ELECTRON-MICROSCOPIC DETECTION OF ADENYLATE CYCLASE IN ENTEROCYTES OF RABBIT SMALL INTESTINE AFTER COMBINED STIMULATION BY CHOLERA TOXIN AND SODIUM FLUORIDE

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Cytochemical tests showed that adenylate cyclase in the enterocytes of the rabbit small intestine is activated both by cholera toxin and by sodium fluoride. As a result of combined stimulation of adenylate cyclase a maximal critical level of cyclic AMP is produced in the enterocyte of the small intestine, and this leads to self-inhibition of adenylate cyclase. In that case, a weak reaction for the enzyme or no reaction whatsoever could be found electron-microscopically.

KEY WORDS: small intestine; choleragen; sodium fluoride; adenylate cyclase.

The role of the cyclic AMP system in the transmission of action of various hormones on target cells is widely known [8].

The specificity of action of hormones is determined by the complement of receptors of the cell plasmalemma and the specificity of the cell response depends on its nature. Besides specific hormonal activators of adenylate cyclase there are also agents which stimulate the enzyme in many mammalian tissues. Cholera toxin and sodium fluoride are among these activators. Some workers consider that an increase in the intracellular cyclic AMP level leads to increased secretion of electrolytes and fluid from the enterocytes of the small intestine in cholera intoxication [6].

Attempts by biochemists to investigate the action of cholera toxin in vitro on isolated epithelial cells of the small intestine have been unsuccessful, presumably because of a disturbance of general cell functions [9]. During a study of the action of cholera toxin in vivo at the biochemical level it was found that cholera toxin lowers adenylate cyclase activity when stimulated by sodium fluoride in the enterocytes of the small intestine [9] and on erythrocyte membranes [2].

Investigations in the writers' laboratory to study the mechanism of pathogenesis of the rapid intestinal dehydration syndrome [1] involved determination of adenylate cyclase at the cytochemical level, so that on the one hand biochemical procedures of isolation of the intestinal enterocytes could be dispensed with, and on the other hand the cyclic AMP system could be investigated directly in the cell, without ruling out the possible effect of hydrolysis products of adenylate cyclase on its activity.

EXPERIMENTAL METHOD

The model of cholera intoxication described by Dutta and Habbu [5] was used in experiments on young rabbits. The animals aged 8-10 days were starved for 24 h before the beginning of the experiments and received only water; the operations were performed under hexobarbital anesthesia. Cholera toxin obtained from Vibrio cholerae 569B, Pakistan strain, Inaba serotype, was injected into the intestine in a dose of 0.3 ml. Material was taken 1 and 2 h after injection of the toxin. At the same time material was taken from control animals.

Pieces of small intestine were fixed in 1% glutaraldehyde made up in 0.05 M cacodylate buffer, pH 7.4, for 1 h at room temperature. After fixation, the tissue was washed in the same buffer and allowed to stand overnight at 4°C. The material was then sorted and incubated at 30°C for 40-50 min. The composition of the incubation mixture (after Howell and Whitfield [7]) was as follows: 80 mM Tris-maleate buffer, pH 7.4,

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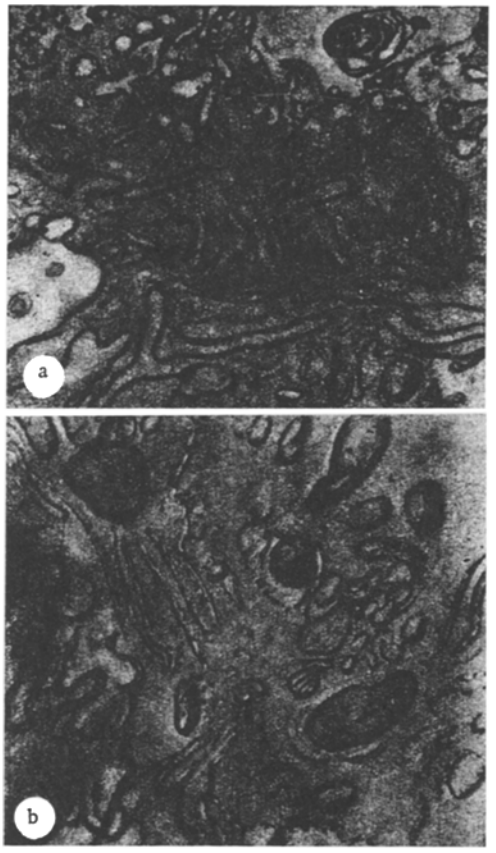

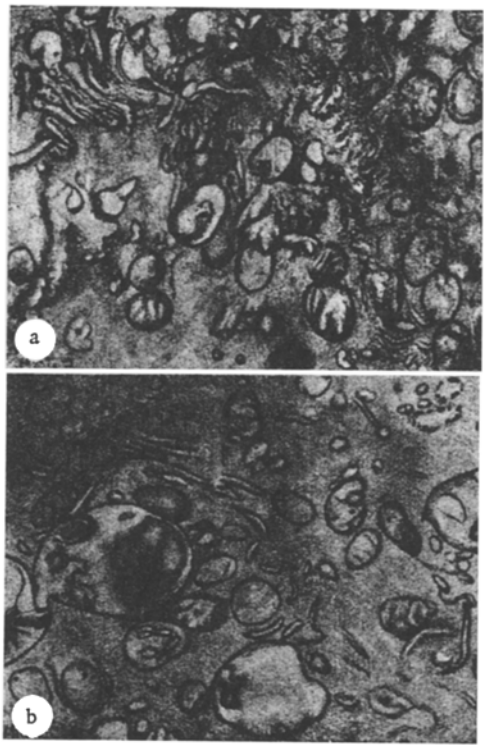

Fig. 1 Fig. 2

Fig. 1. Localization of adenylate cyclase in enterocytes of small intestine of control rabbit: a) weak reaction on lateral plasmalemma. Incubation in medium without addition of sodium fluoride, 30,000×; b) marked reaction on lateral plasmalemma. Incubation in medium with sodium fluoride, 25,000×.

Fig. 2. Localization of adenylate cyclase in enterocytes of small intestine of rabbit 2 h after injection of cholera toxin into intestine. Marked reaction on lateral plasmalemma. Incubation in medium without sodium fluoride, 15,000×.

8% glucose, 2 mM magnesium sulfate, 2 mM theophylline, 0.5 mM ATP, and 4 mM lead nitrate. Incubation was carried out in two incubation mixtures: with or without the addition of 10 mM sodium fluoride.

The control to the detection of adenylate cyclase was carried out in the same stages, but no substrate was present in the incubation mixture.

After incubation the material was quickly washed in 0.05 M Tris-maleate buffer and fixed in 1% osmium tetroxide in 0.05 M cacodylate buffer, pH 7.4, for 1-1.5 h at 4°C. The tissue was then rinsed in the same buffer, dehydrated, and embedded in a mixture of Epon and Araldite. Ultrathin sections were examined in the JEM-100V electron microscope.

EXPERIMENTAL RESULTS

In the control animals the reaction product deposited as electron-dense granules at sites of localization of adenylate cyclase could be seen both on the apical and on the lateral and basal plasmalemma of the enterocytes. If sodium fluoride was added to the substrate the reaction was intensified, as shown by an increase in size of the granules of the hydrolysis product and a more widespread distribution of the granules over the lateral and basal plasmalemma of the enterocytes on the villi of the small intestine (Fig. 1a, b). The irregularity of detection of adenylate cyclase on the plasmalemma of the enterocytes will be noted: It indicates differences in the functional state of the cells and of their membrane-bound enzymes.

One hour after injection of cholera toxin, the reaction on the lateral and basal plasmalemma of the enterocytes in material incubated in substrate without sodium fluoride was more marked and more widespread than in material incubated in substrate with sodium fluoride.

After 2 h of stimulation of adenylate cyclase by cholera toxin alone the reaction still remained strong and widespread (Fig. 2), but after incubation in medium with sodium fluoride the reaction on the lateral and basal plasmalemma was very weak or absent altogether.

These results show that in control animals sodium fluoride activated the adenylate cyclase of the plasmalemma of enterocytes of the rabbit small intestine, whereas after exposure to cholera toxin the addition of sodium fluoride did not cause an increase in the intensity of the reaction on the plasmalemma, and in some cases no adenylate cyclase activity could be demonstrated cytochemically. Meanwhile, stimulation of adenylate cyclase by cholera toxin alone intensified the cytochemical reaction compared with the control. Similar results were obtained in biochemical investigations on cells of the adrenal glands [10, 11] and epithelial cells of the villi of the small intestine [9].

These results can be interpreted as follows. Since medium fluoride is a constant stimulator of adenylate cyclase it is unlikely that it could have an inhibitory action directly on the enzyme in cells exposed to the action of cholera toxin. In that respect, investigations in vivo have the advantage over those in vitro, for in the latter the effect of an increased intracellular cyclic AMP concentration on the catalytic center of the polyenzymic adenylate cyclase system is not taken into account. In mammalian cells, this system involves interaction between three plasmalemmal enzymes: adenylate-cyclase kinase, adenylate cyclase itself, and protein phosphatase. Cyclic AMP-dependent activation of adenylate-cyclase kinase leads to phosphorylation inhibition) of adenylate cyclase and, consequently, to the cessation of synthesis of cyclic AMP from ATP [3, 4]. Additional activation of adenylate cyclase, when already stimulated by cholera toxin, by sodium fluoride causes a sharp increase in the intracellular cyclic AMP concentration, leading to activation of adenylate cyclase kinase. The latter, in turn, phosphorylates adenylate cyclase, i.e., converts it into the inactive form. Self-inhibition of a adenylate cyclase thus takes place.

The cytochemical investigation showed that adenylate cyclase in the enterocytes of the rabbit small intestine is activated both by cholera toxin and by sodium fluoride.

As a result of combined stimulation of adenylate cyclase a maximal critical cyclic AMP level is reached in the cell and this leads to self-inhibition of adenylate cyclase; in that case a weak reaction or no reaction whatsoever for the enzyme is obtained electron microscopically.

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