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PERIPHERAL BLOOD STEROID CONCENTRATIONS IN THE PREOVULATORY RABBIT

J. W. WATERSTON, III and T. M. MILLS

Department of Endocrinology, and Center for Population Studies, Medical College of Georgia, Augusta, Georgia 30902, U.S.A.

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SUMMARY

Peripheral blood samples were collected from mature rabbits immediately following (0 h) and 2, 6, and 12 h after coital stimulation. Serum concentrations of progesterone, 17α-hydroxy-progesterone, testosterone and 17β-estradiol were determined by radioimmunoassay. Blood levels of the latter 3 steroids rose to approximately twice 0 h concentrations by 2 h postcoitus, then declined to nearly 0 h levels by 6 and 12 h following mating. The pattern of blood progesterone was somewhat different; levels showed a 20-fold rise over 0 h concentrations by 2 h postcoitus and remained elevated at 6 h but fell to nearly 0 h levels by 12 h following mating. The results of this study fully confirm previously published postcoital secretion patterns of progesterone, testosterone, and 17β-estradiol. In addition, postcoital levels of 17α-hydroxy-progesterone in the rabbit are reported for the first time.

INTRODUCTION

The blood levels of progesterone, 20α-dihydroprogesterone, testosterone, 17β-estradiol and estrone have been quantitated in the blood of the preovulatory rabbit [1–6] and in the pregnant rabbit [7–9]. Generally, only the ovarian vein levels of these steroids were reported and often only in a few animals. The purpose of this study was to quantitate the levels of 4 steroids in individual peripheral serum samples obtained from groups of several rabbits and to relate these steroid concentrations to the time after coital stimulation. The results show that blood progesterone, testosterone, and 17β-estradiol follow the previously reported pattern of postcoital rise and decline prior to ovulation [3, 4]. In addition, the preovulatory levels of serum 17α-hydroxy-progesterone are reported for the first time in the rabbit and are found to follow a pattern similar to that of testosterone.

EXPERIMENTAL

Antisera utilized in the assays of progesterone and 17α-hydroxy-progesterone were purchased from Dr. G. E. Abraham of Harbour General Hospital, Torrance, California. Antisera used in the assay of testosterone and 17β-estradiol were raised in this laboratory. The cross reactivity and specificity of the Abraham antisera have been detailed [10], while the specificity and properties of the antitestosterone and antiestradiol sera are described elsewhere [11, 12]. The following tritium-labeled steroids were obtained from New England Nuclear Corporation: [2,4,6,7-^{3}H]-progesterone (S.A. 81·1 Ci/mmol), [1,2-^{3}H]-17α-hydroxy-progesterone (S.A. 49·2 Ci/mmol), [1,2,6,7-^{3}H]-testosterone (S.A. 91·1 Ci/mmol), [2,4,6,7-^{3}H]-17β-estradiol (S.A. 95 Ci/mmol). All solvents were reagent grade and most were redistilled prior to use.

Animals

Sexually mature, white, female rabbits of the New Zealand strain weighing 3–4 kg were used. Animals were housed in individual cages with compressed food and water available *ad libitum*. Mating was accomplished by placing the female in a cage with a vigorous buck rabbit. Vaginal washings were checked microscopically; only females showing vaginal spermatozoa were utilized. Animals were killed by a sharp blow to the head and blood samples were collected directly from the heart into tubes containing no anticoagulant. Samples were obtained from 13 rabbits sacrificed immediately after mating (0 h) and from 8, 2-h postcoital; 10, 6-h postcoital; and 8, 12-h postcoital rabbits. Blood samples were allowed to clot and then centrifuged with the resulting sera frozen until analysis.

Steroid analysis

Serum samples were thawed, 2 ml aliquots were taken, and approximately 1000 d.p.m. of ^{3}H steroid added for subsequent method recovery determination. Each sample was then extracted 3 times with 4 vol.

Abbreviations and trivial names used: 1,3,5(10)-estratriene-3,17β-diol (17β-estradiol); 1,3,5(10)-estratriene-3β,17α-diol (17α-estradiol); 3-hydroxy-1,3,5(10)-estratrien-17-one (estrone); 4-pregnene-3,20-dione (progesterone); 17-hydroxy-4-pregnene-3,20-dione (17α-hydroxy-progesterone); 17β-hydroxy-4-androsten-3-one (testosterone); 20α-hydroxy-4-pregnen-3-one(20α-dihydroprogesterone); luteinizing hormone (LH).

of freshly distilled diethyl ether. The ether was evaporated under a N_2 stream and the dried sample dissolved in 0·5 ml isooctane. The steroids were separated chromatographically according to the method of Abraham[13] as modified by Parker[11]. This method utilizes a 5 cm. celite column with propylene glycol–ethylene glycol (1:1 v/v) as the stationary phase and steroids eluted with isooctane and successive elutions with 5%, 15%, and 35% ethylacetate in isooctane. Progesterone was eluted in initial isooctane fractions while both 17α-hydroxy-progesterone and testosterone were eluted in the same 15% ethylacetate washes; 17β-estradiol was eluted with 35% ethylacetate. The solvents were evaporated and each steroid fraction reconstituted in the radioimmunoassay buffer.

Radioimmunoassay

Due to the varying concentrations of steroids present in plasma samples, duplicate aliquots ranging from 12 to 30% of each fraction were taken for assay. In some instances, these aliquots gave values outside of the sensitive region of the standard curve; these samples were reassayed at a different dilution.

A standard curve for each steroid was prepared for every assay with values ranging from 25 to 1000 pg steroid (10–400 pg for the 17β-estradiol assay). Steroid concentrations resulting from these assays were converted to ng/ml and differences between groups analyzed statistically (unpaired Student's *t* tests).

RESULTS

Radioimmunoassay of the serum levels of progesterone, 17α-hydroxy-progesterone, testosterone and 17β-estradiol at varying times after mating are presented in Fig. 1. Quantitation of serum from 0 h rabbits yielded levels of 0·29 ± 0·08 ng progesterone/ml, 0·41 ± 0·09 ng 17α-hydroxyprogesterone/ml, 0·13 ± 0·01 ng testosterone/ml and 0·10 ± 0·01 ng 17β-estradiol/ml. Coital stimulation of these animals resulted in a statistically significant increase at 2 h in the blood levels of all 4 of these steroids with levels of 17α-hydroxy-progesterone, testosterone and 17β-estradiol approximately doubling, while average progesterone levels increased more than 20-fold to greater than 6 ng/ml. By 6 h after mating, the levels of all the steroids except progesterone fell to levels which do not differ from 0 h; progesterone remained elevated at 6 h postcoitus. However, in ovulated rabbits (12 h after mating) the levels of progesterone had fallen to near 0 h levels. Serum concentrations of 17α-hydroxy-progesterone, testosterone, and 17β-estradiol were found to be equal to, or less than, 0 h levels at 12 h after mating.

DISCUSSION

The blood concentrations of progesterone and testosterone presented in Fig. 1 are in good agreement with previously published studies of rabbit plasma steroid levels. Hilliard *et al.*[3] reported peripheral plasma levels of testosterone in the range of 50 to 100 pg/ml. When these rabbits were mated or injected with LH, the blood testosterone level went up to 200–300 pg/ml at 90 min after coitus and then fell to approximately 75 pg/ml by 6 h postcoitus. Our results also generally agree with the study of YoungLai[14] who reported that the total plasma progestin (including presumably progesterone and 20α-dihydroprogesterone) was about 2 ng/ml before mating, rose to a peak of close to 6 ng/ml 3 h after coitus and then fell to unmated levels by 6 and 9 h postcoitus. YoungLai[14] also reported the follicular fluid content of these same steroids after mating and recorded that a peak of progestin concentration occurred at 3 h after coitus. By 9–12 h after mating, the progestin content of the follicular fluid had fallen to levels below the sensitivity of the assay. Although in this study, the peripheral plasma estrogen levels never exceeded 40 pg/ml in all mated rabbits, the follicular fluid concentration at 3 h postcoitus was about 270 pg of estrogen/mg follicular fluid [14].

In the present study, the peripheral serum level of 17β-estradiol is found to be 0·10 ± 0·01 ng/ml in 13 separate determinations of serum from 0 h rabbits. This average value is somewhat higher than the 17β-estradiol range of 15 to 25 pg/ml reported by Hilliard *et al.*[3]; values of less than 40 pg/ml have also been reported [14]. Possibly, our 17β-estradiol fraction may include some 17α-estradiol contamination. This 17α-isomer is not separated from 17β-estradiol by the column chromatography utilized and 17α-estradiol is produced by the rabbit [15], although it is of low estrogenicity in this species [16]. 17α-estradiol has been reported to cross react 13% with the antibody used to measure 17β-estradiol samples in this study [11]. The steroid is, however, readily measurable in ovarian vein plasma. Shaikh and Harper[2] found

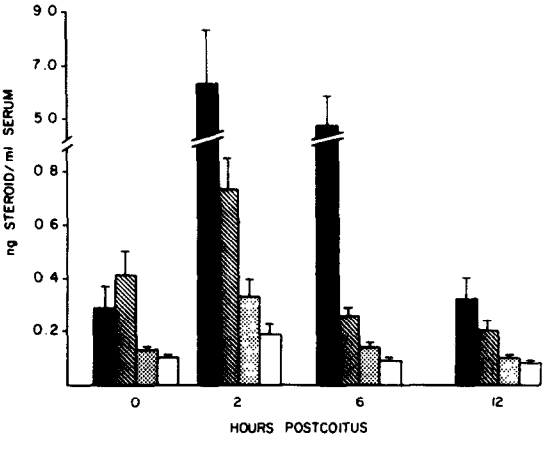

Fig. 1. The concentration of selected steroids in peripheral blood of rabbits immediately after (0 h) and 2, 6, and 12 h after mating. Solid bars, progesterone; hatched bars, 17α-hydroxy progesterone; stippled bars, testosterone; open bars, 17β-estradiol. Each bar represents the mean of 8–13 separate determinations; brackets represent one standard error of the mean.

ovarian vein 17β-estradiol levels averaged 351 ± 76·4 pg/ml in estrus rabbits, while Hilliard *et al.*[1] report values as high as 750 pg/ml in ovarian vein plasma from unmated rabbits.

The patterned secretion of 17α-hydroxy-progesterone has not been previously reported in the preovulatory rabbit. This steroid follows a pattern very similar to that of testosterone; the pattern is not similar, however, to that of progesterone (Fig. 1). Very high levels of the 17 hydroxylated progestin are synthesized *in vitro* by follicles isolated from rabbits mated 2 h prior to sacrifice [17]. Whereas the serum levels of 17α-hydroxy-progesterone approximately double at 2 h after mating (Fig. 1), there is nearly a 200-fold increase in its *in vitro* synthesis in 2 h postcoital follicles compared to follicles from unmated rabbits [17]. In the human female, 17α-hydroxy-progesterone levels in the blood rise in the late follicular phase reaching a maximum coincident with the ovulatory surge of LH [18]. The source of this steroid is considered to be follicular [18]. Very recently, however, Shaikh and Shaikh[19] reported no significant alteration in the levels of 17α-hydroxy-progesterone in either the adrenal vein blood or in peripheral blood of rats over the entire estrous cycle. A peak did occur, however, in ovarian vein levels of 17α-hydroxy-progesterone early on the day of estrus. Despite these studies, however, it is unknown whether the surge in 17α-hydroxy-progesterone secretion plays a physiologic role in the ovulatory process or only represents the spillover of steroid intermediates into the plasma of the preovulatory rabbit. Hilliard *et al.*[20] assign significance to the preovulatory rise in the synthesis and secretion of 20α-dihydroprogesterone and propose a positive feedback of this steroid on the release of LH by the pituitary [20]. Possibly steroids such as 17α-hydroxy-progesterone may have some similar effect on pituitary function.

The underlying basis for the pattern in steroid secretion seen in the preovulatory rabbit ovary is unknown. Undoubtedly, large ovarian follicles are the source of much of the steroid measured in the blood after mating [5]. Previously published *in vitro* studies demonstrate that the pattern of steroidogenesis in isolated ovarian follicles closely parallels changes in blood levels of 17α-hydroxy-progesterone, testosterone, and 17β-estradiol (Fig. 1). The *in vitro* synthesis of these steroids by follicles obtained 2 h after mating is more than 20 times greater than in 0 h follicles [17]. Blood steroids, however, only double in this same 2 h duration (Fig. 1). The increase in *in vitro* steroidogenesis in isolated follicles may reflect changes in the synthetic potential of the follicular tissue under conditions of excess substrate and cofactor.

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