EXPERIMENTAL BIOLOGY

Time Course of Cell Proliferation in Rat Liver in the Early Postnatal Ontogeny and Role of Epidermal Growth Factor in Organization of Proliferative Regimen

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Circadian rhythm of DNA synthesis, mitotic activity, and duration of mitosis in rat liver were studied on days 3, 7, and 12 of life. Age-associated differences in the rhythmic parameters of these characteristics were detected. Epidermal growth factor plays an important role in the formation of cell proliferation rhythm in the early postnatal ontogeny and in the formation of proliferative hepatocyte pool.

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

Circadian rhythm of cell proliferation in mammalian tissues is determined by an intricate complex of regulatory factors acting at the tissue and whole body levels [5]. However, the development of proliferation-regulating system during ontogeny remains virtually not studied. This is explained, first, by not simultaneous maturation of different tissues for reception of regulatory factors and formation of the adequate response to it and second, by not simultaneous beginning of functioning of the systems determining the regimen of cell proliferation specific for each tissue during ontogeny.

Epidermal growth factor (EGF) is one of the factors essential for developmental process and level of cell multiplication in tissues [8,9]. However, little known about the role of this factor in the formation of circadian rhythm of cell division in different tissues. The early stages of postnatal ontogeny characterized by imperfect mechanisms of regulation and diverse tempo of tissue maturation are less studied. We previously showed that injection of EGF to rats did not change DNA synthesizing and mitotic activity in the glandular epithelium of the fundal and pyloric compartments of the stomach, but the intensity of cell proliferation increased appreciably in the small intestinal epithelium [1, 2]. Here we studied the circadian changes in DNA synthesis and mitosis in the liver of rats on days 3-12 of postnatal development.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats on days 3, 7, and 12 of life. The animals were kept under conditions of constant day/night regimen (light from 6.00 to 18.00). In order to evaluate DNA synthesis, the animals were injected with ^{3}H-thymidine in a dose of 3.7 MBq/100 g one hour before sacrifice. The proliferative pool was evaluated by injections of ^{3}H-thymidine every 5 h for 24 h. For evaluating mitotic activity, colchamine in a dose of 1.5 mg/kg was injected 4 h before sacrifice. Four or five rats from the control and experimental groups were examined for each period of the study. A total of 129 animals of different age were used. EGF ("Diagnosticum" Laboratory, Institute of Hematology and Blood Transfusion, Lvov) was injected at 9.30 in a dose of 0.5 mg/kg. The number of labeled cells (RI) and mitoses

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blocked with colchamine (MI_{CC}) were evaluated in histological preparations after examination of at least 15,000 hepatocytes. The label index and mitotic index were expressed in promilles. The duration of mitosis was evaluated as described previously [3]. The significance of differences was evaluated using Fisher—Student *t* test.

The intensity of fluctuations in the cell proliferation values was evaluated by the rhythm amplitude, which was determined as the ratio of the maximum to minimum value.

RESULTS

The liver of 3-day-old rats was studied over 16 h (Table 1). An increase of RI at 13.00 was noted during this time, with the minimum cell labeling index at 21.00 ($p<0.01$).

The amplitude of RI fluctuations was 3.29 for the mean number of 2.15‰ labeled cells during the studied period. Injection of EGF modified the time course of DNA synthesis in hepatocytes. The index of labeled nuclei was maximum at 17.00 and minimum at 21.00-1.00, the mean RI decreased by 38.1%. The amplitude of RI fluctuations somewhat increased (to 3.4), which attests to more synchronous entry of hepatocytes in the DNA production phase under the effect of EGF.

Mitotic activity also varied during the observation. The greatest number of blocked mitoses in the liver of control rats was observed at 13.00-21.00, the minimum number at 21.00-1.00. The amplitude of MI_{CC} fluctuations was 2.5, the total number of mitoses 3.53%. After injection of EGF mitotic activity increased by 31.7%, the increase was most pronounced during hours, when this parameter was the lowest in the control. Due to this, the amplitude of MI_{CC} fluctuations decreased to 1.5.

A clear-cut circadian rhythm of DNA production with the maximum at 9.00, minimum at 1.00 ($p<0.01$), and amplitude of 5.4 was observed in the liver of 7-day-old rats (Table 2). It is noteworthy that the next day at 13.00 RI value did not correspond to the value detected at 13.00 on day 1 of the experiment. Hence, the rhythm of DNA production in hepatocytes of 7-day-old rats should be regarded as circahoralian, with a period of about 21. RI decreased for a short time after EGF injection and then increased to the level 2-fold higher than in the control. The amplitude of RI fluctuations at this term of the study decreased to 3.2.

Changes in mitotic activity in 7-day-old animals over 24 h were characterized by a curve with two peaks at 13.00-17.00 and at 5.00-9.00 and minimum values at 17.00-21.00. The rhythm amplitude equal to 2.8 indicates poor synchronization of mitotic division in rats of this age. The time of mitosis also changed

TABLE 1. Dynamics of DNA-Synthetic and Mitotic Activities of Hepatocytes in 3-Day-Old Rats after Single Injection of EGF

Time of the day	RI (*M*±*m*, ‰)		Changes, %	Time of the day	RI (*M*±*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	+41.0
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 RI	2.15	1.33		Mean MI_{CC}	0.88	1.16	

Note. *$p<0.05$, **$p<0.001$ compared to the control (intact animals). Here and in Tables 2, 3: "-": suppression; "+": stimulation.

TABLE 2. Dynamics of DNA-Synthetic and Mitotic Activities of Hepatocytes in 7-Day-Old Rats after Single Injection of EGF

Time of the day	RI ($M\pm m$, ‰)		Changes, %	Time of the day	RI ($M\pm m$, ‰)		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 (next day)	0.41±0.05	1.24±0.34	+202.4	9.00-13.00 (day 2	0.41±0.03	0.66±0.09	+61.0*
Mean RI	1.29	1.06		Mean MI_{CC}	0.64	0.98	

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control.

TABLE 3. Dynamics of DNA-Synthetic and Mitotic Activities of Hepatocytes in 12-Day-Old Rats after Single Injection of EGF

Time of the day	RI ($M\pm m$, ‰)		Changes, %	Time of the day	RI ($M\pm m$, ‰)		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 (next day)	1.71+0.39	1.50±0.03	-12.3	9.00-13.00 (day 2	1.10±0.16	0.58±0.07	-47.3*
Mean RI	1.15	1.38		Mean MI_{CC}	0.88	0.69	

Note. *$p<0.05$ compared to the control (intact animals).

over 24 h: from 0.3-0.4 h at 13.00-21.00 to 1.3-1.5 h in the night and morning hours (21.00-5.00). The mean circadian duration of mitosis was 0.9 h. The total number of mitoses over 24 h was 4.23‰. After injection of EGF mitotic activity appreciably increased at all terms of the study, but the two-peak pattern of the mitosis rhythm was preserved.

The amplitude of MI_{CC} increased to 4.2. This was paralleled by a sharp increase in the amplitude of fluctuations in mitosis duration over 24 h, the mean circadian value increased to 1.5 h. Total number of cells divided over 24 h was 54% higher than in the control.

The peak of DNA-synthetic activity in 12-day-old rats (Table 3) was observed in the morning hours, similarly as in 7-day-old ones, but the passive phase of the rhythm was shifted to the daytime. Similarly to 7-day-old animals, RI values at 13.00 on days 1 and 2 of the study did not coincide, that is, the period of DNA synthesis rhythm deviated from the circadian. A specific feature of DNA synthesis rhythm was a low level of synchronization: the amplitude of RI fluctuations over 24 h was 2.0. Injection of EGF increses the number of DNA-producing cells at some terms of the study, by 20% increased the mean circadian index of label, an appreciable (to 4.6) increased the amplitude of RI in comparison with the control. The proliferative pool (or circadian growth fraction) was evaluated in this series of experiments; it amounted to 6.99± 1.09‰.

Like in 7-day-old rats, the rhythm of mitotic activity was characterized by an acrophase from 5.00 to 9.00, but the active phase of the rhythm was prolonged and lasted from 21.00 to 13.00. Coincidence of the mitotic index in the same hours of days 1 and 2 of experiment indicates that the circadian rhythm of cell division is established in this age group. The amplitude of MI_{CC} (3.94) indicates a sufficiently high degree of synchronization of mitotic division over 24 h. The total number of cells divided over 24 h (6.37‰) is very close to the proliferative pool value. This fact suggests that DNA synthesis in hepatocytes at this stage of postnatal development mainly provides the growth of the organ at the expense of mitotic division. Injection of EGF did not lead to appreciable changes in the mitosis rhythm amplitude (3.8), but caused a significant (by 24.6%) decrease in the number of cells divided over 24 h.

After injection of EGF the proliferative pool of hepatocytes increased by 222.2% and reached 22.52± 7.41‰ of the control. This paradoxical decrease in mitotic activity against the background of increased hepatocyte proliferative pool can be explained by, first, intensification of liver cell polyploidization under the effect of EGF and second, by the effect of exogenous EGF on mutual transition of hepatocytes between the proliferative and balloting pools, whose presence in proliferating cell systems was previously proven [4].

Hence, the rhythm of cell division forms during the early postnatal period in rats. Postnatal cell proliferation in rat liver is synchronized from day 21 of life and is determined by the emergence of circadian rhythm of corticosterone concentration in the plasma [7]. However, our results indicate that starting from day 3 of life the number of proliferating cells varies over 24 hours. Such rhythm parameters as the time of the acrophase and passive phase, duration of mitosis, mean circadian DNA-producing and mitotic activities are changing. EGF is an important factor regulating hepatocyte multiplication. Its proliferative effects seem to reflect the influence on the mechanisms controlling cell transition into DNA production phase and mitosis and determining the duration of mitosis and hepatocyte release from mitotic cycle into silent populations. The type of these effects depends on the rat age. Ontogenetic characteristics of the effect of EGF can be explained by specific features of its reception. It is known that the binding capacity of receptors for this growth factor in liver cells varies during the first month of life [6].

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