ELECTRON-MICROSCOPIC STUDY OF ADENYLATE CYCLASE LOCALIZATION IN EPITHELIAL CELLS OF THE SMALL INTESTINE OF RABBITS WITH NAG INFECTION

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A primary increase in adenylate cyclase activity in NAG infection is observed in the apical plasmalemma of the villous cells and later this process spreads to the lateral and then to the basal plasmalemma of the enterocytes. After more prolonged exposure to the infection an increase in adenylate cyclase activity is also observed in the cells of the crypts. During the action of the toxin, the activity of the enzyme thus gradually increases in the whole plasmalemma of the affected epithelial cells of the villi and crypts, and the epithelial cells of the small intestine in the zone of the focal lesion become involved in the pathological process.

KEY WORDS: NAG infection; small intestine; adenylate cyclase

In the last decade many papers dealing with the study of the biochemical mechanisms of the disturbance of water and mineral metabolism under the influence of cholera toxin have been published, but far fewer studies have been made of NAG infection.

Investigations have shown [8, 14] that the pathogenic action of cholera toxin and the toxin of NAG vibrios is similar. Accordingly the mechanism of action of cholera toxin and of the toxin of NAG vibrios on the epithelium of the mammalian small intestine can be regarded as analogous to a certain degree.

In the modern view one of the manifestations of the action of cholera enterotoxin is stimulation of the adenylate cyclase system, and the resulting raised level of cyclic AMP leads directly to an increase in the secretion of fluid and electrolytes in the intestine [1-3, 9].

The question of priority of the reaction of activation of the adenylate cyclase system in the epithelial cells in different parts of the small intestine has not yet been settled. Two hypotheses have been put forward to explain the increase in adenylate cyclase activity in the plasmalemma of the epithelial cells of the small intestine. The supporters of one hypothesis [11, 12] consider that the greatest increase in adenylate cyclase activity in response to cholera toxin is observed in the epithelial cells at the apices of the villi, whereas supporters of the other [10] claim that the primary manifestation of the action of cholera toxin is a change in the morphological and functional state of the crypt cells. These different views are based on the results of biochemical investigations.

No reference to the study of the morphological substrate of functional changes in adenylate cyclase activity in the epithelial cells of the villi and crypts of the small intestine can be found in the recent literature. It was accordingly decided to undertake a submicroscopic study of the localization of the enzyme in these cells.

EXPERIMENTAL METHOD

Experiments were carried out on newborn rabbits aged 8-10 days and weighing under 100 g. The animals were starved for 24 h before administration of the NAG vibrios and were given only water to drink. A 16-h broth culture of a toxigenic strain of NAG vibrio No. 10703, in a dose of 0.3 ml (10^8 bacterial cells), was injected into the lumen of a segment of small intestine by the method of Dutta and Habbu [15]. The animals were killed under hexobarbital anesthesia 10 and 30 min and 1, 2, 4, 6, and 18 h after injection of the NAG vibrios.

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Fig. 1. Adenylate cyclase activity on lateral and basal plasmalemma of enterocytes of small intestine of control newborn rabbit (40,000 ×).

Pieces of small intestine were fixed in 1% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, containing 4.5% glucose. Fixation continued for 1 h at room temperature. The tissue was then rinsed several times in the same buffer and allowed to stand overnight at 4°C. Selected fragments of tissue were then incubated by the method of Howell and Whitfield [6], together with a control in which 10 mM sodium fluoride was added to the incubation mixture (control 1).

Simultaneously with material taken from the experimental animals, material was taken from control animals by the same method (control 2). A control for detection of the enzyme activity was set up with the same stages, but the material was incubated without the incubation mixture (control 3).

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

EXPERIMENTAL RESULTS

In the sections used to study the localization of adenylate cyclase in the small intestine of the control rabbits the reaction product was found on the apical, lateral, and basal plasmalemma of cells both of the villi and of the crypts (Fig. 1).

Activation of the adenylate cyclase system was observed in the experimental animals after injection of the NAG vibrios. Activity of the enzyme in the small intestine 10 min after injection of NAG vibrios was most marked on the apical plasmalemma of the epithelial cells of the villi (Fig. 2A). The increase in activity after 30 min was manifested more clearly on the lateral plasmalemma of the enterocytes (Fig. 2B) and on the boundary between the prismatic and goblet cells. Adenylate cyclase activity 1 h after injection of the NAG vibrios was increased in the basal plasmalemma of the enterocytes and in the basement membrane of the epithelial cells. Adenylate cyclase activity 2-4 h after the beginning of action of the toxin was most marked in the

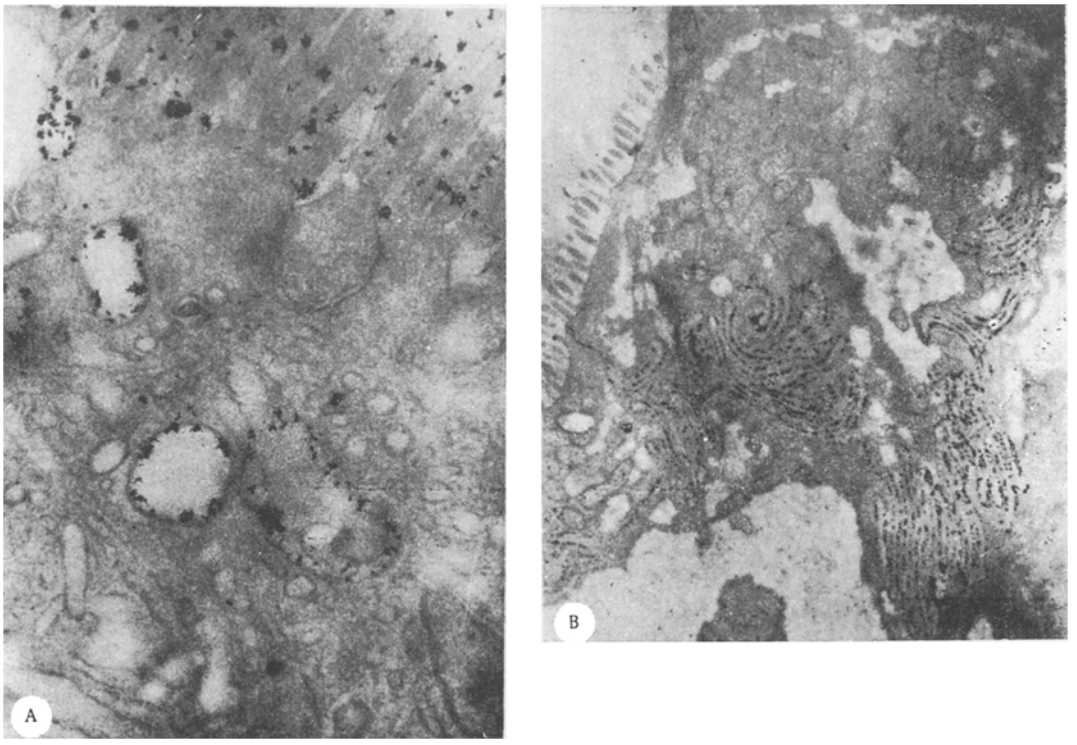

Fig. 2. Adenylate cyclase activity on plasmalemma of enterocytes of small intestine of newborn rabbit. A) Apical plasmalemma 10 min after injection of NAG vibrios, 50,000 ×. B) Lateral plasmalemma 30 min after injection of NAG vibrios, 17,000 ×.

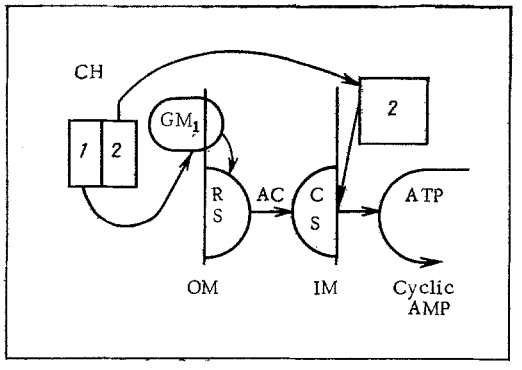

Fig. 3. Modified scheme of action of cholerogen according to data of Robinson et al. [10] and Finkelstein [5]. OM) Outer membrane of enterocyte; IM) inner membrane of enterocyte; CH) cholerogen: 1) 1st subunit, 2) 2nd subunit; GM_1) membrane receptor of cholerogen (monosialosylganglioside), AC) adenylate cyclase, RS) receptor subunit of AC, CS) catalytic subunit of AC.

epithelial cells of the crypts. After 6-18 h no regular pattern of enzyme activation could be found, for its activity at these times was observed in the plasmalemma of different parts of the epithelial cells of the small intestine.

It can accordingly be postulated that the increase in adenylate cyclase activity in this model takes place in a definite sequence: Activity of the enzyme first increases on the apical plasmalemma of the villous cells, indirect evidence that it is the result of primary interaction between the toxin and these membranes. These

observations support the results of biochemical studies [12, 13] which showed that cholera toxin interacts primarily with the membranes of the intestinal microvilli.

Cholera toxin has been shown [7] to interact with the plasmalemma through the intermediary of a hypothetical receptor – monosialosylganglioside (GM_1). In all probability this receptor is in constant functional communication with the receptor subunit of adenylate cyclase, which is activated by the toxin. The activated receptor subunit of adenylate cyclase stimulates the second (catalytic) subunit of this enzyme, which directly affects the level of cyclic AMP formation [10]. According to the most recent data [5], the cholerogen also consists of two subunits, one of which attaches itself to the receptor whereas the other penetrates directly into the cell. This so-called active subunit in turn acts on the catalytic subunit of adenylate cyclase facing the hyaloplasm, and activates it (Fig. 3).

It can tentatively be suggested that the toxin of NAG vibrios consists of two functional subunits analogous to the subunits of the cholera toxin. The results of the present experiments can accordingly be interpreted as follows. The action of the toxin and, in particular, of its first subunit, is mediated through the adenylate cyclase system and its mechanism is of the cis-membrane type, i.e., in this case the toxin does not necessarily penetrate into the cell. The other, biologically active, subunit of the NAG vibrio toxin, on the other hand, penetrates into the cell and promotes additional activation of adenylate cyclase from the hyaloplasm.

The present experiments also showed that during the action of the toxin, adenylate cyclase activity gradually increases throughout the plasmalemma of the affected epithelial cells of the villi and crypts, so that the epithelium of the small intestine in the zone of the pathological focus becomes involved in the pathological process.

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