PROLIFERATIVE RESPONSE OF GASTRIC GLAND EPITHELIUM TO VAGOTOMY AND SYMPATHECTOMY

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The widespread occurrence of diseases of neurogenic etiology endows the general biological problem of neurotrophism, many aspects of which have still received little study, with medical importance. The integrating effect of the nervous system is essential for the action of factors regulating cell renewal in tissues, but data obtained by different workers relative to the role of the parasympathetic and sympathetic innervation in the mechanism of trophic control of proliferative processes are contradictory [3]. In this connection great interest has been aroused by the principles of adaptive morphological and functional changes in denervated tissues, characterizing the mechanisms of interaction between regulators of proliferation at the tissue and whole body levels. They are particularly complex in the glandular epithelium of the stomach, which is distinguished by its structural and functional heterogeneity and, as a result, by the marked polymorphism of cellular responses to disturbance of innervation.

The aim of the present investigation was accordingly to study circadian rhythms and intensity of DNA synthesis and mitotic activity and also changes in the structural state of the epithelium of the gastric glands in the destructive phase of neurogenic dystrophy caused by vagotomy and chemical desympathization.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats with a mean weight of 220 g. The animals were kept on a constant schedule of daylight and darkness (6 a.m. to 6 p.m. in daylight, 6 p.m. to 6 a.m. in darkness), with free access to food and water. In the experiments of series I the parasympathetic innervation was disturbed by bilateral subdiaphragmatic vagotomy, performed under inhalational ether anesthesia on male rats 10 days before the experiment. In series II males and females kept together for 3 days before the experiment to ensure synchronization of the ovarian cycle were used. The experiments began 30 days after desympathization, induced by injection of guanethidine in a dose of 10 mg/100 g body weight daily for 3 weeks, starting from the first day after birth. In both series intact rats of the same age served as the control. Control and experimental animals were killed simultaneously at equal intervals throughout the 24-hour period (3 h in series I, 4 h in series II), having been given ^{3}H-thymidine in a dose of 3.7 MBq (0.1 ml)/100 g body weight (specific activity 0.85 TBq/mmole) 1 h before sacrifice to record DNA-synthesizing cells, vinblastine (2.5 mg/kg into the animals of series I) 3 h before sacrifice, and colchamine (5 mg/kg into the animals of series II) 4 h before sacrifice to obtain a more objective estimate of mitotic activity. The number of blocked mitoses (C-mitoses) and the number of cells with labeled nuclei were counted in autoradiographs of the fundal part of the stomach in 50 longitudinally divided gastric glands in each preparation. The index of labeled nuclei (ILN) and the index of C-mitoses (MI_{col}) were calculated in 1000 epitheliocytes and the cell renewal time was determined by integrating the mitotic activity values over the 24-h period, by the equation:

$$CRT = 1/\Sigma MI_{col}.$$

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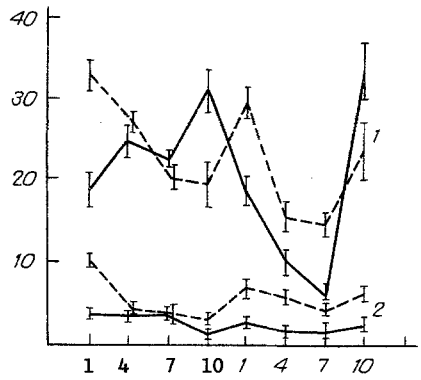

Fig. 1. Circadian rhythm of changes in proliferative activity of epithelial cells of gastric glands of intact and vagotomized rats. Continuous line — control; broken line — vagotomy; 1) ILN (in ‰); 2) MI_{col} (‰) Abscissa, clock time.

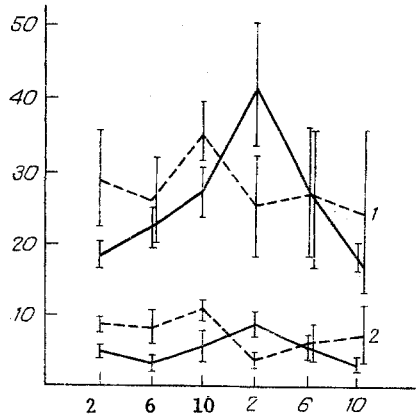

Fig. 2. Circadian rhythm of changes in proliferative activity of epithelial cells of gastric glands of intact and sympathectomized rats.

Changes in the structural state of the glandular epithelium were assessed by means of morphometric criteria, with calculation of an epithelial formula for 20 longitudinal sections through the fundal glands [5].

The numerical results were subjected to statiatical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Fig. 1. A bimodal rhythm of changes in the number of DNA-synthesizing cells with maxima of ILN at 10 p.m. and 10 a.m. was observed in the epithelium of the fundal glands of the intact rats. A low level of DNA synthesis was observed between 1 and 7 p.m. and DNA-synthetic activity became maximal at 7 a.m. The mean daily ILN was 20.98‰. After vagotomy the circadian rhythm of DNA synthesis in the epithelium of the fundal glands changed abruptly: it rose to a maximum at 1 p.m., ILN fell until 7-10 p.m. ($p < 0.05$), and at 1 a.m. a second peak was observed, and was followed by a significant ($p < 0.01$) decrease of this parameter, At times of the 24-h period when the level of DNA synthesis in the intact rats was low (1 p.m., 1-7 a.m.) vagotomy led to a significant ($p < 0.05$) increase in ILN, although its mean value for the 24-h period was virtually unchanged compared with parameters obtained for the control animals, not exceeding 22.9‰. The amplitude of the circadian rhythm of DNA-synthetic activity fell after the operation by 2.5 times (from 5.71 to 2.17), evidence of loss of synchronization of the cells entering the phase after vagotomy.

TABLE 1. Epithelial Formula of Gastric Glands after Denervation

Type of denervation	Animals	Chief cells, %	Parietal cells,%	Parietal cells, %
Vagotomy	Control	53,85±1,51	15,07±2,29	31,08±2,72
	Experimental	47,42±1,97*	15,22±1,86	37,36±2,21
Sympethectomy	Control	52,67±2,34	11,25±0,97	36,08±1,91
	Experimental	53,77±3,65	10,17±0,94	36,06±4,19

Legend. *$p < 0.05$. Significantly different result.

The circadian rhythm of entry of cells of the gastric gland epithelium into mitosis was unimodal with a maximum between 1 and 7 p.m.

The mean 24-hourly values of MI_{col} were 2.74% and the cell renewal time in the epithelium of the fundal glands 45.70 days. After bilateral subdiaphragmatic vagotomy the rhythm of the circadian changes in the number of dividing cells of the glandular epithelium of the stomach became bimodal: a maximum of mitotic activity was recorded at 1 p.m., and was followed by a significant ($p < 0.001$) reduction of its value at 4-10 p.m., a second rise at 1-4 a.m., following by a further fall at 7 a.m. The mean 24-hourly value of MI_{col} in the vagotomized rats was twice as high as in the control, namely 5.55‰, the indices of mitotic activity between 10 p.m. and 7 a.m. being significantly ($p < 0.01$) higher than its level in intact animals. The cell renewal time in the epithelium of the fundal glands fell from 45.7 days in the control to 22.52 days in the experimental rats. Reduction of the amplitude of the circadian rhythm of mitotic activity after vagotomy is evidence of weakening of synchronization of entry of the epithelial cells of the fundus of the stomach into mitosis.

The quantitative and phase changes which we observed in the parameters of proliferation are characteristic of compensatory and regenerative changes in the digestive organs after vagotomy, which other workers also have described [3].

The results of the experiments of series II (Fig. 2) showed that sympathectomy, like vagotomy, is followed by changes in circadian rhythms and in the intensity of cell multiplication in the epithelium of the fundal glands The highest value of ILN in the control rats was observed in the glandular epithelium of the gastric fundus at 2 a.m., compared with 10 p.m. in the experimental rats. The mean 24-hourly value of ILN did not change significantly (only from 26.41 to 28.3‰). Synchronization of the cells before the S-phase was weakened, as shown by the decrease in the amplitude of the circadian rhythm of DNA-synthetic activity (from 2.24 to 1.44) in desympathized animals. Even greater differences were found on analysis of the circadian rhythm of mitotic activity. The largest number of epithelial cells of the gastric glands embarking on mitosis was observed at 2 a.m., compared with 10 p.m. in the case of the experimental rats. The mean 24-hourly MI 1 rose from 5.15 to 7.57‰, and the cell renewal time was reduced by more than 10 days (from 32.39 to 22.03 days). The amplitude of the rhythm of mitotic activity was almost unchanged (3.14 and 3.00 respectively).

The proliferative response of cells of the glandular epithelium of the stomach to desympathization was similar to that in response to vagotomy: synchronization of their entry into the phase of DNA synthesis and into mitosis was reduced, and mitotic activity was increased. Just as after total sympathectomy, in response to injection of antibodies to nerve growth factor [1, 2], intensification of proliferation in the denervated tissue was observed in our experiments also. In our opinion the results are particularly interesting when compared with data of morphometric evaluation of the state of the glandular epithelium of the denervated stomach. The number of cells per gland fell by 14.5% ($p < 0.05$) in the vagotomized animals and had a marked tendency to decline (by 17.9% compared with the control, but $p > 0.05$) in the sympathectomized rats. However, whereas after vagotomy the number of highly specialized chief glandulocytes in the gastric glands was reduced, sympathectomy did not cause any change in the epithelial formula (Table 1). This evidently reflects the specific features of parasympathetic and sympathetic regulation of cell renewal, just as in the denervated liver vagotomy leads to a decrease but sympathectomy to an increase in the cohesive force of the hepatocytes, against a background of increased mitotic activity in both cases [4].

The proliferative response of the glandular epithelium to disturbance of the parasympathetic and sympathetic innervation is thus similar in direction and is manifested as intensification and desynchronization of cell renewal processes, although changes in the histologic structure of the gastric glands differ after the different types of denervation The trophic action of the autonomic nervous system evidently takes place through the predominant influence of one of its parts on cell proliferation, but of the other part on differentiation of cells of the gastric glandular epithelium.

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EFFECT OF ELEUEROCOCCUS ON BIORHYTHMS OF PERIPHERAL BLOOD PARAMETERS IN DOGS

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A general biological law of the undulating course of all phases of adaptation, realized as an oscillating process, has been formulated and explains differences in biological effects obtained by acting on the living organism at different times [1, 4]. The chronobiological analysis of the effect of one of the most popular adaptogenic plant preparations in the Soviet Union, namely eleuterococcus, on peripheral blood parameters, which are known to undergo circadian and seasonal variations, is particularly interesting from this standpoint [3, 7, 8, 10, 11, 13, 15], being concerned with the temporal organization of the system [2, 9, 12]. The widespread use of eleuterococcus to optimize adaptive processes [5, 6, 14] under ordinary and stress situations requires knowledge of its chronotherapeutic action.

This paper describes the study of circadian rhythms of concentrations of erythrocyte, hemoglobin, and leukocytes in the peripheral blood of dogs and the effect on them of eleuterococcus, administered repeatedly at different times of day.

EXPERIMENTAL METHOD

Experiments were carried out in winter on 12 inbred (beagles) and mongrel dogs closely similar in age and body weight, and kept for a long time under standard animal house conditions, with regular alternation of daylight (12 h) and darkness (12 h), and receiving similar diet and exercise. Blood for analysis was taken from the anterior Skachkov veins, keeping strictly to the order of their use for a period of 3 days. In each series tests were carried out in six time cuts: 6 and 10 a.m., 2, 6, and 10 p.m., and 2 a.m. The concentrations of erythrocytes and leukocytes were determined with a hemocytometer, and hemoglobin by a hemoglobinometer. A pharmacopoeial preparation of liquid extract of eleuterococcus was evaporated before use to one-quarter of its volume and diluted to the original volume with physiological saline, after which it was fed to dogs on bread in a dose of 1.5 ml/10 g body weight for 14 days; six dogs received only one dose of the preparation, at 9 a.m., the other six at 3 p.m., after which the program of tests was repeated. The experimental results were analyzed by a nonlinear method of least squares and by Student's statistical test, on a computer. There were two series of experiments In series I the initial rhythms of concentrations of erythrocytes, hemoglobin, and leukocytes were studied in winter in all 12 dogs. The dogs were then divided into two subgroups,

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