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17β-Estradiol and Progesterone Concentrations in Myometrium of Pregnancy and Their Relationships to Concentrations in Peripheral Plasma*

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ABSTRACT. 17β-Estradiol (E_2) and progesterone (P) concentrations in blood and in the myometrium of human pregnancy at term (n = 33) and in a few samples (n = 5) around midterm of pregnancy were determined. E_2 concentration in the myometrium (per g wet wt) at midterm was lower than the concentration in the plasma (per ml) so that the myometrium to plasma (My:Pl) ratio was 0.7. Relative to plasma concentration, the myometrial E_2 increased little from midterm to term so that My:Pl was only 0.2 at term. Although P concentration in the myometrium was much greater than that in the plasma at midterm, My:Pl ratio being 2.2, it was lower than that in plasma at term so that My:Pl ratio was only 0.6. A fairly good correlation between plasma steroids and the myometrial steroids was observed at midterm but was distorted at term, probably due to saturation of the tissue-binding capacity. Steroid concentrations determined on the basis of protein showed a good correlation to the values expressed on the basis of wet weight. Whereas myometrial E_2 concentration was significantly influenced by the distance from placenta, P concentration was not. (*J Clin Endocrinol Metab* **46**: 622, 1978)

IN SPITE of extensive data on the levels of estradiol and progesterone in plasma in various endocrine conditions, there still is very limited information on the concentrations of these steroids in the myometrium. The main reason for this lack is the unavailability of a suitable method for determining steroid concentrations in the rather small samples of human uterine tissues. We have recently reported satisfactory methods for the determination of progesterone and estradiol concentrations in the human and rabbit myometrium with some preliminary data on the concentration of these steroids in the human myometrium (1, 2).

In the present study, data on the concentration of estradiol and progesterone in the pregnant human myometrium have been obtained. Moreover, analysis of the blood samples obtained from the same patients simultaneously with the tissue samples allowed us to examine the relationship between the levels in the plasma and in the myometrium.

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Materials and Methods

Tissues

Uterine samples were obtained from 35 healthy women during deliveries by cesarean section and collected in ice-cold Krebs-Ringer solution as described previously (1). While collecting these samples, the location of the placenta was carefully checked and recorded in each case. Myometrial samples were obtained also from five patients in the 17th to 20th week of pregnancy undergoing abortion by hysterotomy. The tissue pieces were thoroughly rinsed in the Krebs-Ringer medium and, after dissecting away the endometrium, the myometrial samples (weighing 200–400 mg) were stored frozen at −20 C until used for analysis. To check the extent of the loss of steroids with time, before processing of the tissues the pieces were, in some experiments, allowed to remain in the Krebs-Ringer medium at 4 C or at room temperature (20–22 C) for 1 h before they were frozen and stored. Although there was no significant loss of either steroid when the tissue was left at 4 C, both steroids were lost (up to 40%) from the tissue with incubation at room temperature.

Tissue digestion and extraction of steroids

The frozen tissues were thawed and then digested in 0.5 ml mixture containing 5% sodium dodecyl sulfate (SDS) and 0.5 N NaOH as described

previously (1). An aliquot (usually 0.1 ml) of the digested material was taken for protein determination and the remainder was extracted three times with 3 vol ethyl acetate. The combined extracts were evaporated to dryness under air at 40 C. The dried extract, however, contained a significant amount of SDS which was removed before RIA by Sephadex LH-20 chromatography, and 6 ml eluate were collected (1, 2).

RIA

After evaporation of the collected (6 ml) eluate, the residue was dissolved in 1 ml ethyl acetate, and a suitable amount depending on the predicted concentration, of 17β-estradiol (E_2) and progesterone (P) was taken for the respective RIA assay of these steroids. The procedure for RIA of E_2 was that described by Lindberg *et al.* (3). The reliability of this assay for determination of E_2 in tissue extracts has previously been checked by us (2). The recovery of authentic unlabeled E_2 added to digested myometrial samples and calculated at the end of the completed procedure was 88.5% (2). The coefficient of variation in replicate analysis of E_2 was about 10%.

The method for P determination was essentially similar to that described by Youssefnejadian *et al.* (4). The antiserum (FO 22.5.73), which was a gift from Dr. Kjell Martinsson (Royal College of Veterinary Medicine, Stockholm) and found to be highly specific for P, was used in a dilution of 1/1500 (vol/vol). Other details have been described previously (1). Also, E_2 and P concentrations in plasma were determined by these radioimmunoassays. There was an almost complete recovery of not only radiolabeled P, but also of unlabeled P (96.5%) added to digested tissue and determined by RIA after extraction (1).

Protein concentration in the digested tissue was determined by the method of Lowry *et al.* (5) by using bovine serum albumin, dissolved in digestion mixture, as standard.

TABLE 1. E_2 and P concentrations in plasma (Pl) and myometrium (My) samples from pregnant women at midterm and term

Parameter	Midterm (n = 5)	Term (n = 33)
Plasma E_2 (ng/ml)	2.9 ± 1.0	22.2 ± 1.6
Myometrial E_2 (ng/g)	1.6 ± 0.6	3.2 ± 0.31
My E_2/Pl E_2	0.69 ± 0.33	0.18 ± 0.03
Plasma P (ng/ml)	27.2 ± 8.4	175.3 ± 11.3
Myometrial P (ng/g)	54.7 ± 16.9	92.2 ± 6.8
My P/Pl P	2.2 ± 0.42	0.61 ± 0.06
My P/My E_2	40.7 ± 10.1	39.1 ± 5.1

Values are means ± SE.

Chemicals

Sodium lauryl sulfate (SDS) was purchased from Sigma Chemical Co. E_2 and P were purchased from Ikapharm, Israel. Radioactive [2,4,6,7-H^3]estradiol (114 Ci/mmol) and [1,2,6,7-H^3]progesterone (105 Ci/mmol) were obtained from New England Nuclear Corporation, and Sephadex LH-20 from Pharmacia Fine Chemicals, Sweden.

Results

Table 1 shows the mean concentration of E_2 and P in the myometrium and in plasma from pregnant women around midterm and at term. Midterm samples are those which were obtained between the 17th–20th week of pregnancy. E_2 concentration in the myometrium (nanograms per g) around midterm was lower than that in plasma (nanograms per ml), whereas myometrial P concentration was about twice that in the plasma. However, for neither steroid was there a statistically significant difference between plasma and tissue concentrations. This difference at term became significant ($P < 0.001$) for both steroids. Although at term both steroids increased in the myometrium, this increment was small when compared to that in plasma, particularly in the case of E_2. Consequently, the ratio of myometrium to plasma (My:Pl) for E_2 (Table 1) was about 0.7 at midterm and decreased to a very low value (<0.2) at term. For P, this ratio at midterm was 2.2 and decreased to 0.6 at term. The decrease in the ratio (My:Pl) from midterm to that at term for both steroids was highly significant ($P < 0.001$). The relative concentrations of the two steroids in the myometrium at midterm and at term remained virtually unchanged; P concentration was about 40 times greater than that of E_2.

Steroid concentrations in the myometrium were also measured in terms of protein content. Mean concentrations of E_2 in midterm and term samples were 15 and 25 pg/mg protein, and those in the case of P were 0.39 and 0.60 ng/mg protein. There was a good correlation between the values expressed on the

basis of wet weight and those of protein for either steroid; the correlation coefficient was 0.91 and 0.92 for E_2 and P, respectively (n = 37).

Fig. 1 compares steroid concentration in plasma to that in the myometrium at midterm. There was a fairly good correlation between plasma and tissue both in the case of E_2 (r = 0.78) and P (r = 0.93). At term, however, the relationship in the case of E_2 was very weak and, in the case of P, insignificant (not shown).

In order to see whether the concentration of steroids in the myometrium was influenced by proximity to the placenta, samples were arbitrarily classified into three groups (Fig. 2) depending on the location of the placenta. Because the samples were obtained from the same portion of the myometrium (low transverse cesarean section), the location of the placenta, carefully checked and recorded, served as an index for arranging the samples in three groups: one with the placenta very close to the excised myometrial sample, called the proximal group (Prox); another group with placental location as distant as possible from the sample, called the distal group (Dist); and a third group with placental location midway (Mid) between these two extremes. An analysis of these data (Fig. 2) showed that the mean E_2 concentration in the myometria forming the Prox group was significantly ($P < 0.005$) higher than that of the Dist group, whereas no difference could be detected between Mid and Dist group. In the case of P, no statistically significant difference between the three groups was found. Because of the limited number of samples, grouping and analysis of this kind could not be performed on the material from midterm.

Discussion

The results show that the myometrial concentration of E_2 in the pregnant human myometrium is very low as compared to that in the plasma. This is most evident at term, and the findings confirm our preliminary results (2). The My:Pl ratio decreased from about 0.7 to 0.18 from midterm to term. Whether reduced E_2-binding capacity in the myometrial cell at term pregnancy is a result of the P-induced decrease in tissue E_2 receptors (2) cannot be proved until measurements of the E_2 concentration in the non-pregnant myometrium after the administration of E_2, to increase plasma E_2 levels, are made. The bulk of the published evidence (6–8) favors the possibility that P influence is a major factor responsible for the reduction of E_2 binding in tissue. Our recent results (unpublished) also indicate an almost complete absence of high affinity E_2 receptors in the pregnant myometrium at term. A greater binding of E_2 to plasma proteins with advancing pregnancy, as previously considered (2), may also have contributed to the low myometrial E_2 concentrations.

Although the correlation between plasma

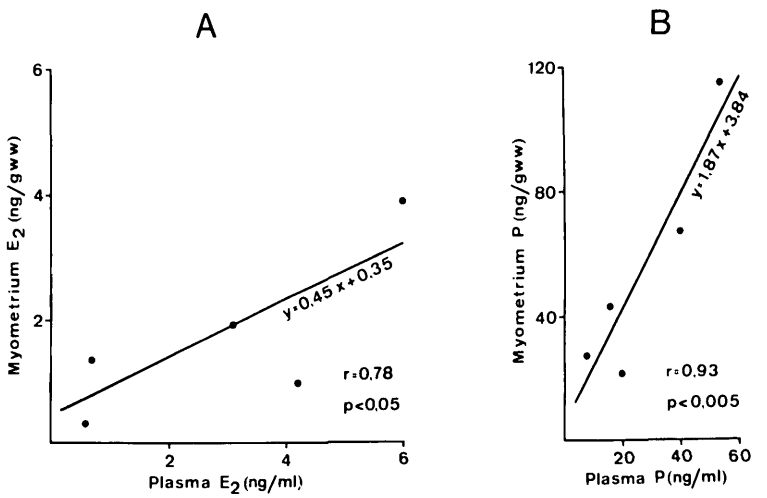

FIG. 1. The relationship between the concentration of steroid in plasma and in the myometrium in samples taken at midterm. A, Estradiol; B, progesterone.

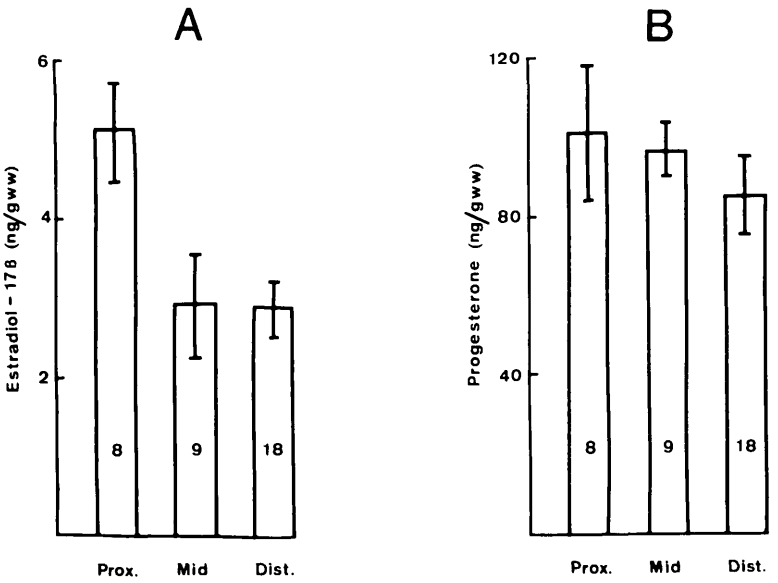

FIG. 2. The influence of placental location on the myometrial concentration of estradiol (A) and progesterone (B). The terms Prox., Mid., and Dist. refer to proximal, midway and distal location of placenta with respect to the excised myometrial sample (see *Materials and Methods*).

and myometrial E_2 at midterm was good, it was distorted at term because of the inability of the myometrium to keep up with the large increase in the blood level with advancing pregnancy. The same appears to be true for P. Similar results have been obtained in a recent but separate study (to be published) where myometrial samples, 10 each in midterm and term of pregnancy, were analyzed.

The concentration of P was significantly higher in tissues obtained at term than those obtained at midterm ($P < 0.05$). Although the concentration of P at term, as determined in the present investigation, compares well with that reported by Runnebaum and Zander (9), the concentration at midterm was lower than that reported by these authors. In fact, the study of Runnebaum and Zander showed that there was no difference in the concentration of P from the first trimester of pregnancy to the end of pregnancy, and the authors concluded that the P-binding capacity of the myometrium was already saturated by the first 15 weeks of pregnancy. It should be pointed out, however, that the number of samples at midterm in the present study was relatively small (n = 5), and the difference between midterm and term samples attained only a low level of statistical significance ($P < 0.05$).

In spite of the minor quantitative differences between our results and those of Runnebaum and Zander, there is a general agreement in the two studies that the increase in the myometrial P does not keep up with the increase in the plasma P, so that the My:Pl ratio, which is considerably greater than unity earlier in pregnancy, decreases to less than unity at the end of pregnancy.

The data of the present study indicate that distance between the placenta and the sample of the myometrium analyzed had no influence on the concentration of P in the myometrium. Runnebaum and Zander (9), who studied this aspect of myometrial P concentration in a more direct manner, also reached this conclusion. Interestingly, these authors also reported that until about the second trimester myometrial P concentration was significantly influenced by the placenta so that the concentration of P in myometrial samples obtained from placental sites was significantly greater than that in samples obtained from so-called antiplacental sites. These differences, however, became insignificant at the term of pregnancy (9).

Of greater interest is the present finding that E_2 concentration, in contrast to P in myometrial samples, was significantly influenced by the proximity of the placenta. These observations suggest that there is a difference in myometrial-placental relationship with respect to the passage (or leakage) of E_2 and P

from placenta. Relatively low myometrial E_2 concentration *per se,* very low concentrating ability for E_2 (My:Pl ratio at term being 0.18), and an increase of E_2 concentrations with proximity to the placenta lead one to wonder about the extent to which myometrial E_2 is derived from blood or simply reflects leakage from placenta. The finding that endometrial (10, 11) and myometrial (Batra *et al.,* unpublished) E_2 concentration during the proliferative phase can be as high as 1 ng/g rules out the possibility that myometrial E_2 in pregnancy could simply be a contamination (or leakage) from placenta.

The present data on the lack of influence of the placenta on myometrial P concentration argue against the concept of a local "block" by a high P concentration at the placental site (12) in myometrial activity. On the other hand, our finding that the E_2 concentration is higher in the myometrium close to the placenta might indicate an estrogen "block." However, the relatively low myometrial E_2 concentration in pregnancy and the indication of only minor changes towards term weaken the arguments for a local E_2 effect on myometrial activity.

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