ACTION OF HYDROCORTISONE ON THE TEMPORAL PARAMETERS OF THE MITOTIC CYCLE IN DUODENAL EPITHELIAL CELLS

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Experiments in which thymidine-H^3 was injected into male C57BL mice weighing 20-30 g showed that after a single injection of 0.1-3 mg hydrocortisone the duration of the period of DNA synthesis and of the whole mitotic cycle of cells of the duodenal crypts was sharply increased. The index of labeled nuclei fell from 29 (normal) to 15-9%. The results show that erosions and ulcers of the intestinal mucosa can arise as a result of marked inhibition of physiological regeneration of the intestinal epithelium by glucocorticoids; consequently, the increase in concentration of glucocorticoid hormones may be a pathogenetic factor in the development of duodenal ulcers.

Hypertrophy of the adrenal cortex and elevation of the blood level of the glucocorticoids are observed in patients with duodenal ulcer [3, 7]. Every exacerbation is associated with an increase in the concentration of glucocorticoids in the blood [6]. It has also been known for a long time that during prolonged states of stress in animals, erosions develop in the mucous membrane of the stomach and small intestine [10]. Administration of glucocorticoids is used as a method of producing erosions and ulcers of the stomach and duodenum experimentally [8]. During the treatment of patients with rheumatoid arthritis and bronchial asthma with glucocorticoids, gastric and duodenal ulcers occurred in 16-31% of cases [7]. Nevertheless the mechanism of action of glucocorticoids in these circumstances is not clear. It was therefore decided to study the action of glucocorticoids on the processes of cell renewal in the duodenal epithelium, the frequent site of ulcers.

The object of this investigation was to study the effect of hydrocortisone on the temporal parameters of the mitotic cycle in duodenal epithelial cells and on the index of labeled nuclei (ILN). These indices reflect the intensity of physiological regeneration of the tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 150 C57BL mice weighing 20-23 g, divided into three groups: group 1 was the control, and the mice of groups 2 and 3 received an intraperitoneal injection of an aqueous suspension of hydrocortisone (Gedeon Richter, Hungary), containing 0.1 and 0.3 mg hydrocortisone respectively at 5 a. m. At 6 a. m. the animals of all groups received an intraperitoneal injection of thymidine-H^3 in a dose of 0.7 μCi/g body weight (thymidine-H^3 of Soviet manufacture, specific activity 1.4 Ci/mmole). The mice were sacrificed in batches of three at the following times: 7, 8, 9, 10 a. m., noon, 3, 4, 6, and 10 p.m. midnight, 3, 7, and 10 a.m., and 1, 4, and 7 p.m. At autopsy the duodenum was removed, fixed in Carnoy's fluid, embedded in paraffin wax, and cut into sections 5 μ in thickness. The sections were dewaxed, coated with type R (NIIKHIMFOTO) emulsion, and 14 days later the sections were developed and stained with Carazzi's hematoxylin. The number of labeled and unlabeled mitoses in the epithelium of the crypts was counted in the duodenal sections and the number of labeled mitoses was expressed as a percentage of the total for each time of sacrifice in the animals of each of the three groups.

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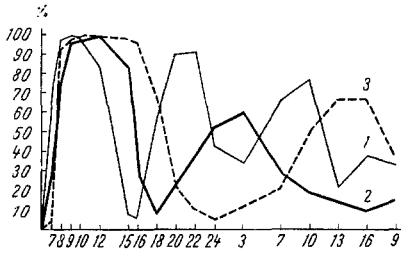

Fig. 1. Changes in percentage of labeled mitoses with time in duodenal crypts under normal conditions (1) and after administration of 0.1 mg (2) and 3 mg (3) hydrocortisone. Abscissa: time after injection of thymidine-H^3 (in h); ordinate: percentage of labeled mitoses. Curves: 1) control, 2) injection of 0.1 mg hydrocortisone, 3) injection of 3 mg hydrocortisone.

The duration of the periods of the mitotic cycle and of the cycle as a whole was determined graphically by the method of Quastler and Sherman [9] in the experimental and control groups. In addition, in each group the ILN was determined for all the animals sacrificed at 8, 9, and 10 a. m.

EXPERIMENTAL RESULTS

The experimental results are shown graphically in Fig. 1. The temporal parameters of the mitotic cycle of the duodenal epithelial cells in the control (curve 1) were as follows: the total duration of the cycle was 11 h, the duration of DNA synthesis (S-period) was 6.5 h, and the mean duration of the premitotic period (G_2) was 1 h. The duration of the presynthetic period (G_1) was calculated by the equation:

$$G_1 = T - (S + G_2 + M) = 11 - (6.5 + 1 + 0.5) = 3\text{h},$$

where T is the duration of the complete cycle, S the duration of the period of DNA synthesis, G_2 the duration of the premitotic period, and M the duration of mitosis. The mean ILM for the intact was 29%.

After administration of 0.1 mg hydrocortisone to the mice the temporal parameters of the mitotic cycle of the duodenal epithelial cells showed significant changes (Fig. 1, curve 2): T = 16 h, S = 8.5 h, G_2 = 1.4 h, G_1 = 5.6 h. After a single injection of hydrocortisone the total duration of the mitotic cycle was thus increased by 5 h, the duration of DNA synthesis by 2 h, and the duration of the presynthetic period by 2.6 h. Under these same conditions the ILN was reduced to 15%, i.e., almost by half.

Injection of 3 mg hydrocortisone retarded the passage of the cells through the phases of the mitotic cycle still more (Fig. 1, curve 3). The total duration of the mitotic cycle reached 26 h, or $2\frac{1}{2}$ times greater than its duration in the duodenal epithelium of intact mice. The duration of DNA synthesis also was increased, to 11.5 h (normal 6.5 h). The mean ILM after administration of this dose of hydrocortisone was reduced still further – to 9%.

Thirty hours after injection of the thymidine-H^3 into intact mice cells with labeled nuclei had managed to reach the tip of the duodenal villi. After this same period in animals receiving 0.1 mg hydrocortisone, labeled nuclei were still found only at the level of the lower third of the villi.

It is interesting to note that the epithelium of the small intestine is particularly sensitive to hydrocortisone, for the same doses of the hormone had no effect on the mitotic cycle or the proliferative pool of the esophageal epithelium [2, 4].

It can be assumed that during a prolonged increase in the concentration of glucocorticoids in the body the marked inhibition of physiological regeneration of the intestinal epithelium must lead to the formation of an epithelial defect, i.e., of an erosion. This increase in the level of the glucocorticoids, in the writers' view, is a primary pathogenetic factor in the development of duodenal ulcer. The role of hypersecretion of gastric juice with an increased content of pepsin and hydrochloric acid in the pathogenesis of the ulcer is important, but not primary.

Whereas some clinicians consider that hypersection of glucocorticoids is the result of the ulcer [1, 3], many others regard adrenocortical dysfunction as one of the pathogenetic factors of peptic ulceration [5, 7].

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