FREQUENCY AND PHASE CORRELATIONS OF BIORHYTHYMS OF SOME METABOLIC PARAMETERS DURING POSTNATAL ONTOGENY IN MICE

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Depending on the state of orderliness of its processes ontogeny can be divided into three stages: the onset of orderliness, its maintenance at the necessary level, and reduction of its degree, which is associated with a decline of adaptability of the organism. One of the basic principles of the orderliness of living systems is their time structure, which may be called temporal rhythmic functional organization. The study of the principles governing this organization in the ontogenetic development of organisms is not only of theoretical, but also of practical importance [1, 2, 11]. One possible way of studying these principles is to analyze circadian rhythms of functionally connected parameters of the body under standard external environmental conditions. Under these circumstances the early and late stages of postnatal ontogeny are particularly interesting for study, because it is at these times that the body is most sensitive to the action of exogenous factors [13].

The aim of this investigation was to study frequency and phase correlations of biorhythms of some metabolic parameters during postnatal ontogeny in mice.

EXPERIMENTAL METHOD

The experiments were carried out on female albino mice (*Mus musculus*, ICR/Shön) aged from 1 to 52 weeks. Mice aged 2 and 3 weeks were taken from their mothers at random. At the age of 3 weeks, animals were distributed randomly into groups with 10 mice in each group. The mice reproduced by rotation and were kept in a room insulated against noise (temperature 21 ± 2°C, humidity 55-65%) with artificial lighting (light from 6 a.m. to 6 p.m.). Food (standard pellet diet) and drinking water were provided ad libitum. The experiments were carried out in February and March. Animals of each age group were decapitated every 2 h for 24 h (10 mice were used at each time). The glucose concentration in deproteinized blood was determined by the glucose oxidase method and the plasma insulin concentration by radioimmunoassay. The insulin determination was carried out jointly with Dr. F.-E. Ulrich (Clinic and Polyclinic for Internal Medicine, Martin Luther University [12] and liver glycogen was determined with the aid of phenol and sulfuric acid after hydrolysis of the liver tissue. The food intake was evaluated as the ratio of the weight of the gastric contents to the weight of the empty stomach. The numerical data were subjected to spectral analysis [5] and cosinor analysis [7].

EXPERIMENTAL RESULTS

When ontogenetic changes in rhythmic functional organization are studied, especially during the period of growth, at least two processes must be taken into account: adaptation and maturation. In laboratory mice a change gradually takes place from diurnal to nocturnal activity [13]. In the course of this change, splitting of the circadian rhythm is observed initially into ultradian components, but this is followed by readaptation, expressed as an increase in amplitude of the 24-hourly oscillations and the formation of ecologically adequate phase correlations with periodic changes of external environmental conditions. This regular pattern is characteristic of various parameters of the body (Fig. 1). At the same time, it has to be noted that even in the case of functionally connected parameters, these changes in rhythms are not necessarily synchronized, and as a result of this, differences in frequency and phase correlations may arise.

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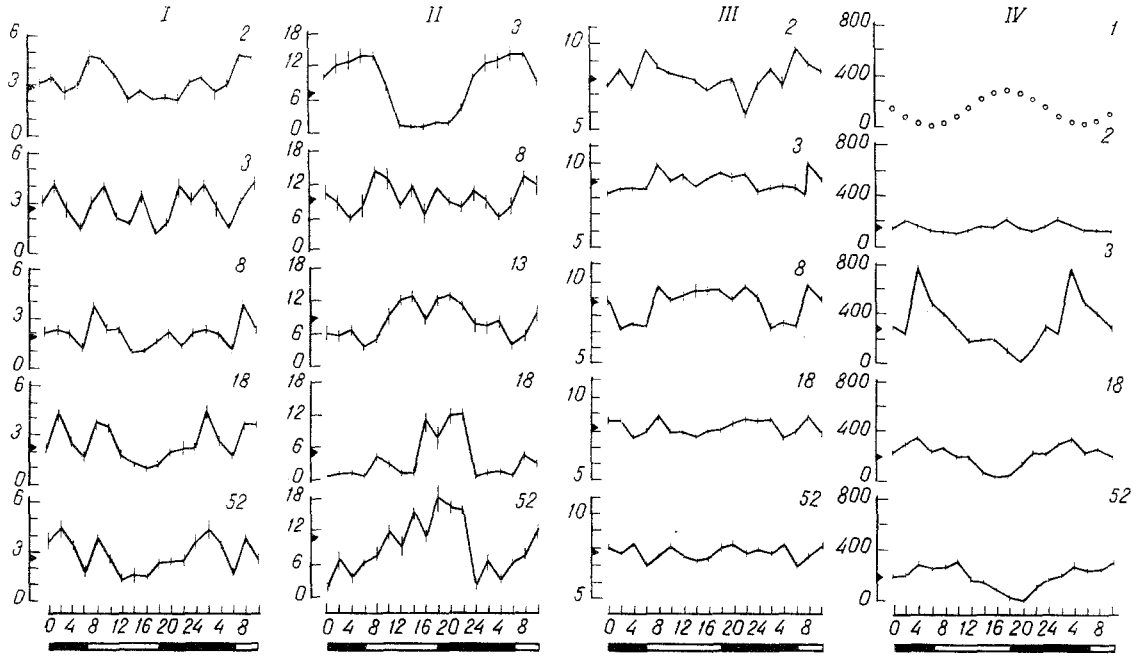

Fig. 1. Twenty-four hourly traces of food intake — ratio of weight of gastric contents to weight of empty stomach (I), plasma insulin concentration (in μg/ml) — II, blood glucose concentration (in mmoles/ml) — III, and liver glycogen concentration (in μg/ml/g body weight — IV, in mice of different ages. Numbers above curves show age of mice (in weeks). Abscissa, values of parameters; ordinate, clock time.

It will be clear from Fig. 1 that the food intake of 2-week-old mice reaches a maximum in the first half of the period of daylight. However, by the age of 3 weeks, three peaks were present on the 24-hourly trace of this parameter, two of them (immediately after the light was switched on and in the middle of the dark period) characterized in ontogeny by a relatively stable phase position, whereas the third has a strong negative shift, and merges with the nocturnal peak in mice aged 18 and, in particular, 52 weeks. A similar pattern, but even more marked, was observed previously in the circadian rhythm of motor activity of mice during ontogeny [13].

Reversal of the position of the acrophase also takes place with age in the rhythm of changes of insulin concentration (Fig. 1), but these changes are not identical with the rhythm of food intake. In adult animals the peak plasma insulin concentration lies in front of the peak of nocturnal food intake, and this may be regarded as due to stimulation of food intake by increased secretion of the hormone [3, 4, 10]. It is interesting to note that the circadian rhythm of feeding split up into ultradian components in mice by the age of 2 or 3 weeks, whereas it takes place in the rhythm of changes of the plasma insulin concentration only after removal of the mice from their mother.

Conversely, quite close phase correlation was observed between the traces of 24-hourly changes in the blood glucose concentration and feeding (at least during the period of growth of the animals (Fig. 1). A similar pattern also was observed previously in adult (18-week) mice. However, in their rhythm of changes in the blood glucose concentration the amplitude was very low and the acrophase poorly reproduced [13]. In adult mice, a high blood glucose level is evidently maintained throughout the 24-h period. Although the amplitude of the rhythm in some cases is statistically significant, biologically it may not be significant, and this may explain the phase instability of the rhythm.

The 24-hourly trace of the liver glycogen concentration in mice aged 3 weeks was almost the same as in adult animals, although 3-week-old mice still exhibited ultradian rhythms of feeding. The bimodal curve of the rhythm of this parameter with low amplitude was found in mice aged 2 weeks. Animals aged 1 week were represented by the drawing of a hypothetical trace of the rhythm. Thus reversal takes place earliest during ontogeny in the rhythm of the liver glycogen compared with rhythms of other parameters studied. The absence of experimental proof of the presence of a rhythm of changes in liver glycogen concentration in mice aged 1 week can be explained by the low value of this parameter at that age. It increases only with the beginning of taking solid food [6]. This hypothesis is confirmed by studies of

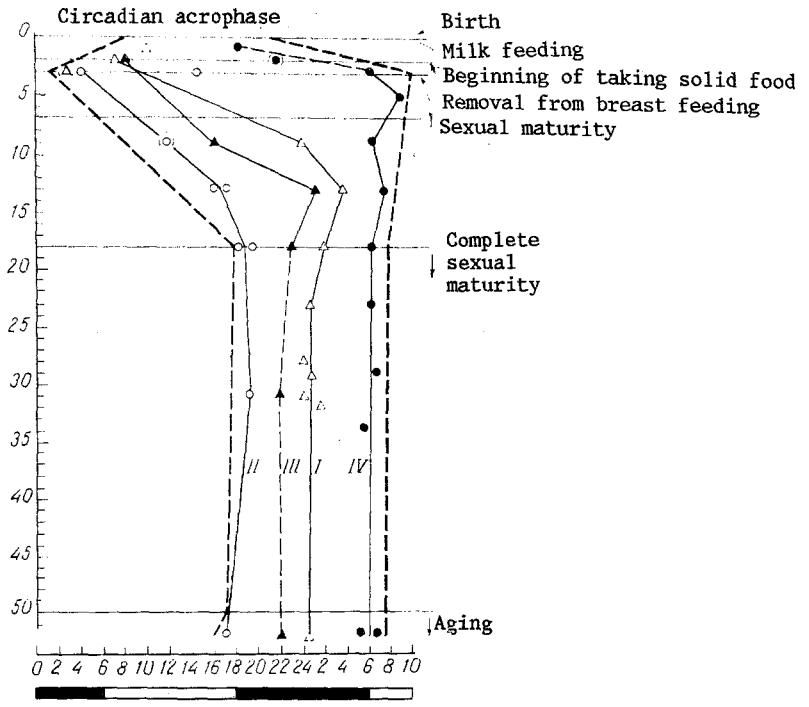

Fig. 2. Changes in position of circadian acrophase depending on age. Abscissa, age of animals (in weeks): ordinate, clock time. I) Feeding, II) plasma insulin, III) blood glucose, IV) liver glycogen.

the level of tyrosine aminotransferase in the rat liver, the circadian rhythm of which is inverted at the end of the period of milk feeding [8]. It must also be noted that the beginning of the circadian rhythm of changes in liver glycogen concentration in mice aged 1 week would contradict the results of investigations [2] and the results of our own unpublished cytophotometric investigations, which showed that the liver does not attain morphological and functional maturity until the age of 3 weeks.

These data are evidence that during the period of growth of mice, "physiological desynchronization" is observed. They do not enable conclusions to be drawn on how this affects the stability of the functional systems of the body, but it can be noted that their rhythmic organization in the course of ontogeny (including at the stage of growth) is very plastic and that so-called sensitive phases exist. One of them is the time when the animals are taken from their mother.

In adult animals for quite a long period the frequency and phase correlations in biorhythms of the functional parameters studied remain relatively stable. The established course of the traces of their circadian changes is frequency preserved until advanced old age. However, acrophases of the rhythms are characterized initially by a small, and later a stronger positive shift [9, 13]. Thus although the different functions of the body are preserved until the late stages of ontogeny, coordination between them and the external environment is impaired. As a result of this the probability and the degree of internal and external desynchronization is increased [11, 14], and this ultimately may lead to total loss of adaptive capacity and to pathological disturbances in the animal.

These arguments are confirmed by data in Fig. 2, which show age changes in the position of the circadian acrophase. A negative phase shift in young animals, differing for different parameters, can be clearly seen. After inversion of the acrophases, approximately at puberty, and with the end of growth, there follows a period when their position is relatively stable. Coincidence in the times of the acrophases is least marked in the period of removal from the mother and most marked in adult animals. In old age, it is again reduced [13].

Thus in the course of ontogeny circadian rhythms are formed initially, in connection with functional maturation of the organs. Later linking mechanisms are formed, which are responsible for optimal internal synchronization of functions and adaptation to a periodically changing environment, including both biotic and abiotic factors. This synchronization is evidently main-

tained by the maturity of the corresponding receptors, receiving adequate temporal information, and forming functional connections. After the period of relative stability of rhythmic organization there follows a phase of reduction of the achieved degree of orderliness; moreover, functional connections are weakened first, and this is reflected in internal and external desynchronization, and not until the end of ontogeny do the circadian rhythms themselves disappear. All the facts described above agree with the general scheme of ontogeny formulated at the beginning of this article.

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