DIURNAL RHYTHM OF CHANGES IN THE NUMBER OF DNA-SYNTHESIZING AND MITOTICALLY-DIVIDING JEJUNAL EPITHELIAL CELLS AFTER SUBDIAPHRAGMATIC VAGOTOMY

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Bilateral subdiaphragmatic vagotomy in rats led to an increase in proliferative activity of the jejunal epithelium 7 days after the operation without any accompanying change in the diurnal rhythm of cell division. Meanwhile, after vagotomy the phase structure of the rhythm of DNA synthesis was modified in the intestinal epithelial cells: from the normal unimodal type it became bimodal.

KEY WORDS: vagotomy; jejunum; DNA synthesis; mitoses.

Experimental and clinical investigations have shown the role of neurotrophic influences in the regulation of renewal of the cell composition of the epithelium of the small intestine [2, 10, 12-14]. However, on the basis of information at present to be found in the literature it is impossible to reach any valid conclusion regarding the role of the nervous system in the regulation of cell division or on the mechanisms by which the nervous system exerts its influence on mitotic activity of the tissues [3].

Accordingly, it is interesting to study the contribution of the autonomic nervous system (and, in particular, its parasympathetic division) in the regulation of cell reproduction. The study of this problem is also of practical importance, bearing in mind the frequency with which vagotomy is performed as a component of many conservative operations used for the treatment of gastric and duodenal ulcers.

EXPERIMENTAL METHOD

Experiments were carried out on 123 male Wistar rats weighing 120-150 g. Under ether anesthesia subdiaphragmatic vagotomy was performed on 51 rats, and the remaining animals served as the control. The animals were killed 7 days after the operation and 24 h after the last meal: This occurred every 3 h in the course of the 24-h period starting from 10 a.m. An intraperitoneal injection of [H^3]thymidine in a dose of 1 μCi/g body weight was given to the rats 1 h before sacrifice. Pieces of the proximal portion of the jejunum were taken for investigation. In histological sections 7 μ thick, the number of mitoses and the number of cells with labeled nuclei in crypts cut along their long axis were counted. The mitotic index (MI) and the index of labeled nuclei (ILN) were expressed in promille. The results were subjected to statistical analysis by the methods of Fisher and Student and of R. B. Strelkov.

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that the largest number of labeled nuclei in the jejunal epithelium of the control animals was found between 10 a.m. and 4 p.m., with a maximum of ILN at 1 p.m. ILN then fell (for the interval between 10 a.m.-4 p.m. and 7 p.m. $P = 0.0001$), and it remained low until 10 p.m. The increase in ILN at 1 a.m. was not significant ($P = 0.064$). By 7 a.m. the number of labeled nuclei had risen again ($P < 0.05$). Consequently, in the course of the 24-h period a period of increased DNA-synthesizing activity of the epithelial cells (from 7 a.m. to 4 p.m.) and a period of reduced activity (from 7 p.m. to 4 a.m.) can be distinguished.

In the vagotomized rats the highest values of ILN were found at 4 p.m. Consequently,

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TABLE 1. Diurnal Changes in Number of DNA-Synthesizing and Mitotically Dividing Jejunal Epithelial Cells After Subdiaphragmatic Vagotomy in Rats (M ± m)

Time of day	ILN, ‰			MI, ‰		
	control	experiment	P	control	experiment	P
10	465,5±70,2	349,9±53,5	>0,05	18,8±3,4	22,6±5,5	—
13	559,9±56,2	463,7±62,4	>0,05	17,5±1,9	20,9±4,6	—
16	405,3±95,9	701,2±2,9	=0,013	18,3±3,2	21,6±5,3	—
19	143,0±30,7	537,1±123,8	=0,011	19,8±4,0	17,7±5,1	—
22	129,3±23,6	309,9±68,8	=0,034	18,2±4,1	30,2±3,7	>0,05
1	387,9±121,1	473,5±175,4	>0,05	24,7±5,7	31,3±3,8	>0,05
4	131,4±12,4	209,2±5,7	=0,003	27,4±9,7	44,4±8,1	>0,05
7	499,3±54,0	679,7±61,7	=0,055	43,4±4,7	61,8±38,8	=0,005
Mean diurnal value	340,2	465,5		23,5	29,2	

the peak of DNA synthesis showed a phase delay of 3 h. From 10 p.m. to 4 a.m. ILN was low, it increased significantly until 7 a.m. (in the interval 4-7 a.m. $P < 0.05$) and fell again until 10 a.m. ($P < 0.05$). In the vagotomized animals the phase structure of the rhythm of DNA synthesis was thus altered: It became biomodal (maxima of ILN at 1-7 p.m. and 7 a.m., minima at 10 p.m.-4 a.m. and 10 a.m.). The amplitude of the waves of ILN also was reduced in the course of the 24-h period from 4.3 in the control to 3.2. This fact points to some degree of desynchronization of the commencement of DNA synthesis by the cells.

The absolute values of ILN in the vagotimized rats were appreciably higher than the corresponding values for the control animals at nearly all times of the investigation. The mean diurnal value of ILN was about 37% higher than the control.

The diurnal dynamics of MI in the jejunal epithelium of the intact and experimental rats was similar in character. The number of mitotically dividing cells reached a maximum at 4-7 a.m. and a minimum at 1-7 p.m. Differences in the values of MI in the intact and vagotomized animals were particularly marked at times during the 24-h period characterized by an increase in mitotic activity in the control animals. Elevation of the level of cell proliferation after vagotomy, incidentally, was manifested much more clearly after the use of colcemid [10]. The use of colcemid has demonstrated an increase in MI after vagotomy in other organs also — in the liver [1] and pancreas [4]. These findings confirm the view that the use of stathmokinetic agents is essential for the objective evaluation of changes in the kinetics of cell proliferation [3, 6].

It will be noted that changes in the rhythm of cell division are observed under the influence of other experimental procedures modifying the state of the autonomic nervous system [7, 11]. The nervous system thus participates not only in the maintenance of homeostasis, but also in the temporal organization of morphogenetic processes.

In the present experiments the general character of the rhythm of mitoses was preserved after vagotomy, whereas the rhythm of DNA synthesis became bimodal. These findings confirm the concept [8] that, in principle, it is possible for the mechanisms of cell synchronization to function separately before the S phase and before mitosis, as has been shown in other objects [5].

It can thus be concluded that vagotomy leads to an increase in the proliferative activity of the epithelium of the small intestine while at the same time it modifies the parameters of the rhythm of cell division. Very probably the increase in mitotic and DNA-synthesizing activity of the intestinal epithelium after vagotomy is based on predominance of sympathetic tone, for stimulation of the splanchnic nerves is known to give the same result [15]. Vagotomy may also lower the threshold of sensitivity of the epithelial cells of the small intestine to the action of other regulatory (stimulating) factors of nervous and humoral nature. The development of destructive processes in the small intestine after vagotomy may also play a definite role in the stimulation of mitotic activity [9].

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IMMUNOREACTIVE LULIBERIN IN THE VISCERAL ORGANS OF RATS

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The distribution of luliberin (luteinizing hormone-releasing hormone, LH-RH) in various organs and tissues of rats was investigated. In the liver, kidneys, duodenum, pancreas, adrenals, and heart of rats a factor analogous to LH-RH in its immunochemical and chromatographic properties was found. The concentration of immunoreactive LH-RH in the liver, kidneys, duodenum, pancreas, and adrenals was about equal (5-7 pg/mg methanol extract obtained from an acetate extract of acetone powder), whereas in the heart it was a little less (2 pg/mg extract). In the blood cells this factor was present in trace amounts. The immunoreactive LH-RH of the visceral organs is either hypothalamic in origin or is synthesized in these organs.

KEY WORDS: luliberin; visceral organs.

Considerable progress has been made in recent years in the study of the peptide hormones of the hypothalamus: thyroliberin (TRH), luliberin (luteinizing hormone-releasing hormone — LH-RH), and somatostatin. They have been isolated in the pure form, their chemical structure has been established, and they have been synthesized chemically [4]. Studies of the mechanism of regulation of secretion and synthesis of anterior pituitary hormones by these hypothalamic factors are making rapid progress [4, 14].

Ever-increasing attention is nowadays being paid to investigations of the extrahypothalamic localization of TRH, somatostatin, and LH-RH and their effect on regulatory processes unconnected with pituitary activity. Recently published work has shown that somatostatin may be found in the pancreas, intestine, and other organs [5, 6] and that this hormone regulates various physiological processes in the body [13]. As regards TRH it is known that, besides in the hypothalamus, it is also found in various parts of the CNS [7, 8, 11], and for that reason a mediator function has been suggested for this tripeptide [7, 8, 11]. Somatostatin and TRH are thus evidently characterized by a broad spectrum of biological action.

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