The Rate of Phase Shift of Plasma Corticosterone Circadian Rhythm during Early Developmental Stages in Neonatally Blinded Rats

Shinji ITOH, Ryoji HIROTA, and Goro KATSUURA

Shionogi Research Laboratory, Osaka, 553 Japan

Abstract The phase shift of circadian periodicity of pituitary-adrenocortical activity was studied in neonatally blinded infant rats. The phase shift of the rhythm was surprisingly rapid during the early stage of the prepubertal period, while changes in the rhythm pattern were rather small after puberty, as indicated by a slight shift of the acrophase. Low amplitude was observed in the group data of blinded rats after puberty.

There have been a number of studies on the circadian rhythm of the pituitary-adrenocortical function which is entrained by environmental light-dark alterations. Constant light or dark and light-dark phase shifts were shown to cause marked changes in plasma corticosterone rhythmicity. In order to eliminate photic input, blinded rats were used in experiments by several investigators (KRIEGER, 1973; RAMALEY, 1974; WILSON et al., 1976; TAKAHASHI et al., 1977; SWAN et al., 1978). According to KRIEGER (1973), the eyes are essential for the maintenance of circadian periodicity, since animals with their eyes enucleated at either 1, 14 or 30 days of age did not show normal periodicity. RAMALEY (1974) also reported that rats blinded at day 1 of age had a 24-hr pattern of corticosterone concentration at 45 days of age that differed from the normal pattern. Recently SWAN et al. (1978) performed a more detailed study on the effect of the age of blinding on the circadian rhythm of pituitary-adrenal function in female rats. In rats whose eyes were removed at 1, 26 or 60 days of age, the circadian rhythm was found to be disrupted, but the presence of free-running rhythms was observed, suggesting that the eyes and their central projection are not essential after birth for the development and maintenance of rhythmic pituitary-adrenal function. These structures are, however, necessary for synchronization with a light-dark cycle.

These observations apparently indicate the occurrence of free-running circadian periodicity in neonatally blinded rats, but the rate of phase shift in the early stages of life has not been clearly demonstrated. Accordingly, the present study was carried out to observe the rate of phase shift, mostly in the prepubertal period in neonatally blinded rats of both sexes.
MATERIALS AND METHODS

Wistar strain albino rats of both sexes were housed at a constant temperature of 25±2°C under a light-dark ratio of 12:12 with lights on at 07:00. Rat biscuits (Oriental Yeast Co.) and water were available ad libitum.

After birth, the litters were culled to 5 male and 5 female pups. Bilateral enucleation of the eyes were performed on the 3rd day of life under ice-cold anesthesia. They were weaned and separated from their mothers on the 21st day of life and maintained with 5 rats per cage, but with advancing age, the number in one cage was decreased to 2. Control intact infant rats were kept under the same conditions.

In the first series of experiments, rats at 3 to 10 weeks of age were sacrificed by decapitation in the morning (09:00) and evening (19:00) and the trunk blood was collected and plasma was kept frozen at −20°C until analysis. Corticosterone concentrations were determined by the method of Zenker and Bernstein (1958) with minor modifications. In the second series, 42- and 70-day-old rats of both sexes were used to observe the 24-hr pattern of plasma corticosterone levels in detail, the animals being sacrificed every 3 hr during the day starting at 09:00, with one exception of determination at 19:00 instead of 18:00 as seen in the figures.

Statistical analysis for the difference between morning and evening levels was conducted by means of Student’s t-test. In the second series of experiments, a 24-hr cosine curve was fitted by the method of least squares to obtain the mesor, amplitude and acrophase, employing an electronic computer (Halberg et al., 1967).

RESULTS

Development of plasma corticosterone rhythm

The normal pattern of plasma corticosterone rhythm, low in the morning and high in the evening, had already developed at 3 weeks of age in both intact and blinded rats, though the amplitude was very small. This pattern might be produced by the influence of maternal behavior. When infant rats were separated from their mothers after weaning, the circadian variation developed rapidly in normal male rats, showing a peak value similar to the adult level at 5 weeks of age (Fig. 1), while in normal female rats the peak of rhythm rose more slowly, reaching the adult level at 8 weeks of age (Fig. 2). In contrast to the above, in blinded rats a reversed pattern of corticosterone rhythm appeared soon after weaning. In both sexes a complete reversal was observed at 5–6 weeks of age. Thereafter morning and evening levels became nearly the same at 8 weeks in male blinded rats and at 10 weeks in female ones.
Fig. 1. Plasma corticosterone levels determined at 09:00 and 19:00 in normal and neonatally blinded male rats. *P<0.05, ***P<0.001.

Fig. 2. Plasma corticosterone levels determined at 09:00 and 19:00 in normal and neonatally blinded female rats. *P<0.05, ***P<0.001.
Twenty-four hour pattern of plasma corticosterone rhythm

In normal control rats of both sexes, as shown in Figs. 3 and 4, the peak values of plasma corticosterone were observed at 19:00 and the troughs at 09:00 both on the 42nd and 70th days of life. On the other hand, in neonatally blinded rats, the highest levels were seen at 09:00 on the 42nd day and at 06:00 on the 70th day of life in both sexes, with the lowest values at 21:00 on the 42nd and 70th days in

Fig. 3. Circadian rhythms of plasma corticosterone on the 42nd day of life in normal (open circles) and blinded (closed circles) rats of both sexes. Numbers in the figure represent the number of rats.

Fig. 4. Circadian rhythms of plasma corticosterone on the 70th day of life in normal (open circles) and blinded (closed circles) rats of both sexes. Numbers in the figure represent the number of rats.
Table 1. Circadian changes in mesor, amplitude and acrophase of plasma corticosterone in neonatally blinded rats.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (day)</th>
<th>Mesor (µg/dl)</th>
<th>Amplitude (µg/dl)</th>
<th>Acrophase</th>
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<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Blinded</td>
<td>Normal</td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>10.1</td>
<td>—</td>
<td>1.4</td>
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<tr>
<td></td>
<td>42</td>
<td>12.2</td>
<td>10.6</td>
<td>9.9</td>
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<td></td>
<td>70</td>
<td>14.4</td>
<td>12.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>10.4</td>
<td>—</td>
<td>2.8</td>
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<tr>
<td></td>
<td>42</td>
<td>30.7</td>
<td>20.3</td>
<td>19.1</td>
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<tr>
<td></td>
<td>70</td>
<td>51.7</td>
<td>45.6</td>
<td>28.8</td>
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</table>

Fig. 5. Circadian rhythm patterns drawn by an electronic computer by means of least square method in normal (straight lines) and blinded (dotted lines) rats of both sexes on the 42nd and 70th days. Open and closed circles are mean values of plasma corticosterone concentrations at each time of a day in control and blinded rats, respectively.

males, and at 19:00 on the 42nd day and 21:00–03:00 on the 70th day in females.

The data obtained, together with our previously reported data in infant normal rats at the 21st day of life (Itoh and Hirota, 1976), were analyzed by fitting cosines with the method of least squares. As summarized in Table 1, the mesor of the blinded rats was somewhat lower than that of controls, and amplitude was greatly reduced in the blinded rats on the 70th day. The acrophase of normal male rats was around 20:00 and that of females around 22:00, while that of blinded male rats was 08:57 on the 42nd day, which advanced to 07:35 on the 70th day, and in female blinded ones it changed from 08:20 to 10:46 during the 4 weeks. The computed curves are illustrated in Fig. 5. Variations of the waves were significant in every case on the 42nd and 70th days, but not on the 21st day, when analysis of variance was applied.

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DISCUSSION

In previous observations blinded rats were shown to have a free-running pituitary-adrenal circadian rhythm. Krieger (1973) found that rats blinded at day 1 of age exhibited a different number of peaks on successive days, in some, as many as 3 irregularly spaced peaks over a 24-hr period. Swan et al. (1978) also observed disrupted corticosterone patterns in rats blinded at 1, 26 or 80 days of age. The blinded group did not show the morning troughs and evening peaks in corticosterone levels characteristic of the controls when examined at 84, 112 or 142 days of age. However, corticosterone patterns of individual rats suggested the presence of free-running 24-hr rhythms, since alignment of the steroid peaks of the blinded rats produced patterns comparable to those of controls.

As to the development of free-running rhythms in blinded rats, Wilson et al. (1976) reported that normal rhythmicity was retained at 3 weeks after blinding, but a phase shift was present at 10 weeks. Takahashi et al. (1977) also demonstrated that in blinded rats a fairly constant phase shift was seen after the 3rd week and phase reversal of the rhythm was found at the end of the 7th week. They noted that even after the end of the 7th week a clear periodicity was observed in the majority of blinded rats when the patterns were determined individually.

In the present study the circadian periodicity of plasma corticosterone in neonatally blinded rats at the time of weaning was quite similar to that of normal controls; that is, the evening level was significantly higher than the morning one. This might be due to the influence of maternal behavior, as demonstrated by Levin et al. (1976). They showed that blind litters reared by normal mothers do display significant daily fluctuations in the plasma levels of corticosterone. In the present study, however, after weaning when pups were separated from their mothers, the blinded infant rats exhibited a tremendously rapid phase shift of rhythm and within 2–3 weeks the pattern reversed completely, displaying highly elevated morning levels and markedly reduced evening ones (Figs. 1 and 2). This finding indicates that photic input is essential for the normal development of a 24-hr pattern of pituitary-adrenal circadian periodicity. After this period of the prepubertal stage, the phase shift of the rhythm may become asynchronous between rats since entrainment by a light-dark cycle is not present in blinded rats. The low amplitude of the corticosterone rhythm in blinded rats observed at 70 days of age (Table 1 and Fig. 5) probably results from different patterns of rhythms in individual rats. This possibility has been clearly shown by Swan et al. (1978). However, rather small changes in the acrophase during the 4-week period from the 42nd to 70th day suggest that the phase shift is not so large after puberty compared with the surprisingly rapid shift during prepubertal stages.

The reason why such a rapid phase shift occurred during the prepubertal period remains to be clarified. It is generally believed that circadian rhythms are generated within the central nervous system in which environmental light serves as the synchronizer for determining phase. The suprachiasmatic nucleus (SCN)
has been suggested to be the central pacemaker responsible for the generation of several biological rhythms, since destruction of this nucleus caused not only a loss of rhythmicity of adrenocortical activity (Moore and Eichler, 1972; Raismann and Brown-Grant, 1977), but also abolished other circadian rhythms.

The circadian rhythm of corticosterone secretion in the rat has been shown to develop by about weaning age (Itoh and Hirota, 1976) and the pattern is closely correlated with the appearance of direct retinohypothalamic projection to the SCN (Campbell and Ramaley, 1974). However, removal of the eyes on postnatal day 2, which would prevent formation of the retinohypothalamic projection, has no effect on the development of the circadian rhythm in locomotor activity and drinking (Stephan and Nunez, 1977). Since neonatally blinded rats showed free-running rhythms in drinking and locomotor activity, it was assumed that development of the retinohypothalamic innervation of this nucleus is not essential to the development of endogenously generated rhythmicity (Mosko and Moore, 1979). This may be true in the development of rhythmicity in the pituitary-adrenal axis. It might be possible to speculate that a loss of entrainment by a light-dark cycle during the developing stages of early life may manifest endogenously generated rhythmicity which differs from the 24-hr cycle. The existence of a secondary synchronizer to generate a new circadian rhythmicity in a previously arrhythmic animal with a lesion of the SCN was suggested by Krieger et al. (1977). In neonatally blinded rats such a secondary synchronizer may develop over the time course of central neural maturation. Further studies are required to define the central mechanism for the generation of the rapid phase shift in neonatally blinded rats. Moreover, the reason why large individual differences in the phase shift occur in postpubertal blinded rats reared under the same environmental conditions, which has been demonstrated by Swan et al. (1978), remains to be clarified.

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REFERENCES


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