EFFECT OF CHOLERA TOXIN ON ULTRASTRUCTURE OF CELLS OF THE RATE ENTERO-ENDOCRINE SYSTEM

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Many investigations have been devoted to the study of the pathogenesis of choleragenic toxicosis, and they have revealed that cholera toxin (CT) binds irreversibly with the monosialoganglioside receptor (Gm_1) on the apical plasmalemma of prismatic intestinal epitheliocytes, and this is followed by activation of adenylate cyclase and elevation of the cAMP level in these cells [4, 7]. Ultrastructural changes in the prismatic epitheliocytes and goblet cells of the small and large intestines of various animals also have been described, and changes in the small intestine are frequently nidal in character [1, 2]. Changes in the intestinal endocrine cells under the influence of CT have not been adequately studied. We know that the entero-endocrine system includes a number of hormone-producing cells, diffusely distributed among the epitheliocytes of the gastrointestinal tract. Secretory products of these cells (amines, polypeptides) play an important role in the physiology of the digestive system [3]. Nine types of endocrine cells have been discovered by immunohistochemical methods in the intestine of man, the rat, and other mammals [6]. As yet there are no reliable data on changes in the ultrastructure of entero-endocrine cells under the influence of CT.

In the investigation described below, the Ec, G, D, and F cells of the rat intestine were investigated at the ultrastructural level in choleragenic toxicosis.

EXPERIMENTAL METHOD

Mature Wistar rats weighing 140-160 g were anesthetized with pentobarbital and unpurified CT (0.4 ml/100 g body weight) and purified CT (20 μg/100 g, diluted in 0.5 ml of physiological saline) were introduced directly into the duodenum. Control animals received 0.5 ml of physiological saline or of inactivated CT respectively. Pieces of intestine were taken 10 and 30 min, and 1, 2, 3, 4, 6, 12, and 24 h after administration of CT. These times were chosen so as to make absolutely certain that cells of the same population of the intestinal mucosa were being studied during the period before their possible replacement, and also because of the convenience of comparing the results with those of a previous investigation of epitheliocytes of the small intestine under the influence of CT. Material was fixed in a mixture of 4% paraformaldehyde solution of 1.25% glutaraldehyde solution in 0.1M cacodylate buffer (pH 7.3), and then postfixed in 1.33% OsO_4 solution. Dehydration was carried out in acetones of increasing strengths and the material was embedded in Vestopal W. Ultrathin sections were stained with uranyl acetate and lead citrate. To determine absorption of CT on endocrine cells, CT labeled with horseradish peroxidase was injected into an isolated loop of intestine 5-6 cm long. Material for study was taken at the same times. Pieces of tissue of experimental and control animals were fixed under identical conditions. Cryostat sections 25 μ thick were used for detection of peroxidase activity by Karnovsky's method. The sections were then postfixed in 1% OsO_4 solution, dehydrated, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on a Reichert (Austria) ultramicrotome. Ultrathin sections were examined in the JEM-100B electron microscope (Japan).

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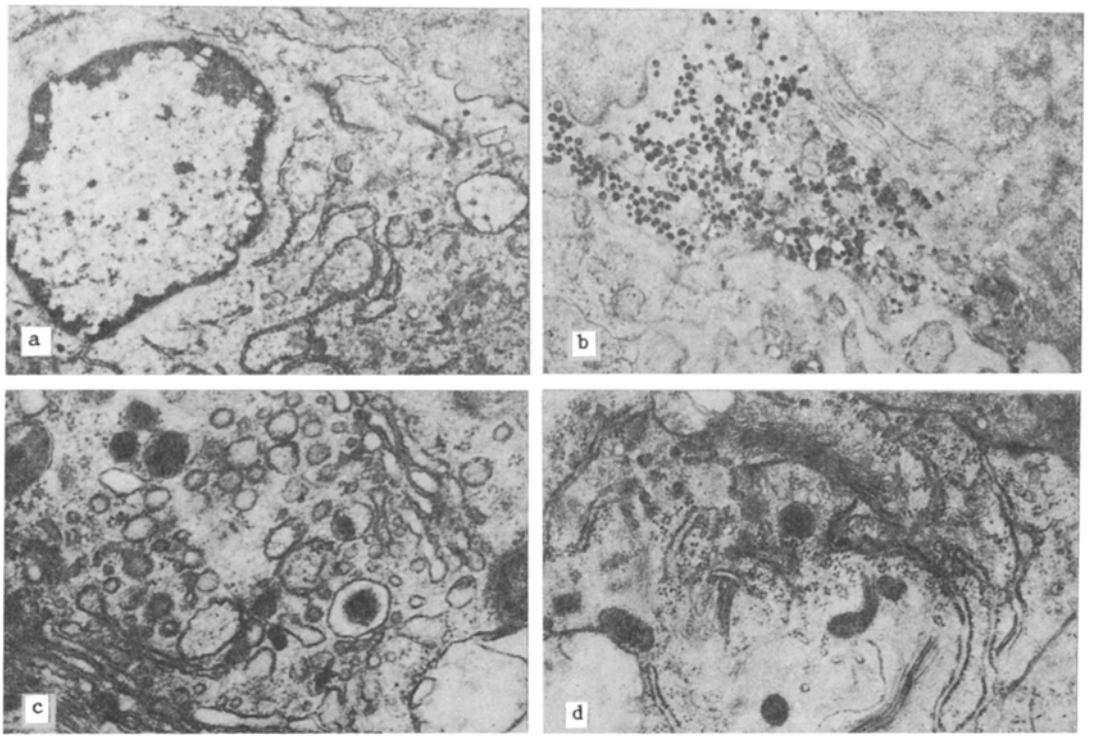

Fig. 1. Ec cells of the rat small intestine. a) Degranulated Ec cell of duodenum 10 min after injection of CT (6000 ×); b) content of secretory granules in duodenal Ec cell of control animal (7000 ×); c) hypertrophy of lamellar apparatus of Ec cell in jejunum 1 h after injection of CT (25,000 ×); d) lamellar apparatus of Ec cell in normal jejunum (26,000 ×).

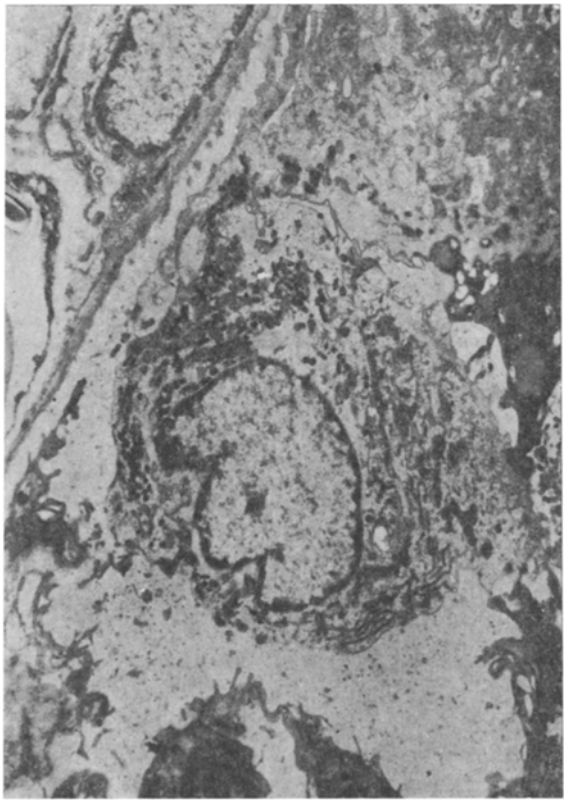

Fig. 2. Duodenal Ec cell in pathological focus 6 h after injection of CT (5000 ×).

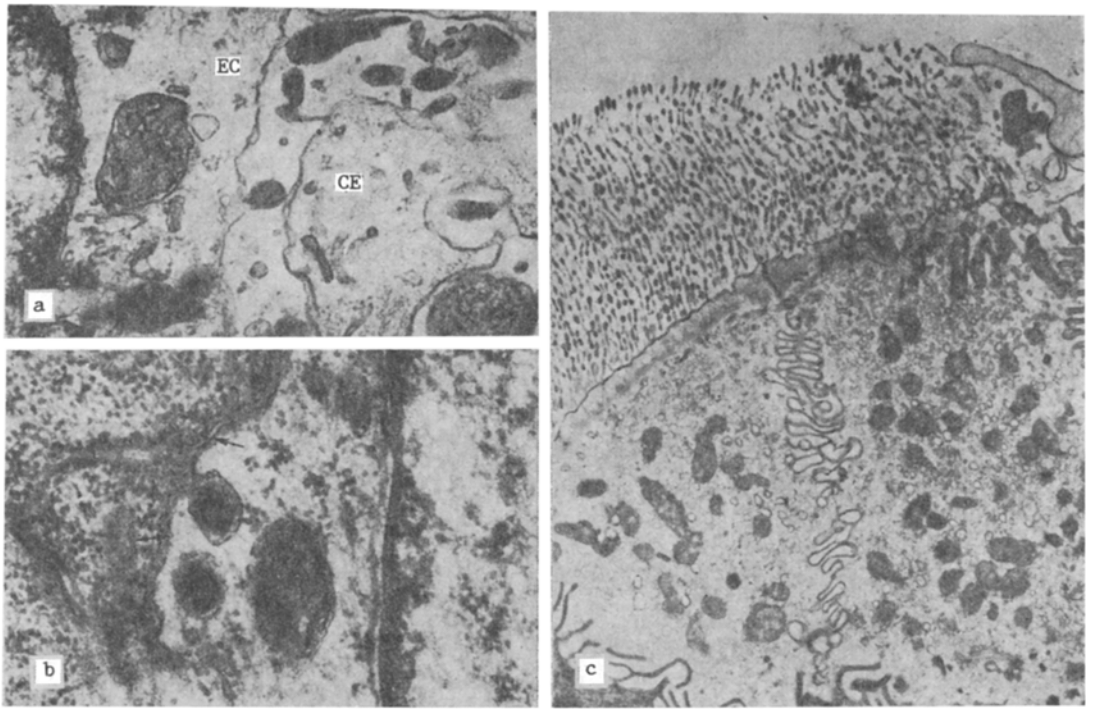

Fig. 3. Ec cells of rat small intestine. a) Secretory granules of Ec cell (Ec) of jejunum in widened intercellular space 4 h after injection of CT (40,000 ×). CE) Cytoplasm of epitheliocyte; b) intercellular space (arrow) and secretion of granules of Ec cell in normal jejunum (53,000 ×); c) microvilli, mitochondria, and endoplasmic reticulum of duodenal Ec cell 6 h after injection of CT (6700 ×).

EXPERIMENTAL RESULTS

Electron-cytochemical study of the localization of peroxidase-labeled CT revealed the reaction product on the membrane of microvilli of Ec cells 10 min after incubation. Examination of serial semithin and ultrathin sections showed that only 10-30 min after administration of CT some Ec cells were affected by degranulation (Fig. 1a, b). Distribution of their secretory granules along the basal and lateral plasmalemma was observed, together with degranulation of these cells, 1-2 h after introduction of CT into the intestinal lumen, and the lamellar apparatus was hypertrophied (Fig. 1c, d). Widened intercellular spaces appeared around the Ec cells. After 3-6 h of choleragenic toxicosis, the Ec cells in all pathological foci lost their longitudinal orientation. The lamellar apparatus showed hypertrophy and hyperplasia, and its elements were distributed throughout the cytoplasm. The number of secretory granules in them increased again. The smooth and rough endoplasmic reticulum was fragmented. The cytoplasm of the Ec cells, compared with the control, contained many microfilaments as well as free monosomes and polysomes. The intercellular spaces were greatly widened, and sometimes the spaces were wider than the cells themselves (Fig. 2). As the distance from these altered endocrine cells increased within the limits of the epithelial layer, the intercellular spaces between the epitheliocytes gradually narrowed. It must be pointed out that we observed secretory granules around Ec cells at these same times in the widened interepithelial spaces (Fig. 3a), which is not the case normally (Fig. 3b). After 6 h of choleragenic toxicosis almost completely degranulated Ec cells were seen in the epithelial layer. The smooth and rough endoplasmic reticulum and the lamellar apparatus in them were vesicular. Mitochondrial cristae were tubular in structure and the microvilli had a twisted, bead-like shape with alternation of regions of greater and less electron density (Fig. 3c). The foci described above were found on both villi and crypts. They were less numerous 12-24 h after administration of CT and were found mainly on villi. The lamellar apparatus in the S cells of the epithelial layer 3-6 h after administration of CT was just as hypertrophied as in the Ec cells, but the intercellular spaces were not widened. Many empty secretory granules could be seen in the G cells of the duodenum. No changes were found in the D cells. We attribute nidality of the pathological changes in the intestinal epithelium in choleragenic toxicosis to the presence of endocrine

cells of Ec type in these foci. On the basis of the results it can be postulated that Ec, by binding to the apical plasmalemma of Ec cells, potentiate their functional activity. Their secretory products (serotonin, substance P, and motilin), secreted into the intercellular space, act on neighboring epitheliocytes and nearby vessels (the paracrine effect). These substances can play the role of diarrhea-inducing factors in choleragenic toxicosis, as is confirmed by data in the literature [9-11]. Hormones secreted by S cells (secretin) and by G cells (gastrin) may also participate in intensifying secretion of fluid into the intestine [5, 8].

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COMPENSATORY AND ADAPTIVE CHANGES IN THE SMALL INTESTINE AND LIVER OF RABBITS ON SHORT-TERM CHOLESTEROL FEEDING

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Since cholesterol (Ch) is a risk factor of ischemic heart disease, much attention has been paid in the literature to regulation of the blood Ch level under normal conditions and in various disturbances (excess Ch in the diet, emotional stress, hereditary hypercholesterolemia – HCh, progressive atherolsclerosis, and so on). The discovery of low-density lipprotein (LDL) receptors explained the mechanism controlling the concentration of Ch-carrying lipoproteins (LP). It was shown that the blood LDL level is determined by the balance between activity of 3-hydroxy-3-methylglutaryl-CoA reductase, which participates in intracellular Ch synthesis, and the number of LDL receptors [6]. Competitive relationships have been found between chylomicrons (ChM) and very low density lipoproteins (VLDL) during binding with liver receptors [7, 9]. It has been shown that Ch is accepted from liver cell membranes by high density lipoproteins (HDL) [5]. Regulation of Ch transport at the level of the small intestine is less clear. Yet it can be postulated that regulation at that level is particularly important in the the case of insufficiency of liver function. Experiments

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