PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 75–77 (1974)

Pituitary-Adrenal Function in Adult Androgenized Female Rats[1] (38046)

JON D. DUNN
(Introduced by M. Hess)
Department of Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana 70112

Although there seems to be little doubt that the genesis for the observed circadian periodicity in pituitary-adrenal function and the cyclic phenomena associated with the female pattern of gonadotropin release has a central neural origin, there are relatively few data to indicate the part of the brain involved. However, on the basis of the striking similarities between the cyclic phenomena associated with the respective systems, it has been suggested that the neuroendocrine mechanisms involved in each may be closely related (1). Both are subjected to phase regulation by environmental lighting (1, 2), are interrupted or altered by continuous illumination (1, 2) and involve pentobarbital-sensitive neural mechanisms (1).

Since it is well established that administration of androgen to neonatal female rats (androgenized) alters the cyclic release of gonadotropins (3), the following study was undertaken to evaluate whether the circadian periodicity in pituitary-adrenal function is likewise compromised in the androgenized female rat.

Materials and Methods. Animals used in the present study were adult (200–280 g), androgenized, female rats (Sprague-Dawley derived). Rats were androgenized with 1.25 mg of testosterone propionate (TP) suspended in 0.5 ml oil; injections were administered sc on day 5. The young were then returned to the mothers and left undisturbed, except for necessary routine care, for 17 days. At 21 days of age, rats were separated according to sex and housed two per cage under conditions of controlled lighting (fluorescent illumination from 04:00–18:00) and temperature (24 ± 1C). Thirty days after weaning, vaginal smears were taken 6 days a week. Only animals that showed 5 consecutive days of vaginal cornification and that had been housed for at least 3 wk under the environmental conditions described above were included in the present study. Three days prior to each experiment, rats were transferred to individual cages and handled daily in order to familiarize animals with entry to animal quarters, cage opening and removal from cage. To further standardize conditions, the animal quarters were locked and not entered during the 18 hr preceding the experiment. Additionally, all treatment and collection procedures, assigned and carried out according to completely randomized design, were done outside the animal quarters. Purina laboratory chow and tap water were available *ad libitum*.

The method used to assess nonstress and stress levels of serum and adrenal corticosterone was similar to that described previously (4). In the first study, nonstress blood samples were obtained by removing rats individually from the animal quarters to an adjacent preparation room where they were rapidy decapitated (<20 sec after cage opening) and trunk blood was collected. Following clotting, blood samples were centrifuged and the serum collected. Individual serum samples were frozen and stored for subsequent fluorometric determination of corticosterone concentration. Immediately after decapitation, adrenals of animals were rapidly removed, cleaned and weighed to

[1] Supported by N.I.H. Grant NS08929.

the nearest 0.1 mg and frozen for subsequent corticosterone determinations.

In the second study, nonstress blood samples were obtained, 4 hr after rats were injected sc with 100 μg/kg of dexamethasone (DECADRON) or 0.5 cc of normal saline (NaCl), by removing rats from individual cages in the animal quarters and taking them to the preparation room where they were rapidly anesthetized with ether. The external jugular vein was exposed and 1.0–1.5 ml of blood was collected in a heparinized syringe within 3 min of initial handling. Following 3 min exposure to ether vapor, rats were placed in individual holding cages; 12 min later (15 min after onset of stress), rats were anesthetized with ether and a stress blood sample was taken from the jugular vein. Blood samples were centrifuged and plasma collected. Plasma or serum and adrenal corticosterone concentrations were measured fluorometrically (5). Correction for residual fluorescence was not made. However, fluorescence measured in plasma from adrenalectomized female rats is equivalent to approximately 6 μg corticosterone/100 ml plasma.

The first study, designed to evaluate the effect of neonatal androgen treatment (TP, 1.25 μg/rat) on the circadian periodicity in adrenocortical activity, was performed during a single 24 hr period. The second study, conducted during the time of peak nonstress corticosterone levels, was undertaken to evaluate other parameters of pituitary-adrenal function.

Statistical probabilities were derived from analysis of variance and Student's t test.

Results and Discussion. As evident in Figs. 1 and 2, adult androgenized female rats showed a highly significant rhythm in nonstress serum ($F(7,32) = 6.31$, $P < 0.01$) and adrenal ($F(7,32) = 3.91$, $P < 0.01$) corticosterone levels. Neither the phase nor the amplitude appeared different from that reported previously for intact female rats housed under similar environmental conditions (4) serum (Fig. 1) and adrenal (Fig. 2) corticosterone levels began increasing prior to 2 pm with peak levels occurring at 5 pm. Thus, adrenocortical periodicity remained intact in the face of altered ovarian function. Androgenized female rats showed persistent vaginal cornification and polyfollicular ovaries, both indicative of interrupted ovarian cyclicity.

These observations suggest that the neuroendocrine mechanisms involved in the cyclic release of ACTH may be separate from those producing cyclic release of gonadotropins. However, the absence of overt changes in vaginal exfoliative cytology does not necessarily reflect an absence of circadian changes in gonadotropin release. Rather persistent vaginal cornification may be indicative of altered feedback mechanisms. Consistent with the latter possibility are recent studies which indicate that serum LH levels in androgenized female rats fluctuate with a predictable 24 hr periodicity (2).

The results of the second study offer further evidence that pituitary-adrenal func-

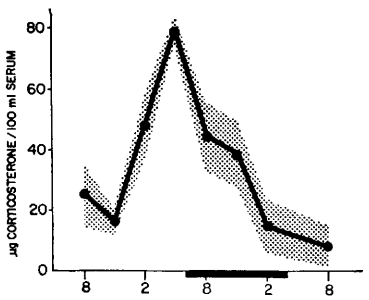

FIG. 1. Twenty-four hour periodicity in nonstress levels of serum corticosterone in androgenized female rats. In this and subsequent figure, black circles represent mean corticosterone values of 5 animals. Stippled area indicates SE and horizontal black bar indicates periods of darkness. Time of day is indicated by numbers on abscissa.

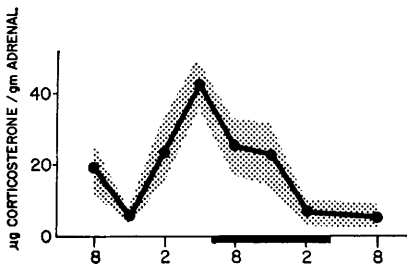

FIG. 2. Twenty-four periodicity in nonstress adrenal corticosterone concentration.

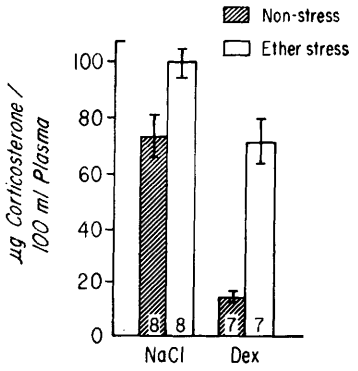

FIG. 3. Effect of Dex (100 μg/kg) on plasma corticosterone levels in androgenized female rats subjected to ether stress. In this illustration, number of animals per treatment group is indicated at the base of the columns; vertical lines indicate SE.

tion was not altered by neonatal exposure to testosterone. As demonstrated in Fig. 3, 4 hr after dexamethasone (Dex) treatment, nonstress plasma corticosterone levels were lower ($P < 0.01$) than those of saline-treated animals and both saline- and Dex-treated rats showed a significant response ($P < 0.01$) to ether stress.

Although the present study offers no information regarding the relatedness of the central neural mechanisms involved in the cyclic release of ACTH or gonadotropins, these data provide considerable evidence that pituitary-adrenal function in the female rat is not compromised by neonatal treatment with testosterone. Neither nonstress nor stress aspects of pituitary-adrenal function were altered, and mechanisms involved in feedback suppression of ACTH release appeared intact.

Summary. The effect of neonatal exposure to androgen (1.25 mg testosterone propionate, sc) on pituitary-adrenal function was assessed by evaluating the 24 hr fluctuations in nonstress levels of serum and adrenal corticosterone and by determining the adrenocortical response to ether stress. At 3 hr intervals over a 24 hr period, blood samples for fluorometric determination of serum corticosterone concentration were obtained by rapid decapitation. Adrenals were removed, weighed to the nearest 0.1 mg and frozen for subsequent corticosterone determination. In a second study nonstress and stress blood samples were taken 4 hr after dexamethasone (Dex, 100 μg/kg) or saline injection. Blood samples for determinations of nonstress levels of corticosterone were obtained rapidly (<3 min) from the jugular vein; stress samples were obtained 15 min following onset of 3 min ether stress. In the first study a 24 hr periodicity, similar to that reported previously for intact females, was observed in both serum and adrenal corticosterone levels. Serum and adrenal corticosterone values began increasing prior to 2 pm and peak levels occurred at 5 pm. In the second study, non-stress plasma corticosterone levels in Dextreated rats were lower ($P < 0.01$) than those of saline-treated animals and both groups showed a significant response to stress ($P < 0.01$). Collectively, these data provide evidence that pituitary-adrenal function in the female rat is not compromised by neonatal treatment with testosterone.

I would like to express my appreciation to Laurel Patterson and Jackie Skaggs for their excellent technical and secretarial assistance.

1. Critchlow, V., *in* "Advances in Neuroendocrinology" (A. Nalbandov, ed.) p. 377. University of Illinois Press, Urbana (1963).

2. Dunn, J., *in* "Biological Rhythms in Neuroendocrine Activity" (M. Kawakami, ed.) Igakushoin, Tokyo (in press).

3. Gorski, R. A., *in* "Frontiers in Neuroendocrinology" (L. Martini and W. Ganong, ed.) p. 237. Oxford University Press, New York (1971).

4. Dunn, J., Scheving, L., and Millet, P., Amer. J. Physiol. **223**, 402 (1972).

5. Guillemin, R., Clayton, G. W., Lipscomb, H. S., and Smith, J. D., J. Lab. Clin. Med. **53**, 830 (1959).

Received Oct. 22, 1973. P.S.E.B.M., 1974, Vol. 146.