EXPERIMENTAL BIOLOGY

INTEGRATION OF TISSUE-SPECIFIC INHIBITORS AND THYROID HORMONES IN REGULATION OF EPITHELIAL CELL PROLIFERATION IN GASTRIC GLANDS

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Cell proliferation in renewing tissues is regulated by interacting factors of systems at different hierarchic levels. The integrative approach to the study of regulatory processes indicates how different controlling mechanisms cooperate with each other into a balanced system [2, 7]. The biological effects of tissue-specific inhibitors of cell proliferation (chalones) depend on the concentrations of many different hormones, which are factors of the regulatory system at the whole body level [1, 3, 5]. Meanwhile, the principles governing interaction between chalones and thyroid hormones have not yet been investigated. In the investigation described below the role of tissue-specific inhibitors of cell proliferation in the regulation of epithelial cell division in the gastric glands was studied in the presence of natural and raised thyroid hormone levels. The particular value of the stomach as a test object is due to the heterogeneity of its cell composition and also the opportunity which it gives of undertaking a comparative analysis of phenomena observed in the fundal and pyloric parts of the organ.

EXPERIMENTAL METHOD

Experiments were carried out on 173 noninbred male albino mice weighing initially 20 g, and kept on a schedule of 12 h of daylight (6 a.m. to 6 p.m.) and 12 h of darkness, and on a standard diet The animals received their last meal 18 h before decapitation. A raised thyroid hormone level was created in 84 mice by intraperitoneal injection of L-thyroxine ("Reanal," Hungary). The hormone was injected daily at 11 a.m. for 7 days, and was dissolved immediately before injection. This injection schedule led to a persistent rise of the serum thyroxine and tri-iodothyronine levels [6]. Mice of the control group received the corresponding volume of solvent of the hormone. At 9 a.m. on the 8th day after the first injection of thyroxine into 40 mice with a natural hormonal background and into 44 mice with a raised thyroid hormone level, a chalone-containing extract (CCE) obtained from the total mucous membrane of the hog stomach by alcoholic precipitation, was injected intraperitoneally in a dose of 10 mg per mouse, in 0.5 ml of physiological saline. The remaining animals received the corresponding volume of solvent of the extract at the same time. The animals were killed four or five at a time, 4 h after the injection and thereafter every 3 h until 1 p.m. the following day. Vinblastine was injected into the mice 3 h before sacrifice in a dose of 4 mg/kg, and 1 h before sacrifice they received an injection of ^{3}H-thymidine in a dose of 3.7 MBq/100 g body weight. Histological sections and autoradiographs of the stomach, tongue, and small intestine were prepared by the usual methods. DNA-Synthesizing activity was estimated by determination of the radioactive index (RI), by calculating the ratio of the number of cells with labeled nuclei and the total number of cells counted. Mitotic activity was judged by the mitotic index (MI), the ratio of the number of cells blocked in mitosis, to which the number of prophases

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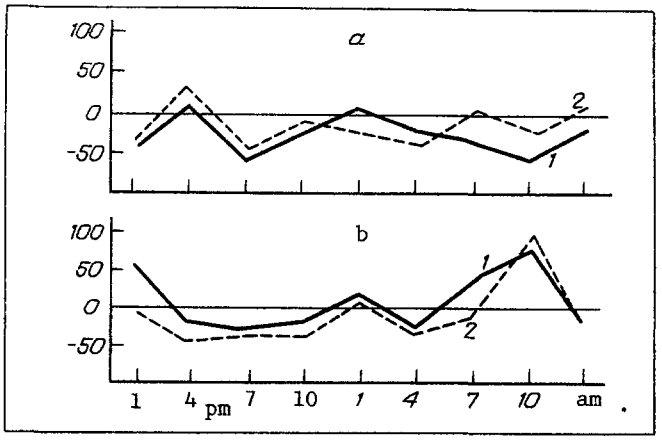

Fig. 1. Dynamics of RI and MI in epithelium of gastric glands of mice receiving CCE. Here and in Fig. 2: abscissa, clock time; ordinate, changes (in %) a) Fundus of stomach, b) pyloric part. 1) RI, 2) MI.

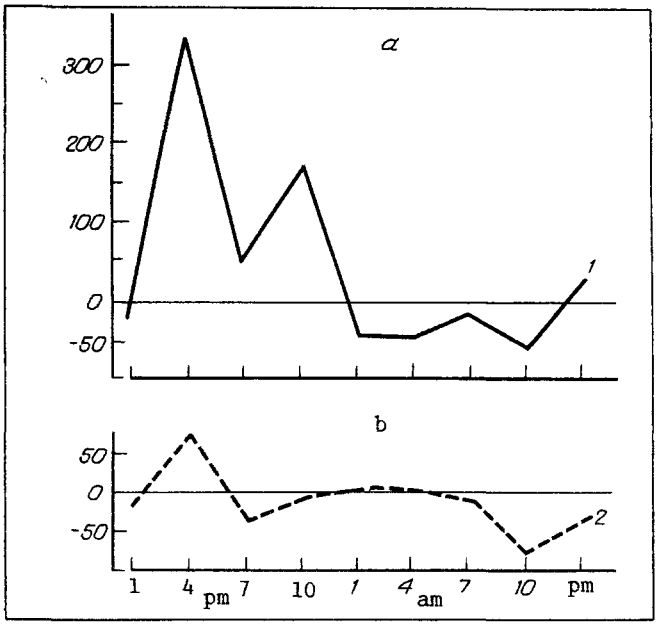

Fig. 2. Dynamics of RI and MI in gastric epithelium of mice receiving thyroxine and CCE.

was added, and the total number of cells counted. In transverse sections through the gastric mucosa, the small intestinal mucosa, and the epithelium of the tongue, at least 5000 cells were counted. RI and MI were expressed in promille. The numerical results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney test.

EXPERIMENTAL RESULTS

Analysis of the trend of cell division showed that against a natural hormonal background, CCE induced depression of DNA synthesis and of mitotic activity in the epithelium of the gastric glands of the mice. RI in the epithelium of the fundal glands of the stomach was reduced 4 and 10 h after injection of the extract by 45.7 and 58.9% ($p < 0.05$), whereas MI was reduced at the same times by 42.5 and 44.3% respectively ($p < 0.05$). Later, there was no difference between DNA-synthesizing and mitotic activity of the control and experimental animals (Fig. 1). The rapid onset of inhibition of

DNA synthesis points to the presence of G_1-chalone activity in CCE and the rapid onset of inhibition of mitotic activity indicates the existence of G_2-chalone activity.

In the epithelium of the pyloric glands RI was reduced 10 h after injection of CCE by 23.1% ($p < 0.05$) and MI was reduced after 7, 10, and 19 h by 40.0, 37.0, and 36.5% respectively ($p < 0.05$). In the latter stages, DNA-synthesis and mitotic activity of the epithelial cells of the gastric glands of intact mice and mice receiving CCE did not differ significantly (Fig. 1). However, after removal of the chalone block a tendency was observed for waves of RI and MI to be formed. The intensity of cell division in the epithelium of the tongue and small intestine was unchanged after injection of CCE.

Thus against a natural hormonal background injection of CCE led to a species nonspecific but tissue-specific inhibition of DNA-synthesizing and mitotic activity in the epithelium of the gastric glands of the mice, evidence of the important role of chalones in the regulation of proliferation of the gastric epitheliocytes. The time of onset and intensity of the inhibitory effect of CCE in the glandular epithelium of the fundus and pyloric part of the stomach did not coincide. The reactivity of the epithelial cells of the glands in these parts of the stomach to the regulatory influence of chalones evidently differs.

The dynamics of changes in cell proliferation in the epithelium of the gastric glands of mice receiving frequent injections of thyroxine, in response to injection of CCE differed significantly from the trend of changes in animals with a natural hormonal background (Fig. 2). In the presence of an excess of thyroid hormones, a single injection of CCE was not accompanied by any significant decrease in RI in the epithelium of the pyloric glands and MI in the epithelium of the fundal glands. DNA-synthesizing activity in the glandular epithelium of the gastric fundus was reduced only after 12 h of circulation of CCE in the mice, by 39.3% ($p < 0.05$). In the glandular epithelium of the pyloric part of the stomach, 10 and 25 h after injection of CCE a decrease of MI by 33.2 and 78.6% respectively ($p < 0.01$) was observed. Consequently, the inhibitory effect of CCE in this case was of shorter duration than that against a natural hormonal background.

Thus under conditions of an excess of thyroid hormones the intensity of inhibition of proliferation by CCE in the glandular epithelium of the stomach was reduced. This rule is manifested differently in the epithelium of the fundal and pyloric glands. It is important to point out that the action of thyroid hormones on the cell kinetics may be realized through their effect on manifestation of the regulatory effects of tissue-specific inhibitors of cell proliferation.

It can be concluded from the results of this investigation that a role in the regulation of proliferation of the epithelial cells of the gastric mucosal glands is played by interacting factors of systems at different hierarchic levels: chalones (local tissue regulation) and thyroid hormones (regulation at the whole body level).

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