EXPERIMENTAL BIOLOGY

Study of Biological Rhythms of Small Intestinal Cryptic Epithelial Mitosis of Different Periodicity by Fourier Analysis

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Rhythms of cell division with different periods in the mouse small intestinal cryptic epithelium were studied using Fourier analysis. It was found that the proliferative system of the crypt is characterized by an intricate spatial and temporal organization. The amplitude of low-frequency rhythms increases, while the amplitude of high-frequency rhythms decreased in the direction from the crypt bottom to the neck.

Key Words: *spatial and time organization; proliferation; Fourier analysis; crypt*

Spatial and temporal organization of proliferative systems remains an important biological problem. A relationship between rhythms of cell division in the crypt and spatial location of cells in it was previously demonstrated [3]. However, this is true for only diurnal rhythms. This is obviously insufficient, because temporal organization includes the totality of rhythms with different periods [1,2]. Rhythms of different periods were described for the proliferative system of the epithelium of the small intestinal crypt, but the entire spectrum of oscillations was presented [4].

The aim of this study was to demonstrate the low-frequency and high-frequency rhythms and their relationship with the location of cell population in the crypt.

MATERIALS AND METHODS

The study was carried out on 40 outbred male albino mice (21-23 g) kept at 23°C and 12:12 h day: night regimen (light from 6.00 to 18.00) at 250-300 lux. The object of the study was cryptic epithelium from the proximal part of the small intestine: 20 longitudinally dissected crypts, the wall of each of them consisting of 25 cells. Total mitotic index (TMI, %) was evaluated per 1000 cells. For evaluation of circahoralial fluctuations in the number of mitoses, all mitotic indexes determined every 20 min throughout 24 h. The results were processed by Fourier's analysis, due to which the entire spectrum of biological rhythms in each cell subpopulation of the mouse small intestinal cryptic epithelium was detected and analyzed. According to Kotelnikov's theorem, the interval of measurements T (20 min in our study) allows to detect harmonics with a period longer than 2T (40 min) in the periodical process.

The algorithm of Fourier analysis was developed from the assumption according to which the proliferative activity conforms to a rhythm with 24 h period and experimental data were the instantaneous values of activity indexes changing in accordance with this rhythm. Due to this assumption, it was possible to apply Fourier transform analysis

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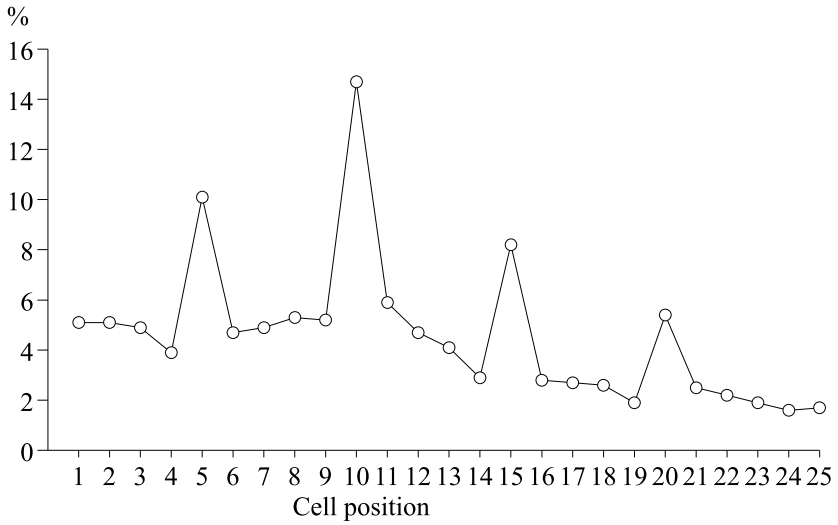

Fig. 1. Spatial gradient of the mean periodical TMI in the circadian rhythm of mitotic activity in the mouse small intestinal cryptic epithelium.

to harmonic expansion of the circadian rhythm. The amplitude and phase shift values in comparison with the starting moment of measurements were determined for each of these harmonics. Since the experimental values are discrete by their nature (known only for the period of measurement), discrete Fourier transform analysis was used in the algorithm.

The algorithm was realized in the form of a program using the table of experimental results as the input data and releasing the table of amplitudes and phases of individual harmonics (zero: mean index; first harmonic curve: sinusoid with a 24-h period; second curve: sinusoid with a 12-h period, *etc.*). The sum of all harmonics is a periodical function with a 24-h period passing strictly through the mean values of experimental measurements at respective moments. The FFTW3 library of Fourier transform analysis is used in the program. Higher values of the amplitude corresponded to greater impact of harmonic with the corresponding period on the integral picture of circadian rhythm. The last harmonic, *i.e.* harmonic with the highest frequency (in our case 40-min) is calculated with a low reliability of the amplitude and without phase evaluation, because of specific features of the algorithm of discrete Fourier transform analysis, and therefore is not analyzed in this study.

RESULTS

Previous studies carried out at Department of Biology (Medico-Biological Faculty of Russian State Medical University) demonstrated spatial and time organization of the proliferative system of the mouse small intestinal cryptic epithelium. Circadian rhythms of cell division were detected and their relationship with the location of epitheliocytes in the cryptic wall was revealed [5]. Elevation of mi-

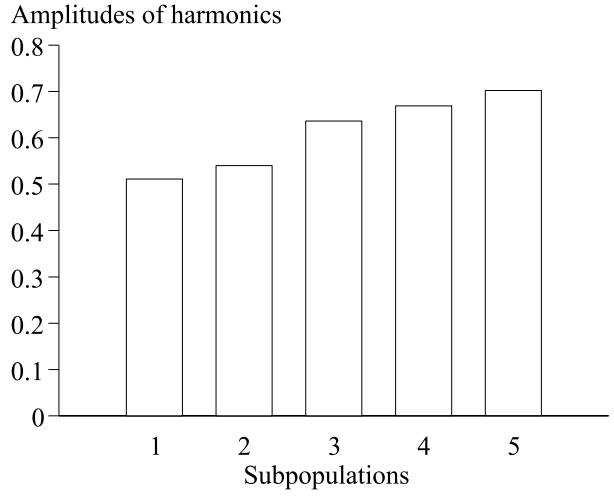

Fig. 2. Parameters of 24-h rhythm harmonics in cell subpopulations of mouse small intestinal cryptic epithelium.

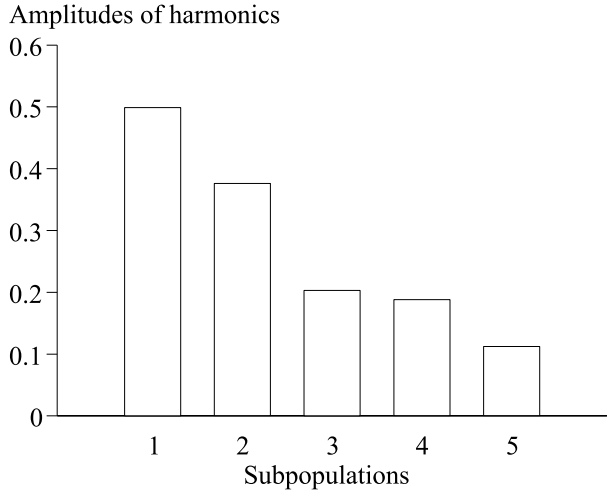

Fig. 3. Parameters of 2-h rhythm harmonics in cell subpopulations of mouse small intestinal cryptic epithelium.

totic activity was noted for every 5 cells (cell positions 5, 10, 15, and 20; Fig. 1). The closer to the cryptic neck, the rarer were periodical rises of mitotic activity. Due to the detected spatial distribution of mitoses along the cryptic wall, 5-cell subpopulations were singled out in it: subpopulation 1 (cell positions 1-5), subpopulation 2 (cell positions 6-10), subpopulation 3 (cell positions 11-15), subpopulations 4 (cell positions 16-20), and subpopulation 5 (cell positions 21-25; Fig. 1).

The study revealed the following regularities. The circadian (24-h) rhythm of mitotic activity of epitheliocyte was most pronounced for all subpopulations (Fig. 2).

The greatest amplitude of fluctuations was observed in subpopulation 5 (0.706). The amplitudes of subpopulations 3 and 4 were lower (0.683 and 0.682, respectively). In subpopulations 1 and 2 it was even lower (0.577 and 0.573, respectively).

Significant fluctuations were observed for the 2-h rhythm, their amplitude however decreased from subpopulation 1 to subpopulation 5 (0.511, 0.467, 0.372, 0.234, and 0.122; Fig. 3).

Hence, the sum of the rhythmic processes in the proliferative system of the mouse small intestinal cryptic epithelium depends on the spatial distribution of cells in the crypt. The amplitude of low-frequency rhythms increases in the direction from the bottom to the neck, while the amplitude of high-frequency rhythms decreases in this direction. If we assume that the constituents with greater amplitude are more important for the system, then the high-frequency rhythms determine proliferative activity of cells of the cryptic bottom, while the cryptic neck cells conform to low-frequency rhythms. These data indicate an intricate spatial and time organization of this system, manifesting by the existence of biological rhythms with different periods, and a relationship between these fluctuations and the location of cell subpopulations in the cryptic wall.

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