PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1146–1149 (1975)

Control of cell Division in the Cornea of Rats II. Age-Dependent Effects of Dexamethasone upon Circadian Mitotic Activity[1] (38705)

P. GOODRUM CRUZE[2] AND S. S. CARDOSO

Department of Pharmacology, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163

In newborn organisms certain circadian rhythms are notably absent (1–5). In addition, it is of interest to note that there seems to be a chronological coincidence for the onset of some of these rhythms. The circadian mitotic rhythm in the corneal epithelium of immature rats is not present before the third week of life (6), while a circadian rhythm for plasma corticosterone has not been detected before the twenty-first or the twenty-fifth day of life (3, 5).

Studies on the interaction of the adrenal rhythm and the circadian mitotic rhythm in rodents have shown a relationship may exist between these two parameters (7–9). Evidence has been presented indicating that dexamethasone, a synthetic glucocorticoid, profoundly alters the circadian mitotic rhythm in the corneal epithelium of adult rats (8, 10, 11). Thus, to further verify whether a relationship exists between the corticosterone rhythm and the circadian mitotic rhythm, an attempt was made to induce or in some way alter the circadian pattern of mitotic activity in the corneal epithelium of immature rats by the administration of dexamethasone before the onset of the natural rhythm. The data obtained from these studies are reported in this communication.

Materials and Methods. Pregnant female rats (Holtzman Company) were standardized for periodicity studies in our animal quarters for at least a period of 1 wk before the birth of their young. The lighting schedule for the animal quarters was a 12 hr:12 hr LD schedule. Light was maintained from 0600 hr to 1800 hr and darkness from 1800 hr to 0600 hr by a timing device. Each female was singly caged. A commercial diet and tap water were made available *ad libitum*. The temperature was maintained at 24 ± 2°.

Only those pups born between 0600 hr and 1800 hr on the same day were used to facilitate uniformity in the age of the experimental animals in these studies. Animals of both sexes were employed in these experiments.

Dexamethasone[3] was administered in a single dose of 40 μg to experimental animals. Saline-treated animals were given 0.1 cc of 0.85% saline, the same volume of drug administered to the dexamethasone-treated animals. All injections were made by the intraperitoneal route at 1500 hr (the circadian phase of peak corticosterone levels in adult rats).

Animals were then sacrificed at 4-hr intervals for the 24 hr following the injection. Swift decapitation was the mode of sacrifice.

At sacrifice both eyes were removed and fixed in Alfac (a mixture of 85 ml of 80% ethanol, 10 ml of formalin, and 5 ml of glacial acetic acid) for at least 24 hr. Dissection followed, and whole corneas were stained by the Feulgen technique (12). One hundred microscopic fields defined by an ocular micrometer were studied under oil and the number of actively dividing cells recorded.

The data were then analyzed by Student's *t* test with significance accepted as 0.05 or less.

Results. A single dose of dexamethasone or saline was administered at 1500 hr to animals of several ages. Untreated controls accompanied the respective studies. Sacrifice was begun at 1900 hr, 4 hr after the injection, and continued at 4-hr intervals over

[1] Supported in part by U.S. Public Health Service Grant No. CA11332-03.

[2] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Tennessee College of Medical Sciences, Memphis, TN.

[3] Dexamethasone (Decadron) was kindly provided by Dr. C. Stone of Merck, Sharpe, and Dohme.

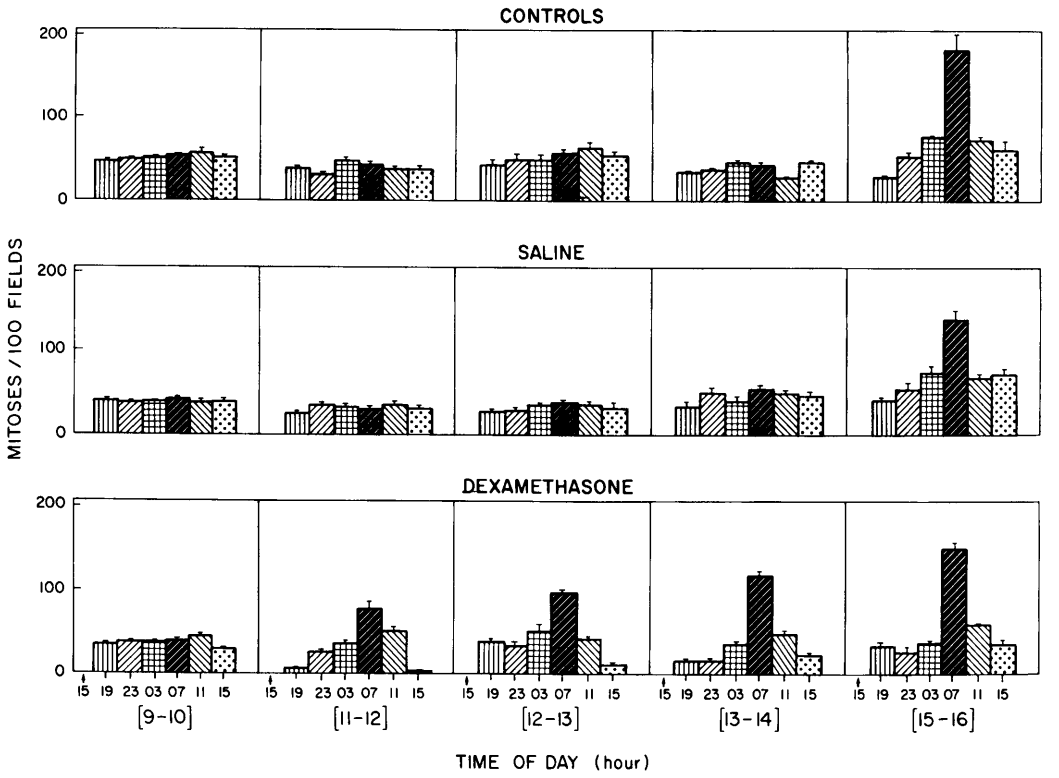

FIG. 1. The effect of dexamethasone administration at 1500 hr upon mitoses in the cornea of immature rats of various ages. Each bar represents the mean value of mitoses per 100 microscopic fields ± SE for a minimum of five animals. Brackets indicate the age of the animals in days at sacrifice.

the following 24 hr. The data collected from these experiments are shown in the accompanying figure.

Observation of all the control data reveals that a nonrhythmic distribution of mitoses prevails prior to the third week of life as we had reported earlier (6). The mitotic rhythm in the cornea as it first appears is qualitatively similar to that exhibited by the adult animal (13).

The mitotic activity reported for dexamethasone-treated animals on day nine and studied through 1500 hr on the tenth day of life exhibited no rhythmic component. However, when compared with their control groups, dexamethasone-treated animals demonstrated significantly lower levels of mitoses for each time point. Saline-treated animals also demonstrated significantly lower levels of mitoses than the control animals while not differing significantly from the dexamethasone-treated animals. Such an effect seen with the saline administration may be attributed to the young rat's response to stress with increased levels of corticosterone as immature animals have been shown to so respond as early as the second day of life (14, 15).

In contrast to the observations made on animals treated with dexamethasone on the ninth day after birth, the animals treated with 40 μg of dexamethasone at 1500 hr on the eleventh postnatal day revealed what appeared to be a dexamethasone-induced change with a resultant circadian profile resembling that found in the corneas of untreated fifteen day old or older control animals. The dexamethasone-induced peak of mitotic activity and trough value were seen respectively at 0700 hr and at 1900 hr–2300 hr. Consequently, a comparison of eleven day old dexamethasone-treated animals with their respective saline-treated and untreated controls revealed a significant

increase in mitotic activity at 0700 hr and 1100 hr, while significant decreases were recorded for 1900 hr and 1500 hr.

Studies on rats injected with dexamethasone at 1500 hr on the twelfth day of life as well as those treated on the thirteenth day of life yielded results much like those seen in animals treated at 1500 hr on day 11 after birth. With increasing age a significant increase in peak mitotic activity was noted in dexamethasone-treated animals of 11, 12, and 13 days of age.

Fifteen-day old animals treated with dexamethasone, untreated controls, and saline-treated counterparts of the same age all demonstrated a synchrony of mitoses around 0700 hr. However, values of mitotic activity at 2300 hr and 0300 hr in dexamethasone-treated animals were significantly decreased from control and saline-treated levels. Therefore, the peak of mitoses appeared to rise more steeply at 0700 hr. Nevertheless, the general circadian mitotic rhythm in the 15- 16-day old animal treated with dexamethasone was comparable to that of controls.

Discussion. Many environmental stimuli have been shown to act as conditioners for a number of rhythms. Several external factors such as light have been shown to act as cues by which colony synchronization is accomplished. In the prolonged absence of such cues, individuals free run, that is, maintain their individual circadian rhythms in a fashion desynchronized from other members of the colony (16). It is of interest to note that the opening of the eyes which occurs around the fifteenth day of life, coincides in time with the onset of the circadian mitotic rhythm in the corneal epithelium of rats. However, unpublished data from this laboratory reveal that immature rats kept in constant darkness from day 11 through day 17 after birth experienced no retardation in the onset of the normal circadian mitotic rhythm of the cornea.

While it has been reported that immature rats exhibit no detectable rhythm in plasma corticosterone levels before the twenty-first day of life, apparently they are capable of responding to many forms of stress by the second day of life (14, 15). Thus it is possible that rhythmic functions like the circadian mitotic rhythm depend on the maturation of different physiological parameters including that of the hypothalamic-pituitary-adrenal axis. Our doses of the pharmacological agent, dexamethasone, could be creating an artificial circadian cycle for glucocorticoids leading eventually to the non-random distribution of mitotic activity in the corneal epithelium.

In conclusion, the results obtained from these studies demonstrate the ability of dexamethasone to induce changes in the circadian mitotic pattern in the corneas of immature rats. That this effect is strongly age dependent is indicated by the responsiveness of the 11-day old animal but not the 9-day old animal to dexamethasone. Such data would indicate the possible involvement of a maturation process in the steroid target tissue as well as a possible role for the steroids in promoting and maintaining (17) circadian rhythms such as the mitotic rhythm.

Summary. Data are presented here which verify that immature rats exhibit no circadian fluctuation of mitoses in the corneal epithelium prior to the third week of life. These data indicate that dexamethasone administration induces age-dependent alterations in the circadian mitotic pattern of the corneal epithelium. Nine day old animals still revealed no rhythmic component in circadian mitotic activity after dexamethasone treatment. Eleven-, 12- and 13-day old animals treated with dexamethasone exhibited circadian cycles which looked qualitatively like the circadian rhythm of the 15-day old animal. Fifteen-day old animals responded with a synchrony of mitotic activity at 0700 hr and a generalized decrease in mitotic activity. The possible dependence of these responses upon existing circadian mitotic activity and maturation of the central nervous system and/or the glucocorticoid target tissue is discussed.

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Received November 19, 1974. P.S.E.B.M. 1975, Vol. 148.