THYROID HORMONES REGULATE EPITHELIAL CELL PROLIFERATION IN GASTIC GLANDS

S. N. Smirnov, S. N. Fedchenko, and S. G. Mamontov

UDC 616 33-018.73-02:577.175.44]-07

KEY WORDS: thyroid hormones; cell proliferation; stomach.

Thyroid hormones have a significant influence on proliferation [6, 8, 10]. There is little information about the action of these hormones on cell kinetics in the epithelium of the gastric glands [1] and we have no complete idea of their contribution to the regulation of epithelial cell division in the gastric glands. Meanwhile an explanation of the principles governing thyroid regulation of cell multiplication in the glandular epithelium of the stomach would help to shed light on some difficult problems in the pathogenesis and correction of human diseases linked with disturbances of proliferation in the epithelium of the gastric glands. This paper describes the study of the pattern of cell division in the glandular epithelium of the fundal and pyloric parts of the mouse stomach in the presence of natural and elevated thyroid hormone levels.

EXPERIMENTAL METHOD

Experiments were carried out on 96 noninbred male albino mice initially weighing 20 g, kept under conditions of a fixed period of daylight (from 6 a.m. to 6 p.m.) and on a standard diet. The last meal was given 18 h before sacrifice. Raised levels of thyroid hormones in the experimental animals (46 mice) were produced by intraperitoneal injection of L-thyroxine (from Reanal, Hungary) in a dose of 0.1 mg/kg. The hormone was injected daily at 11 a.m. for 7 days. A solution of thyroxine was prepared immediately before injection [5]. Animals of the control groups received the solvent of the hormone at the same times. This pattern of injection of thyroxine ensured continuous hyperthyroidism, The raised plasma thyroid hormone level was maintained for at least 96 h after the last injection [7].

To assess cellular proliferation we used autoradiographic and stathmokinetic methods, The circadian rhythm of proliferation was studied by decapitating animals four or five at a time from the control and experimental groups every 3 h, All mice were given an intraperitoneal injection of vinblastine 3 h before sacrifice in a dose of 4 mg/kg body weight, and ^{3}H-thymidine in a dose of 3.7 MBq/100 g body weight 1 h before sacrifice. To determine the size of the proliferative pool, ^{3}H-thymidine was injected into six control and six experimental mice every 5 h during the 24-h period. Specific radioactivity of the ^{3}H-thymidine was 0.85 TBq/mmole. Histological preparations and autoradiographs were obtained by traditional methods and stained with Mayer's hematoxylin. The number of labeled nuclei and the number of blocked mitoses were counted among at least 5000 epitheliocytes in longitudinal sections through the gastric mucosa, DNA-synthesizing activity was characterized by the autoradiographic index (RI) and mitotic activity by the mitotic index (MI); RI and MI were expressed in promille, the proliferative pool as a percentage. Circadian rhythms of DNA synthesizing and mitotic activity were assessed by a graphic-parametric method [9].

The results were subjected to statistical analysis by Student's t test.

Department of Medical Biology and Genetics, Lugansk Medical Institute Laboratory of Cell Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 649-651, June, 1991. Original article submitted December 17, 1990.

0007-4888/91/0006-0859$12.50

TABLE 1. Circadian Rhythms of DNA-Synthesizing and Mitotic Activity in Epithelium of Fundal Gastric Glands of Mice with Natural Hormonal Background and Mice Repeatedly Receiving Thyroxine (M ± m)

Clock time	RI, %, intact mice	Mice receiving thyroxin	p	MI, ‰, intact mice	Mice receiving thyroxine	p
13	25,8±4,2	8,5±2,5	<0,05	15,4±1,8	5,1±1,8	<0,05
16	12,1±2,8	3,6±1,2		5,4±1,2	6,5±1,1	
19	10,7±2,3	11,7±6,9		13,1±1,7	3,9±1,1	<0,01
22	14,9±1,8	8,8±3,1		8,6±0,9	4,1±1,0	<0,01
1	11,3±2,8	13,5±8,2		5,5±1,2	5,9±1,9	
4	21,6±3,3	8,4±0,6	<0,05	6,2±1,1	2,8±0,8	<0,05
7	9,1±3,6	6,0±2,1		6,0±1,6	3,8±0,9	
10	17,7±7,1	13,7±2,5		3,6±0,9	3,5±0,7	
13*	18,6±4,0	11,9±3,2		5,6±1,4	4,7±0,8	
	15,3	9,3	—	8,0	4,4	—

Legend. Here and in Table 2, asterisk indicates 2nd day of investigation.

TABLE 2. Circadian Rhythms of DNA-Synthesizing and Mitotic Activity in Epithelium of Pyloric Glands of Mice with Natural Hormonal Background and Mice Repeatedly Receiving Thyroxine (M ± m)

Clock time	RI, ‰		p	MI, ‰		p
	intact mice	mice receiving thyroxine		intact mice	mice receiving thyroxine	
13	43,2±9,9	31,6±3,1		28,3±2,3	34,2±6,8	
16	66,8±3,5	26,2±5,6	<0,01	26,2±3,1	14,5±2,0	<0,05
19	31,3±8,5	48,8±18,8		27,7±3,3	18,7±1,8	
22	45,1±2,9	48,5±23,0		19,9±3,5	18,8±2,7	
1	33,2±11,6	34,7±4,8		16,1±1,4	14,3±1,4	
4	79,4±4,8	35,4±4,3	<0,01	30,7±2,7	17,7±4,8	<0,05
7	23,1±7,9	19,2±2,7		16,5±4,2	16,3±3,8	
10	24,8±8,7	32,2±7,1		9,9±2,1	20,1±3,3	
13*	48,2±8,8	31,6±3,1		13,5±1,6	21,2±3,6	<0,05
Mean for 24-h period	43,4	34,6	—	21,9	19,3	—

EXPERIMENTAL RESULTS

Against a natural hormonal background the size of the proliferative pool in the glandular epithelium of the fundal part of the stomach was 9.73 ± 1.79%, and in the epithelium of the pyloric glands it was 18.69 ± 1.84%, Most cells with lab'eled nuclei in the gastric epithelium of the fundal part of the stormach were located in the floor of the pits and in the necks of the glands, whereas in the pyloric glands, they were found in the basal segments.

In the glandular epithelium of the fundal part of the stomach the acrophases of the rhythm reflecting changes in RI were observed at 1 p.m. and 4 a.m. ($p < 0.05$), acrophases of the rhythm of mitotic activity at 1 and 7 p.m. ($p < 0.05$). The average coefficient of synchronization of the passage of the cells into mitosis was 1.1, and of passage into the S phase 0.25. The main 24-hourly values of RI and MI were 15.3 and 8.0‰ respectively (Table 1). The acrophases of the circadian rhythm of DNA synthesis in the epithelium of the pyloric glands were recorded at 4 p.m. and 4 a.m. ($p < 0.05$), acrophases of the rhythm of mitosis at 1 p.m. and 4 a.m. ($p < 0.05$). The average coefficient of passage of cells into the phase of DNA synthesis was 0.3, and of passage into mitosis 0.79. The mean 24-hourly values of RI and MI were 43.4 and 21.9‰ respectively (Table 2).

Thus transition of the epithelial cells of the gastric glands into the phase of DNA synthesis and into mitosis is characterized by a high level of synchronization. Circadian rhythms of cellular proliferation in the glandular epithelium of the stomach were bimodal, which is not a characteristic of the other tissues of the gastrointestinal tract of adult animals [2, 4]. The intensity and the circadian rhythms of proliferation in the epithelium of the fundal and pyloric parts of the stomach differed. These differences evidently reflect the functional and structural heterogeneity of the glandular epithelium of the stomach, and also differences in reactivity of the epithelial cells of the fundal and pyloric glands to the action of regulatory factors.

After repeated injections of thyroxine, the pattern of cell proliferation in the epithelium of the gastric glands changed considerably. The proliferative pool in the glandular: epithelium of the fundal part of the stomach decreased by 62.6% ($p < 0.05$), and in the epithelium of the pyloric glands by 42.4% ($p < 0.05$). The mean 24-hourly mitotic activity of the epithelial cells decreased in the fundal part of the stomach by 39.2% and in the pyloric part by 20.3%. Mean 24-hourly values of MI decreased correspondingly by 44.2 and 11.3%. The circadian rhythms of DNA synthesis and mitotic activity became unimodal in the presence of an excess of thyroid hormones (Tables 1 and 2). The coefficient of synchronization of entry of the epitheliocytes of the fundal and pyloric glands into the phase of DNA synthesis was 0.21. The coefficient of synthesis of entry of cells of the glandular epithelium of the fundal glands into mitosis was 0.19, whereas for cells of the pyloric glands it was 0.2.

The regulatory effects of thyroid hormones in the epithelium of the gastric glands. are thus manifested as changes in the intensity and circadian rhythms of proliferation. An excess of thyroid hormones desynchronizes cell division. We know that exogenous thyroxine did not cause smoothing of the peaks of the circadian rhythms of cell proliferation in the epithelium of the esophagus and in an Ehrlich's ascites tumor [4, 5]. These differences in the type of action of the hormone on the temporal organization of proliferation in these tissues can probably be explained by differences in the phase structure of the circadian rhythm of DNA synthesis and mitosis The desynchronizing effect of thyroid hormones on circadian rhythms of cell proliferation in the glandular epithelium of the stomach is per.haps connected with absence of the G_0- and G_2-effects of these hormones These effects consist essentially of stimulation of passage of the cells from reserve populations into the proliferative pool [3]. This hypothesis is supported by the decrease in size of the proliferative pool which we found after repeated injections of thyroxine.

Differences in the intensity of inhibition of cell proliferation in the epithelium of the fundal and pyloric glands are particularly interesting. In the presence of an excess of thyroid hormones, the pattern of cell proliferation changes more especially in the glandular epithelium of the fundal part of the stomach, possible evidence that its epitheliocytes are more sensitive to thyroid hormones than those of the epithelium of the pyloric glands Differences in the action of thyroid hormones on division of epitheliocytes of the fundal and pyloric glands also are manifested in the fact that a decrease in the intensity of proliferation in the epithelium of these glands was observed at different times of day (Tables 1 and 2). These observations suggest differences in the rhythms of sensitivity of the epithelial cells of the fundal and pyloric glands to the regulatory influence of thyroid hormones.

The results of this investigation indicate that thyroid hormones play an essential role in the formation of the proliferative pattern of epithelial cells of the gastric glands. Thyroid regulation of cell division is effected through mechanisms determining the exchange of cells between the proliferative pool and reserve populations, and also through mechanisms responsible for passage of the cells into the phase of DNA synthesis and into mitosis. The regulatory effects of thyroid hormones in the fundal and pyloric parts of the stomach are exhibited differently.

The research was conducted in accordance with the FPI-3 program of the State Committee for Education of the USSR.

LITERATURE CITED

1. A. A. Belopashko, Byull. Éksp Biol. Med., No. 7, 104 (1972).
2. I. K. Rakhmatullina, Circadian Rhythms of Physiological Processes [in Russian], Moscow (1972), pp. 53-56.
3. Yu. A. Romanov, Morphological and Functional Bases of Regulation and Restoration of Physiological Functions [in Russian], Moscow (1970), pp. 112-113.
4. V. P. Rybakov, Biology of Cell Reproduction [in Russian], Moscow (1972), pp. 103-113.
5. V. A. Stepanenko and Yu. A. Romanov, Byull. Éksp. Biol. Med., No. 12, 715 (1984).
6. M. Ernst and E. R. Froesh, FEBS Lett., No. 1, 163 (1987).
7. P. Langer, O. Foldes, and K. Gschwendtova, Exp. Clin. Endocrinol., **90**, No 3, 301 (1987).
8. S. Okamoto, M. Ito, Y. Kitamura, and K. Matsumoto, Cell Tissue Kinet., **18**, No. 6, 583 (1985).
9. Yu. A. Romanov and S. S. Filippovich (J. A. Romanow and S. S. Filippowitsch), Vorträge des Deutsch-Sowjetischen Symposiums Chronobiologie-Chronomedizin, Berlin (1981), pp. 485-500.
10. T. Smeets and S. van Buul-Offers, Cell Tissue Kinet., **19**, No. 2, 161 (1986).