SUBMICROSCOPIC DETECTION OF GUANYLATE CYCLASE IN MAMMALIAN EPITHELIAL AND MYOCARDIAL CELLS

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The electron-cytochemical method of detection of guanylate cyclase has several advantages and enables differentiation of the enzyme by its location in the plasmalemma, cytoplasm, nucleoplasm, and nucleolus. It was shown by this method that the enzyme has species and organ specificity of distribution in the cell and can be activated by sodium azide, which is a specific activator. The distribution of guanylate cyclase is strictly dependent on the functional state of the cell.

KEY WORDS: mammalian cells; electron-cytochemical method; guanylate cyclase.

The role of cyclic nucleotides is well known in metabolic regulation, in processes of hormone synthesis, hormonal induction of enzymes, activation and inhibition of enzymes, RNA, DNA, and protein synthesis, processes of cell growth and differentiation, nervous transmission, the cellular permeability of secretion, and many other intracellular processes.

To understand the pathogenesis of many hormonal, infectious, and other diseases it is necessary to study the whole system of cyclic nucleotides and, in particular, their indicator enzyme systems which participate directly in nucleotide synthesis.

The role of the cyclic AMP and cyclic GMP systems in some hormonal and infectious diseases has been most intensively studied [2, 4, 6]. The qualitative characteristics of the enzymes which participate in the synthesis of these nucleotides, i.e., adenylate and guanylate cyclases, have not been studied under pathological conditions.

Until recently the only known method of determining the cyclic GMP level and activity of guanylate cyclase was a biochemical method [1, 5], but since this is an *in vitro* method, it can be used only for quantitative analysis of enzyme activity.

It was accordingly decided to study the intracellular localization of guanylate cyclase in mammals. An original method was devised and used for this purpose.

EXPERIMENTAL METHOD

Sexually mature rats weighing 140-160 kg and rabbits aged 8-12 days were used. The animals were killed under hexobarbital anesthesia. The small and large intestine, liver, pancreas, lungs, and heart were investigated.

Small pieces from the organs were fixed in 1% glutaraldehyde in 0.05 M cacodylate buffer with glucose (pH 7.4) for 1 h at room temperature. The material was then washed in the same buffer for 24 h at 4°C. After sorting, the material was incubated.

Incubation conditions were optimized for each species of animal and for each separate organ. To optimize detection of the enzyme, six variants of the incubation medium and also different incubation temperature regimes (from 27 to 37°C) for between 30 and 60 min were tested.

The composition of the incubation medium was: 80 mM Tris-maleate (pH 7.4) with the addition of 8% glucose, 1-2 mM theophylline, 0.1-0.5 mM manganous chloride, 0.1-0.5 mM GTP, and 4 mM lead nitrate.

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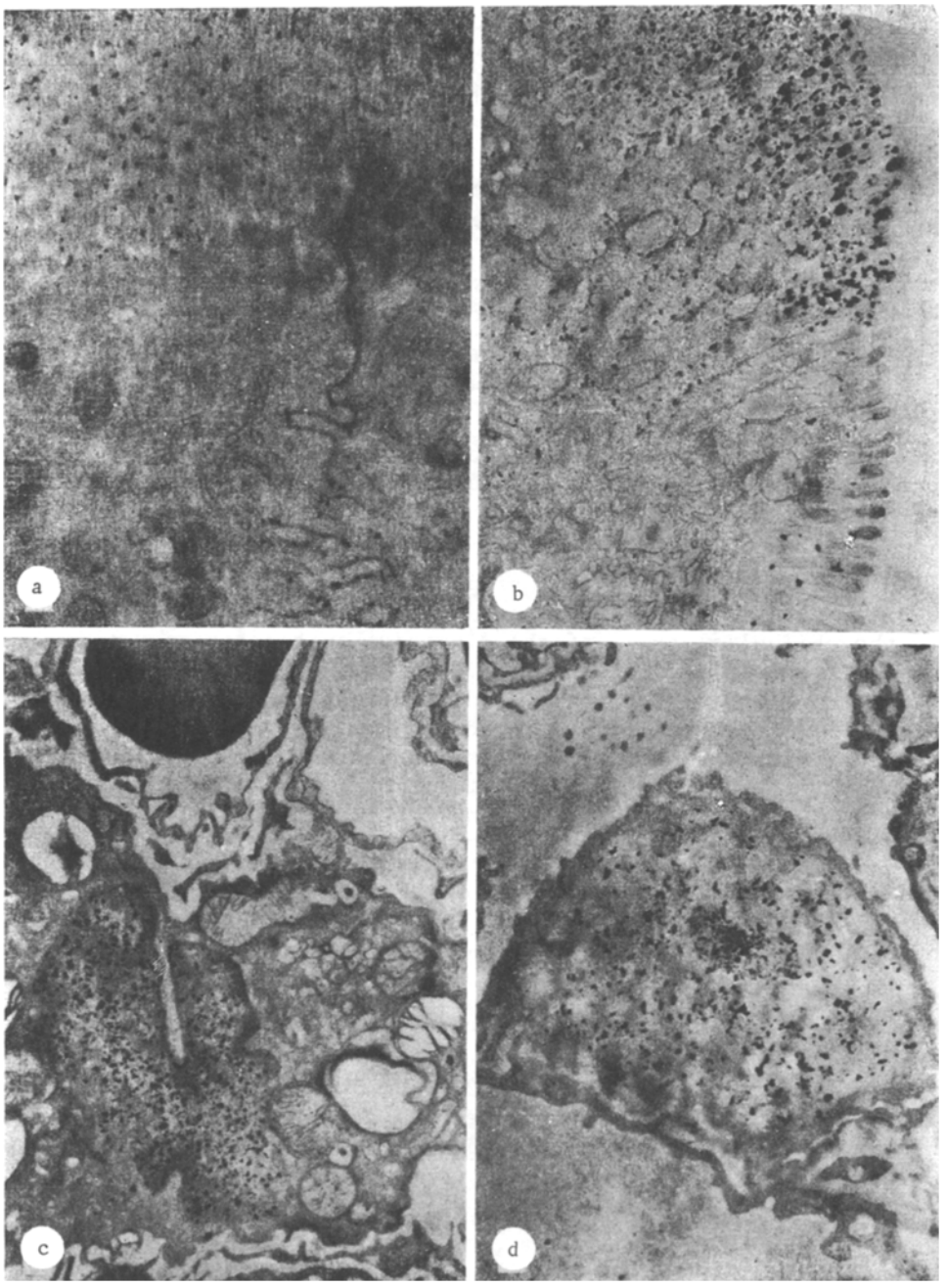

Fig. 1. Guanylate cyclase activity. A) On apical and lateral plasmalemma of epithelial cells of rat small intestine, 20,000×; B) on apical and lateral plasmalemma and also in cytoplasm of epithelial cells of rabbit small intestine. Sodium azide added to incubation medium; 9000×; C) in nucleus of rat type II pneumocyte, 8000×; D) in nucleus and nucleolus of rat type I pneumocyte, 12,000×.

The control for detection of the enzyme was set up in incubation medium without substrate (control 1) and in incubation medium with the addition of sodium azide (control 2).

After incubation the material was quickly washed in 0.08 M Tris-maleate buffer with glucose (pH 7.4) and postfixed in 1% osmium tetroxide solution in 0.05 M cacodylate buffer with glucose for 1.5 h at 4°C, then washed in the same buffer, dehydrated in acetone (30, 50, 70, 96, and 100%), and embedded in a mixture of Epon and Araldite. Unstained ultrathin sections were examined in the JEM-100V electron microscope.

EXPERIMENTAL RESULTS

The experiment showed differences in the localization of guanylate cyclase. The most common site was in the plasmalemma, which is more clearly defined in the epithelial cells of the digestive system (Fig. 1A). After addition of sodium azide to the incubation medium hydrolysis of the substrate was increased, and consequently the reaction for detection of the enzyme was more intensive and more widespread (Fig. 1B).

In the epithelial cells of the lungs guanylate cyclase was located not only on the plasmalemma, but also in the nucleoplasm (Fig. 1C) and, mainly, in the nucleolus (Fig. 1D), whereas in the epithelial cells of the small and large intestine it was located in the cytoplasm (Fig. 1B).

Cells in different functional states also differed in their content of the enzyme. The localization of the enzyme in a functionally active cell was fairly widespread, whereas in an inactive cell it was sometimes impossible to detect the enzyme cytochemically.

Some workers [3] have found that guanylate cyclase activity in mammalian epithelial cells is highest in the lungs, where they detected the enzyme biochemically both in membrane fractions and in the cytoplasm, although a more concrete localization was not established.

The present experiments showed that guanylate cyclase is located in the lungs and heart both in the plasmalemma and in the nuclei of pneumocytes and myocardiocytes. It was also found that guanylate cyclase possesses a species- and organ-specific distribution in the cell. For example, in the epithelial cells of the digestive system the enzyme is located more frequently in the plasmalemma and cytoplasm (Fig. 1A, B), whereas in the epithelial cells of the lungs and in the myocardiocytes it is located more frequenty in the nucleoplasm and nucleolus (Fig. 1C, D). To conclude, the electron-cytochemical method of detection of guanylate cyclase has many advantages for it enables the enzyme to be differentiated by localization into plasmalemmal, cytoplasmic, nucleoplasmic, and nucleolar membranes, the latter being the predominant type. By means of this method it was found that the distribution of the enzyme in the cell is species- and organ-specific and that it can be activated by a specific activator, namely sodium azide. The distribution of the enzyme in the cell depends strictly on the functional state of the cell.

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