Cell Technologies in Biology and Medicine, Vol. 1, No. 1, January, 2005

Diurnal Dynamics of Cell Proliferation in Rat Liver during Early Postnatal Ontogeny and Effect of Epidermal Growth Factor on Hepatocyte Proliferative Activity

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine,* Vol. 1, No. 1, pp. 52-55, January, 2005
Original article submitted December 27, 2004.

Circadian rhythms of DNA synthesis, mitotic activity, and duration of mitosis in rat liver were studied on postnatal days 3, 7, and 12. Some age-related peculiarities of these rhythms were revealed. Epidermal growth factor was found to play an important role in the formation of cell proliferation rhythm during the early postnatal ontogeny and in the regulation of the pool of proliferating hepatocytes.

Key Words: *circadian rhythm; proliferation; epidermal growth factor*

In mammals, circadian rhythm of cell proliferation is determined by a complex of control factors acting at the tissue and organism levels [5]. However, the development of proliferation control system during ontogeny remains poorly studied, because of, first, non-simultaneous maturation of tissues perceiving regulatory factors and forming adequate response and second, non-simultaneous triggering of systems determining tissue-specific mode of cell proliferation during ontogeny.

Epidermal growth factor (EGF) significantly affect developmental processes and the level of cell reproduction in tissues [8,9]. Little is known on its role in the formation of circadian rhythm of cell division in tissues. It is especially true for the early stages of postnatal ontogeny characterized by imperfection of the control mechanisms and different rates of tissue maturation. We showed that injection of EGF to rats did not change DNA-related synthetic and mitotic activity in epithelial cells of the gastric fundus and pylorus, but markedly increased cell proliferation rate in small intestinal epithelium [1,2]. The present paper describes the data on diurnal changes in DNA synthesis and mitotic activity in rat liver from postnatal days 3 to 12.

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MATERIALS AND METHODS

Experiments were performed on random-bred albino rats on postnatal days 3, 7, and 12. The animals were kept under conditions of 12:12 dark-light regimen (daylight from 6:00 to 18:00). To assess the level of DNA synthesis, the rats were injected with ^{3}H-thymidine (3.7 MBq/100 g body weight) 1 h before sacrifice. The size of proliferation pool was assessed by injection of ^{3}H-thymidine every 5 h during a day. For evaluation of mitotic activity, colchamine (1.5 mg/kg) was injected 4 h before sacrifice. In each examined period, the control and experimental groups comprised 4-5 rats, the total amount of rats being 129. EGF (Research Institute of Hematology and Blood Transfusion, Lvov) was injected at 9:30 in a dose of 0.5 mg/kg. Labeled cells (labeling index, LI) and colchamine-blocked mitoses (mitotic index, MI_{col}) were counted on histological preparations; not less than 15,000 hepatocytes were examined (both indices were expressed in ‰). Duration of mitosis was determined as described

0007-4888/05/1391-0150

previously [3]. The results were analyzed statistically using Fisher—Student's *t* test. The degree of variation of cell proliferation indices during a day was evaluated by rhythm amplitude (ratio of maximum to minimum values for the specified parameter).

RESULTS

The liver of 3-day-old rats was examined during 16 h (Table 1). In control rats, the maximum and minimum LI were observed at 13:00 and 21:00, respectively ($p<0.01$).

LI variation amplitude was 3.29, the number of labeled cells was 2.15‰ during this period. Injection of EGF altered the dynamics of synthetic processes in hepatocytes. The maximum and minimum LI values were observed at 17:00 and 21:00-1:00, respectively. The mean LI decreased by 38.1%. LI variation amplitude increased to 3.4, which attests to more synchronous entering of hepatocytes into DNA-synthesizing phase under the action of EGF.

Mitotic activity also varied during the examined period. In the liver of the control rats, the number of blocked mitoses was maximum from 13:00 to 21:00, and minimum from 21:00 to 1:00. MI_{col} variation amplitude was 2.5. The total count of mitoses was 3.53‰. Administration of EGF increased mitotic activity by 31.7%, and this increase was most pronounced in the period characterized by minimum values of this parameter. MI_{col} variation amplitude decreased to 1.5.

In the liver of 7-day-old rats (Table 2) we observed a clear circadian rhythm of DNA synthesis with maximum at 9:00 and minimum at 1:00 ($p<0.01$). LI variation amplitude was 5.4. However, at 13:00 on the next day LI differed from the value observed at the same time on the first day. Consequently, rhythm of the DNA synthesis in 7-day-old rats should be considered as nearly circadian with a period of 21 h. After administration of EGF, LI decreased, but then increased again and surpassed the control by 2 times. LI variation amplitude decreased (to 3.2) compared to the control.

In 7-day-old rats, the diurnal changes of mitotic activity were described by a two-peak curve with maximum at 13:00-17:00 and 5:00-9:00 and minimum at 17:00-21:00. LI variation amplitude (2.8) reflected low degree of synchronization of mitotic division in rats of this age. Duration of mitosis also changed

TABLE 1. Effect of Single Injection of EGF on Dynamics of Synthetic and Mitotic Activity of DNA in Hepatocytes of 3-Day-Old Rats

Time	LI ($M\pm m$), ‰		Changes, %	Time	MI_{col} ($M\pm m$), ‰		Changes, %
	control	experiment			control	experiment	
13:00	3.62±0.39	0.97±0.29	-73.2**	9:00-13:00	0.78±0.12	1.10±0.25	+41
17:00	1.83±0.29	2.73±0.60	+49.2	13:00-17:00	0.94±0.18	0.97±0.07	+3.2
21:00	1.10±0.21	0.80±0.15	-27.3	17:00-21:00	1.30±0.38	1.49±0.39	+14.6
1:00	2.06±0.29	0.81±0.14	-60.7	21:00-1:00	0.510±0.007	1.09±0.13	+113.7*
Mean daily LI	2.15	1.33		Mean daily MI_{col}	0.88	1.16	

Note. *$p<0.05$, **$p<0.001$ compared to the control; "-" suppression; "+" stimulation.

TABLE 2. Effect of Single Injection of EGF on Dynamics of Synthetic and Mitotic Activity of DNA in Hepatocytes of 7-Day-Old Rats

Time	LI ($M\pm m$), ‰		Changes, %	Time	MI_{col} ($M\pm m$), ‰		Changes, %
	control	experiment			control	experiment	
13:00	1.34±0.23	0.59±0.09	-55.8*	9:00-13:00	0.60±0.06	0.79±0.17	+31.7
17:00	0.94±0.20	1.8±0.2	+91.5*	13:00-17:00	0.97±0.20	1.36±0.70	+40.2
21:00	0.82±0.17	0.45±0.09	-45.1	17:00-21:00	0.35±0.05	0.30±0.01	-14.3
1:00	0.44±0.02	1.18±0.04	+168.2***	21:00-1:00	0.43±0.06	1.27±0.02	+195.3**
5:00	1.80±0.26	1.43±0.47	-20.5	1:00-5:00	0.49±0.03	0.96±0.11	+95.9*
9:00	2.38±1.47	0.93±0.21	-60.9	5:00-9:00	0.98±0.26	1.18±0.24	+20.4
13:00^{+}	0.41±0.05	1.24±0.34	+202.4	9:00-13:00^{+}	0.41±0.03	0.66±0.09	+61*
Mean daily LI	1.29	1.06		Mean daily MI_{col}	0.64	0.98	

Note. Here and in Table 3: *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control; "-" suppression; "+" stimulation; $^{+}$experimental day 2.

TABLE 3. Effect of Single Injection of EGF on Dynamics of Synthetic and Mitotic Activity of DNA in Hepatocytes of 12-Day-Old Rats

Time	LI (*M*±*m*), ‰		Changes, %	Time	MI_{col} (*M*±*m*), ‰		Changes, %
	control	experiment			control	experiment	
13:00	0.80±0.13	1.42±0.70	+77.5	9:00-13:00	1.12±0.28	0.500±0.002	-55.3
17:00	0.88±0.11	0.76±0.11	-13.6	13:00-17:00	0.37±0.03	0.66±0.10	+78.4*
21:00	1.12±0.18	1.720±0.005	+53.6	17:00-21:00	0.56±0.11	0.39±0.01	-30.3
1:00	1.19±0.04	2.45±0.38	+105.9*	21:00-1:00	0.98±0.02	1.47±0.10	+50.0*
5:00	1.32±0.23	0.53±0.09	-59.8*	1:00-5:00	0.78±0.23	0.40±0.01	-48.7
9:00	1.59±0.26	1.40±0.48	-11.9	5:00-9:00	1.46±0.25	0.75±0.13	-48.6
13:00^{+}	1.71±0.39	1.50±0.03	-12.3	9:00-13:00^{+}	1.10±0.16	0.58±0.07	-47.3*
Mean daily LI	1.15	1.38		Mean daily MI_{col}	0.88	0.69	

throughout a day from 0.3-0.4 h at 13:00-21:00 to 1.3-1.5 h at night and in early morning (21:00-5:00). The mean daily duration of mitosis was 0.9 h. The total number of mitoses per day was 4.23‰. EGF significantly increased the mitotic activity in almost all periods of the study, although the mitotic activity curve retained both peaks.

EGF increased MI_{col} variation amplitude to 4.2. The variation amplitude of the duration of mitosis markedly increased during a day, and its mean value increased to 1.5 h. The total number of cells divided per day surpassed the control by 54%.

Similarly to 7-day-old rats, the peak of DNA synthetic activity in 12-day-old rats was observed in the morning hours (Table 3), although the passive phase of this rhythm shifted to the daytime. During the first and second days of the study, the values of LI measured at 13:00 were different, so the period of DNA synthesis deviated from the diurnal one. In this age group, the peculiar feature of the rhythm of DNA synthesis was low level of its synchronization: LI variation amplitude during the day was only 2. EGF increased the number of cells synthesizing DNA in some periods of the study, increased the mean daily LI by 20%, and markedly increased LI variation amplitude to 4.6. In this series, the proliferative pool (daily growth fraction) was measured (6.99±1.09‰).

Similarly to 7-day-old rats, the acrophase of mitotic activity in 12-day-old rats occurred in a period from 5:00 to 9:00, although the active phase of the rhythm became longer (from 21:00 to 13:00). The coincidence of mitotic values at the same hours of the first and second experimental days showed that the circadian rhythm of cell division was established at this age. MI_{col} variation amplitude (3.94) indicates a rather high degree of synchronicity of mitotic division in the cells during a day. The total number of the cells divided during a day (6.37 ‰) was very close to the proliferative pool value. Probably, at this stage of the early postnatal ontogeny, DNA synthesis in rat hepatocytes is mainly directed to the development and growth of liver due to increase in the number of cell by mitotic division. Therefore, injection of EGF at this period produced no significant changes in the variation amplitude of mitotic rhythm (3.8), although it pronouncedly decreased the total daily count of divided cells (by 24.6%).

EGF increased the proliferative pool of hepatocytes by 222.2% compared to the control (to 22.52± 7.41‰). The paradoxical decrease in mitotic activity against the background of increased proliferative pool of hepatocytes could be explained, first, by enhanced polyploidization of hepatocytes under the effect of EGF, second, by the effect of exogenous EGF on mutual transitions of hepatocytes between the proliferative and balloting pools, whose existence in proliferating cell systems was established [4].

Therefore, a certain rhythm of cell division is formed in the early postnatal ontogeny. There are data that the postnatal cell proliferation in rat liver is synchronized starting from postnatal day 21 and is controlled by the formation of circadian rhythm of blood corticosterone concentration [7]. We showed that starting from postnatal day 3, the number of proliferating cells varies during a day. The following parameters of the rhythm vary: times of acrophase and passive phase, amplitude and duration of mitosis, and mean daily values of DNA synthesizing and prolipherative activities. Its proliferative effects can reflect modulation of mechanisms regulating cell transition into the phases of DNA synthesis or mitosis, the duration of mitosis, and transition of hepatocytes from the mitotic cycle into resting state. The character of this modulation depends on animal age. Ontogenetic peculiarities of EGF action can be related to specificity of its reception. It is noteworthy that binding capacity of EGF re-

ceptors in hepatocytes varies during the first month of life [6].

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