EXPERIMENTAL GENETICS

Chronobiological Characteristics of the Activity of the Cellular Genetic Apparatus in Surviving Sections of Rat Brain

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Diurnal variation of the incorporation of labeled precursors of RNA and proteins into spinal, stem, and cortical populations from the nervous system of rats is studied on surviving sections of the brain. It is shown that in the absence of an effect of other parts of the nervous system, afferentation, and humoral factors isolated nerve cell populations preserve the diurnal rhythm of activity of the genetic apparatus that correlates with the motor activity and the light reception (light-dark cycle) of animals.

Key Words: *diurnal rhythms of ^{3}H-uridine and ^{3}H-leucine incorporation; brain sections; cosinor analysis*

Cells from different parts of the nervous system of mammals under conditions of surviving sections are a promising model which permits in-depth research into the mechanisms of biological rhythms and into their adaptive role at the level of cells and tissues [1]. In this study we explored the diurnal time course of the formation of primary genetic products by cortical, stem, and spinal populations of rat nerve cells *in vitro*.

MATERIALS AND METHODS

Surviving sections of the somatosensory cortex (SC), visual cortex (VC), cerebellar vermis cortex (CVC), suprachiasmatic hypothalamic nucleus (SHN), lumbar spinal cord (LSC), spinal L_5 ganglia (SG), and cranial cervical sympathetic ganglia (CCSG) derived from male Wistar rats preadapted to a 12:12-h light-dark cycle served as the object of investigation. Measurements were performed at 10:00, 13:00, 16:00, 19:00, 22:00, 01:00, 04:00, 07:00, and 10:00 h; one hour prior to each time point parts of organs (80-100 mg) were placed in medium 199 and minced with a razor to obtain pieces not larger than 1×0.5×0.5 mm. Sections were cultured in 2 ml of medium 199 containing 20% bovine serum, 70 μg/ml vitamin C, and 5 mg/ml glucose in Petri dishes at 37°C at the interface of the liquid nutrient medium and air [5]. Following a 40-50-min culturing, some sections of each organ were incubated with ^{3}H-uridine (0.6 MBq/ml, specific activity 550 GBq/mmol) and the remaining sections with ^{3}H-leucine (0.8 MBq/ml, specific activity 925 GBq/mmol) for 15 min. Sections were then washed in a cold (4°C) medium containing excess unlabeled nucleoside or amino acid and treated with 5% trichloroacetic acid at 4°C for 90 min, after which sections were washed with ethanol, transferred to vials, and solubilized with thiamine, and scintillator was added to the solution. The radioactivity of total RNA and proteins (*i*) and of their acid-soluble fractions (*p*) was measured on a scintillation counter at each time

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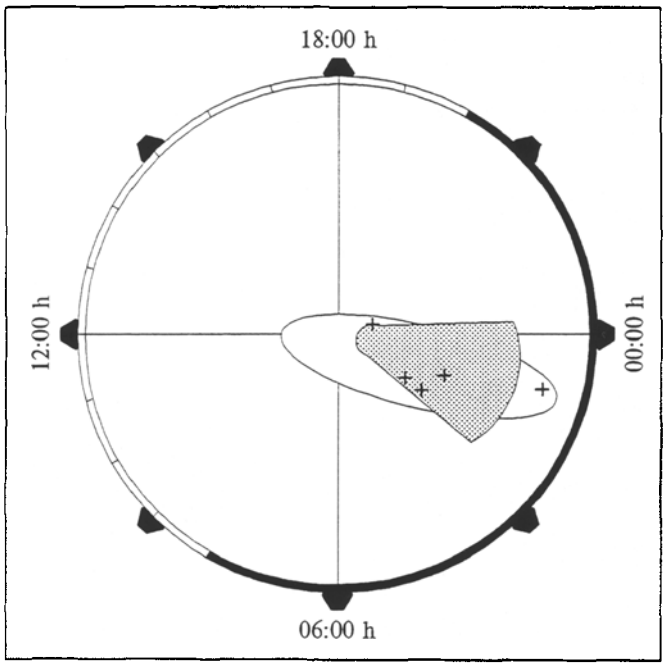

Fig. 1. Cosinor diagram of diurnal variation of relative incorporation of ^{3}H-leucine in surviving sections of rat brain visual cortex. The position of an individual cosinor is marked by a "plus."

point. The sum of these indexes of radioactivity $P_i=p+i$ shows the permeability of tissue for precursor at each time point, while the relative incorporation corrected for permeability is $I_i=i\times(P/P_i)$, where P is the mean permeability throughout the experiment and P_i is the permeability at some given time point [3]. Calculations were performed on the basis of the specific radioactivity of sections (cpm/mg). The results were treated using a modification of the method of group cosinor-analysis.

RESULTS

Analysis of the level of radioactivity in sections of the organs from different parts of the nervous system incubated with ^{3}H-uridine shows that the intensity of synthesis of total RNA, the pool of RNA precursors, and the permeability of tissue for precursors change during the course of the day. The acrophases of relative incorporation of labeled uridine into cells of CVC, LSC, SG, and CCSG were observed at night, while that into cells of SC and VC early in the morning (Table 1). Thus, the isolated populations of nerve cells unaffected by other parts of the nervous system and in the absence of afferentation and humoral factors preserve the diurnal rhythm of activity of the genetic apparatus that correlates with the motor activity and the light reception (light-dark cycle) of animals. The calculated indexes of incorporation of ^{3}H-leucine into proteins of nerve cells peaked at certain intervals (the intrinsic acrophases were observed from 04:00 to 10:00-15:00 h, depending on the type of organ). The unequal intervals between the maxima of incorporation of ^{3}H-uridine and ^{3}H-leucine into cells of several organs of the nervous system are evidently indicative of different durations of transcription and translation. Thus, the temporal parameters of diurnal variation of the activity of transcription and translation *in vitro*, in sections derived from different parts of the nervous system, correlate well with our findings obtained in similar experiments, characterizing the diurnal activity of the genetic apparatus of nerve cells *in vivo* [6], thus positively answering the question as to whether brain sections can be used as a model for a study of the biorhythms at the cell and tissue level. The mean values of acrophase (ϕ) and amplitude (A) were assessed using a modified method of group cosinor-analysis. The second step in this widely used method of processing chronobiological data [7] is to average the individual data and to determine the confidence intervals for fluctuations of A and ϕ. The mean values of ϕ and A were calculated as follows:

$$\varphi_{cp.}=\frac{1}{n}\sum_{i=1}^{n}\varphi_i;\quad A_{cp.}=\frac{1}{n}\sum_{i=1}^{n}A_i \qquad (1)$$

where ϕ_i and A_i are the parameters of individual cosinors. These equations are not identical to those used by Bagrinovskii *et al.* [2] and Emel'yanov [4]:

$$A_{cp.}=\sqrt{x_0^2+y_0^2};\ \varphi_{cp.}\begin{cases}-\text{arctg}\,(y_0/x_0) & \text{при } x_0\geqslant 0\\ \pi-\text{arctg}\,(y_0/x_0) & \text{при } x_0\leqslant 0\end{cases} \qquad (2)$$

where

$$x_0=\frac{1}{n}\sum_{i=1}^{n}x_i;\quad y_0=\frac{1}{n}\sum_{i=1}^{n}y_i \qquad (3)$$

where x_i and y_i are the Cartesian coordinates of individual cosinors. Equations (1) and (2) are not identical due to nonlinear transformations from Cartesian to polar coordinates.

The use of equations (2) may lead to significant errors. Let us, for example, take two points (C_1 and C_2) of the follow-up with the coordinates $x_1=a>0$ and $y_1=0$ and $x_2=0$ and $y_2=b<0$. Since in the first point $\phi=0°$ and in the second $\phi=90°$, the mean value of group ϕ is 45°, irrespective of the values of a and b. Meanwhile, the use of equations (1) yields the following group value of ϕ:

$$\phi_{mean}=-\text{arctg}(b/a) \qquad (4)$$

which, depending on the given values of a and b, may range from 0 to 90°.

The same is observed for the group value of A. Indeed, if two individual cosinors have equal A,

and their ϕ differ by 180°, the calculation performed by equation (2) yields a zero mean value of A for the group cosinor, which is unnatural. In addition, the confidence intervals for $\phi > \pm 6$ h cannot be calculated by the classical method at all, since the angle formed by tangents to the ellipse passing through the origin of the coordinates cannot exceed 180° (12 hours). It is suggested that the confidence probability be determined within a sector or a segment limited in the phase by rays $\phi_{1,2}=\phi_0 \pm \Delta\phi$ and in the amplitude by circles with a radius $r_{1,2}=r_0 \pm \Delta r$, where ϕ_0 and r_0 are the acrophase and the mean amplitude of the group cosinor, and $\Delta\phi$ and Δr are the confidence intervals of their measurement. The differences between some results obtained by different methods of quantitation of the parameters of the group cosinors (Table 1) are shown in Fig. 1, which presents the parameters of 5 individual cosinors, characterizing the phase and the amplitude of ^{3}H-leucine incorporation into cells of VC. A 95% ellipse of the confidence zone of simultaneous variation of A and ϕ, calculated by formulas (2) and (3), encompasses the origin of the coordinates, which makes the parameters of the group rhythms undetectable. On the other hand, determination of the confidence probability

TABLE 1. Diurnal Variation of Relative Incorporation of ^{3}H−Uridine and ^{3}H−Leucine (cpm/mg) into Surviving Sections of Different Parts of the Rat Nervous System

Part of nervous system	Mesor ($M \pm m$)	Amplitude		Acrophase	
		A	95% confidence interval	A	95% confidence interval
SHN					
^{3}H−uridine	1957.9±107.1	516.2	383.6−648.7	20:58 h	20:18 h−21:38 h
		509.3*	363.8−670.3	21:04 h	19:51 h−21:52 h
^{3}H−leucine	3859.0±92.6	968.4	761.8−1174.9	01:17 h	23:25 h−03:09 h
		909.1*	608.9−1359.8	01:02 h	23:58 h−03:35 h
SG					
^{3}H−uridine	907.9±40.9	248.5	47.4−449.7	00:59 h	00:21 h−01:37 h
		247.4*		01:02 h	
^{3}H−leucine	326.4±17.4	99.7	63.7−135.8	14:28 h	13:14 h−15:41 h
		96.6*	51.0−144.9	14:32 h	12:16 h−16:11 h
CCSG					
^{3}H−uridine	645.2±43.3	504.1	391.2−616.9	4:59 h	04:34 h−05:25 h
		502.2*	357.4−562.1	05:01 h	04:15 h−05:33 h
^{3}H−leucine	343.9±23.3	103.6	58.5−148.8	14:57 h	13:02 h−16:51 h
		96.6*	43.4−164.7	15:14 h	11:30 h−17:17 h
LSC					
^{3}H−uridine	351.1±24.3	102.8	14.9−190.7	23:18 h	21:25 h−01:11 h
		99.2*		23:40 h	
^{3}H−leucine	190.9±10.5	67.2	32.5−101.8	07:40 h	06:17 h−09:04 h
		65.1*	18.7−112.9	07:30 h	05:50 h−10:16 h
CVC					
^{3}H−uridine	4500.7±480.7	1529.1	576.0−2482.2	02:13 h	01:04 h−03:23 h
		1486.5*	422.2−2751.3	02:20 h	22:07 h−03:43 h
^{3}H−leucine	606.1±51.5	249.4	133.6−365.3	09:58 h	08:58 h−10:58 h
		243.4*	134.7−400.8	09:47 h	08:35 h−12:56 h
VC					
^{3}H−uridine	755.2±19.9	167.5	114.8−220.2	10:51 h	09:02 h−12:39 h
		157.2*	84.9−229.6	10:47 h	08:17 h−13:19 h
^{3}H−leucine	336.6±17.2	87.1	10.1−163.9	00:58 h	23:40 h−02:15 h
		85.2*		00:55 h	
SC					
^{3}H−uridine	5085.1±146.7	903.4	431.7−1375.1	06:14 h	05:26 h−07:01 h
		890.5*	324.1−1460.0	06:14 h	04:52 h−07:14 h
^{3}H−leucine	10 161±701.9	1330.9	819.5−1842.3	05:41 h	04:07 h−07:15 h
		1247.9*	760.1−1963.2	05:18 h	03:53 h−08:16 h

Note. An asterisk denotes the values of A and p obtained on the basis of unmodified group cosinor analysis. The absence of confidence intervals of A and p means that the origin of coordinates is inside a 95% ellipse.

for A and ϕ within the segment (1) yields definite values of the group A and ϕ.

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Stem Hematopoietic Cells with Inserted Foreign Gene: Proliferative Activity and Proliferative Potential in the Long Term after Transplantation into Irradiated Mice

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The transfer of the human adenosine deaminase gene to murine stem hematopoietic cells is performed on an irradiated sublayer of a long-term bone marrow culture by the conventional method of retroviral transduction with cytokines and by stimulation of stem cells without cytokines. The efficiency of gene transfer into colony-forming units (CFUs) with the aid of cytokines is 72% and without them it is 50%. In irradiated mice reconstituted with the retrovirus-infected bone marrow cells the donor hematopoietic activity is preserved during a 1-year period. The proliferative activity of CFUs of chimeric cells 6 months after the reconstitution was the same and did not depend on the mode of gene transfer. The spleen repopulation activity is lowered in all the groups of chimeric mice 6-12 months after reconstitution.

Key Words: *stem hematopoietic cell; retrovirus gene transfer; proliferative activity; proliferative potential; adenosine deaminase; CFUs*

Methods have now been developed for transferring foreign genes to the genome of mammalian somatic cells. The most effective among these methods is retroviral transfection, the technique allowing for the insertion of foreign DNA in the genome of stem hematopoietic cells (SHC). It has been demonstrated that SHC containing a neutral gene are capable of repopulating the hematopoietic system of irradiated animals and of maintaining hematopoiesis over significant periods of time [3,7]. However, the main properties of SHC, such as the development potential, proliferative potential, and proliferative activity of the posterity, have not been compared in sufficient detail for cells carrying a cell marker and intact cells. Meanwhile, cells with a cell marker differ from intact cells not only in the presence of foreign DNA in their genome but

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