PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 216–220 (1981)

The Influence of Estrogen Administration on Plasma Prolactin Levels in the Neonatally Androgenized (NA) Female Rat[1] (41049)

RICHARD R. GALA

Department of Physiology, Wayne State University, School of Medicine, Detroit, Michigan 48201

Abstract. Diurnal plasma prolactin levels were examined in intact neonatally androgenized (NA) female rats and NA rats ovariectomized (OVX) as adults and given estrogen. The administration of 0.5 mg of polyestradiol phosphate (PEP) to NA–OVX animals resulted in a diurnal plasma prolactin pattern similar to that of intact NA animals. When compared with normal OVX PEP-treated animals the plasma PRL levels of NA animals were higher at 0900, 1100, and 1300 hr and lower at 1500, 1700, 1900, and 2100 hr. In intact NA and NA–OVX estrogen-treated animals plasma prolactin values were significantly higher in the afternoon when compared with morning values. Plasma estradiol was twice as high in intact NA animals than in NA–OVX animals while PEP-injected animals had a value 14 times higher than that of intact NA rats. Despite the very high estradiol value the plasma PRL pattern of NA–OVX + PEP animals was comparable to intact NA animals. The ovaries were smaller and the oviducts larger in NA animals when compared to controls. Uterine growth to exogenous estrogen was significantly reduced in NA animals when compared to normal animals while adrenal and anterior pituitary growth and thymus involution were similar.

Serum prolactin (PRL) levels in neonatally androgenized (NA) female rats are elevated (1–3). We have observed higher basal (morning) plasma PRL levels in intact NA rats when compared to ovariectomized (OVX), estrogen-treated normal animals (4). We have also observed higher plasma PRL levels in NA animals during the afternoon when compared to morning values (4). Ovariectomy of the NA rat will decrease serum PRL (2) while exogenous estrogen will increase it (5); in the latter study the time of blood sampling was not noted. The purpose of this study was to compare the diurnal plasma PRL pattern of NA–OVX and normal OVX rats with and without the administration of exogenous estrogen.

Materials and Methods. Midpregnant Sprague–Dawley rats were purchased from Spartan Research Animals, Inc. (Haslett, Mich.) and housed separately in a temperature (23 ± 2°) and light (L14: D10; lights on 0600–2000 hr) controlled room. On the day of parturition, litters were adjusted to 10 pups favoring female young whenever possible. On Day 3 of life (approximately 48 hr after birth) female pups in the litter were injected sc with 1.25 mg of testosterone proprionate (TP) in 0.1 ml of sesame oil. Three weeks after birth the young were weaned and placed two per cage. At 11 weeks of age animals were checked for vaginal patency. Only 1 animal out of 30 was vaginally patent and had a cornified smear for the week period prior to OVX. At 12 weeks of age two groups of 10 animals each were OVX as were two groups of normal females. At 15 weeks of age all animals were fitted with aortic catheters as described previously (6). A single sc injection of 0.5 mg polyestradiol phosphate (PEP—1.0 mg of Estradurin, Ayerst Lab., N.Y.) was given to one group of NA-OVX animals and one group of normal-OVX animals at the time of aortic catheterization. At 16 weeks of age blood samples were taken from all animals. At this time the intact NA animals were still not vaginally patent. A total of five groups were sampled: intact NA, normal OVX, NA–OVX, normal OVX + PEP and NA–OVX + PEP. A catheter extension was attached to the animals at 0800 hr on the day of sampling and blood samples (0.3 ml) were obtained at 0900, 1100, 1300, 1500, 1700, 1900, and 2100 hr replacing the fluid each time with warm (37°) heparinized

[1] Supported in part by NIH Grant CA-21597 and Wayne State University Prolactin Radioimmunoassay Fund 206-0606.

0037-9727/81/020216-05$01.00/0

saline (6). The blood samples were immediately diluted with an equal volume of phosphate-buffered saline (the RIA buffer), centrifuged, and the diluted plasma was removed and stored frozen at −20° until assayed.

All animals were weighed and sacrificed the day after blood sampling. The following organ weights were recorded: anterior pituitary, thymus, adrenal, uterus, and the ovary and oviducts for the animals in the intact NA group; ovarian and oviductal weights were recorded for the OVX groups at the time of OVX (12 weeks of age).

Plasma prolactin levels were determined at two dilutions in duplicate by a double-antibody RIA as previously described (7). The iodination standard was NIAMDD RP I_2 and assay standard was NIAMDD RP-1 (11 IU/mg). Samples from OVX animals were assayed at 1:10 and 1:20 dilutions while all other groups were assayed at 1:20 and 1:40 dilutions. Plasma estradiol levels were determined in the laboratory of Dr. Neena B. Schwartz of Northwestern University by RIA without column separation (8); a 0.1-ml plasma aliquot from each time period sampled was pooled for each animal and assayed in duplicate at 1:3 dilution.

Body and organ weights were statistically analyzed using a one-way analysis of variance and differences among means were determined using Duncan's new multiple range test (9). Plasma prolactin levels between groups were statistically analyzed using a two-way analysis of variance with computer assistance; comparisons within a group were assessed statistically using a one-way analysis of variance and the paired t test. The difference in plasma estradiol between intact NA and OVX animals was assessed statistically using Student's t test. A level of probability of $P < 0.05$ was considered statistically significant for all tests.

Results. The plasma PRL level of the intact NA animal did not show a significant ($P > 0.05$) diurnal rhythm although there is a tendency for PRL to be higher in the afternoon than in the morning (Fig. 1). In the NA−OVX animal the PRL level was significantly decreased ($P < 0.001$) when compared to the intact NA animal and no significant ($P > 0.05$) diurnal rhythm was

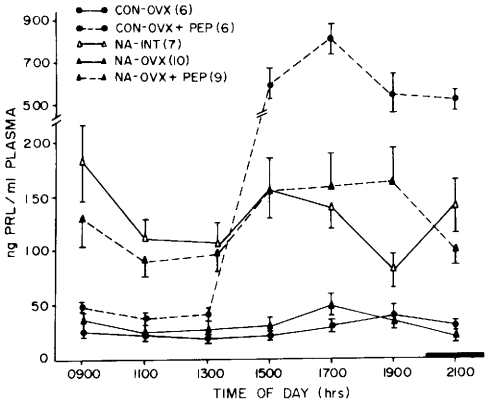

FIG. 1. Plasma prolactin levels (means ± SEM) of neonatally androgenized (NA) and normal (CON) female rats with and without estrogen administration. Animals were 16 weeks old at time of blood sampling and ovariectomy (OVX) was performed 4 weeks prior to sampling. A single injection of 0.5 mg of polyestradiol phosphate (PEP) was injected sc 1 week prior to sampling. NA-INT are NA animals with intact ovaries. Number of animals/group is presented in parentheses. Dark bar on horizontal axis represents dark period of the light cycle. The results of statistical analyses are presented in the text.

apparent but again a tendency did exist for higher values in the afternoon when compared with those in the morning. The administration of estrogen to the normal-OVX animal resulted in a marked and significant ($P < 0.001$) increase in plasma PRL in the afternoon while estrogen administration to the NA−OVX animal increased both morning and afternoon values to a comparable degree resulting in a diurnal pattern of PRL secretion which was not statistically significant ($P > 0.05$) and was similar to that observed for the intact NA animal. However, when the data at 1100 hr from intact NA and NA−OVX animals given estrogen were combined and compared by the paired t test to the combined data at 1700 hr, the 1700-hr value was significantly higher (153.3 vs 99.6 ng/ml; $P < 0.025$). A similar comparison of combined OVX values also revealed a significantly higher PRL level at 1700 hr when compared to that at 1100 hr (42.6 vs 21.5 ng/ml; $P < 0.01$).

NA animals had smaller ovaries and larger oviducts ($P < 0.05$) when compared with normal animals (Table I). NA−OVX and normal-OVX animals had similar uter-

TABLE I. BODY WEIGHT AND ENDOCRINE ORGAN WEIGHTS OF NORMAL (CON) AND NEONATALLY ANDROGENIZED FEMALE (NA) RATS AFTER OVARIECTOMY (OVX) ALONE AND OVARIECTOMY PLUS ESTROGEN ADMINISTRATION

Experimental groups	No. of animals	Body wt (g)	Ovarian wt (mg)		Oviductal wt (mg)		Uterine wt (mg)	
			Actual	Per 100 g body wt	Actual	Per 100 g body wt	Actual	Per 100 g body wt
NA intact	9	313 ± 10[a]	54.2 ± 4.2	16.8 ± 1.3[a]	41.0 ± 3.0	13.1 ± 1.0[b]	412 ± 23	132.6 ± 9.2[b]
CON–OVX	7	331 ± 6[a]	98.4 ± 4.7*	35.6 ± 1.4[b]	25.1 ± 2.3*	9.2 ± 0.9[a]	119 ± 7	35.9 ± 1.7[a]
NA–OVX	10	329 ± 5[a]	42.6 ± 3.4*	15.3 ± 1.3[a]	34.2 ± 2.5*	12.2 ± 0.8[b]	148 ± 11	45.2 ± 3.3[a]
CON–OVX + PEP[d]	8	305 ± 6[a]	93.6 ± 4.0*	34.3 ± 1.4[b]	20.7 ± 0.7*	7.6 ± 0.3[a]	435 ± 34	143.4 ± 12.3[b]
NA–OVX + PEP[d]	10	301 ± 10[a]	35.2 ± 5.2*	12.8 ± 1.9[a]	29.3 ± 1.5*	10.7 ± 0.6[b]	286 ± 19	95.2 ± 6.4[c]

Experimental groups	No. of animals	Body wt (g)	Adrenal wt (mg)		Thymus wt (mg)		Anterior pituitary wt (mg)	
			Actual	Per 100 g body wt	Actual	Per 100 g body wt	Actual	Per 100 g body wt
NA intact	9	313 ± 10[a]	116.1 ± 4.2	37.2 ± 1.5[b]	384 ± 26	121 ± 6[a]	19.7 ± 0.8	6.3 ± 0.3[a,b]
CON–OVX	7	331 ± 6[a]	97.1 ± 3.0	29.3 ± 0.6[a]	651 ± 49	196 ± 14[b]	17.5 ± 1.1	5.3 ± 0.3[a]
NA–OVX	10	329 ± 5[a]	96.5 ± 4.6	29.4 ± 1.4[a]	543 ± 51	167 ± 18[a,b]	16.9 ± 1.4	5.1 ± 0.4[a]
CON–OVX + PEP[d]	8	305 ± 6[a]	104.5 ± 3.2	34.5 ± 1.7[a,b]	379 ± 43	126 ± 16[a]	24.0 ± 1.8	7.9 ± 0.6[b]
NA–OVX + PEP[d]	10	301 ± 10[a]	100.6 ± 4.7	33.4 ± 1.2[a,b]	387 ± 23	129 ± 8[a]	21.1 ± 1.6	6.9 ± 0.4[b]

Note. Measurements taken at 12 weeks of age are marked with an asterisk. Values are means ± SEM.

[a–c] Different superscripts indicate significant differences among values at $P < 0.05$.

[d] Single sc injection of 0.5 mg polyestradiol phosphate (PEP) 1 week earlier.

ine, adrenal, thymus, and anterior pituitary weights. The administration of estrogen induced a comparable increase in adrenal and pituitary weight and a decrease in thymus weight for both NA and normal animals and the final weights were similar to those of intact NA animals. The increase in uterine weight after the administration of estrogen to the NA–OVX animal was significantly less ($P < 0.05$) than that observed for the normal animal (Table I) and the final weight was significantly less ($P < 0.05$) than that of the intact NA rat.

The plasma estradiol level of the intact NA rat was approximately twice that observed for the NA–OVX and normal OVX animals ($P < 0.05$) (Table II). The administration of 0.5 mg of PEP resulted in a marked and similar increase in plasma estradiol for the NA–OVX and normal-OVX animal when compared to the OVX or the intact NA animals ($P < 0.001$).

Discussion. We have reported that in the intact NA rat the plasma PRL level was elevated in the morning and that there was a slight but significant elevation in the afternoon when compared to morning values (4). We have confirmed this observation in the present study and showed further that the morning elevation in plasma PRL was not due to some unusual steroid secreted by the NA ovary. The exogenous administration of estrogen to NA–OVX animals resulted in a diurnal PRL pattern that was similar to that of the intact NA animal. Further, we observed that the magnitude of the afternoon surge in the intact NA animal was not due to the low level of estradiol secreted by the NA ovary since exogenous estrogen increased plasma estradiol 14-fold but did not alter the PRL diurnal pattern. In the normal OVX animal given estrogen, the morning PRL level was approximately half that, and the afternoon value four times that of estrogen-treated NA–OVX animals while plasma estradiol was similar. These results emphasize that the lack of a normal diurnal PRL pattern does not lie in the amount of estrogen produced by the NA ovary.

The slight but significant increase in plasma PRL observed in normal and NA–OVX animals in the afternoon is similar to that reported previously by us for normal-OVX animals (6). A recent report (10) indicated that the suprachiasmatic nuclei (SCN) of the hypothalamus is responsible for generating the afternoon PRL surge. We have reported that in NA rats corticomedial amygdaloid lesions reduced the elevated plasma PRL level but did not alter the diurnal rhythm and suggested that the corticomedial amygdala may be the amplifier of the estrogen-induced diurnal PRL surge (4). It appears then that the defect in PRL surge generation in the NA rat does not lie with the primary oscillator (the SCN), but with the amplification system.

It is interesting to note that vaginal patency and cornification in the NA rat are not prerequisites for elevated basal PRL levels. In our previous study TP was administered approximately 72 hr after birth and vaginal patency and cornification was evident in all animals when adults (4). In the study reported here, TP was given approximately 48 hr after birth and when adult the vagina was not patent in 29 of 30 animals. When the vagina was surgically opened the smears contained predominately leukocytes. In both studies, however, the diurnal plasma prolactin pattern was similar as was the estradiol level indicating that vaginal patency and cytology do not always reflect the endocrine mileu of the NA rat.

The influence of NA on decreasing ovarian weight has been observed previously by us (4) and by other investigators (11). The small increase in uterine weight of the NA animal administered estrogen has been pre-

TABLE II. PLASMA ESTRADIOL LEVELS OF NORMAL (CON) AND NEONATALLY ANDROGENIZED FEMALE (NA) RATS AFTER OVARIECTOMY (OVX) AND ESTROGEN ADMINISTRATION

Experimental groups	No. animals	Plasma estradiol (pg/ml)
NA intact	6	31.4 ± 9.7*
NA–OVX	7	14.0 ± 1.9
CON–OVX	5	13.3 ± 1.4
NA–OVX + PEP[a]	9	442.0 ± 26.7
CON–OVX + PEP[a]	5	443.6 ± 43.9

[a] Single sc injection of 0.5 mg polyestradiol phosphate (PEP) 1 week earlier.

* Plasma estradiol value significantly higher ($P < 0.05$) than combined values for NA–OVX and CON–OVX groups.

viously reported (12, 13) and it appears that the major defect lies in the rate of cytosol estradiol receptor replacement after it is translocated into the nucleus (14). However, under chronic estrogen stimulation, as in the intact NA animal, the uterus can maintain its weight comparable to that of the normal animal. This defect in estrogen responsiveness appears to exist for the uterus and the CNS but not for the adrenal, anterior pituitary, and thymus since estrogen's action on these organs was not altered by NA (Table I). In these three organ systems, however, the actions of estradiol are indirect and require the participation of other hormones.

The author would like to express his appreciation to Mr. Daniel J. Haisenleder for surgical assistance and blood sampling, to Mrs. Christa Lafayette for prolactin radioimmunoassays, and to Ms. Bridgett Mann in Dr. Neena B. Schwartz's laboratory at Northwestern University for estradiol radioimmunoassays. I also appreciate receiving as a gift from the National Pituitary Agency of the National Institute of Arthritis, Metabolism and Digestive Diseases, the rat prolactin used for iodination (RP I_2) and standards (RP-1).

1. Mallampati, R. S., and Johnson, D. C., Neuroendocrinology **11**, 46 (1973).
2. Ratner, A., and Peake, G. T., Proc. Soc. Exp. Biol. Med. **146**, 680 (1974).
3. Mallampati, R. S., and Johnson, D. C., Neuroendocrinology **15**, 255 (1974).
4. Peters, J. A., and Gala, R. R., Endocrinology **106**, 1740 (1980).
5. Mallampati, R. S., and Johnson, D. C., J. Endocrinol. **59**, 209 (1973).
6. Lawson, D. M., and Gala, R. R., J. Endocrinol. **62**, 75 (1974).
7. Kuo, E. Y. H., and Gala, R. R., Biochim. Biophys. Acta **264**, 462 (1972).
8. Nequin, L. G., Alvarez, J. A., and Campbell, C. S., Endocrinology **97**, 718 (1975).
9. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill Book Company, New York (1960).
10. Kawakami, M., Arita, J., and Yoshioka, E., Endocrinology **106**, 1087 (1980).
11. Gorski, R. A., and Barraclough, C. A., Endocrinology **73**, 210 (1963).
12. Lobl, R. T., Trotta, P., and Burmberger, B., J. Endocrinol. **60**, 371 (1974).
13. Lobl, R. T., Maenza, R. M., Biol. Reprod. **13**, 255 (1975).
14. Aihara, M., Kimura, T., and Kato, J., Endocrinology **107**, 224 (1980).

Received September 1, 1980. P.S.E.B.M. 1980, Vol. 166.