ACTION OF AN ESOPHAGEAL CHALONE PREPARATION ON PROLIFERATION OF ESOPHAGEAL EPITHELIAL CELLS

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An essential role in the regulation of cellular homeostasis of many tissues is played by chalones, which are endogenous inhibitors of proliferation [1, 3, 5]. However, this problem has been inadequately studied in relation to the esophageal epithelium.

The aim of this investigation was to study the action of a chalone-containing preparation (CCP) from the esophagus on proliferation of esophageal epithelial cells.

EXPERIMENTAL METHOD

Experiments were carried out on 135 noninbred male albino mice weighing 21-22 g, adapted for 3 weeks to standard conditions: temperature 20 ± 1°C, food provided ad libitum, 12 h of daylight from 6 a.m. to 6 p.m., 12 h of darkness, intensity of illumination 250-300 lx.

CCP was used in the form of a lyophilized aqueous extract from mouse esopbagus [10, 13]. For this purpose pieces of esophagus were frozen in liquid nitrogen, and ground to powder in an agate mortar. The powder was suspended in distilled water in the ratio of 1:7. The cell suspension was then vigorously homogenized in a glass—Teflon homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was lyophilized. All procedures were carried out at 0-4°C.

Mice of one group received CCP by intraperitoneal injection in a dose of 5 mg per mouse, those of the second group received 10 mg per mouse. Control animals were given an injection of 0.5 ml of physiological saline. The injections were given at 8 p.m. The animals were sacrificed 30 min, and 1, 2, 4, 9, 12, and 15 h later. At each stage of the investigation five animals were used. The mice were given an intraperitoneal injection of ^{3}H-thymidine in a dose of 0.75 μCi/g body weight in 0.5 ml physiological saline (specific activity 4.8 Ci/mmole) 1 h before sacrifice. The esophagus and small intestine were taken for investigation. The tissues were fixed in Carnoy's fluid, material was dehydrated by the standard method in alcohols, and embedded in paraffin wax. Sections 5 μ thick were cut. The sections were dewaxed with chloroform and covered with type M liquid photographic emulsion (Photographic Chemical Research Institute), diluted with distilled water in the ratio of 1:1. After exposure of the sections for 3 weeks at 4°C they were developed with Metol developer. The sections were then stained with Ehrlich's hematoxylin. To determine the mitotic index (MI) and index of labeled (with ^{3}H-thymidine) nuclei (ILN) the number of mitoses and the number of labeled cells were counted in 5000 cells of the basal layer of the esophageal epithelium and the epithelium of the small intestinal crypts. The results were expressed in promille. Cells with four or more grains of reduced silver above them were considered to be labeled. Differences between the results were assessed by the Fisher—Student test at a $P \leq 0.05$ level of significance.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the values of Miand ILN in the basal layer of the esophageal epithelium in the control varied during the period of investigation. Maximal values of MI were recorded at 5 a.m. The maximum of ILN occurred before the peak of MI, namely at 9 p.m., in agreement with data in the literature [4, 6].

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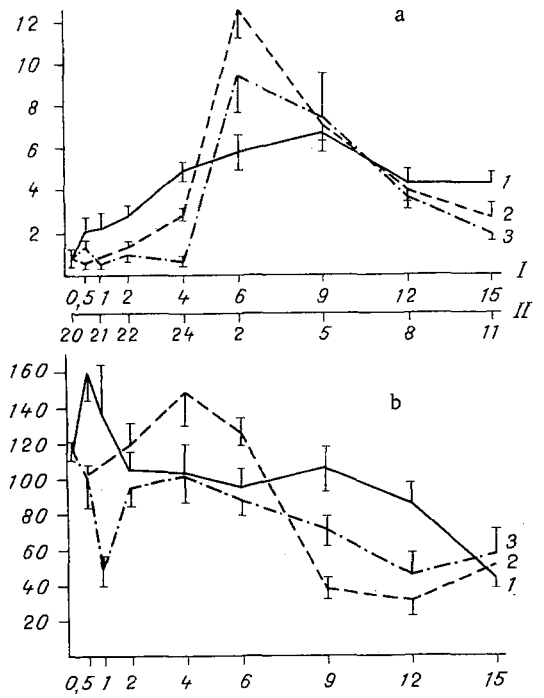

Fig. 1

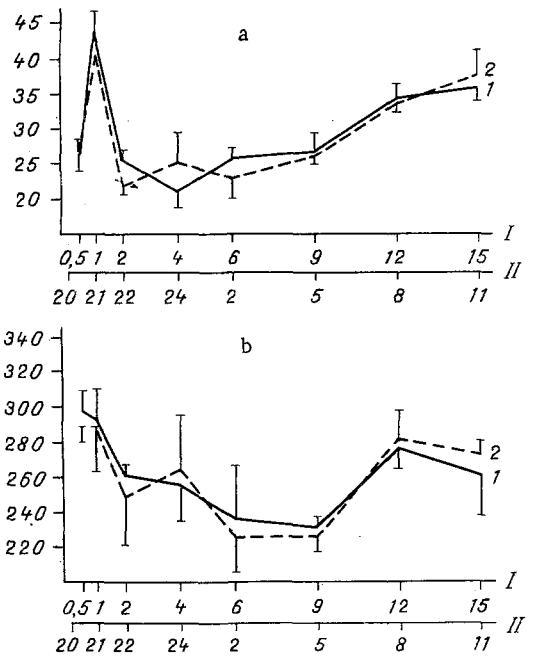

Fig. 2

Fig. 1. Effect of different doses of CCP, isolated from esophageal cells, on mitotic activity (a) and level of DNA-synthesizing cells (b) in basal layer of mouse esophageal epithelium. a) Abscissa: 1) time after injection of preparation (in h), II) clock time; ordinate, MI (in °/₀₀); b) abscissa, time after injection of CCP (in h); ordinate, ILN (in °/₀₀). 1) Control; 2) CCP in dose of 5 mg per mouse; 3) CCP in dose of 10 mg per mouse.

Fig. 2. Effect of CCP isolated from esophageal cells on mitotic activity (a) and level of DNA-synthesizing cells (b) of epithelium of small intestinal crypts. a) Abscissa: I) time after injection of CCP (in h), II) clock time; ordinate, MI (in °/₀₀); b) abscissa: I) time after injection of preparation (in h), II) clock time; ordinate, ILN (in °/₀₀). 1) Control 2) CCP in a dose of 10 mg per mouse.

A characteristic feature of chalones from the majority of tissues is that their action is directly dependent on the dose given [8, 9, 11]. The results of the present experiments confirmed this conclusion. The choice of doses for studying dependence of the action of CCP on cell division and DNA synthesis in the esophageal epithelium on dose was based on data in the literature indicating marked inhibition of MI and ILN under the influence of similar doses of the preparations obtained in a similar manner [13]. It will be clear from Fig. 1a that 30 min after injection of each dose of CCP entry of the esophageal epithelial cells into mitosis and into the phase of DNA synthesis was delayed. The maximal fall of MI (by 88.4%, $P < 0.001$) was produced by a dose of 10 mg per mouse 4 h after injection; a dose of 5 mg per mouse of CCP depressed mitosis at this time by only 43% ($P < 0.01$). Inhibition of cell division in the esophageal epithelium persisted for 4 h. We know from the literature that the duration of the G_2 phase in the esophagus is 1 h [2]. Consequently, inhibition of mitotic activity in the esophageal epithelium can be explained by delay of the cells by CCP in the G_2 phase.

Six hours after injection of CCP MI exceeded the control value by 120.5% ($P < 0.01$) when the dose given was 5 mg per mouse, and by 66.3% ($P = 0.05$) when the dose was 10 mg per mouse; this result can evidently be explained by the synchronizing action of the chalone on the emergence of the cells from the G_2 phase into mitosis. At the later stages of the experiment mitotic activity did not differ significantly from the control values. Consequently, the duration of action of G_2 chalone from the esophagus on cell division in its epithelium is 9 h. The effect of esophageal G_2 chalone on cell division in the esophageal epithelium is clearly reversible and characterized by a phasic pattern. Mitotic activity 15 h after injection of CCP in doses of 5 and 10 mg per mouse was reduced by 38% ($P = 0.05$) and 55% ($P < 0.02$), respectively. Since the duration of the phases of the mouse esophageal epithelial cells (S +

+ G_2 + $\frac{1}{2}$M) is 9.7 [2], the change in MI can be explained by the influence of the G_1 component of the preparation on the passage of cells into the S phase, which later is reflected also in their passage into mitosis. The existence of this effect is shown by analysis of the level of DNA-synthesizing cells in the esophageal epithelium after exposure to CCP.

ILN fell after 30 min by 37% ($P < 0.02$, $P < 0.05$) after injection of doses of 5 and 10 mg per mouse (Fig. 1b). After 1 h the number of DNA-synthesizing cells had fallen by 67% ($P < 0.02$) when 10 mg of the preparation was injected, and after 2 h their number had reached the control value. When a dose of 5 mg per mouse was used, the number of DNA-synthesizing cells after 4 and 6 h was 45% ($P = 0.05$) and 33% ($P < 0.05$), respectively, higher than the control. After 9-12 h ILN in both cases again was significantly reduced, but after 15 h the number of DNA-synthesizing cells was back to normal. This biphasic character of the curve showing the number of DNA-synthesizing cells in the esophageal epithelium of the mice after injection of CCP is evidence of its action on the cell in the late G_1 phase and early G_2 phase (or on resting cells). The question of reversibility of the action of G_1 chalone in the form of synchronization of the entry of the cells into the S phase, requires further study.

The results of a study of tissue specificity of esophageal chalone are given in Fig. 2. To assess the tissue specificity of the inhibitory action of esophageal CCP on the number of dividing and DNA-synthesizing cells of this tissue, its action on MI and ILN was studied in the epithelium of the small intestinal crypts. No effect of esophageal CCP was found on MI (Fig. 2a) and ILN (Fig. 2b) in the epithelium of the small intestinal crypts, evidence of tissue specificity of the biological action of esophageal CCP.

In the opinion of most investigators [7, 8, 12, 13], the preparations tested may be considered to contain chalone if it is water-soluble, is produced by and contained in the tissue on which it acts tissue-specifically, briefly, and reversibly, on the late G_1 phase, blocking passage into the S phase and (or) the G_2 phase, delaying passage of the cells into mitosis, and has an inhibitory action on cell reproduction both in vivo and in vitro. For most chalones characteristic features are thermolability and dependence of effect on dose. It follows from the data in this paper that the preparation which we used possesses all the fundamental properties listed above.

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