CHRONOBIOLOGICAL CHARACTERISTICS OF THE ACTION OF THYROXINE OF REPRODUCTION OF EHRLICH'S ASCITES TUMOR CELLS

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KEY WORDS: thyroxine; mitotic index; radioactive index; Ehrlich's ascites tumor.

Among the hormones controlling cell reproduction in normal tissues an important role is played by the thyroid hormone thyroxine [1, 3]. However, the effect of thyroxine on division of tumor cells has so far received little study. Some workers [2] observed inhibition, others [9, 10] stimulation of tumor growth after administration of thyroid hormones to animals. The action of thyroxine on cell division in normal tissues has been shown [3, 8] to depend on clock time. It is not known whether a similar principle applies to tumor cells.

The aim of the present investigation was to study the action of thyroxine on cell proliferation in a hyperdiploid strain of Ehrlich's ascites tumor (EAT) depending on clock time.

EXPERIMENTAL METHOD

Experiments were carried out on 260 noninbred male albino mice weighing 18-20 g. A hyperdiploid strain of EAT was transplanted intraperitoneally into all the animals. In the experiments of series I mice were kept at a temperature of 18°C with natural illumination and with food *ad lib*. Starting from the first day of development of the tumor, at 11 a.m. the animals were given an intraperitoneal injection of the sodium salt of L-thyroxine (from "Reanal," Hungary). The thyroxine was dissolved in 1.0 ml of 0.1 M NaOH solution immediately before injection and diluted to the necessary concentration with physiological saline. The hormone was injected in doses of 1, 10, 30, 60, and 100 μg/100 g body weight in a volume of 0.2 ml during 6 days of tumor growth. The solvent of the thyroxine only was injected into control animals in a volume of 0.2 ml. Mice were killed at noon and 9 p.m. on the 5th day, and at noon on the 6th day of the growth cycle of EAT. The action of thyroxine was judged by changes in the mitotic index (MI) in the control experimental animals.

The animals of series II were kept at a temperature of 18°C, with a 12-h period of daylight (from 4 a.m. to 4 p.m.), and with food *ad lib*. Thyroxine in a dose of 10 μg/100 g and the solvent of the hormone were injected into the animals just as in series I. The mice were killed starting from the 96th hour of tumor development, every 3 h for 2 days (the 5th and 6th days of EAT development), five animals at each point of the investigation. The animals were given an injection of [^{3}H]thymidine in a dose of 0.5 μ Ci/g body weight (specific radioactivity 4.1 Ci/mmole) 1 h before sacrifice. The technique of preparing the specimens of tumor cells was described previously [4]. On autoradiographs from each animal, during examination of 3000-5000 cells the number of dividing and DNA-synthesizing cells was counted and MI and the radioactive index (RI) were calculated and expressed in promille. Parameters of the rhythms of these indices were determined by a graphic-parametric method [6], the duration of mitosis of EAT cells being taken to be 1.5 h [5]. The results were subjected to statistical analysis by the Fisher–Student method. Differences were considered significant at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

As Fig. 1 shows, on the 5th day of the experiment MI in EAT in the control animals at 9 p.m. was 3.8 times higher than MI at noon ($P < 0.05$). At different times of the 24-hour

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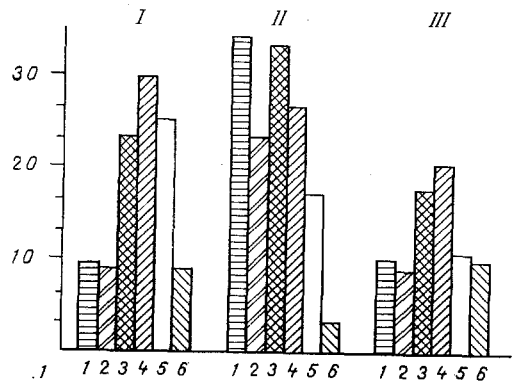

Fig. 1. Changes in MA in hyperdiploid strain of EAT on 5th-6th day of tumor growth after injection of various doses of thyroxine and in control. Ordinate, MI (in °/₀₀). 1) Control, 2) 1 μg, 3) 10 μg, 4) 30 μg, 5) 60 μg, 6) 100 μg thyroxine. I) Noon, II) 9 p.m., III) noon.

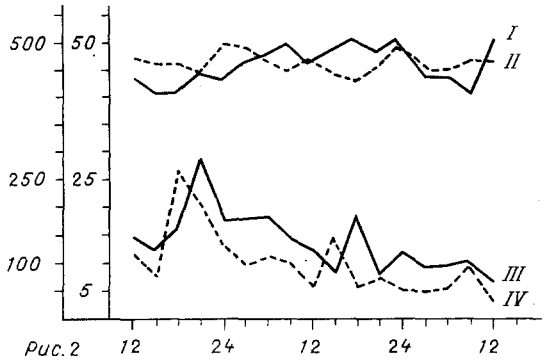

Fig. 2. Changes in MA (III, IV) and number of DNA-synthesizing cells (I, II) in hyperdiploid strain of EAT on 5th-6th day of tumor growth in control (I, III) and experiment (II, IV). Abscissa, clock time; ordinate, RI and MI (in °/₀₀).

period thyroxine differed in its action on reproduction of tumor cells. At noon on the 5th day of EAT development, for instance, the hormone had a stimulating effect on mitotic activity (MA) when given in doses of 10, 30, and 60 μg (MI was increased by 2.6, 3.2, and 2.8 times respectively, $P < 0.05$). Doses of 1 and 100 μg at noon caused no change in MA compared with the control. At 9 p.m. on the 5th day, when MI of the control animals was increased, doses of 1, 60, and 100 μg thyroxine reduced MI by 1.5, 2.0, and 11.5 times respectively ($P < 0.05$). Changes in MI under the influence of thyroxine in doses of 10 and 30 μg were not significant, although MI also was less than in the control. At noon on the 6th day thyroxine in doses of 10 and 30 μg increased MI by 1.7 and 2.0 times respectively ($P < 0.05$), but in doses of 1, 60, and 100 μg, it caused no change in MI.

It can be concluded from these results that the action of thyroxine on division of EAT cells depends, first, on its dose and, second, on the clock time. Since after daily administration of thyroxine for several days to mice the blood hormone level remains consistently high [7], the absence or presence of stimulation of cell division depending on clock time is evidence of the existence of rhythmic changes in sensitivity of proliferating EAT cells to the action of thyroxine in the course of the 24-hour period. The stimulating effect of thyroxine on cell reproduction was observed during the minimum of MA (afternoon), and in inhibitory effect during the maximum of MA in EAT (the evening).

The results of the experiments of series II showed (Fig. 2) that MA does not remain constant during 5-6 days of growth of EAT either in the control or in the experiment. In the

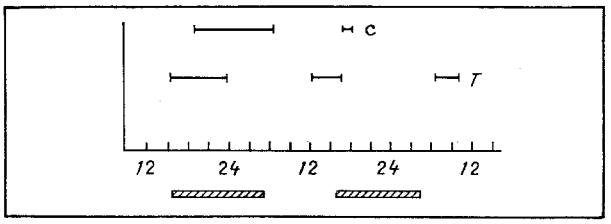

Fig. 3. Phase diagram of biological rhythms of cell division in hyperdiploid strain of EAT on 5th-6th days of growth in control (C) and under the influence of thyroxine (T). Abscissa, clock time, in hours.

control animals maximal values of MI were observed at 9 p.m. on the 5th day and at 6 p.m. on the 6th day ($P < 0.05$). The rhythm of MI during the 24-hour period was monophasic, with a period of 21 h. The active phase of the rhythm of MI on the 5th day occupied virtually all the dark portion of the 24-hour period, but on the 6th day it occupied only its beginning; on that day, moreover, the active phase of the rhythm of mitosis was much shorter than on the 5th day (Fig. 3). During growth of the tumor a shift of the acrophase of MI was observed to the left by 3 h. The relative amplitude of the rhythm was 2.4 and 2.5 and the coefficient of synchronization 0.4 and 0.8 respectively. The midrhythm values of MI on the 6th day were significantly lower than on the 5th day: 17.5 and 11.5% respectively ($P < 0.05$). The pool of dividing cells during the period of the rhythm on the 6th day also was less than on the 5th day (169 $^{0}/_{00}$ and 264 $^{0}/_{00}$ respectively). The fraction of mitoses in the active phase of the rhythm was 69% on the 5th day and 25% on the 6th day.

Changes in the number of DNA-synthesizing cells in the control during the 5th and 6th days of EAT development were totally different in character from those in MI (Fig. 2). RI increased gradually from 6 p.m. until 9 a.m. on the 5th day ($P < 0.05$), after which it showed little change until midnight on the 6th day, fell until 9 a.m., and then rose again until noon on the 7th day of tumor growth ($P < 0.05$). The mean daily values of RI on the 5th and 6th days of the experiment were virtually the same (442 and 461 $^{0}/_{00}$ respectively). The 24-hourly pools of DNA-synthesizing cells also were closely similar on these days (502 and 524 $^{0}/_{00}$ respectively).

First, therefore, no regular rhythmic fluctuations of RI were found during the 24-hour period and, second, changes in RI and MI throughout the experiment were not synchronized. The writers obtained similar results previously [4]. They show that the circadian rhythm of mitosis in EAT is evidently unconnected with fluctuations in the number of DNA-synthesizing cells, but is due to a mechanism of synchronization of cell movement, in the mitotic cycle immediately before mitosis.

In the case of repeated injection of thyroxine into animals, in the course of the experiment three acrophases of MI were observed: at 6 p.m. on the 5th day, at 3 p.m. and 9 a.m. on the 6th day ($P < 0.05$). The period of the rhythm was 21-18 h. The active phase of the rhythm on the 5th day occurred at the beginning of the dark period, but on the 6th day at the end of the light period, i.e., earlier than in the control (Fig. 3). The value of MI after injection of the hormone was significantly higher than the control at 6 p.m. on the 5th day and at 3 p.m. on the 6th day of growth of EAT ($P < 0.05$). At other times of the experiment MI either did not differ from the control values (9 a.m. and 9 p.m. on the 6th day), or it was significantly lower (at 3 a.m. on the 5th day and at noon, 6 p.m., midnight, and noon on the 6th day ($P < 0.05$). Thus stimulation of cell multiplication in EAT by thyroid hormone, as in the first series of experiments also, did not take place during the acrophase of the MI rhythm in the control, but at times of reduced MA, 3 h before the acrophase of MI in the control animals, indicating that thyroxine may have a synchronizing action on rhythmic entry of cells into mitosis. This is confirmed by data showing higher values of relative amplitude and coefficient of synchronization of successive waves of MI (3.86, 2.5, and 1.67) compared with the control (1.29, 0.83, and 0.56 respectively). The midrhythmic values of MI were higher (13.8 $^{0}/_{00}$) on the 5th day than on the 6th day (7.6 $^{0}/_{00}$), but lower than the control values. The number of cells dividing during the period of the rhythm (208 and 107 $^{0}/_{00}$ respectively) also was less than in the control. The relative number of cells dividing in the active phase of the rhythm on the 5th day was smaller than in the control (50%), and this evidently affects the level of MA during thyroxine administration. These observations indicate that under the influence of thyroxine the number of proliferating cells

does not increase during the period of the rhythm but, on the contrary, it has a tendency to fall.

Injection of thyroxine caused no significant change in the mean 24-hourly number of DNA-synthesizing cells compared with the control (469 ‰ on the 5th day and 454 ‰ on the 6th day). The 24-hourly pools of DNA-synthesizing cells differed only a little from each other (530 and 517 ‰) and from the control. During the 2nd day of the experiment some fluctuations were observed in the value of RI, but they were not significant. At all points of the investigation RI did not differ significantly from the control. Thus during administration of thyroxine also, changes in MI and RI with time were not synchronized.

The results of this investigation indicate that similar chronobiological principles govern the response of dividing tumor cells to the action of thyroxine as in the case of cells of normal tissues, and are expressed as rhythms of sensitivity of the tissues to this hormone. Meanwhile thyroxine has no appreciable effect on the number of DNA-synthesizing tumor cells, changes in which did not have the character of a circadian rhythm in either the control or the experimental animals. Cells characterized by rhythmic entry into mitosis cells entering the S period from the G_1 phase did so nonrhythmically and they had no such rhythm of sensitivity to thyroid hormone. Injection of thyroxine into animals likewise is not accompanied by an increase in the pool of dividing cells in EAT, whereas such an increase does occur in normal tissue [3].

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EFFECT OF *SCHIZANDRA CHINENSIS* LIGNANS ON CELL DIVISION IN THE CORNEAL EPITHELIUM AND TONGUE OF ALBINO RATS EXPOSED TO CHRONIC COLD STRESS

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In previous investigations the writers showed that chronic exposure to cold stress causes activation of DNA synthesis in the corneal and lingual epithelium of albino rats [6]. This phenomenon was interpreted as a structural trace of adaptation [5], aimed at restoring tissue homeostasis, when disturbed as a result of the damaging effect of stress [1, 7]. The increase in frequency of pathological mitoses (PM) was characterized as a cellular manifestation of disadaptation [10]. Data in the literature indicate that preparation of *Schizandra chinensis* (Chinese magnolia vine) alleviate the course of the general adaptation syndrome (GAS) and possess adaptogenic properties [4].

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