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CHRONOBIOLOGICAL STUDY OF THE COURSE OF EXOGENOUS GLUCOSE RELEASE INTO THE BLOOD STREAM

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UDC 615.154.94:547.455.623]-033.1"52"+ 615.31:547.455.623].033.018.5"52"

KEY WORDS: biorhythm; assimilation, glucose; muscle.

The study of circadian biological rhythms (BR) can provide a deeper understanding of the principles governing the course of biological processes and can lead to greater therapeutic effectiveness of drugs [1, 5]. An urgent problem in chronobiology is the study of BR of carbohydrate metabolism in health and disease. This is particularly true of tissues whose activity depends on insulin.

The aim of this investigation was to study BR of release of exogenous glucose (G) into the blood stream, and the effect of an electromagnetic field and of latent insulin deficiency on it.

EXPERIMENTAL METHOD

Altogether 360 noninbred male albino mice weighing 20-25 g were used. The animals were first deprived of food for 24 h. There were three series of experiments. Mice of series I were given an intramuscular injection of 0.1 ml of 40% G solution. The animals were decapitated every 5 min after the beginning of the injection, blood was collected, and the G level determined in the plasma obtained from it. Mice of series II were divided into three groups: group 1) intact mice, group 2) mice with latent insulin deficiency, and group 3) healthy mice exposed to an electromagnetic field. At 9.30 a.m. all mice of the experiments of series II were given an intramuscular injection of 0.1 ml of 40% G solution, and all the manipulations described for series I were carried out, except that these animals were decapitated 30 min after the beginning of the injection. The experiment was repeated 12 times at 2-hourly intervals in the course of the 24-h period. In series III (control) intact mice received an injection of 0.1 ml of 40% sucrose solution. Latent insulin deficiency was induced by the method described previously [2], and the plasma glucose level (PGL) was determined by the glucose oxidase method [9]. The animals were exposed to an electromagnetic field by placing them between two parallel magnet coils, to which square pulses were applied. The pulse frequency was 15 Hz and the intensity of the electromagnetic field was 5 Oe. Exposure lasted 90 min before injection of G and 30 min thereafter.

EXPERIMENTAL RESULTS

The experiments of series I showed that the PGL of the mice reached a maximum 25-30 min after injection of G (Fig. 1). This time was chosen for taking samples of series II and III.

Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 481-483, October, 1985. Original article submitted January 18, 1985.

 0007-4888/85/0010-1422$09.50

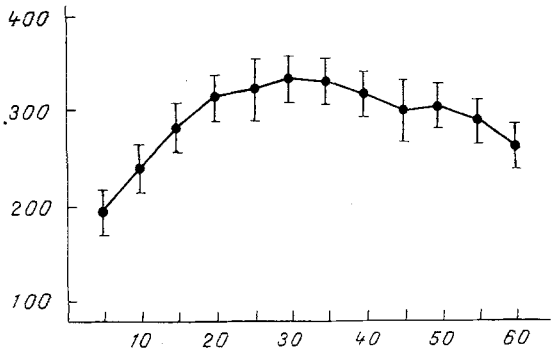

Fig. 1. Time course of plasma glucose level after intramuscular injection of G. Abscissa, time after injection of G (in min); ordinate, PGL (in mg %).

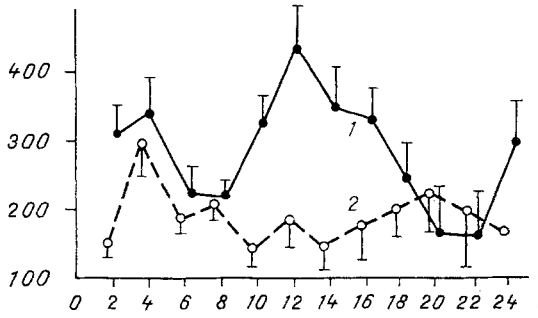

Fig. 2. Time course of changes in PGL after injection of G in intact mice (1) and in mice exposed to an electromagnetic field (2). Here and in Fig. 2: abscissa, time (in h); ordinate, PGL (in mg %).

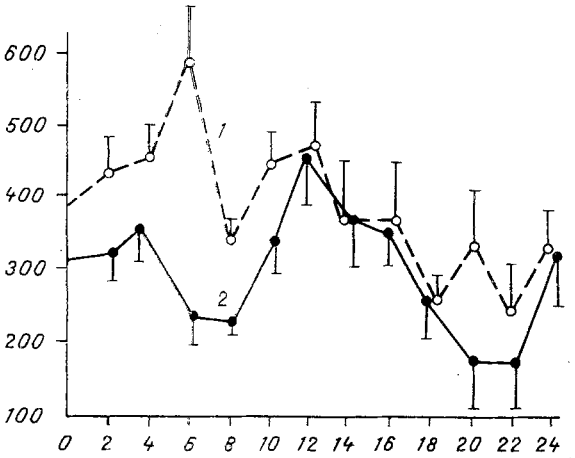

Fig. 3. Dynamics of changes in PGL after injection of G into mice with latent insulin insufficiency (1) and into intact mice (2).

Intramuscularly injected G induced painful shock, accompanied by release of catecholamines into the blood stream [8], followed by breakdown of glycogen to G and release of endogenous G into the blood stream. Consequently, the PGL of the mice was made up of exogenous (injected) G and endogenous G. In the experiments of series III sucrose was used, and according to data in the literature [4], sucrose is not assimilated by striated muscle (SM). No difference was observed under these circumstances between the PGL at different times of day and night. It was 153 ± 11.5 mg % at 6 p.m., 116 ± 12.7 mg % at 8 p.m., 205 ± 12.5 mg % at 10 p.m., 171 ± 19.7 mg % at midnight, 205 ± 8.1 mg % at 2 a.m., 200 ± 16.1 mg % at 4 a.m., 156 ± 12.3 mg % at 6 a.m., 181 ± 10.9 mg % at 8 a.m., 163.9 ± 9.8 mg % at 10 a.m., 169 ± 6.5

mg % at noon, 172 ± 19.0 mg % at 2 p.m., 166 ± 17.0 mg % at 4 p.m., and 153 ± 11.5 mg % at 6 p.m. again.

In intact mice after injection of G two phases of a rise of its concentration in the plasma were observed with maxima at 4 a.m. and noon (Fig. 2). Differences between values of the G concentration in the experiment and control were significant at the time points of 2, 4, and 6 a.m. ($P < 0.01$), and at the points 10 a.m. ($P < 0.01$), and at 10 a.m. ($P < 0.01$), at noon ($P < 0.001$), and at 2, 4, and 6 p.m. ($P < 0.01$).

The graph of PGL of mice exposed to an electromagnetic field as a function of clock time had a single maximum ($P < 0.05$) at 4 a.m. (Fig. 2). Thus PGL in mice exposed to an electromagnetic field did not rise in the period from 10 a.m. to 6 p.m. The mean PGL for the 24-h period for intact mice was higher than for mice exposed to an electromagnetic field: 227 ± 31 and 187 ± 22 mg % ($P < 0.05$). In mice with latent insulin insufficiency a single peak was observed with a maximum at 6 a.m. (Fig. 3). Differences between PGL values were significant at 8 and 6 a.m. and 6 a.m. and 10 p.m. ($P < 0.05$). A tendency toward preservation of the rhythm was observed in these mice, although this rhythm was manifested at a different level — with higher values of PGL. The mean PGL for the 24-h period for intact mice was lower than for mice with latent insulin insufficiency: 277 ± 31 and 350 ± 45 mg %.

The results of the experiments of series II show that although the same quantity of exogenous G was injected into the intact animals at different times of the 24-h period, the PGL varied throughout the 24-h period. These changes cannot be explained by fluctuations in the level of endogeneous G released into the blood stream on account of a stress reaction, for a special experiment in which sucrose was injected showed only minor changes in this parameter during the 24-h period. Consequently, it can be tentatively suggested that rhythmic fluctuations in PGL during the 24-h period are connected with the intensity of release of G into the blood stream from SM. We known that if muscle is incubated in G solution [4] or during perfusion of SM [7], its cells can assimilate G. Sodium currents into the muscle cell and potassium currents from the cell increase considerably under these circumstances [6]. In a previous investigation the writers showed [3] that after intramuscular injection of G the plasma potassium concentration changes parallel with PGL, and changes in the sodium concentration are opposite in phase to changes in the G and potassium concentrations. This means that with an increase in PGL, after intramuscular injection of G the potassium level rises whereas the sodium level falls. During active assimilation of exogenous G by SM cells the plasma potassium level falls and its sodium level rises, and the concentration of exogenous G released into the blood stream also is reduced. During the period of weak assimilation of exogenous G by SM cells, on the other hand, the blood calcium level rises whereas the sodium level falls. Under these circumstances the exogenous G concentration in the muscle is high and the unassimilated glucose will be relased into the blood stream, thereby raising its blood level.

There are thus grounds for considering that circadian fluctuations in PGL after intramuscular injection of exogenous G may be due to changes in assimilation by SM cells.

The results showed that this process is influenced by an electromagnetic field. The result of the action of this factor on circadian fluctuations in PGL took the form of a change in their character and, in particular, a decrease in the release of exogenous G into the blood stream in the period from 10 a.m. until 6 p.m., due to an increase in its assimilation by SM cells. It was also shown that exposure to an electromagnetic field leads to an increase in assimilation of exogenous G by SM cells.

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CHRONOTOXICITY OF CYCLOPHOSPHAMIDE UNDER DIFFERENT CONDITIONS OF LIGHT AND DARKNESS

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UDC 615.277.3.099"52"

KEY WORDS: chronotoxicity; biological rhythms; cyclophosphamide.

Various substances, including chemotherapeutic preparations, differ in their therapeutic and toxic action when administered at different times of the 24-h period [1, 5]. Data have been published to show that the toxicity of cyclophosphamide varies at different times of day and night [6, 8-11, 14]. Mice are known to be most resistant to sub- and superlethal doses of cyclophosphamide at midnight and 6 a.m. [2-4]. During desynchronosis, caused by keeping animals in continuous darkness or, in particular, in continuous daylight, the character of the circadian rhythm of toxicity may vary, and in some cases it may flatten out [7, 12, 13].

This paper describes the study of the chronotoxicity of cyclophosphamide during the 24-h period in animals kept under conditions of natural alternation of daylight and darkness or in continuous illumination.

EXPERIMENTAL METHOD

Two series of experiments were carried out in which mature noninbred male albino mice weighing 21-26 g were used. In series I 360 mice were divided into four groups, with 90 animals in each group, and were kept under conditions of natural light and darkness and with free access to food and drink. In series II, 144 mice also were divided into four groups with 36 animals in each group and kept for 14 days under isolation in a closed (with no windows) but well ventilated room, in continuous artificial light (average about 60 lx) and with food and water *ad lib*. During the first days when animals of this series were kept under conditions of continuous daylight, they showed increased excitability, and some mice had bites on their tail, in agreement with observations made by other workers [12] on animals kept in the same way. Cyclophosphamide (USSR origin), in a dose of 900 mg/kg body weight, calculated individually for each mouse, was injected intraperitoneally 4 times a day at 6 p.m., midnight, 6 a.m., and noon. Death of the animals was recorded every 30 min during the 24-h period. Toxic effects of cyclophosphamide were determined by observing the survival rate of the experimental animals. The results were subjected to statistical analysis by the chi-square test.

EXPERIMENTAL RESULTS

In series I the animals died 4 h after injection of cyclophosphamide. As will be clear from Fig. 1, at all times of the investigation more animals survived when the drug was injected at midnight and 6 a.m. than when it was injected at 6 p.m. and noon. The differences became significant ($P < 0.05$) 7 h after injection, between injections at midnight (82% of mice survived), at 6 p.m. (67%), and at noon (55%). At later times of observation the differences still remained, and after 24 h their significance after injections at midnight (22%) and 6 a.m. (21%) compared with injection at 6 p.m. (10%) was real or close to reality ($P \leqslant 0.05$). Later this tendency continued. Consequently, mice become chronoresistant to the toxic action of cyclophosphamide and reach their peak of resistance at midnight and 6 a.m., with a minimum at 6 p.m. These results agree with data in the literature [2-4]. However, they do not confirm the results of investigations [8, 10] in which the maximum and minimum of toxicity of cyclophosphamide were observed at different times of the 24-h period.

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0007-4888/85/0010-1425$09.50