exposure, which indicates the activation of cambial elements of the epithelium. In all studied cases, D_1 cells were more frequently found in the epithelium than other types of endocrinocytes.

Keywords: intestine, epithelium, endocrinocytes, melatonin, doxylamine succinate, ultrastructure

DOI: 10.1134/S1990519X20010058

INTRODUCTION

The hormone melatonin (Melaxen drug) (Hack et al., 2003; Lemoine, Zisapel 2012) and doxylamine succinate, H-1 histamine receptor blocker (Donormyl drug) (Vande Griend and Anderson, 2003; Levin, 2008) are popular somnological drugs. Melatonin is also used in the treatment of digestive diseases (Konturek et al., 2011; Ermachenkov et al., 2012). It was demonstrated that melatonin and doxylamine succinate can lead to the emergence of digestive disorders (nausea, vomiting, diarrhea, coprostasis) (Hausser-Hauw et al, 1995; Hack et al., 2003; Videla et al., 2013). The functioning of the digestive organs is closely related to the activity of endocrine cells of the intestinal mucosal epithelium and is regulated by hormones produced by these cells. Disorders observed in patients treated with melatonin or doxylamine succinate orally are probably associated with a change in endocrinocytes of the epithelium of digestive system mucosa, in particular, in the small and large intestine. However, no data on this issue are available in works published to date.

The aim of the present work was to study reactive changes in endocrine cells of mucosal epithelium of the duodenum, colon, and rectum in rats with the administration of melatonin and doxylamine succinate.

MATERIALS AND METHODS

The samples of mucous membrane of the duodenum, colon, and rectum were studied in 20 Wistar line male rats. In total, two series of the experiments were carried out (five rats in each experimental group and five rats in each corresponding control group).

The solutions of melatonin (first series of experiments) or doxylamine succinate (second series of experiments) were daily administered intragastrically to animals from the experimental groups for 1 month using a probe. Melatonin was administered at a dose 0.5 mg/kg of anima weight (0.83 mL melatonin solution per average animal weight 500 mg). Melaxen (Unipharm Inc., United States) was used in this series of the experiments: one tablet containing 3 mg of melatonin was dissolved in 10 mL of physiological solution. Doxylamine succinate was administered at a dose 13 mg/kg animal weight (0.97 mL of doxylamine succinate solution per average animal weight 500 mg). Donormyl (UPSA, SAS, France) was used in this series of the experiments: one tablet containing 15 mg of doxylamine succinate was dissolved in 10 mL of physiological solution. In the control groups, physiological solution was administered in the appropriate volume to rats.

The day after the last administration of melatonin or doxylamine succinate (32nd day from the beginning of the experiment), euthanasia was performed in

accordance with European Directive 2010/63: rats were inhalationally euthanized with halothane solution (0.5%), and then decapitation was carried out. The experimental protocols were approved by the local Ethics Committee of Mechnikov Northwestern State Medical University, Ministry of Health of Russia (protocol no. 3 of March 14, 2018).

The intestine samples were fixed in 2.5% glutaraldehyde on a phosphate buffer (pH 7.4) (Sigma-Aldrich, Switzerland) for 2 h at 4°C, washed in 0.01 M phosphate buffer (three times for 30 min), additionally fixed with 1% osmium tetroxide according to Caulfield's protocol (fixator pH 7.4; fixation time, 1 h at room temperature) and poured into araldite M (Fluka, Switzerland) (1 day, at 37°C; 3 days, at 56°C).

Ultrathin serial sections (40–60 nm) obtained using a LKB III ultramicrotome (BROMMA, Sweden) were contrasted with plumbum citrate prepared according to Reynolds from plumbum nitrate (II) (LenReactiv, Russia) and uranyl acetate (GRANHIM, Russia) and studied in a JEM-100 S electron microscope (JEOL, Japan).

Morphometric analysis of the size (diameter) of secretory granules of endocrine cells in experimental and control animals was carried out in electron microscopic images with magnification of 20000× and 28000×. The type of endocrine cells was determined according to the size, form, and structure of secretory granules based on international classification of endocrine cells (Solcia et al., 1981). The estimation and comparison of the size (diameter) of secretory granules were conducted in rats from experimental and control groups. Statistical processing of the obtained data was carried out using Statistica 10 (StatSoft Inc.). The Mann–Whitney criterion was used to estimate the significance of differences between the groups ($p < 0.05$).

RESULTS

The endocrine part of the duodenum, colon, and rectal mucous membrane epithelium is mainly presented by the following types of endocrinocytes: EC cells synthesizing serotonin and melatonin; L cells, intestinal glucagon; D cells, somatostatin; and D_1 cells, vasoactive intestinal peptide. Other types of endocrine cells were found extremely rarely. In the control animals, endocrinocytes had a typical structure described previously (Solcia et al., 1981). In EC cells (Fig. 1a), endocrine granules (diameter 130 ± 5 nm) had an irregular shape and contained electron-dense secretory material surrounded by closely adjacent membrane, which was not traced in its entirety. D cells (Fig. 1b) contained large rounded secretory granules (diameter 202 ± 10 nm). The secretory material of the granules was electron-dense, finely granulated, surrounded by closely adjacent membrane. D1 cells contained the smallest (diameter 109 ± 11 mm) oval endocrine granules and a finely granulated secretory material surrounded by a discontinuous membrane separated from it by a considerable distance (Fig. 1c). The granules of L cells (diameter 168 ± 7 nm) are round and electron-dense, and the secretory material is surrounded by the membrane separated from it by a narrow light gap (Fig. 1d). Secretory granules in endocrinocytes of animals from the control groups occupy a significant amount of the cytoplasm. Other organelles (mitochondria, granular endoplasmic reticulum, polysomes, Golgi complex) are not numerous and are localized between the granules singly.

In the intestinal epithelium of rats from the experimental groups, endocrine cells were found rarely and were located singly among exocrinocytes, connected with them by means of desmosomes. The ultrastructure of different types of endocrine cells in all studied intestine departments (administration of melatonin or doxylamine succinate) was characterized by similar changes that differ in degree of severity. The sizes of secretory granules contained in the cytoplasm of different types of animal endocrinocytes from the experimental and control groups were comparable. A decrease in the number of secretory granules; change in the amount of polysomes, mitochondria, granular endoplasmic reticulum tanks; and the emergence of significant enlightened cytoplasm regions indicate the functional activity of endocrine cells, the development of processes of regeneration or destruction in them in animals exposed to the experimental effects. The absence of any differences of the ultrastructure of animal endocrinocytes from the experimental groups as compared with the control indicates that the cells are at rest.

With administration of melatonin or doxylamine succinate, the cytoplasm in some endocrinocytes was filled with multiple secretory granules, in which the membrane surrounding them is separated from the secretory material by a light gap. Mitochondria and granular endoplasmic reticulum, without numerous polysomes and without pronounced changes, were located between the granules. The bundles of tonofilaments were seen in the cytoplasm (demonstrated in Fig. 2a using the example of EC cell). The cell nuclei were oval with smooth contours and contained a narrow rim of heterochromatin near the nuclear membrane, as well as in the form of small clusters in its middle part.

In other endocrine cells (demonstrated in Fig. 2b, using the example of an L cell), secretory granules also occupy most of the cytoplasm. A few mitochondria with pronounced traits of swelling and altered cristae (reduced in number and fragmented), often with myelin-like structures, were registered in the cells. These changes in mitochondria indicate the initial stage of the development of destructive changes in them. Tanks of granular endoplasmic reticulum were narrow, without changes. Tonofilaments in the cyto-

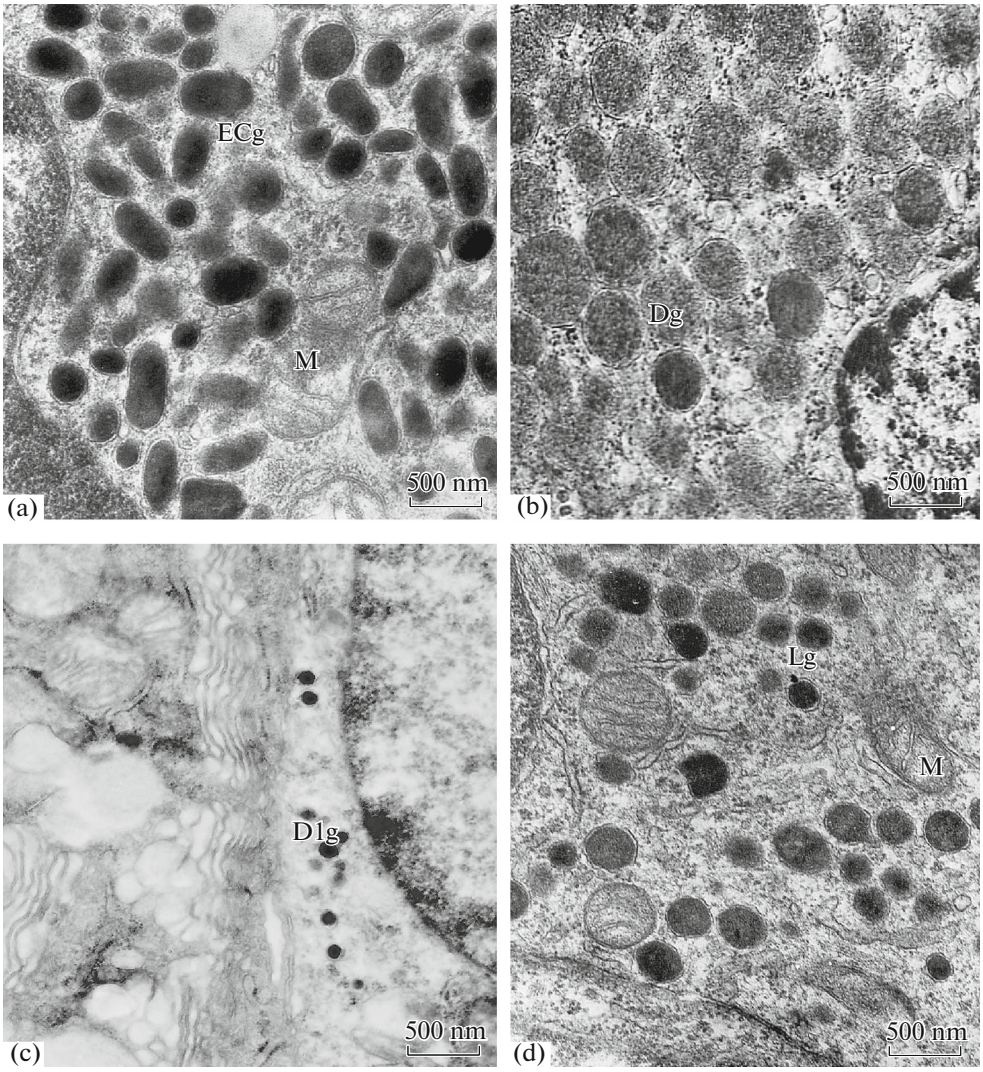

Fig. 1. Secretory granules in endocrine cells of duodenal mucosa epithelium in the control animals. (a) EC cells, (b) D cells, (c) D1 cells, and (d) L cells. Dg, secretory granule of D cell; D1g, secretory granule of D1 cell; ECg, secretory granule of EC cell; Lg, secretory granule of L cell; and M, mitochondrion.

plasm of endocrinocytes were single. The cell nuclei were unchanged compared with the control.

A visual decrease in the content of secretory granules in the cytoplasm were observed in most endocrine cells (Figs. 2c, 2d). Swollen mitochondria with reduced or single cristae were located between the granules (with the administration of doxylamine succinate, and cristae were not traced most frequently in individual mitochondria). Changes of varying severity were registered in the structure of granular endoplasmic reticulum (more frequently, with the administration of melatonin): visual expansion of tanks and an increase in the surface area of the organelles (with uneven distribution of ribosomes associated with its membrane) were observed (Fig. 2c).

Endocrine cells, in which significant areas of the cytoplasm were very rarefied and contained only single secretory granules, were mainly found with the administration of doxylamine succinate (Fig. 2d). A decrease in the content of endocrine granules in the cell cytoplasm, the structure of mitochondria and granular cytoplasmic reticulum, along with the detection of single polysomes, indicate the presence of the cells in a state of increased functional activity.

D_1 cells were most frequently found in both series of the experiments in all studied types of endocrinocytes. These cells were in a state of increased functional activity. In addition, endocrinocytes, a significant part of the cytoplasm of which was filled with mitochondria with well-developed transversely oriented cristae, narrow granular endoplasmic reticulum tanks, and polysomes, were found quite often in the epithelium (Fig. 3a). Endocrine cells sometimes generated the groups (cell clusters) connected by desmo-

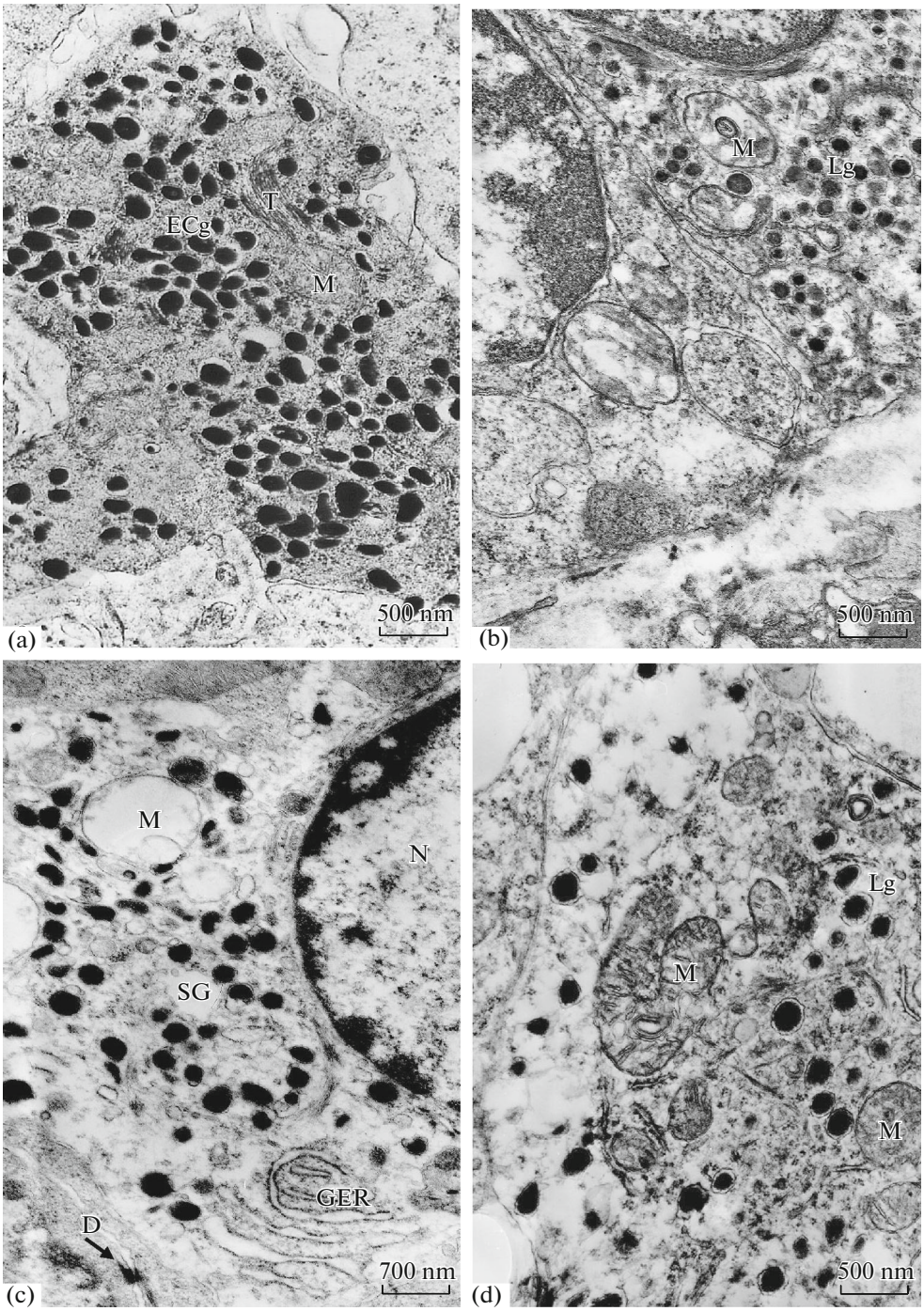

Fig. 2. Endocrine cells of mucosal epithelium of (a) colon, (b) rectum, and (c, d) duodenum after daily administration of (a–c) melatonin or (d) doxylamine succinate within a month. (a) EC cells, (b, d) L cell, and (c) endocrinocyte; the expansion of granular endoplasmic reticulum is noteworthy. D, desmosome; T, tonofilaments; ECg, secretory granule of EC cell; Lg, secretory granule of L cell; GER, granular endoplasmic reticulum; M, mitochondrion; SG, secretory granule; and N, nucleus.

somes. These cells contained single granules, multiple polysomes, and swollen mitochondria. Such structure and group distribution of endocrinocytes indicates their formation from cambial cells. A relatively high frequency of the detection of destructive changes in mitochondria was a peculiarity of the ultrastructure of endocrinocytes (Fig. 3b). As a result of swelling, the size of these organelles was sharply increased, single cristae were barely visible, and external membranes were not detected.

Cambial cells were detected with the administration of melatonin or doxylamine succinate in the epi-

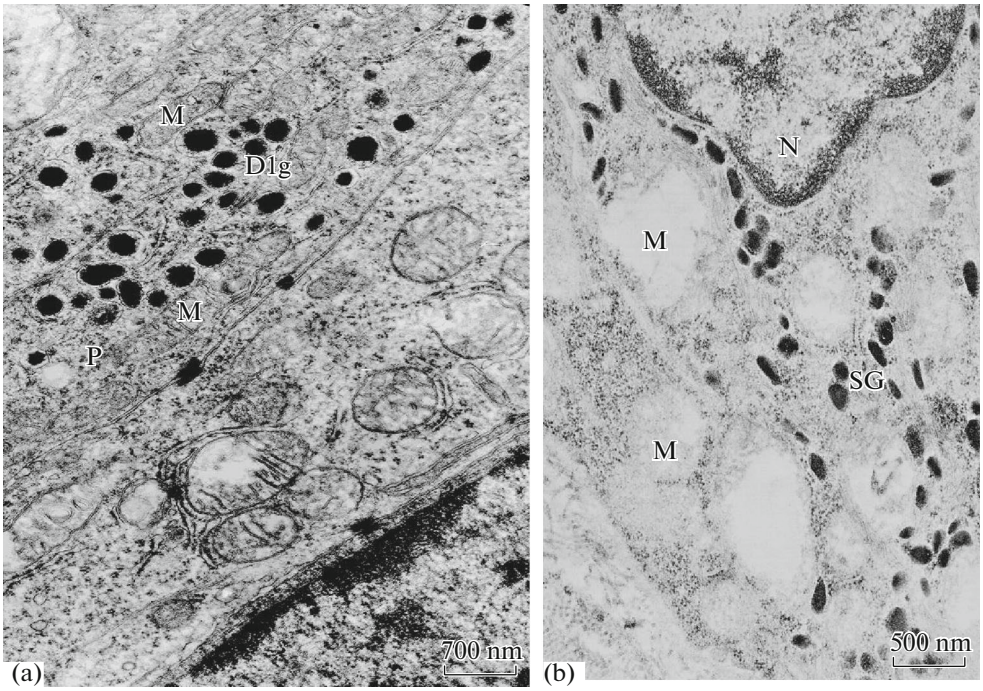

Fig. 3. Differentiated endocrinocytes in mucous epithelium of (a) duodenum and (b) colon after administration of (a) melatonin and (b) doxylamine succinate. D1g, secretory granule of D_1 cell; and GER, granular endoplasmic reticulum; M, mitochondrion; and P, polysomes.

thelium of the mucous membrane of all intestine departments. Multiple polysomes, among which oval, small mitochondria with well-developed correctly oriented cristae were located, were the main structures in the cytoplasm of these cells. In addition, multiple poorly differentiated endocrinocytes, which were often arranged in groups, were found in the epithelium. In these cells, nuclei with uneven contours occupied a significant part of the cytoplasm and contained evenly distributed chromatin with a narrow strip of heterochromatin near the nuclear envelope. Single endocrine granules or small groups of them, the type of which is difficult to establish at this stage of differentiation, were observed in some poorly differentiated cells (Figs. 4a–4c). Mitochondria were in a state of swelling in individual similar endocrinocytes.

DISCUSSION

The study of reactive changes in endocrine cells of intestinal mucosal epithelium in rats when exposed to doxylamine succinate or melatonin was carried out after the administration of these somnological drugs in therapeutic doses (intermediate between those used in a clinical practice for the treatment of a child and an adult) (Levin, 2008; Lemoine and Zisapel, 2012; Tolmacheva, 2019). The dose selection was determined by the fact that, as expected, the effect of doxylamine hydrogen succinate on the organism is proportional to the therapeutic dose range 12.5–25 mg (Videla et al., 2013), as well as the fact that, depending on the dose, melatonin has a different effect on duodenal motility, stimulating or slowing down the chyme transit (Drago et al., 2002; Sommansson et al., 2013).

As a result of an electron microscopic study of the epithelium of the mucous membrane of the studied intestine departments, it was established that changes were observed in most endocrinocytes after the administration of melatonin or doxylamine succinate: in the structure of mitochondria, there were a granular endoplasmic reticulum and a decrease in the content of secretory granules in the cytoplasm. Swelling of mitochondria, expansion of granular endoplasmic reticulum tanks, and emergence of a large number of polysomes (registered in endocrinocytes of animals from the experimental groups) are the "response" of the cells to experimental exposure (melatonin or doxylamine succinate). Previously, such ultrastructural transformations were described in the cecum epithelium with experimental escherichiosis (Barkhina et al., 1992), in rat duodenal epithelium when exposed to organofluorine compounds (Puzyrev et al., 1997), and in the epithelium of duodenum and rectum in rats during starvation (Ivanova et al., 2009) and are considered morphological traits of intracellular regenerative processes (Ivanova, 2013).

Dystrophic changes of varying severity, which were more often found with the administration of doxylamine succinate, first appeared in endocrinocyte mitochondria and were accompanied not only by

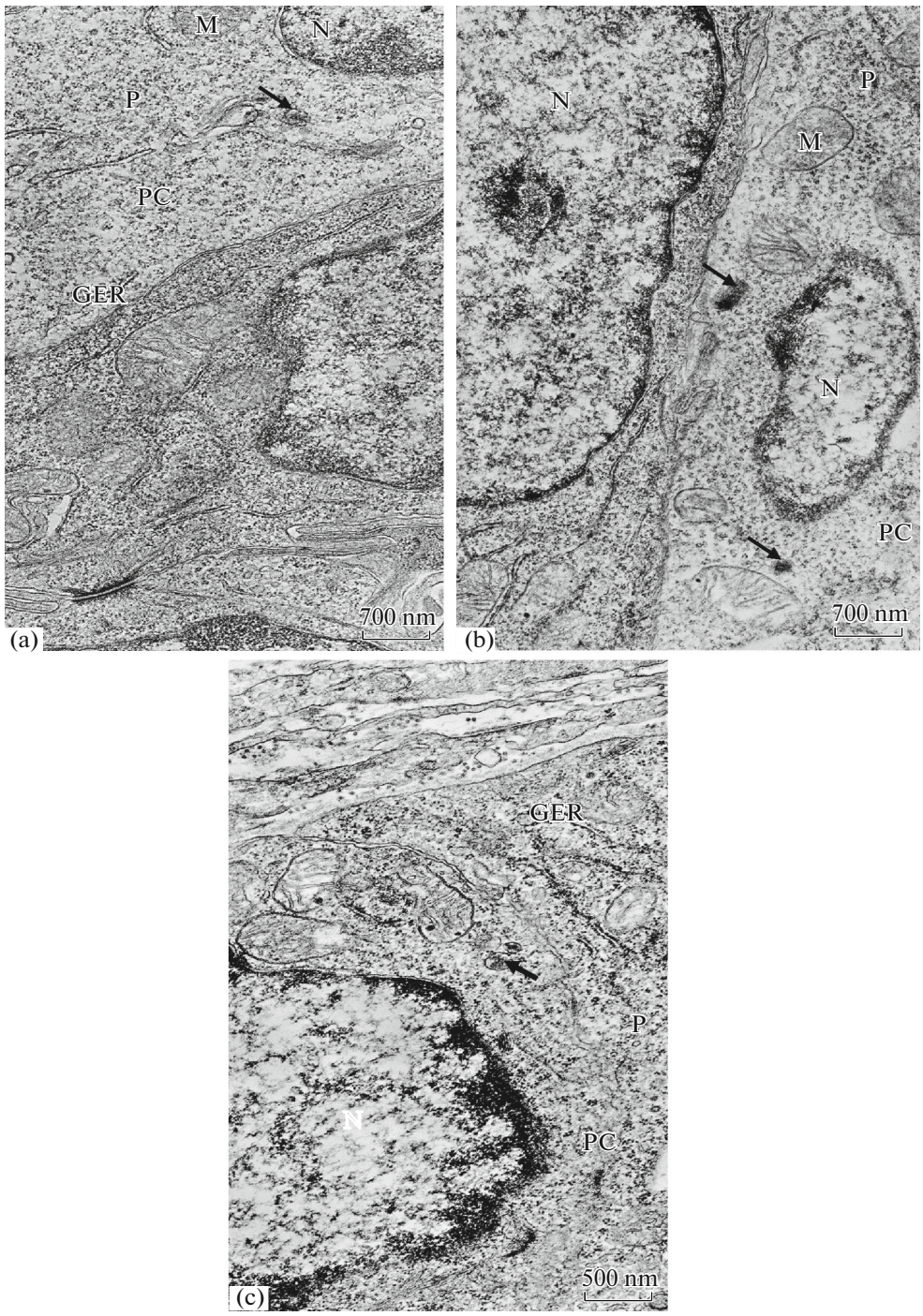

Fig. 4. Poorly differentiated cells (PC) forming endocrine granules (arrows) in mucosal epithelium of (a, b) duodenum and (c) colon after administration of (a, b) melatonin or (c) doxylamine succinate. For designations, see Figs. 1–3.

matrix enlightenment and disorganization of cristae, but also by their destruction. Such a structure of mitochondria in the cells of colon epithelium has been described with an experimental administration of Freund's adjuvant (killed tuberculous mycobacteria suspended in oil–water emulsion, arthritis modeling) (Kaladze, 2013). The pronounced ultrastructural changes of endocrinocytes with the administration of doxylamine succinate in rats can be regarded as morphological grounds for a possible disruption of the digestive system in animals. Apparently, the differences that we found in changes in the ultrastructure of endocrinocytes in different series of the experiments (with the administration of melatonin (hormone) or

Table 1. Comparison of changes in the ultrastructure of endocrinocytes after administration of melatonin or doxylamine succinate

Observed changes	Administration of melatonin	Administration of doxylamine succinate
Decrease in the number of mitochondria	+	+
Swelling of mitochondria	+	+
Fragmentation of mitochondrial cristae and reduction of their number	+	+++
Enlightenment of the mitochondrial matrix	+	+
Decrease in the number of secretory granules	+	++
Expansion of granular endoplasmic reticulum tanks, increase in surface area, uneven distribution of ribosomes associated with its membranes	++	+
Emergence of significant number of enlightened regions in the cell cytoplasm	–	+
Decrease in the content of polysomes	+	+

+, presence of these changes in animal cells; ++, pronounced changes; +++, most pronounced changes; –, absence of changes.

doxylamine succinate (histamine receptor blocker, Table 1) are due to the fact that introduced substances have different mechanisms of action.

In addition to altered differentiated endocrinocytes, numerous poorly differentiated cells were found in the epithelium of the mucous membrane of the studied intestine departments. It may be that factors that have affected the development of reactive changes in the structure of endocrine cells with the administration melatonin or doxylamine succinate contribute to the activation of the "stem cell niche" of the intestine criptae (Nimiritskii, 2018). In turn, the activation of "niche" cells led to the emergence of a large number of poorly differentiated cells in the epithelium. Previously, poorly differentiated endocrine cells with multiple polysomes with a reduced content of other organelles were described in the epithelium of digestive organs during the starvation (Ivanova et al., 2009). The emergence of these cells indicates the development of regenerative processes in the tissues in response to the administration of melatonin or doxylamine succinate.

Thus, an increase in the functional activity, which in some cases led to the development of destructive changes, took place in endocrine cells of intestinal mucosal epithelium after daily administration (within a month) of melatonin or doxylamine succinate to rats. Mitochondria first reacted to the introduced substances. More pronounced reactive changes in the structure of endocrinocytes of all studied intestine departments were observed when exposed to doxylamine succinate.

FUNDING

This work was supported by the grant of the Government of St. Petersburg for students, postgraduate students, young scientists, and young PhDs 2016, PSP series no. 16553.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All experiments were carried out in accordance with generally accepted ethical international standards for the use of laboratory animals. The experimental protocols were approved by the local Ethics Committee of Mechnikov Northwestern State Medical University, Ministry of Health of the Russian Federation, protocol no. 3 of March 14, 2018.

REFERENCES

Barkhina, T.G., Ali-Riza, A.E., and Parkhomenko, Yu.G., The ultrastructural characteristics of the endocrine cells of the normal murine cecum and in experimental escherichiosis, *Biull. Eksp. Biol. Med.*, 1992, vol. 114, no. 10, pp. 429–432.

Drago, F., Macauda, S., and Salehi, S., Small doses of melatonin increase intestinal motility in rats, *Dig. Dis. Sci.*, 2002, vol. 47, pp. 1969–1974.

Ermachenkov, M.N., Guliaev, A.V., and Anisimov, V.N., Melatonin and colorectal cancer: the rise of standard treatment efficacy, *Vestn. Severo-Zapad. Gos. Med. Univ. im. I.I. Mechnikova*, 2012, vol. 4, no. 3, pp. 78–83.

Hack, L.M., Lockley, S.W., Arendt, J., and Skene, D.J., The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects, *J. Biol. Rhythms*, 2003, vol. 18, pp. 420–429.

Hausser Hauw, C., Fleury, B., Scheck, F., Pello, J.Y., and Lebeau, B., Effect on sleep architecture and residual effect of a dose of 15 mg of doxylamine in healthy volunteers, *Sep. Hop. Paris*, 1995, vol. 71, pp. 742–750.

Ivanova, V.F., Regeneration of endocrine gastroenteropancreatic system in experimental and clinical pathology: concept development and current problems, *Morfologiia*, 2013, vol. 144, no. 6, pp. 73–84.

Ivanova, V.F., Puzyriov, A.A., Kostiukevitch, S.V., and Drai, R.V., Structural changes in rat intestinal wall during starvation, *Morfologiia*, 2009, vol. 136, no. 6, pp. 62–68.

Kaladze, N.N., Filonenko, T.G., and Sizova, O.A., Features of ultrastructural changes in colonic mucosa in experimental modeling of adjuvant arthritis, *Zdor. Rebenka*, 2013, vol. 44, no. 1, pp. 18–21.

Konturek, P.C., Brzozowski, T., and Konturek, S.J., Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options, *J. Physiol. Pharmacol.*, 2011, vol. 62, pp. 591–599.

Lemoine, P. and Zisapel, N., Prolonged-release formulation of melatonin (circadin) for the treatment of insomnia, *Expert. Opin. Pharmacother.*, 2012, vol. 13, pp. 895–905.

Levin, Ya.I., Joy and sadness of sleep, *Ross. Med. Zh.*, 2008, vol. 16, no. 30, pp. 27–31.

Nimiritsky, P.P., Sagaradze, G.D., Efimenko, A. Yu., Makarevich, P.I., and Tkachuk, V.A., The stem cell niche, *Tsitologiia*, 2018, vol. 60, no. 8, pp. 575–586.

Puzyrev, A.A., Ivanova, V.F., and Maimulov, V.G., Adaptation of the organism to the action of environmental factors at the cellular and subcellular levels, *Morphologiia*, 1997, vol. 112, no. 4, pp. 23–28.

Solcia, E., Capella, C., Buffa, R., Usellini, L., Fiocca, R., Frigerio, B., Tenti, P., and Sessa, F., The diffuse endocrine-paracrine system of the gut in health and disease: ultrastructural features, *Scand. J. Gastroenterol. Suppl.*, 1981, vol. 70, pp. 25–36.

Sommansson, A., Nylander, O., and Sjöblom, M., Melatonin decreases duodenal epithelial paracellular permeability via a nicotinic receptor-dependent pathway in rats in vivo, *J. Pineal Res.*, 2013, vol. 54, pp. 282–291.

The Vidal Directory. Medicinal Products in Russia, Tolmacheva, E.A., Ed., Vidal Rus., 2019.

Vande Griend, J.P. and Anderson, S.L., Histamine-1 receptor antagonism for treatment of insomnia, *J. Am. Pharm. Assoc.*, 2012, vol. 52, pp. 210–219.

Videla, S., Cebrecos, J., Lahjou, M., Wagner, F., Guibord, P., Xu, Z., Cabot, A., Encabo, M., Encina, G., Sicard, E., and Sans, A., Pharmacokinetic dose proportionality between two strengths (12.5 mg and 25 mg) of doxylamine hydrogen succinate film-coated tablets in fasting state: a single-dose, randomized, two-period crossover study in healthy volunteers, *Drugs R D*, 2013, vol. 13, pp. 129–135.

Translated by A. Barkhash