CIRCADIAN RHYTHM OF RESPONSE OF MOUSE LINGUAL AND ESOPHAGEAL EPITHELIAL CELLS TO HYDROXYUREA

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KEY WORDS: hydroxyurea; index of labeled nuclei; circadian rhythm

Investigations by several workers have demonstrated a circadian rhythm of response of proliferating cells to various exogenous factors [2, 6-8], but the inadequate volume of research in this direction is reflected in discussions of the mechanisms of this rhythm [1].

The aim of this investigation was to study the circadian rhythm of sensitivity of the epithelial cells of the tongue and esophagus to hydroxyurea, which blocks transition of cells into the S phase of the mitotic cycle.

EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing 22-25 g. The animals were kept in 12 h of daylight (8 a.m. to 8 p.m.) and 12 h of darkness. After 17 days, the mice were given a single intraperitoneal injection of hydroxyurea (HU, "Serva") 3 h before sacrifice in a dose of 250 mg/kg body weight [5], at 10 p.m., 2, 6, and 10 a.m., and 45 min before sacrifice they also were given an injection of ^{3}H-thymidine in a dose of 0.75 μCi/g body weight (specific activity 10.1 Ci/mmole). At each time point five or six mice were used. The samples of the animals' tongue were taken at these times and of the esophagus twice a day. Histological sections 4-5 μ thick were exposed with type M emulsion for 30 days, and by analysis of 8000-10,000 cells of the stratum basale of the esophagus and the dorsal surface of the tongue in each case, the index of labeled nuclei (ILN) was determined and expressed in promille ($^0/_{00}$).

EXPERIMENTAL RESULTS

Table 1 shows that fluctuations of ILN occurred in the lingual epithelium of the control animals with a maximum at 1 a.m. and a minimum at 1 p.m. ($p < 0.001$). A decrease in ILN ($p \leq 0.005$) was found in the experimental animals at all times of the investigation. The degree of inhibition of entry of the cells into the S-phase of the mitotic cycle under the influence of HU was expressed as a linear function of the initial level of ILN (coefficient of correlation 0.98). During the period of maximal values of ILN, for instance, it was 63% in the lingual and 64.5% in the esophageal epithelium, but only 39.5% in the lingual and 42% in the esophageal epithelium during the minimum of ILN. Consequently, the results indicate the existence of a circadian rhythm of the response of both kinds of epithelial cells to HU.

Mechanisms of circadian rhythms of cell response to external factors have been linked with the effect of the substance on one period of the mitotic cycle, and also with the number of cells present in that period of the cycle at the given time of day [1, 4]. The results of the present investigation likewise confirm this view. For instance, according to data in [7, 8], adrenalin and vinblastine, which affect roughly the same part of the mitotic cycle (vinblastine causes a block in mitosis, whereas adrenalin prevents entry of the cells

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TABLE 1. Changes in ILN (M ± m; in ‰) in the Lingual (a) and Esophageal (b) Epithelium 3 h after Injection of HU into Mice

Clock time	Control	HU
	(a)	
1 p.m.	41,8±2,7	25,2±2,0 (—39,5)
5 p.m.	66,3±9,0	30,5±3,1 (—54)
9 p.m.	115,5±8,9	56,1±5,7 (—51,5)
1 a.m.	228,4±7,9	84,4±8,0 (—63)
5 a.m.	147,3±7,8	66,3±7,1 (—55)
9 a.m.	73,2±5,8	41,7±2,6 (—43)
	(b)	
1 p.m.	40,4±3,4	23,4±2,0 (—42)
1 a.m.	202,5±9,1	71,7±5,9 (—64,5)

Legend. Changes in ILN compared with control shown in parentheses (in %).

into mitosis from the G_2-phase), had the strongest action on proliferation of the corneal cells of mice of the same age in opposite phases (adrenalin in the morning, in the period of maximal values of the mitotic index, vinblastine in the evening). A G_2-chalone-containing extract of Ehrlich's ascites tumor has a stronger action (Block in the G_2-period) if used at the time of lowered mitotic activity of the cells of this tumor [2]. Consequently, the circadian rhythm of response of proliferating cells to the action of substances affecting the same period of the cycle may be different, for it is not determined solely by the number of cells in that period of the cycle.

In case of correlation between the circadian rhythm of sensitivity and the circadian rhythm of the number of cells in a given phase of the mitotic cycle, it can be concluded from the actual numerical parameters of cell proliferation that relations between these rhythms are rather complex. For instance, the results of the present experiments indicate that the degree of inhibition of proliferative activity by HU, although correlating with circadian changes in ILN, is not directly proportional to them. In fact, ILN of the basal cells of the lingual epithelium in the control animals at 1 p.m. and 1 a.m. differ from one another by a factor of 5.5 times, whereas the percentage inhibition of cell proliferation differ by only 1.6 times, so that ILN in the experimental animals at these times differ by 3.4 times. Similar results are given also by analysis of ILN of esophageal epithelial cells [7, 8]. In addition, the percentage inhibition of cell proliferation may be the same in animals with different values of ILN. For instance, at 5 p/m and 5 a.m. the percentage reduction of ILN under the influence of HU is the same, namely 54-55, whereas ILN values in the control animals at these times differ by 2.2 times ($p < 0.005$).

The results are thus evidence that the number of cells responding to a single exposure to inhibitors of cell proliferation changes in the course of the 24-h period. Rhythmic changes in the number of sensitive cells evidently exhibit different phase relations with the circadian rhythm of the index of cells proliferation. This may be one cause of the circadian rhythm of sensitivity found when it is studied only twice a day [3, 4]. Possible noncoincidence of the phases of these two rhythms indicates that they may be relatively independent of each other even when the two phases do coincide, as is shown, for example, by the absence of a directly proportional response to the external factor.

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ARTIFICIAL SELECTION FOR HIGH METASTATIC POTENTIAL IN TRANSPLANTABLE RAT RHABDOMYOSARCOMA RA-2 CELL POPULATION

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Strains of transplantable tumors of varied histogenesis, whose cells possess affinity for a particular target organ have now been obtained with the aid of selection in vitro and in vivo [4-6]. The affinity of the cells is manifested as both spontaneous and experimental metastasization [4]. In the latter case, intravenously injected cells invade only that organ of the recipient animal for affinity to which they have been selected, and experimental metastases are formed only in the target organ. This particular feature of tumors with organ affinity enables the fraction of cells capable of forming experimental metastases to be determined with the highest possible degree of accuracy, i.e., enables the metastatic potential (MP) of the tumors to be determined.

In all studies of organotropic primary tumors an increase in MP has been observed during selection for organotropism. However, after completion of selection for organotropism (2-15 cycles), no further selection was carried out to increase MP, so that it is not possible to judge on a sufficiently sound basis the character of inheritance of the MP trait in tumor cell populations or the possibility of increasing MP of organotropic malignant tumors by means of artificial selection.

This paper gives the results of long-term (10 years), repeated (180 cycles) selection in vivo for increasing MP, recorded by the method of lung colonies, in a population of rhabdomyosarcoma cells, induced by 20-methylcholanthrene, and which yielded evidence to show that selection for high MP is effective even after the cells have acquired organotropism.

On the basis of data on the efficacy of selection for high MP, and on the degree of phenotypic and hereditary heterogeneity of the population selected on the basis of MP and its karyotypic heterogeneity, the inheritance of the MP trait was analyzed in a tumor cell population.

EXPERIMENTAL METHOD

Rhabdomyosarcoma RA2 was induced by injection of an oily solution of 20-methylcholanthrene into the thigh muscle of noninbred female albino rats (from the "Rappolovo" nursery).

Selection for increasing the ability of RA2 cells to form experimental metastases in the lungs was carried out in vivo by the lung colonies method [7]. For this purpose, suspensions of single tumor cells were prepared from subcutaneous transplants of a primary tumor, the number of viable cells was counted, and they were injected into the lateral caudal vein

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