EFFECT OF DAYLIGHT—DARKNESS ALTERNATION PATTERN ON CHRONOSENSITIVITY TO ABSOLUTE HYPERCAPNIA

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In recent years much attention has been paid to the study of the effect of oxygen lack, of hypoxic hypoxia, and of hypercapnia on animals and man, and in particular, on their biorhythms [1, 5, 8, 9, 11, 12]. Meanwhile it has become evident that the rhythmic nature of physiological processes itself, and the rise and fall of the functions of organs, organ systems, and the body as a whole, determine unequal resistance and sensitivity of the body in the course of the day or year, and in different years [2, 3, 6, 7]. However, discoordination of biological rhythms caused by changes in activity of the synchronizers may also lead to changes in the chronoresistance and chronosensitivity of the body [4, 10]. It is therefore interesting to compare the principles governing the response of the organism to toxic factors under normal conditions, with the onset of desynchronization, and during readaptation.

Since one of the most important synchronizers for animals is the alternation of daylight and darkness, the present investigation was carried out with the aim of studying the circadian rhythm of sensitivity to absolute hypercapnia under conditions of natural alternation of daylight and darkness, and during adaptation to continuous artificial lighting and on the return of the animals to natural conditions.

METHODS

Altogether 7 series of experiments were carried out in which mature noninbred male mice weighing 18-21 g at the beginning of the experiment were used. The sensitivity of the mice to hypercapnia was determined by recording the time of recovery of the animals from the side position. The mice were lowered into a jar, into which a constant flow of carbon dioxide was provided from a cylinder through an adapter. The level of saturation with CO_2 in the jar was 98.88%. After 10 sec the animal was quickly removed from the jar and placed on a table. A stop watch was set in motion and the time when the animal adopted the side position was recorded. The stop watch was stopped when the mouse abruptly stood up. In this way the time of recovery of the physiological posture was recorded. This individual time (in seconds) served as the criterion of sensitivity of the animal to the toxic action of hypercapnia.

In the experiments of series I with natural daylight and free access of the animals to food and drink, the investigation was conducted at intervals of 4 h, starting from 6 p.m., 7 times a day (at 6 and 10 p.m., 2, 6, and 10 a.m., and 2 and 6 p.m.), 10 mice being used at each time. In series II and III the investigation was conducted during adaptation of the animal to continuous artificial lighting (about 60 lx): after 2 weeks in series II, after 1 month in series III. The experiments of series II were carried out in the same way as those in series I, except the second test at 6 p.m. In series III the animals were investigated at 6-h intervals 4 times a day (at 10 p.m., 4 and 10 a.m., and 4 p.m.), 16 animals being used at each time.

Next day the animals were transferred to natural alternation of daylight and darkness. In series IV and V, the investigation was carried out 2 weeks and 1 month respectively after the return to natural conditions of daylight 6 times a day, 10 animals being used at each time. The tests were carried out after 2 and 2.5 months respectively, 4 times a day, with 10

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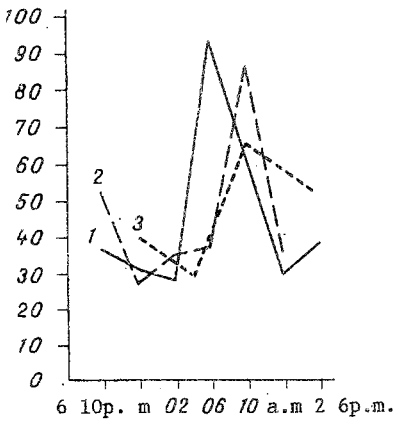

Fig. 1. Circadian rhythm of toxicity of hypercapnia under natural conditions and with continuous daylight: 1) natural alternation of daylight and darkness (control): $P_{18-2} > 0.05$, $P_{2-6} < 0.05$, $P_{6-10} > 0.05$, $P_{6-14} < 0.05$, $P_{14-18} > 0.05$; 2) continuous daylight, after 2 weeks: $P_{18-22} > 0.05$, $P_{22-6} > 0.05$, $P_{6-10} < 0.05$, $P_{10-14} < 0.05$, $P_{14-18} > 0.05$; 3) continuous daylight, after 1 month: $P_{22-4} < 0.05$, $P_{4-10} < 0.05$, $P_{10-16} > 0.05$, $P_{16-22} > 0.05$. Here and in Figs. 2 and 3: abscissa, time of day; ordinate, time of recovery of physiological posture (in sec).

animals used on average each time. The results were subjected to statistical analysis by the Fisher–Student method.

RESULTS

It will be clear from Fig. 1 that in mice kept under natural conditions, a distinct circadian rhythm of sensitivity to hypercapnia was found. The toxicity curve was unimodal in character, with the greatest sensitivity between 6 and 10 a.m., and a maximum at 6 a.m. (time of recovery from the side position 93 sec), and a period of least sensitivity from 2 p.m. through 6 p.m. to 2 a.m. (the maximum was more than three times greater than the minimum). This corresponds to data in [12], in which the peak sensitivity of mice to hypoxia was observed at noon (53.5% of animals died), and the minimum at midnight (33.3%). In animals kept for 2 weeks under conditions of continuous daylight, the character of the hypercapnia toxicity curve was in general very similar. However, there were differences also. First, the maximum of sensitivity was shifted from 6 a.m. to 10 a.m., and sensitivity at 6 a.m. was least, whereas in the previous experiment it was greatest. Second, the time of recovery from the side position at 6 p.m. was increased (53 sec), although this difference was not significant.

Investigation of toxicity after 1 month revealed a further phase shift of the maximum of sensitivity toward daylight hours (10 a.m. to 4 p.m.), and also an increase in the period of increased sensitivity throughout the 24-h period. Whereas in the original experiments maximal sensitivity was observed at 6-10 a.m., and at other times relative resistance was observed, after 1 month of keeping the mice in continuous daylight, on the other hand, the period of increased sensitivity occurred at 10 a.m. to 4-10 p.m., and the maximum of resistance was observed only at 4 a.m. Consequently, in continuous daylight, there was a phase shift in the rhythm of sensitivity as well as lengthening of the time zone of increased sensitivity during the 24-h period.

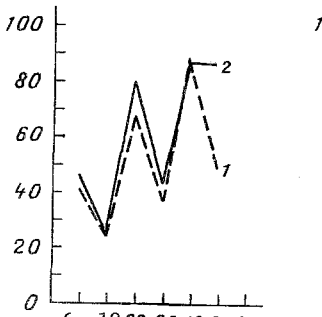

Fig. 2

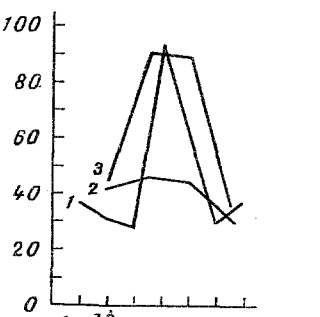

Fig. 3

Fig. 2. Circadian rhythm of toxicity of hypercapnia on returning animals to conditions of natural alternation of daylight and darkness. 1) Readaptation for 2 weeks: $P_{18-22} < 0.05$, $P_{22-2} > 0.05$, $P_{2-6} > 0.05$, $P_{6-10} < 0.05$, $P_{10-14} > 0.05$, $P_{14-18} > 0.05$; 2) readaptation, after 1 month: $P_{18-22} \leqslant 0.05$, $P_{22-2} < 0.05$, $P_{2-6} \leqslant 0.05$, $\leqslant P_{14-18} < 0.05$.

Fig. 3. Circadian rhythm of toxicity of hypercapnia under conditions of continued readaptation. 1) original curve (the same as Fig. 1, control); 2) adaptation for 2 months: $P_{22-4} > 0.05$, $P_{10-16} < 0.05$, $P_{16-22} < 0.05$; 3) readaptation for 2.5 months: $P_{22-4} < 0.05$, $P_{10-16} < 0.05$, $P_{16-22} > 0.05$.

When the animals were returned to natural alternation of daylight and darkness the character of the circadian rhythm changed abruptly, and the toxicity of hypercapnia curves became bimodal in appearance with a maximum of sensitivity at 2 and 10 a.m. (Fig. 2). Incidentally, whereas after 2 weeks of readaptation the rise at 2 a.m. and the fall at 6 p.m. were not yet significant, although close to that level ($P = 0.06$–0.09), after 1 month the circadian rhythm of toxicity was distinctly bimodal in character with a maximum of sensitivity at 2 a.m. and 10 a.m. to 2 p.m., and a minimum at 10 p.m. and 6 a.m.

After 2 months of readaptation the curve changed its character once more: increased sensitivity was observed at 10 p.m. to 4 a.m. to 10 a.m., and minimal at 4 p.m. (Fig. 3). Meanwhile the flattening of the curve and the reduction by half of the time taken for recovery from the side position at times of maximum sensitivity (46 sec at 4 a.m., 45 sec at 10 a.m.) compared with the 6-h point on the original curve (93 sec) will be noted. After 2.5 months the toxicity of hypercapnia curve was unimodal in character with a maximum of sensitivity at 4–10 a.m. and a minimum at 10 and 4 p.m., and was very similar to the curve plotted from the results of the experiments of series I, in which the animals were kept under natural alternation of daylight and darkness (Fig. 3).

The results confirm the view that alternation of daylight and darkness is one of the leading, perhaps the most important synchronizer of biological rhythms in animals. When this is abolished, the acrophase of sensitivity to hypercapnia shifts and the period of increased sensitivity in the course of the 24-h period increases. However, more marked disturbances of the character of the rhythms are found under conditions of readaptation, when the rhythm was initially bimodal in character, but later the curve flattened out, and not until 2.5 months had elapsed was the original character of the circadian rhythm of sensitivity to absolute hypercapnia restored.

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EFFECT OF ESTRONE ON FORMATION OF A FOCUS OF HETEROTOPIC HEMATOPOIESIS

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The considerable reduction in the cell content of the bone marrow in patients with myelofibrosis and in animals with an experimental model of that condition [1, 4] is generally associated with mechanical displacement of hematopoietic cells by newly formed bone tissue from the medullary cavity. To test this hypothesis, a model of estrone-induced myelofibrosis has been developed [5], in which relations between bone tissue and hematopoiesis in a heterotopic hematopoietic focus beneath the renal capsule in mice, where there is no closed medullary cavity, is assessed.

METHODS

Female (C56Bl × CBA)F_1 mice were used. Heterotopic transplantation of syngeneic bone marrow beneath the renal capsule was carried out on recipients anesthetized with hexobarbital, 7 days after ovariectomy. Myelofibrosis was induced by giving the experimental animals an injection of an oily solution of estrone in a dose of 0.5 mg daily for 4-6 weeks. The first injection of estrone was given one day after bone marrow transplantation. Control animals were given injections of peach oil in accordance with a similar scheme.

The newly formed focus of ectopic hematopoiesis was removed after one month. The dried bony capsule was weighed, and the number of myelokaryocytes counted in the focus and in the femur. The effect of estrone on formation of the focus of heterotopic hematopoiesis also was studied morphologically.

RESULTS

After a 4-week course of estrone injections active proliferation of bone trabeculae of the metaphysis was observed in the femur, accompanied by thickening of the diaphysis. A morphologically similar pattern was observed during the formation of a focus of heterotopic hematopoiesis after transplantation of syngeneic bone marrow beneath the renal capsule of experimental animals. Whereas the bony capsule in the focus in the control animals consisted of a comparatively thin lamina of bone tissue with very few trabeculae, lying only at the edges of the focus facing the kidney tissue, four injections of estrone caused the active formation of cancellous bone, the trabeculae of which filled the whole space of the newly formed organ. Hematopoietic tissue cells were located between the trabeculae (Fig. 1).

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