CHALONES G_1 AND G_2 OF THE GASTRIC MUCOSA

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Chalones are a universal system of regulation of tissue homeostasis, comparable in their importance with the endocrine or immune systems [5]. More than 20 types of tissue-specific chalone are now known [4, 9]. However, gastric chalones have hardly been studied at all. Only Philpott [15] has shown, on chick embryos, that the gastric mucosa contains an inhibitor with selective suppression of cell division *in vitro*.

The gastric mucosa in man also contains chalones, which possess marked tissue specificity [2]; pyloric chalone, moreover, has been found to be more active than fundal chalone. The inhibitory effect of chalone is weaker in duodenal ulcer than in gastric ulcer. Two chalone-like tissue-specific substances, active against phases G_1 and G_2 of the cell cycle, have been found in the epidermis [12] and mucous membrane of the small intestine [10]. In the small intestine the first of these has a molecular weight of between 120,000 and 150,000 daltons, the second — less than 2000 daltons. The duration of action of chalones on cells of different organs and tissues varies from 5 to 19 h [7, 10, 17]. The dynamics of the inhibitory effect of chalones on the epithelium of the gastric mucosa has not been studied. Investigation of factors regulating the G_1 phase in the gastric mucosa is particularly important for the understanding of the pathogenesis of many of its diseases. The reason is that the G_1 phase is the most labile, and the cell cycle is lengthened or shortened on account of changes in its duration. In atrophic gastritis shortening of the G_1 phase determines more rapid cell renewal, whereas in Zollinger—Ellison syndrome during administration of pentagastrin and also, evidently, in hyperplasiogenic polyps, lengthening of the G_1 phase is the cause of retarded cell formation [1, 3, 8, 11]. Chalone G_1 can direct cells along the path of differentiation. Cells held up by it in the G_1 phase do not subsequently enter the S phase of the cell cycle. It is considered that G_2 chalone transfers cells into the elective pool, without thereby preventing them from returning to the proliferative pool. Meanwhile chalone G_1, by inhibiting proliferation, directs some cells into the fixed pool [6].

The combined use of G_2 chalones and cytostatics acting on cells in mitosis is promising in cancer treatment [6]. In chronic atrophic gastritis and peptic ulcer, the use of chalone G_1 can be expected to prove beneficial.

The aim of this investigation was to detect chalones G_1 and G_2 in the gastric mucosa and to study their effect on cell renewal in the gastric mucosa of mice, and also to determine the time of the peak effect of the action of chalones.

EXPERIMENTAL METHOD

To obtain chalones the gastric mucosa of 21 patients after resection of the stomach for peptic ulcer was used. Regions of mucosa from different parts of the stomach were separated from the submucosa, and an aqueous homogenate was prepared from weighed samples in the ratio of 1:4. The homogenate was frozen at −20°C, to ensure preservation of the active component [9], and after 18 h it was thawed and filtered. Ethyl alcohol was added to the filtrate to obtain 55% from a 72% concentration. The solution was allowed to stand at −4°C for 1 h and then centrifuged for 10 min at 3000 rpm. The residue was discarded and the supernatant used to prepare an 80% alcoholic solution which, in turn, was allowed to stand for 1 h at −4°C. The residue obtained after the second centrifugation was separated and lyophilized. By fractional alcoholic precipitation, the chalones could be obtained separately.

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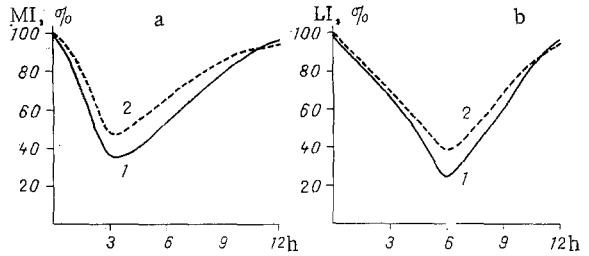

Fig. 1. Action of fundal chalone on regeneration of the gastric mucosa. Here and in Fig. 2: 1) fundal, 2) pyloric region.

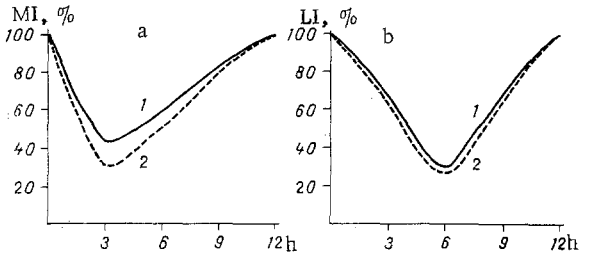

Fig. 2. Action of pyloric chalone on regeneration of the gastric mucosa.

Under these circumstances a mixture of chalones G_1 and G_2 was precipitated from the 55% supernatant, whereas the lighter G_2 fraction was precipitated from the 72% supernatant [13, 14, 16]. The lyophilized product was dissolved in physiological saline at the rate of 20 mg to 1 ml and injected intraperitoneally into 64 noninbred albino mice.

The dynamics of the inhibitory effect of chalone G_2 was studied in the experiments of series I. For this purpose 0.2 ml of chalone with 0.1 mg of colchicine was injected into 20 mice. The animals were killed 3, 6, 9, and 12 h later (five mice at each time). Mice of the control group received an injection of 0.2 ml physiological saline with 0.1 mg colchicine and they were killed in groups of three at the same times. Pieces were excised from the fundal and pyloric regions of the stomach and also from the jejunum.

In series II, 24 mice were given an intraperitoneal injection of 0.2 ml of the solution of the lyophilized product, containing a mixture of chalones G_1 and G2, and they were killed at the same times. A solution of tritiated thymidine, in a dose of 1 μCi/g body weight, was injected intraperitoneally 1 h before sacrifice. Mice of the control group (three animals at each time) received physiological saline instead of the chalones.

Since only a mixture of chalones G_1 and G_2 could be obtained by the method of fractional alcoholic precipitation, experiments of series III were carried out, in which the action of chalone G_2 on DNA synthesis was studied. The animals of this series (20 mice) were killed 6 h after injection of the chalone solution and received an injection of [^{3}H]thymidine 1 h before sacrifice.

The stomach and small intestine were fixed in 10% formalin solution, and strips of mucosa were embedded in paraffin wax. Sections for autoradiographic study were coated with grade M photographic emulsion, exposed for 28 days, developed, and stained with Caracci's hematoxylin. The mitotic index (MI) and labeling index (LI) were calculated in 1000 epithe-

liocytes in the sections by a "blind" method. The final result was expressed as a percentage of the control, which was taken as 100 in each group of experiments.

EXPERIMENTAL RESULTS

Statistical analysis of the data showed that the inhibitory effect of G_2 chalone reached a maximum after 3 h. When fundal chalone was injected, MI in the mucosa of the fundal region fell to 37.5% compared with the control, and in the pyloric region to 48% (Fig. 1). The corresponding figures after injection of pyloric chalone were to 43 and 31% (Fig. 2). After 6 h MI became higher but differed significantly from the control ($P < 0.05$). The difference from the control after 9 h was not significant, and after 12 h values of MI were identical in the control and experimental series.

The inhibitory action of G_1 chalone reached a maximum after 6 h. LI in the mucosa of the fundal and pyloric region 3 h after injection of the chalone was 70% of the control value. After 6 h LI in the fundal region fell to 25% and in the pyloric region to 39% under the influence of chalones from the fundal part of the stomach (Fig. 1), and to 30 and 27% respectively after injection of pyloric chalone (Fig. 2).

LI in the gastric mucosa of animals receiving an injection of G_2 chalone solution did not differ significantly from the control. In all series of experiments neither MI nor LI in the mucosa of the small intestine differed from the control.

The results of these experiments thus show that the epithelium of the human gastric mucosa produces two fractions of a species-nonspecific inhibitor of cell renewal: G_1 chalone, which inhibits DNA synthesis, and G_2 chalone, which delays entry of cells into mitosis.

Both G_1 and G_2 chalones possess some specificity of action, depending on the part of the gastric mucosa from which they are obtained. The antimitotic action of fundal chalone is more marked against the fundal region of the stomach, whereas pyloric chalone is more active against the pyloric part of the stomach.

Regulation of cell division by intratissue inhibitors (chalones), with definite properties of tissue- and cell-specificity, is evidently one of the important factors in the complex system of control of regeneration of the gastric epithelium.

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