EXPERIMENTAL BIOLOGY

Effect of Radiation on the Functioning of Neuron Chromatin of Mice Exposed in the Zone of the Chernobyl Nuclear Power Plant

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Circadian fluctuations in the activity of endogenous RNA polymerases were examined in the nuclei of L_v spinal ganglion neurocytes, spinal α-motoneurons, and in neurons of the sensorimotor and visual cortex of mice exposed to radiation in the zone of the Chernobyl power plant. The circadian organization of the functioning of the genetic apparatus of neurons in various compartments of the nervous system was altered in irradiated mice. A shift of the acrophases of nucleoplasmic and nucleolar labeling in the course of the 24-hour cycle was observed after a 40-day exposure under conditions of a high radioactive background. The degree of synchronization of the level of transcription of ribosomal and structural genes of the studied cell populations diminished. The mesors and amplitudes of circadian rhythms of chromatin matrix activity decreased. The status of the histone component of chromatin also changed in exposed animals.

Key Words: *circadian rhythms; transcription activity of neuronal chromatin; radioactive background in the Chernobyl zone*

The response of the central nervous system to radiation differs fundamentally from the reactions of other organs in the absence of cell loss [3]. However, exposure results in damage to virtually all intracellular structures and macromolecules [4]. Meanwhile, the rhythmic organization of physiological systems of the organism is known to be related to the efficacy of their functioning and to govern the resistance to various unfavorable environmental factors [1].

The goal of this study was to investigate the reactions of neuronal chromatin in some structures of the nervous system of mice exposed to radiation in the zone of the Chernobyl nuclear power plant. This paper presents the results of studies carried out in 1987-1989.

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MATERIALS AND METHODS

C57Bl/6 mice were exposed for 40 days in the Chernobyl accident zone with a radioactive background of 300 to 350 mR/h (total absorbed dose 226 rad). The examinations were carried out 4 times a day because of high animal mortality; 4-5 experimental and 8-9 control mice were examined. Control animals were kept at the base camp of the Academy of Sciences of the USSR beyond the 30-km zone (with a radioactive background of 100 to 120 μR/h).

The transcription activity of chromatin was assessed by autoradiography of the activity of endogenous RNA polymerases in fixed cells [8]. Neurocytes of the L_v spinal ganglion (SG), α-motoneurons of the anterior spinal horns, and neurons of

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the sensorimotor (SMC) and visual (VC) cortex (layers III and IV) were examined. The resultant values were statistically processed after Fisher and Student and the differences were considered reliable at $p<0.05$. For assessing the relationship between various parameters in the course of the 24-hour cycle, linear correlation coefficients of the time series were estimated. The mesors, amplitudes, and estimated acrophases of the rhythms were found using group cosinor analysis software [6].

The status of the chromatin histone component was evaluated using differential staining of histone fractions rich in lysine and arginine with ammonium silver [7].

RESULTS

Morphological examinations of the lungs and liver of mice exposed in the Chernobyl accident zone revealed disorders of the microcirculatory system (hyperemia, hemorrhages, and edema). In addition, there were small sites of leukocytic infiltration and signs of destruction of individual cells. Histological study of the testicles showed virtually total involvement of the spermatogenic epithelial cells, only Sertoli cells being preserved.

Treatment of the cells with ammonium silver showed that there were no cells with darkyellow nuclei and clear cytoplasm in the population of spinal motoneurons in irradiated animals, whereas in control animals such cells were present, though in negligible (3%) amounts. The disappearance of such cells may be due to the high sensitivity of these cells to damaging factors and to their selective death after exposure to ionizing radiation. The percent ratio of cells with orange, yellow, and brown nucleoli was altered in the SMC neurons of exposed mice: in control animals the majority (57%) of the population was represented by cells with orange nucleoli, and in exposed mice 69% of cells contained yellow nucleoli. It is quite possible that some of the cells became transformed under the effect of ionizing radiation and from one class to another in type of nuclear staining.

The data characterizing the circadian dynamics of the matrix activity of neuronal chromatin in control and exposed animals are presented in Table 1. A monophasic 24-hour rhythm of the number of

TABLE 1. Time Course of the Activity of Endogenous RNA Polymerases of Nucleoplasm (NP) and Nucleoli (N) in the Nuclei of Cells from Different Compartments of the Nervous System in Control Animals and Mice Exposed to Radiation in the Chernobyl Accident Zone (Mean Number of Recovered Silver Grains, *M±m*)

Neuron population		Time of day, h			
		10	16	22	4
Spinal ganglion					
Control	NP	16.4±1.2	13.4±0.5	13.7±0.9	16.6±0.9
	N	6.7±0.3	6.2±0.3	6.4±0.5	8.7±0.4
Experiment	NP	13.6±1.4	12.4±1.5	13.3±3.2	12.7±1.0*
	N	6.6±1.0	5.9±0.7	6.8±1.7	6.2±0.2*
Spine					
Control	NP	34.2±3.5	25.0±1.8	39.1±2.0	31.1±1.8
	N	20.7±2.4	19.8±2.1	26.1±1.3	15.5±0.8
Experiment	NP	23.7±7.0	26.2±1.3	29.9±1.2*	30.5±2.1
	N	13.3±5.0	21.1±1.5	19.3±0.3*	15.8±1.6
Sensorimotor cortex					
Control	NP	10.3±0.5	14.2±1.1	18.2±1.5	18.9±0.9
	N	4.3±0.3	5.7±0.2	7.4±0.5	8.0±0.5
Experiment	NP	11.1±0.9	12.2±1.2	15.1±3.2	13.3±0.9*
	N	4.9±0.8	4.6±0.7	6.2±1.6	4.7±0.3*
Visual cortex					
Control	NP	21.0±0.3	13.8±1.6	14.1±1.6	14.5±0.6
	N	9.7±1.2	7.0±0.9	5.7±0.6	7.4±0.6
Experiment	NP	11.6±2.8*	11.0±1.2	13.6±4.2	16.1±1.4
	N	4.9±1.1*	5.1±0.6	6.6±2.1	5.8±1.4

Note. *$p<0.05$ in comparison with the control.

readable DNA matrices in the studied cell populations is seen in both groups of animals. The highest incorporation of ^{3}H-uridine in the spinal neuron nuclei was recorded at 22:00 h, and in the SG and SMC at 04:00 h, whereas the minimal levels were observed from 10:00 to 16:00 h. In the VC the maximal label incorporation was recorded at 10:00 and the minimal at 16:00 h. The degree of ^{3}H-uridine incorporation was decreased in the exposed mice *vs.* the controls during the majority of the examined periods of the 24-hour cycle. However, reliable differences in the level of label incorporation in the control and experiment were observed during the hours of the maximal matrix activity of chromatin. Hence, the follow-up of the circadian course of endogenous RNA polymerases of the neuronal nucleoplasm and nucleoli of animals exposed in the zone of the Chernobyl accident showed a higher radiation sensitivity of the matrix activity of chromatin during the period of increased polymerase activity. This is in line with published reports indicating that the intensity of chromatin transcription is liable to change during the repair of damage in exposed cells [2].

The parameters of the circadian rhythms are presented in Table 2. In control animals the acrophases of activity of endogenous RNA polymerases of the neuronal nucleoplasm of SG (06:36 h), spine (01:25 h), and SMC (00:10 h) were observed during the dark hours. The maximal values of nucleolar labeling were also observed in the dark hours, approximately coinciding with the acrophases of nucleoplasmic labeling: 04:40 h in the SG, 19:57 h in the spine, and 00:22 h in the SMC. The confidence intervals for these populations are similar or overlap. The correlation coefficients (0.5-0.2) of the time series of the activity of endogenous RNA polymerases of the nucleoplasm and nucleoli of the SG, spinal, and SMC neurons demonstrate a positive relationship of varying intensity, indicating their overall synchronization in the course of the day. This jibes with an earlier discovery — that there is a circadian system of nerve cell populations, the maximal levels of whose genetic activity coincide with the peaks of animal activity [5].

The acrophase of nucleoplasmic labeling of VC neurons is observed at 10:02 h and that of the nucleoli at 09:31 h, this evidently being related to day-

TABLE 2. Parameters of Circadian Rhythms of Activity of Endogenous RNA Polymerases of Nucleoplasm (NP) and Nucleoli (N) of Cell Populations from Various Compartments of the Nervous System in Mice Exposed to Radiation in the Chernobyl Accident Zone (Mean Number of Recovered Silver Grains)

Neuron population		Mesor (*M*±*m*)	Amplitude		Acrophase*	
			value	95% confidence interval	value	95% confidence interval
Spinal ganglion						
Control	NP	15.1±1.7	2.4	0.9-3.8	06:36	04:31-08:41
	N	7.0±0.5	1.5	1.1-2.0	04:40	02:18-07:01
Experiment	NP	13.7±2.7	3.9	3.1-4.8	03:50	16:57-14:42
	N	6.7±1.9	2.0	1.4-2.6	02:50	16:35-13:04
Spine						
Control	NP	32.3±1.5	7.8	5.8-9.9	01:25	21:07-05:42
	N	20.5±0.5	5.7	3.1-8.2	19:57	14:33-01:20
Experiment	NP	27.5±7.0	6.5	0.0-13.1	00:18	15:30-09:06
	N	17.4±2.5	5.3	0.0-14.7	17:51	09:40-02:02
Sensorimotor cortex						
Control	NP	15.3±1.3	5.3	3.3-7.3	00:10	22:18-02:02
	N	6.3±0.3	2.1	1.2-3.1	00:22	22:50-01:55
Experiment	NP	13.7±0.2	4.2	0.0-9.2	00:35	17:49-07:21
	N	5.5±0.1	2.1	0.0-4.8	23:40	14:14-09:05
Visual cortex						
Control	NP	15.9±3.0	4.5	2.6-6.4	10:02	07:10-12:54
	N	7.4±1.1	2.7	1.5-3.8	09:31	05:13-13:50
Experiment	NP	11.3±4.7	3.8	0.0-8.0	06:49	03:52-09:46
	N	6.6±2.0	1.9	0.6-3.1	22:39	12:12-09:06

Note. *Time, h.

light reception. These estimated peaks are in counterphase to the acrophases of SG, spinal, and SMC neuronal populations. The internal acrophases of the nucleoplasmic and nucleolar labeling of VC and SG neurons were, respectively, 03:26 and 04:51 h, those for VC and the spine 09:37 and 13:28 h, and for VC and SMC 09:52 and 09:09 h (correlation coefficients from -0.8 to -0.2). Differential analysis of the intensity of nucleoplasmic and nucleolar labeling demonstrates a uniform pattern of circadian changes in the expression of structural and ribosomal genes (correlation coefficients from 0.6 to 0.9), this probably indicating a similar regulation of polymerase activity of nerve cell chromatin in the course of the day.

The mice retain the rhythmic pattern of activity of endogenous RNA polymerases of neurons for 24 hours after radiation exposure, but the acrophases of nucleoplasmic and nucleolar labeling are shifted, this disrupting the synchronization observed in the course of 24 hours in control animals. The mesors and amplitudes of circadian rhythms are decreased (Table 2). As a result of exposure to the radioactive background in the accident zone, the acrophases of expression of structural genes of SG and VC neurons are shifted back 2.5-3 hours (correlation coefficients -0.2 and -0.4, respectively). The most pronounced changes are observed in the daily dynamics of ribosomal gene expression. The shift of acrophases of nucleolar labeling of different neuron populations varies from 1 to 12 h. The most manifest changes were observed in the VC, spine, and SG (r=-0.8, -0.3, and -0.12, respectively). The internal acrophases between the nucleoplasmic and nucleolar labeling of spinal, SMC, and VC neurons in the experiment were 06:27 h, 00:55 h, and O7:10 h, this being appreciably higher than the difference in the control (04:28 h, 00:12 h, and 00:33 h, respectively). Obviously, the mechanisms regulating the polymerase activity of structural and ribosomal genes in the studied populations of neurons in animals exposed to the radiation background lower the degree of synchronization in the course of the circadian cycle.

These data suggest that a 40-day exposure of animals in the zone of the Chernobyl accident under conditions of a high radiation background affects the histone component of chromatin and alters the circadian organization of the formation of primary gene products. Data on the normal and altered functioning of the genetic apparatus of neurons of various compartments of the nervous system under conditions of radioactive contamination will give us a clearer idea about the mechanisms shaping the long-acting specialized adaptive reactions of the organism, facilitating analysis of the pathogenesis of metabolic shifts and for developing recommendations for correcting these shifts.

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