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Prenatal Interference with the Onset of Puberty, Vaginal Cyclicity and Subsequent Pregnancy in the Female Rat[1] (37804)

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In mammals, masculine development and control of adult reproductive function is established during fetal and neonatal life through the "organizing" action of testicular hormones on masculine differentiation of the brain (1). In addition, the pituitary–testicular axis appears to function during this critical period (1,2). Prenatal administration of cyanoketone (2α-cyano-4,4-17α-trimethyl-androst-5-en-17β-ol-3-one), antiserum to testosterone, or gonadotropin antisera block masculine differentiation, as reflected by hypospadias and by nipple development (3). Cyanoketone specifically binds stoichiometrically to Δ^5-3β-hydroxysteriod dehydrogenase, and blocks the production of biologically potent steroid hormones (4) while antibodies to hormones apparently interfere with the action of specific hormones (5).

Feminine differentiation of reproductive structures requires no hormonal control and proceeds in the absence of pituitary and ovarian function (1). However, the data in the present report shows that interference with gonadotropin action or steroidogenesis during fetal and neonatal life does cause latent effects on pubertal development, reproductive cyclicity and subsequent pregnancy in female rats.

Materials and Methods. Groups of six pregnant Sprague–Dawley rats mated by the supplier (Charles River Breeding Laboratories, Wilmington, MA) were injected, intramuscularly, with either cyanoketone (60 mg/kg) in 0.1 ml of dimethylsulfoxide (DMSO) or 0.1 ml of DMSO on Day 13 of gestation; or 0.3 ml of undiluted rabbit antiserum to bovine LH or 0.3 ml of nonimmune rabbit serum on Days 13 to 21 of gestation.

Following parturition, litters were reduced to 8 pups. At 2 days of age, rats from cyanoketone-treated mothers were injected, subcutaneously, with either 9 μg of the inhibitor in 50 μl of DMSO or 50 μl of the diluent. Rats prenatally treated with antiserum to bovine LH were injected, subcutaneously, with either 75 μl of the antibody on Days 2 through 5 of age or were treated with nonimmune rabbit serum alone.

Rats were weaned at 22 days of age and 6 to 10 females from each treatment group were chosen at random for further study. The phases of the estrous cycle were determined from vaginal smears. However, the estrogen phase of the cycle, consisting of estrus and proestrus was designated estrus and the progesterone phase, consisting of diestrus and metestrus, was designated diestrus.

At 6 mo of age the rats were mated overnight with fertile Sprague-Dawley males and laparotomized between 1 and 3 P.M. 15 days after finding spermatozoa in the vaginal smears.

The animal quarters were maintained on a 12 hr light/12 hr dark lighting schedule and

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rats had continuous access to Purina Lab Chow and water.

Statistical evaluation was Student's *t* test.

Results. The antisera did not cross-react with rat prolactin or rat growth hormone, but did bind to rat LH and rat FSH (0.93 and 1.20 mIU/ml, respectively) and thus is referred to as anti-LH:FSH.

No discernible differences could be found between control rats treated with DMSO or nonimmune rabbit serum, and thus, data from these groups were combined.

Prenatal administration of anti-LH:FSH induced early vaginal canalization and premature first estrus (Table I). Additional postnatal treatment with the antibody resulted in vaginae opening 1 wk before controls. Prenatal administration of cyanoketone delayed vaginal opening and the first vaginal estrus by more than 3 days, and an additional neonatal injection of the inhibitor enhanced the effect to 6 days.

Since the first complete estrous cycle was highly irregular, ranging from 3 to 10 days, measurements were made on the next three cycles. The length of pubertal cycles of vehicle-treated control rats was 4.1 days, (2 days of estrus and 2.1 days of diestrus) but experimental rats had 5 to 6 day cycles. Cyanoketone injections caused a lengthening of both diestrus and estrus. Prenatal administraton of anti-LH:FSH increased only diestrus by 1 day, and the combined pre- and postnatal treatment with antibody resulted in 4 days of diestrus (Table I).

When female offspring were adults at 4 to 5 mo of age, vaginal smears of control rats had 2 days of estrus and 2 days of diestrus. As in the case with the first few pubertal cycles, rats injected prenatally with cyanoketone had prolonged periods of both estrus and diestrus. Pre- and postnatal treatment with the inhibitor resulted in a small, but significant increase in the length of estrus. The prolonged cycles of the anti-LH:FSH-treated adult rats were due solely to a lengthened period of estrus. When the antibody was administered before and after birth, the rats had a 5 day cycle with 3 days of estrus (Table I).

When pregnant at 6 mo of age, female rats treated postnatally and/or prenatally with either cyanoketone or anti-LH:FSH were capable of maintaining pregnancy, but the weights of their fetuses and in some cases placental weights were reduced from control values (Table II). Enlarged pituitaries and subnormal ovarian weights were also observed in the pregnant rats injected prenatally with anti-LH:FSH and cyanoketone, respectively. An additional injection of the inhibitor on the second day of life resulted in significantly larger pituitaries more than 6 mo later. Furthermore, adrenal weights were smaller in pregnant rats treated both postnatally and or prenatally with cyanoketone.

Discussion. Our results suggest that the neuroendocrine axis functions in the female fetus, as it appears to function in the neonatal female (6) as well as in the fetal (2,3) and neonatal male (6).

Gonadotropin antisera can advance the first vaginal estrus (7) and cause male infertility (8) when administered after birth. In the adult, gonadotropin antibodies interrupt the estrous cycle (5) and pregnancy (9) by interfering with the secretion of ovarian estrogens (5) and progestins (9). However, it is difficult to ascribe the subsequent effects of postnatal and/or prenatal injections of anti-LH:FSH to interference with ovarian steroidogenesis because the fetal and neonatal ovary are apparently not involved in female sexual differentiation (1) and Δ^5-3β-hydroxysteroid dehydrogenase does not appear in the ovary until 8 to 10 days of age (10). Furthermore, immunization with FSH or LH of prepubertal rabbits had little effect on ovarian development (11). The short half-life of exogenous antibodies in rats (5,12) precludes the possibility in the present study that anti-LH:FSH was retained until 8 to 10 days of age. Although it is possible that the prenatal injections of anti-LH:FSH affected puberty and the estrous cycle by interfering with the maternal endocrine system, it is difficult to explain how these same effects were enhanced with additional antibody treatment after birth. Thus, the antibodies may have interfered directly with gonadotropin control of feminine hypothalamic differentiation (13).

What is perhaps most unusual about the effects of anti-LH:FSH treatment is that an antigonadotropin advanced a gonadotropin dependent event (puberty). It is possible that

TABLE I. Effects of Postnatal and/or Prenatal Treatment with Cyanoketone or Anti-LH:FSH on the Onset of Puberty and Cyclicity.

Treatment		No. of rats	Age (days) at		Body wt (g) at vaginal opening	Pubertal estrous cycles (day)			Adult estrous cycles (day)		
Prenatal	Postnatal		Vaginal opening	First estrus		Total	Diestrus	Estrus[a]	Total	Diestrus	Estrus[a]
Vehicle	Vehicle	6	35.9[b] ±0.26	37.1 ±0.46	117 ±3.2	4.1 ±0.07	2.1 ±0.06	2.0 ±0.06	4.0 ±0.0	2.0 ±0.0	2.0 ±0.0
Cyanoketone	Vehicle	6	39.3[d] ±0.76	40.7[c] ±1.61	149[e] ±5.7	4.9[d] ±0.28	2.6[c] ±0.20	2.4[d] ±0.11	4.7[e] ±0.14	2.5[d] ±0.16	2.3[c] ±0.13
Cyanoketone	Cyanoketone	6	43.0[e] ±1.24	43.2[e] ±1.19	120 ±6.0	4.7[d] ±0.16	2.5[c] ±0.15	2.2 ±0.11	4.3[d] ±0.11	2.0 ±0.04	2.2[c] ±0.09
Anti-LH:FSH	Vehicle	10	32.2[c] ±1.10	33.7[d] ±0.76	104 +7.1	5.1[e] ±0.35	3.0[e] ±0.33	2.2 ±0.09	4.3[d] ±0.07	2.0 ±0.04	2.3[d] ±0.09
Anti-LH:FSH	Anti-LH:FSH	6	29.1[e] ±0.86	31.1[d] ±1.35	99[d] ±5.3	6.0[d] ±0.62	4.2[d] ±0.70	1.8 ±0.13	4.9[e] ±0.07	2.0 ±0.0	2.9[e] ±0.07

The pubertal cycle was an average of the second, third and fourth cycles and the adult estrous cycle was an average of four continuous cycles at 4 to 5 mo of age.

[a]Estrus and proestrus phases of the cycle were designated estrus.

[b]Mean ± standard error.

[c]$P < 0.05$.

[d]$P < 0.01$.

[e]$P < 0.001$.

TABLE II. Effects of Postnatal and/or Prenatal Treatment with Cyanoketone or Anti-LH:FSH on Subsequent Pregnancy on Day 15 of Gestation.

Treatment		No. of rats	Maternal body wt (g)	Maternal organ wt (mg/100 g body wt)			No. of fetuses or placentae	wt (mg)	
Prenatal	Postnatal			Ovaries	Adrenals	Pituitary		Fetal	Placental
Vehicle	Vehicle	6	391[a] ±13	34.0 ±2.3	22.4 ±1.6	4.2 ±0.2	79	177 ±2.2	208 ±3.2
Cyanoketone	Vehicle	5	410 ±14	25.8[b] ±2.7	16.5[c] ±0.5	4.3 ±0.2	66	160[de] ±3.0	173[de] ±3.6
Cyanoketone	Cyanoketone	5	345[b] ±7	31.2 ±1.0	18.1[b] ±0.2	5.9[b] ±0.8	64	166[c] ±2.7	218 ±3.7
Anti-LH:FSH	Vehicle	10	387 ±10	31.3 ±0.9	19.3 ±0.8	4.9[b] ±0.2	133	171[b] ±1.7	212 ±2.9
Anti-LH:FSH	Anti-LH:FSH	5	389 ±7	35.1 ±1.1	20.2 ±1.0	4.8 ±0.3	57	152[de] ±3.5	187[ce] ±5.6

[a]Mean ± standard error.
[b]$P < 0.05$.
[c]$P < 0.01$.
[d]$P < 0.001$.
[e]$P < 0.05$ when weights are calculated as the average of the mean fetal or placental weights of each pregnant rat.

the antibody advanced puberty by increasing the half-life of the endogenous gonadotropins by forming a soluble complex with the hormones and reducing catabolism (7,14).

A prenatal injection of cyanoketone interferes with the onset of puberty, prolongs estrous cycles and during pregnancy ovarian and adrenal weights are subnormal as are fetal and placental weights. Cyanoketone, unlike anti-LH:FSH, delays vaginal opening and the first estrus.

In the adult rat, cyanoketone can block ovarian steroidogenesis and ovulation (15) but the fetal and neonatal ovary has no Δ^5, 3β-hydroxysteroid dehydrogenase (10) and does not accumulate injected inhibitor (16). However, the fetal adrenal does secrete hormones (17), and is inhibited by cyanoketone (4). Despite severe and persistent inhibition of Δ^5,3β-hydroxysteroid dehydrogenase activity in fetal adrenals with cyanoketone (4,18), corticosteroid secretion appears to recover in the hypertrophied glands promptly (18). It is possible that an accumulation of Δ^5,3β-hydroxysteroids resulting from the cyanoketone-induced blockade of fetal adrenal steroidogenesis (19), could interfere with the normal development of the neuroendocrine axis in a manner similar to the androgen-sterilized rat (20).

Recent reports have shown that sex steroids of adrenal origin, along with ovarian hormones, can mediate reproductive behavior and cyclicity in adult female rats (21). During fetal and neonatal life when ovarian hormone production is undetectable, adrenal secretion of estrogens and/or progestins may be responsible for feminine "programming" of the brain and temporary blockade of adrenal steroidogenesis during this critical period with cyanoketone could have resulted in delayed puberty and prolonged cyclicity.

The significantly smaller adrenals, fetuses, placentae and ovaries of pregnant rats treated with cyanoketone 6 mo previously, demonstrate the persistent effects of the inhibitor.

Our results indicate that interference with the hypothalamic–pituitary–ovarian axis of the fetus, as with anti-LH:FSH and cyanoketone, can affect the normal onset of puberty, adult reproductive cyclicity and subsequent pregnancies. These defects may have resulted from an interruption in the normal "programming" of the neuroendocrine reproductive system during fetal differentiation.

Summary. Prenatal administration of anti-LH:FSH advances puberty and increases the diestrus phase of pubertal estrous cycles and the estrus phase of adult cycles. A prenatal injection of cyanoketone, by contrast, delays puberty and prolongs both phases of pubertal and adult estrous cycles. An enhancement of the effects of either agent is obtained with additional neonatal treatment. Each treatment affects subsequent pregnancy by reducing placental and/or fetal weights. Thus, programming of adult reproductive function in the female rat can be disturbed during fetal life.

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