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Effects of Corticosterone Treatment on Puberty in Female Rats (39581)

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Adrenal status has been shown to play a role in the timing of puberty onset (1). Adrenalectomy prior to 26 days of age delays both vaginal opening and ovulation (2, 3), while later adrenalectomy diminishes the ovarian weight response to gonadotrophin (4) and the number of ova shed at the first ovulation (5). Excessive adrenal activation due to adrenocorticophin (ACTH) administration is also associated with delayed puberty (6). We have recently noted that stimuli that altered the timing of the corticosterone rhythm in the blood were associated with changes in puberty onsert in immature mice (7). Most studies of the effects of excessive corticoids on puberty have involved stressful routes of administration such as daily injections or pellet implants. Since the stress itself might have altered the secretion of other pituitary hormones (8, 9), another route of administration seemed desirable. To this end, corticosterone dissolved in the drinking water and available *ad libitum* was provided as a nonstressful source of steroid in order to determine what effects exogenous corticosterone would have upon puberty onset in intact rats.

Materials and methods. Female Sprague–Dawley derived rats were obtained at 21 days of age from Sasco Laboratories, Omaha, Nebr., and were housed three per cage in hanging stainless steel cages in controlled light (lights on 0500 hr and off 1700 hr) and temperature 21 ± 1.2° [SE]). The day after they arrived in the lab, the rats began to receive corticosterone in their drinking water (16, 24, 160, and 240 μg/ml) provided *ad libitum* as a solution of 4% ethanol in tap water. Controls received 4% ethanol in tap water, tap water only, or 0.9% physiological saline. The rats were weighed daily between 0800 and 1000 hr and vaginal smears by lavage were started on the day of vaginal opening. Laparotomy was performed on the day of the first estrous smear (solely cornified cell), and ovulation was confirmed by the presence of hemorrhagic follicles and a dilated oviductal ampulla. All animals examined in this way were found to have ovulated. Rats that did not ovulate were killed at 60 days of age. To determine the blood levels of corticosterone produced by the available corticoid in the drinking water, 22-day-old rats were given various concentrations of corticosterone (Sigma) and killed by decapitation on the fourth day after the start of steroid administration at 0800, 1600, or 2300 hr of colony time. Corticosterone in the serum was measured by a fluorometric procedure previously described (10). The experiment was performed in November when puberty in our colony is later than in summer (2).

Results. In controls maintained on tap water, vaginal opening took place at 39.0 ± 2.2 days and ovulation within 2 days (41.8 ± 2.6 SE) (Table I). Puberty occurred at practically the same time in rats given 4% ethanol in tap water to drink. The lowest concentration of corticosterone (16 μg/ml of 4% ethanol in tap water) did not alter the time of vaginal opening or ovulation. The next concentration (24 μg) did not significantly delay vaginal opening but did delay ovulation. The two high concentrations of corticosterone (160 and 240 μg) delayed both vaginal opening and ovulation ($p = 0.02$). At the highest concentration (240 μg) ovulation had occurred in only one rat by 60 days of age when the experiment was terminated. The other animals had undergone vaginal opening but retained infantile uteri (92.4 ± 14.5 mg; Table I) and ovaries with primarily small follicles.

The average daily water consumption increased from 10 ml/day to approximately 20 ml/day in all groups during the course of the experiment.

Rats given 24 μg of corticosterone/ml of drinking water grew at normal rates (inferred from body weight data collected at 2-day intervals). Their body weight at ovula-

TABLE I. INDICES OF PUBERTY IN INTACT RATS GIVEN CORTICOSTERONE DAILY IN THEIR DRINKING WATER.[a]

Treatment	Number of rats	Age at		Body weight at ovulation	Organ weights on day of ovulation		
		Vaginal opening	Ovulation		Ovaries	Uterus	Adrenals
Tap water	6	39.0 ± 2.2	41.8 ± 2.6	128.6 ± 6.5	35.3 ± 3.6	163.8 ± 5.4	32.6 ± 5.4
Ethanol, 4%	7	39.3 ± 1.7	40.7 ± 2.2	136.0 ± 6.6	44.5 ± 2.8	206.3 ± 16.9	32.2 ± 2.5
16 μg of corticosterone	7	39.6 ± 1.8	41.6 ± 1.7	129.0 ± 5.4	36.9 ± 2.3	178.8 ± 13.0	33.8 ± 2.4
24 μg of corticosterone	10	42.9 ± 1.6	49.8 ± 2.3	152.7 ± 7.7	46.6 ± 3.5	199.9 ± 19.4	36.9 ± 3.1
160 μg of corticosterone	9	46.3 ± 2.4	49.5 ± 2.5	120.0 ± 9.6	39.0 ± 3.9	200.9 ± 30.8	14.6 ± 2.0
240 μg of Corticosterone	10	51.2 ± 4.7	> 60 days	92.1 ± 4.8[b]	25.9 ± 3.1[b]	92.4 ± 14.5[b]	15.1 ± 1.3[b]

[a] Data expressed as mean ± SE. Underlined values different from intact controls given 4% ethanol in tap water ($p < 0.05$), Student's t test, nonpaired variables).

[b] Organ weights at 60 days of age since ovulation did not occur. Concentrations of corticosterone (16 μg, etc.) refer to micrograms per milliliter in 4% ethanol in tap water. All treatments were begun at 22 days of age.

tion was heavier than that of controls maintained on 4% ethanol in tap water ($p < 0.05$) (Table I). Despite a normal growth curve, ovulation was retarded and dissociated from vaginal opening. The adrenal weights of the rats that received corticosterone at 160 or 240 μg/ml were decreased in comparison to controls receiving tap water ($p = 0.01$, Student's t test, nonpaired variables). Corticosterone (160 μg) did retard body weight gain for a few days, but body weights in these rats at vaginal opening and ovulation were not significantly different from controls.

The normal pattern of serum corticosterone in our 14-hr light, 10-hr dark cycle consists of a peak just before dark onset (1600-hr sample time). Rats maintained on tap water, 0.9% saline, or 4% ethanol in tap water, showed the usual 1600-hr peak (Fig. 1). Rats given the two lower concentrations of corticosterone did not have the 1600-hr peak and analysis of variance failed to reveal a significant difference at 0800, 1600, and 2300 hr of colony time. Rats given the two higher concentrations of corticosterone had lower corticosterone values at 1600 hr than normal ($p = 0.05$) and elevated corticosterone at 0800 and 2300 hr ($p < 0.01$).

Discussion. Three results emerge from this study. First, despite the suppression of the usual late afternoon corticosterone peak, puberty as expressed in vaginal opening and the time of the first ovulation occurred at a normal age in rats given the lowest concentration (16 μ/ml) of exogenous steroid in their drinking water. Second, vaginal opening and ovulation were dissociated in rats given 24 μg of corticosterone/ml despite the normal rate of weight gain. Third, at higher doses of exogenous steroid, the daily rhythm of serum corticosterone was altered and perhaps inverted while both vaginal opening and ovulation were retarded. In these rats growth was also retarded.

If the 24 μg of corticosterone treatment had not been included, it would be appropriate to attribute the puberty-delaying effects of exogenous steroid to growth retardation. The association between metabolic status, growth, and sexual maturation has been developed by Frisch and Revelle (11) who have proposed a critical weight hypothesis for puberty in humans. According to this concept, puberty takes place when an individual reaches a threshold growth or metabolic status. Growth retardation due to underfeeding will dissociate vaginal opening and ovulation in rats (12). The dissociation between vaginal opening and ovulation in the presence of a normal growth rate in rats given 24 μg of corticosterone suggests that another factor may be operating as well. Corticoids act as estrogen antagonists in the uterus (13) and it is possible that the sensitivity of vaginal tissue to this effect is less than that of the uterus, allowing for estrogenic stimulation of vaginal opening in the absence of ovulation. We have not yet measured serum estrogens or progestins in these

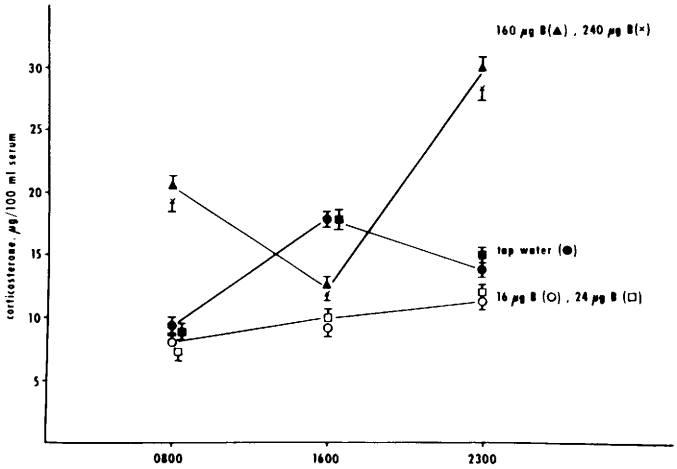

FIG. 1. Effects of corticosterone in the drinking water upon serum corticosterone levels. Rats were given various concentrations of corticosterone dissolved in 4% ethanol in tap water *ad libitum* for 3 days beginning at 22 days of age, and samples were collected by decapitation on the fourth day (at 25 days of age). Controls received either tap water or 4% ethanol in tap water (●). The results of the control groups are combined. The experiment was done at the same time as the study represented in Table I on litter mates or the animals in that experiment. Data expressed as mean ± SE, six rats per group. The time of day at autopsy is shown on the abscissa.

animals to determine whether they are normal or diminished. The exogenous steroid could be acting at any point in the gonadal system to diminish target organ sensitivity or steroid secretion. Corticoids do alter pituitary sensitivity to releasing hormones and gonadal sensitivity to gonadotrophins [reviewed in (14)].

The other key finding is that normal puberty can occur in rats with disrupted adrenal rhythmicity as a result of very low dose exogenous corticosterone (16 μg/ml of tap water), lending support to our previous indications that the adrenal rhythm itself is not required for normal puberty onset although adrenal steroids must be present (15).

The growth retardation produced by higher doses of corticosterone was associated with retarded sexual maturation. This finding supports the Frisch and Revelle hypothesis (11).

In conclusion, two results of this study cast some light on the process of puberty onset. First, the presence of a normal adrenal rhythm is not a requirement for normal vaginal opening and ovulation. Second, higher doses of corticosterone (24 μg) can cause a dissociation between vaginal opening and ovulation without resulting in significant growth retardation. It is possible that these levels mimic the effects of chronic stress which can also lead to a separation between vaginal opening and ovulation (unpublished data). The response to the 24-μg dose of corticosterone clearly suggests that the effect of excess corticosterone is not mediated solely by interference with normal growth rates.

Summary. To study the role of excess corticosterone on regulating puberty onset without the added component of a stressful route of administration, corticosterone was made available *ad libitum* in the drinking water beginning at weaning age (22 days of age). The lowest dose (16 μg of corticosterone/ml of 4% ethanol in tap water) did not delay puberty but did suppress the normal peak of corticosterone at 1600 hr. The next dose (24 μg) also suppressed the evening corticosterone peak. In these rats, vaginal opening was normal but ovulation was delayed in comparison to controls given 4% ethanol in tap water or tap water alone. Growth rates in the rats given 16 or 24 μg were normal. The two high concentrations (160 and 240 μg of corticosterone/ml 4% ethanol of in tap water) delayed both vaginal opening and ovulation, produced high levels of corticosterone in the blood at 0800 and 2300 hr and a depression of corticoster-

one at 1600 hr, and were associated with significant growth retardation. It is concluded that a normal adrenal rhythm is not required for puberty onset and that the ability of exogenous corticosterone to delay puberty onset can be mediated through routes other than growth retardation alone.

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