Dihydrotestosterone Secretion in Pregnant and Pseudopregnant Rats (42998)

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Abstract. **The objective of this study was to determine levels of dihydrotestosterone in the peripheral circulation and the source of its secretion throughout pregnancy and pseudopregnancy. Rats were bled from the jugular vein and ovarian or uterine vein from Day 8 through Day 22 of pregnancy and from Day 8 through the end of pseudopregnancy in various types of pseudopregnant rats. Jugular vein samples alone were obtained on the day of parturition and every fourth day thereafter during the lactational period. Our results indicate that the levels of dihydrotestosterone in the peripheral circulation were high throughout pregnancy and pseudopregnancy then declined during the lactational period. The sources of dihydrotestosterone were primarily the ovary in pseudopregnant rats and the ovary plus uterus in pregnant rats.** [P.S.E.B.M. 1989, Vol 192]

The rat ovaries and placenta secrete significant amounts of testosterone during pregnancy and pseudopregnancy (1). In addition, the ovaries contain significant levels of dihydrotestosterone at the end of pregnancy and release considerable amounts in the ovarian vein at the end of pseudopregnancy (2). However, the levels of dihydrotestosterone in peripheral circulation or the source of its secretion is unknown throughout pregnancy and pseudopregnancy. Therefore, the purpose of this study was to determine levels of dihydrotestosterone in the jugular vein and the source of its secretion throughout pregnancy and pseudopregnancy.

Materials and Methods

Pregnant and pseudopregnant rats of the Sprague-Dawley strain were purchased from the Holtzman Co. in Madison, Wisconsin. They were housed one per cage and exposed daily to 14 hr of photoperiod (lights on at 0400 hr and off at 1800 hr EST) in a temperature-controlled room at 24–26°C. Purina rat chow and water were provided *ad libitum*. The day of insemination and the day of estrus were designated as Day 1 of pregnancy and pseudopregnancy, respectively. On Day 5 of pseudopregnancy, rats were either hysterectomized by midventral abdominal incision or subjected to decidual reaction by scratching the uterine horns with a sharp needle. The pseudopregnancy normally ends on Day 12. However, the duration of pseudopregnancy is prolonged until Day 18 by hysterectomy or by inducing decidual reaction on Day 5 of pseudopregnancy.

Rats were bled from the jugular vein under ether anesthesia by tapping the jugular vein through the unbroken skin using a heparinized syringe. The ovarian blood in all rats and the uterine blood in pregnant and decidua-bearing pseudopregnant rats were collected for 15 min (1, 3). These blood samples were collected only on even days of pregnancy or pseudopregnancy, as shown in Results. About 5–6 hr after the lights went on, jugular blood samples were obtained from these rats prior to the ovarian or uterine bleeding. The jugular vein blood samples were obtained daily for the duration of pregnancy or pseudopregnancy. However, since the rats were terminated on alternate days for the ovarian or uterine vein bleeding starting Day 8 of pregnancy or pseudopregnancy, no more than three (and in some cases five) jugular vein blood samples were obtained from a particular rat. Daily blood samples, withdrawn for as many as 7 days using this procedure, had no noticeable ill effect on the rats (4). The ovarian or uterine blood was measured and all blood samples were centrifuged at 4°C. Plasma was stored frozen at −20°C. Uterine blood samples were not obtained from the ordinary (untreated) pseudopregnant rats due to their very thin uterine veins.

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Radioimmunoassay of Dihydrotestosterone. Plasma concentrations of dihydrotestosterone were measured after ethyl acetate:hexane (3:2) extraction by a radioimmunoassay using a highly specific antiserum (catalogue no. 155, lot r-140; Radioassay System Laboratories, Carson, CA). The antiserum crossreacts 8% with testosterone and none with estradiol, progesterone, and corticosterone. The specificity, validity, and reliability of this assay have been reported previously (2). The standard curve ranged from 0.005 to 1.0 ng and all working standards were in a volume of 0.5 ml. The sensitivity of the assay was 10 pg/assay tube. The coefficient of variation for intraassay was 11.6% and that for interassay was 19.3%.

Statistics. The data were analyzed by one-way analysis of variance followed by the Tukey test when differences were significant. The probability values of less than 0.05 were considered statistically significant.

Results

The concentrations of dihydrotestosterone in the ovarian and uterine vein were four to seven times and two to three times higher ($P < 0.05$), respectively, than the levels in the peripheral circulation on Days 12–22 of pregnancy (Fig. 1). In addition, the concentrations of dihydrotestosterone in the ovarian vein markedly exceeded ($P < 0.05$) the levels in the uterine vein throughout the time course of the study, indicating a dual origin of dihydrotestosterone secretion. The uterine vein concentrations of dihydrotestosterone on Day 18 were higher than its levels in the uterine vein on Day 8, 10, 21, or 22 of pregnancy. Dihydrotestosterone levels in the uterine vein declined steadily between Days 18 and 22 of pregnancy. Similar decreases were observed in the ovarian and peripheral circulation, although these were not statistically different. The concentrations of dihydrotestosterone in the ovarian or jugular vein were not different between days within each group. The secretory rate of dihydrotestosterone by ovary or uterus (Table I) basically reflect a similar pattern as their absolute levels in the circulation (Fig. 1). The concentrations of dihydrotestosterone in the jugular vein were 152 ± 8, 186 ± 19, 146 ± 16, 151 ± 16, 129 ± 8, and 126 ± 20 pg/ml on the day after parturition and every fourth night subsequently through Day 21 during the period of lactation.

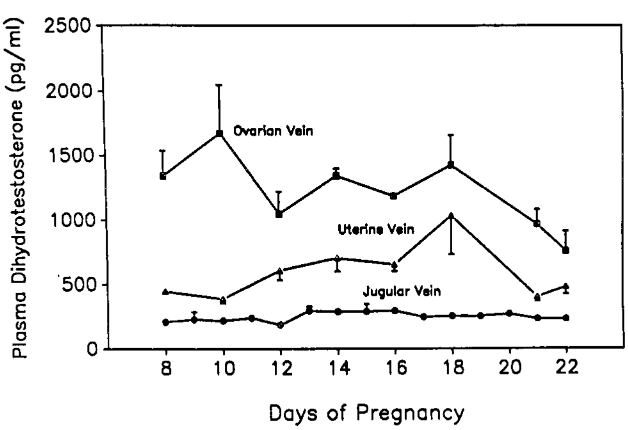

Figure 1. Plasma levels of dihydrotestosterone in ovarian, uterine, and jugular veins throughout pregnancy. Each point represents the mean ± SE (n = 4–18 rats).

Table I. Secretory Rate (pg/hr/Ovary or Uterus) of Dihydrotestosterone during Pregnancy

Day of pregnancy	Ovarian vein plasma	Uterine vein plasma
8	4,594 ± 991[a] (10)	2,768 ± 593 (4)
10	7,528 ± 2,744 (6)	1,514 ± 183 (5)
12	10,796 ± 4,530 (4)	3,042 ± 913 (4)
14	5,652 ± 855 (4)	5,546 ± 2,779 (4)
16	11,137 ± 2,252 (4)	2,571 ± 485 (4)
18	10,397 ± 2,661 (4)	5,297 ± 2,952 (4)
21	2,932 ± 490 (6)	1,996 ± 407 (4)
22	3,769 ± 1,099 (5)	4,692 ± 567 (4)

[a] Values are mean ± SE (n).

The concentration of dihydrotestosterone in the ovarian vein in all three groups of pseudopregnant rats was four to seven times higher ($P < 0.05$) than the levels in the peripheral circulation (Fig. 2). Dihydrotestosterone levels in the uterine vein plasma in decidua-bearing pseudopregnant rats were 437 ± 21, 401 ± 31, and 330 ± 11 pg/ml on Days 8, 10, and 12 of pseudopregnancy, respectively. These levels of dihydrotestosterone in the uterine vein plasma were slightly higher ($P > 0.05$) than the levels in peripheral circulation (Fig. 2), albeit these levels were much lower than the levels of dihydrotestosterone in the ovarian vein plasma during this period. The secretory rate of dihydrotestosterone by ovary in these rats (Table II) reflects a similar pattern as their absolute levels in the circulation (Fig. 2). The actual levels of dihydrotestosterone in jugular vein blood from pregnant and pseudopregnant animals were not statistically different on Days 10–14.

Discussion

Our results clearly indicate that the levels of dihydrotestosterone in the peripheral circulation are in significant amounts throughout pregnancy and pseudopregnancy. The source of dihydrotestosterone is primarily the ovary in pregnant and pseudopregnant rats and also the uterus in pregnant rats. It is also important to notice that dihydrotestosterone levels in the uterine vein plasma of pregnant rats were much lower than the levels in the ovarian vein plasma throughout pregnancy.

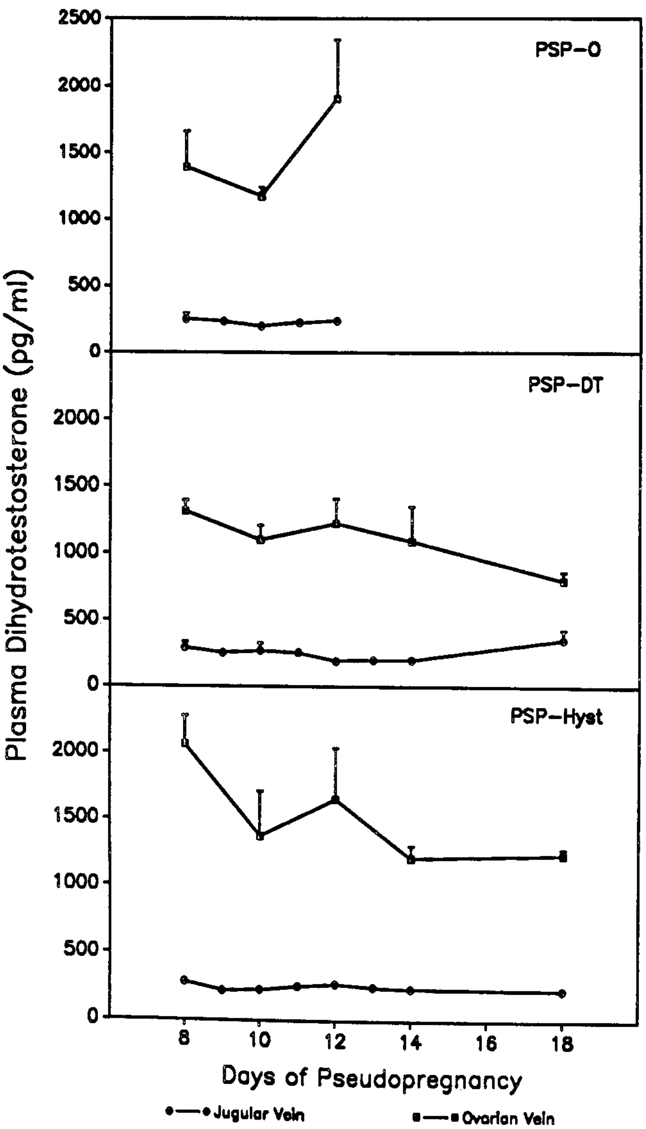

Figure 2. Plasma levels of dihydrotestosterone in jugular and ovarian veins throughout pseudopregnancy in ordinary (PSP-O), decidua-bearing (PSP-DT), and hysterectomized (PSP-Hyst) pseudopregnant rats. Each point represents the mean ± SE (n = 3–9 rats). Induction of decidua or hysterectomy was performed in these rats on Day 5 of pseudopregnancy as described in Materials and Methods.

Table II. Secretory Rate (pg/hr/Ovary) of Dihydrotestosterone during Pseudopregnancy

Day of pseudopregnancy	PSP-O[a]	PSP-DT	PSP-Hyst
8	3,140 ± 937[b] (3)	4,595 ± 2,707 (3)	4,630 ± 1,314 (3)
10	7,303 ± 2,584 (3)	5,117 ± 1,395 (3)	
12	12,001 ± 5,760 (4)	5,565 ± 1,873 (3)	2,390 ± 208 (3)
14		3,742 ± 1,091 (4)	2,771 ± 831 (3)
18		1,953 ± 795 (3)	3,522 ± 231 (3)

[a] PSP-O, ordinary pseudopregnant; PSP-DT, decidua-bearing pseudopregnant; PSP-Hyst, hysterectomized pseudopregnant.
[b] Values are mean ± SE (n).

We have previously (1) shown that testosterone, secreted by the ovaries, decreases from Day 12; that between Days 12 and 18, the placentas were the principal source of this androgen. Results of this investigation indicate that in contrast to testosterone secretion, dihydrotestosterone is secreted from the ovary in abundance during this period. Furthermore, while the dihydrotestosterone levels in the ovarian as well as in the uterine venous plasma were declining in this study on Days 21 and 22 of pregnancy, the testosterone (1) levels in the ovarian vein plasma increased dramatically during the same period, indicating that 5α-reductase activity decreased at the end of pregnancy.

It is well established that rats during pregnancy or pseudopregnancy rarely display lordosis behavior when placed with males (5). However, administration of estradiol benzoate on Days 10–14 causes lordosis behavior in decidua-bearing or in hysterectomized pseudopregnant rats in which the duration of the pseudopregnancy is prolonged, whereas it has no such effect in pregnant rats during that period. Based on these observations, Baum *et al.* (6) proposed the hypothesis that the sexual refractoriness associated with pregnancy is related to (or caused by) the elevated concentrations of dihydrotestosterone present. It has been demonstrated in their study that pseudopregnant females have lower blood concentrations of reduced androgens than pregnant females. The data presented here are in disagreement with their hypothesis where the actual levels of dihydrotestosterone in jugular vein blood from pregnant and pseudopregnant animals on Days 10–14 were not statistically different. Furthermore, ovarian concentrations of dihydrotestosterone increased 2- to 3-fold during the 4-day estrous cycle with the peak levels occurring on the day of proestrus (7, 8) and the levels of dihydrotestosterone remained elevated for the 12-hr period of proestrus (7) when the animal displayed intense lordosis behavior. These data support the hypothesis that endogenous dihydrotestosterone may be positively correlated with the expression of lordosis. Erskine *et al.* (9) failed to augment the behavioral responsiveness to ovarian steroids in pregnant rats either by inhibiting the formation of dihydrotestosterone or by daily treatment with flutamide, an androgen receptor blocker. Collectively, these data indicate that the levels of dihydrotestosterone in the circulation and the expression of lordosis behavior are not correlated and that the high levels of plasma dihydrotestosterone during pregnancy could not be a factor responsible for inhibiting the expression of sexual behavior.

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