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CIRCADIAN RHYTHM OF MITOSIS IN THE CORNEAL EPITHELIUM OF *Microtus arvalis* PALLAS

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KEY WORDS: mitosis; mitotic index; corneal epithelium; vole.

Many factors and mechanisms participate in the regulation of mitotic activity (MA) of cells [4-6]. Cell multiplication processes, it must be assumed, are under the control of a combination of various factors, both external (environmental) and internal (in the body itself). In the study of mitosis great attention must be paid to ecologic factors and to the type of behavior of the animals themselves, which determine the character of manifestation of various processes at the tissue and cell level, i.e., to the motor activity of the animal itself [2].

Despite closer attention to the study of MA, it has not yet been adequately studied in certain organs of wild animals [3, 7].

The aim of this investigation was accordingly to analyze the dynamics of the circadian rhythm of mitosis in the corneal epithelium of adult male and female common voles (*Microtus arvalis* Pallas) during the summer in relation to the circadian rhythm of their motor activity.

EXPERIMENTAL METHOD

Voles caught in the Trakai District of Lithuania in July, 1981, were used. The cornea was chosen as test material because it is the easiest object to analyze. Meanwhile processes taking place in the cornea reflect many aspects of energy metabolism in the organism as a whole [8].

MA in the corneal epithelium was determined in adult animals. Voles weighing 20 g or more were considered to be adult. The mean weight of the animals was 30.0 ± 0.8 g (n = 64) for males and 31.7 ± 1.32 g (n = 91) for females. The animals were decapitated immediately after being caught and thereafter every 3 h for 24 h: at 3, 6, and 9 a.m., 12 noon, 3, 6, and 9 p.m., and midnight, and additionally at 1, 4, and 8 p.m. Total preparations of the corneal epithelium were obtained and the mitotic index (MI, in promille) was calculated by the usual methods [1].

EXPERIMENTAL RESULTS

In the corneal epithelium of the common vole MA is observed by both day and night and it has a tendency to exhibit a polyphasic rhythm (Tables 1 and 2). Altogether 18,786 mitoses were found in total preparations, of which 13,272 (70.6%) were in the period from 9 a.m. to 9 p.m. inclusive. In males, of the 6652 mitoses observed 4229 (63.6%) occurred during daylight, compared with 9043 (74.5%) of 12,134 mitoses recorded in females at that time. The mean MI at night for males was 5.67 ± 1.10‰ and during the day 3.65 ± 1.04‰, and for females 5.62 ± 1.43 and 4.75 ± 1.10‰ respectively. In the interval between darkness (3 and 6 a.m., and midnight) and daylight (9 a.m., noon, 1, 3, 4, 6, 8, and 9 p.m.) differences were not statistically significant, evidence that the level of MI is the same at these times.

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TABLE 1. Circadian Changes in MA in Corneal Epithelium of Adult Male Common Voles in Summer

Time of day	No. of animals	No. of mitoses studied	Phase of mitosis								MI, ‰	P
			prophase		metaphase		anaphase		telophase			
			abs.	%	abs.	%	abs.	%	abs.	%		
3 a.m.	4	556	198	35,6	233	41,9	60	10,8	65	11,7	5,45±0,57	<0,05
6	4	785	226	28,8	310	39,5	139	17,7	110	14,0	7,70±0,65	<0,001
9	6	441	125	28,4	143	32,4	54	12,2	119	27,0	2,88±0,28	<0,001
Noon	7	1771	628	35,5	541	30,5	256	14,5	346	19,5	9,92±0,45	<0,001
1 p.m.	6	160	52	32,5	40	25,0	14	8,7	54	33,8	1,05±0,05	<0,05
3	3	484	221	43,6	171	35,3	32	6,6	70	14,5	6,38±2,05	<0,25
4	6	459	97	21,1	146	31,8	123	26,8	93	20,3	3,00±1,13	<0,5
6	5	244	119	48,8	63	25,8	21	8,6	41	16,8	1,91±0,12	<0,5
8	8	506	190	37,6	205	40,5	38	7,5	73	14,4	2,48±0,65	<0,5
9	4	164	38	23,2	39	23,8	27	16,5	60	36,5	1,61±0,20	<0,001
Midnight	11	1082	284	26,3	393	36,3	92	8,5	313	28,9	3,86±0,16	—
Mean				32,6		34,2		12,9		20,2	4,07	

TABLE 2. Circadian Changes in MA in Corneal Epithelium of Adult Female Common Voles in Summer

Time of day	No. of animals	No. of mitoses studied	Phase of mitosis								MI, ‰	P
			prophase		metaphase		anaphase		telophase			
			abs.	%	abs.	%	abs.	%	abs.	%		
3 a.m.	6	1331	418	13.4	469	35,2	135	10,2	309	23,2	8,50±0,81	<0,001
6	5	535	223	41,7	168	31,4	78	14,6	66	12,3	4,10±0,34	<0,002
9	7	421	115	27,3	107	25,4	73	17,4	126	29,9	2,30±0,20	<0,001
Noon	10	3071	618	20,1	1084	35,3	642	20,9	727	23,7	11,77±0,55	<0,001
1 p.m.	4	310	75	24,2	100	32,3	44	14,2	91	29,3	2,97±0,12	<0,001
3	8	1264	396	31,3	442	35,0	172	13,6	254	20,1	6,05±0,36	<0,001
4	8	405	125	30,9	134	33,1	46	11,3	100	24,7	1,94±0,10	<0,001
6	10	1253	435	34,7	435	34,7	162	12,9	221	17,7	4,80±0,16	<0,1
8	12	1098	333	30,3	327	29,8	159	14,5	279	25,4	3,51±0,76	<0,5
9	10	1221	290	23,8	347	28,4	148	12,1	436	35,7	4,68±0,71	—
Midnight	11	1225	358	29,2	392	32,0	99	8,1	376	30,7	4,27±1,10	—
Mean				27,9		33,0		14,5		24,6	5,10	

The mean 24-hourly MI for the common vole was 4.60‰ (4.07 ± 0.83‰ for males, 5.10 ± 0.86‰ for females). MA in animals of both groups studied reached a maximum at noon, and a minimum in males at 1 p.m. and in females at 4 p.m. When additional samples were taken at 1, 4, and 8 p.m. it was found that the curve of circadian MA assumed a more marked polyphasic character as a result of more frequent sacrifice of the voles.

It follows from this account that on the whole the MA level was the same in the periods of daylight and darkness, and this was connected with the fact that voles are active all round the clock. According to our observations, in Trakai District in July the common vole population has a 24-hourly form of activity with only slight predominance during daylight. The coefficient of correlation between motor activity and MA was $r = -0.04$.

The intensity of cell division during the 24-h period was more dynamic in males. It changed by a lesser degree in females, due to the ecologic features of the group compared. Motor activity of voles is very closely dependent on the environment and on the individual characteristics of the animals, and in turn this is reflected in processes at the cell level, including MA. The absence of data on MA during exposure to different external factors and with different levels of motor activity of the animals does not allow a more detailed analysis of these very important processes of adaptation of common voles to the environment.

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CIRCACIAN RHYTHMS OF ADRENALIN AND NORADRENALIN EXCRETION IN MAN UNDER NORMAL CONDITIONS AND AFTER TAKING ALCOHOL

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KEY WORDS: circadian rhythms; alcohol; adrenalin; noradrenalin.

The study of the effect of toxic substances on the body from the standpoint of chronobiology has led to the creation of a new discipline, namely chronotoxicology. Chronotoxicologic aspects of alcohol intoxication are only just beginning to be studied [3, 7].

The aim of this investigation was to study disturbances of biorhythms caused by alcohol in man, with special reference to circadian rhythms of adrenalin and noradrenalin, the principal parameters reflecting the state of function of the sympathicoadrenal system.

EXPERIMENTAL METHOD

Adrenalin and noradrenalin were determined by a fluorometric method [5] in 20 healthy male volunteers aged 20-26 years, in samples of urine excreted during 4-hourly intervals at 7 and 11 p.m., 3, 7, and 11 a.m., and 3 p.m. in a 24-h period under normal conditions (control) and during 3-day cycles (72 h) after consumption of a single dose of 6.2 ml/kg body weight of 40° alcohol between 5 and 6 p.m. at the end of the control day. The maximal blood ethanol concentration was 3.9 ± 0.19 mM, which corresponds to an average degree of intoxication [6]. During the period of the investigation all subjects were kept under identical conditions of daily routine and on a standard diet. Irrelevant stress was reduced to the minimum. The investigation was conducted in March.

The results were subjected to statistical analysis by computer on a "Kosinor" program [1], with determination of mesors, amplitudes, and calculated acrophases of rhythms.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that normally the maximal excretion of adrenalin and noradrenalin is observed in the active period of the day (from 11 a.m. to 7 p.m.) and minimal during the period of sleep (from 11 p.m. to 7 a.m.), in agreement with data in the literature [2, 4]. The normal adrenalin/noradrenalin ratio (Table 2) has highest values at night (from 11 p.m. to 7 a.m.), and lowest during the first half of the day (from 7 a.m. to 3 p.m.).

Values of mesors, amplitudes, and calculated acrophases of adrenalin and noradrenalin after analysis of the data by the "Kosinor" program are given in Table 3.

Alcohol brings the sympathicoadrenal system into a state of strain, as shown by the considerable and sufficiently stable increase in catecholamine concentrations in the urine. In the first portion of urine after consumption of alcohol (7 p.m.) the adrenalin level was 10.6 times higher than normal ($P < 0.001$) and the noradrenalin level was 3.25 times higher ($P <$

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