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Sex Steroids in Plasma and Reproductive Tissues of the Female Guinea Pig

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ABSTRACT

Progesterone and estradiol-17β concentrations were determined in peripheral plasma, uterine, ovarian and placental tissues in nonpregnant (diestrous) and pregnant (mid- and fullterm) guinea pigs. Plasma progesterone concentration increased 56-fold at midterm but decreased at term to 28-fold of that in the diestrous animals. Although plasma estradiol increased during pregnancy, the changes compared with progesterone were minor.

There was no significant change in uterine progesterone concentration during pregnancy. Uterine estradiol concentrations nearly doubled at midpregnancy but decreased again at term to a value similar to that at diestrous. In the nonpregnant animals, the progesterone concentrations in ovaries containing corpora lutea were not significantly different than those in the pregnant animals and these concentrations were ∿4 times greater than those in ovaries without corpora lutea. Ovarian estradiol concentration in diestrous animals was ∿10 times that of the uterine concentration. It increased 5-fold at midterm and 15-fold at term. Placental progesterone and estradiol concentrations were much lower than the ovarian concentrations of these steroids. The placental estradiol concentration was even lower than the uterine estradiol concentration whereas in the case of progesterone, the concentrations were 2–3 times higher in the placenta than in the uterus. The very low levels of progesterone in guinea pig uterus and placenta might be explained by the secretion in the plasma of progesterone binding globulin. However, the reason for the concentration of ovarian progesterone remaining very high is not clear.

INTRODUCTION

Reproductive biology of the female guinea pig has several features in common with that of women. In both guinea pigs and women spontaneous ovulation occurs and progesterone is secreted by corpora lutea for ∿14 days (Hilliard, 1973). Removal of the ovaries in early pregnancy does not affect the course of gestation (Sisk, 1976); parturition is not preceded by a fall in peripheral plasma progesterone (Illingworth et al., 1974; Liggins et al., 1977). The last two mentioned characteristics contrast with those in the rabbit and the rat (Hilliard, 1973), two mammalian species commonly used as experimental animals in studies dealing with reproductive endocrinology. The guinea pig is therefore generally regarded as a most suitable animal model for elucidating the role of steroid hormones in pregnancy and parturition in humans (Sisk, 1976). However, there is an important difference in the endocrinology of pregnancy in guinea pigs and humans. Guinea pig plasma, unlike human, contains a high affinity progesterone-binding globulin during gestation as well as a corticosteroid binding globulin which is common to both species. Since it is the concentration of the free hormone in plasma that largely determines the concentration of the hormone in the target tissue, one would expect to find a marked difference between guinea pig and human in the concentration of progesterone in a target tissue such as the uterus.

In previous studies data on the concentration of progesterone and estradiol-17β in the human and rabbit reproductive tissues have been reported (Batra et al., 1977; Batra and Bengtsson, 1978; Batra et al., 1979a; Thorbert et al., 1976). In the present study we have examined the relationship between plasma and uterine levels of progesterone and estradiol in both nonpregnant and pregnant guinea pigs. Furthermore, since there is some evidence showing that placental progesterone concentration in the guinea pig is low (Heap and Deanesly, 1966), we have also determined the concentration of

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the above steroids in placental and ovarian tissues.

MATERIALS AND METHODS

Animals

Guinea pigs of a mixed strain were used. Nonpregnant animals were killed on Days 8–11 of the estrous cycle, determined as described previously (Thorbert et al., 1977). Pregnant guinea pigs were placed in 2 groups depending on the stage of pregnancy, which was judged by palpation. The animals killed were under light anesthesia and the day of pregnancy was checked by measurement of mean weights and mean crown-rump lengths of the fetuses (Draper, 1920; Kaufmann, 1969). One group was at 26–34 days (midterm) and the other at 56–64 days (fullterm) of pregnancy. The following tissues were dissected out for tissue steroid analysis: one uterine horn and ipsilateral ovary and a piece of the placenta. The uterine horn was examined microscopically to make sure that all the placenta was removed. While the animals were under anesthesia, blood was drawn from the left ventricle and used for the steroid assay described below.

Steroid Assays

Plasma. Progesterone in plasma of nonpregnant animals was determined by the method described previously (Thorbert et al., 1976; Batra and Bengtsson, 1978). This method was not satisfactory, however, for progesterone determinations in pregnant guinea pigs. Progesterone in these samples was therefore measured with the method for tissue determinations (see below) with the exception that only 3.5 ml eluate from Sephadex LH-20 columns were collected. The antiserum (FO 22-5-73), which was a gift from Dr. Kjell Martinsson and found to be highly specific for progesterone, was used in a dilution of 1:1500 (Batra et al., 1976).

The procedure for radioimmunoassay of estradiol-17β was that described by Lindberg et al. (1974). The antiserum was raised in sheep against estradiol-17-α-6-oxime conjugated with bovine serum albumin and used at a dilution of 1:100,000. After extraction of 1 ml plasma with diethyl ether, antiserum and [^{3}H]-estradiol-17β was added. The mixture was equilibrated at 4°C overnight. The unbound steroid was removed by addition of dextran-coated charcoal. Extensive data on the specificity of the antiserum have been published previously by Lindberg et al. (1974).

Tissues. The assay procedures for the determination of tissue progesterone and estradiol-17β have been reported previously (Batra, 1976; Batra and Bengtsson, 1976). Briefly, the frozen tissues were thawed and then digested in 0.5 ml of a mixture containing 5% sodium dodecyl sulphate (SDS) and 0.5 N NaOH. The digested material was extracted 3 times with 3 volumes ethyl acetate. The combined extracts were evaporated to dryness under air at 40°C. Removal of sodium dodecyl sulphate from the extracted samples before radioimmunoassay was accomplished by Sephadex LH-20 gel chromatography. After evaporation of the collected (6 ml) eluate, the residue was dissolved in 1 ml ethyl acetate and suitable amounts, depending on the predicted concentrations of estradiol and progesterone, were taken for their respective radioimmunoassay. Extensive data on the reliability of these methods have been reported previously (Batra, 1976; Batra and Bengtsson, 1976; Batra et al., 1979a). Almost complete recoveries of estradiol and progesterone added to digested placental and ovarian tissues were obtained. Tissues of one type were analyzed in the same assay.

Free and Bound Progesterone

Free and protein-bound progesterone was measured by the membrane filter (Millipore) technique described previously (Batra, 1974; Batra et al., 1976).

Statistics

In the text and tables values from multiple observations are given as means ± SEM; number of observations when given is shown within parentheses. Student's t test was used in the evaluation of observed differences in mean values.

RESULTS

When plasma progesterone from pregnant guinea pigs was extracted with petroleum ether as previously done successfully for human and rabbit plasma, it was found that the solvent extracted 37–91% (n = 8) of tritiated progesterone added to guinea pig plasma. However, when plasma was first treated with SDS and NaOH as used for tissue steroid analysis, the recovery of added tritiated progesterone was 97.9 ± 1.28% (mean ± SEM; n = 16).

Incomplete and variable extraction of progesterone from plasma of pregnant guinea pigs was probably due to the presence of high affinity progesterone binding globulin (PBG). A measurement of the free and bound fraction of plasma progesterone (Table 1) showed that virtually 100% was in the bound form in the pregnant guinea pig, whereas in the nonpregnant guinea pig the mean value was 77.6%.

Accuracy and Precision of Steroid Analyses

Percent mean recoveries (± SEM) of added estradiol and progesterone, respectively, in guinea pig plasma and various tissues were as follows (n = 10–12): 94.7 ± 4.5 and 95.2 ± 3.6 for plasma, 94.9 ± 3.0 and 92.8 ± 4.9 for uterus, 90.4 ± 4.0 and 99.5 ± 3.2 for ovary, 88.5 ± 4.2 and 104.0 ± 2.8 for placenta. The interassay coefficient of variation calculated from duplicate determinations of estradiol and

TABLE 1. Concentrations of free and protein-bound progesterone in plasma of nonpregnant and pregnant guinea pigs (mean ± SEM).

Reproductive state	n	Progesterone (ng/ml) Total	Free	% Bound
Nonpregnant	4	3.61 ± 0.69	0.76 ± 0.10	77.6 ± 2.57
Pregnancy (mid- and fullterm)	9	160.0 ± 39.40	0.51 ± 0.28	99.6 ± 0.23
Difference: statistical significance		P<0.005	NS	P<0.001

progesterone, respectively, was 11.1 and 10.4 for plasma, 7.7 and 10.2 for uterus, 8.9 and 12.8 for ovary and 6.3 and 12.1 for placenta.

Plasma

Plasma progesterone concentration in the diestrous animals (Fig. 1) was ∿4 ng/ml and it increased by 56-fold at midterm but decreased at term of pregnancy so that it was now 28-fold of that in the diestrous animal. The decrease from midterm to term was significant ($P<0.05$). Estradiol concentrations in plasma at midterm decreased by 30% but the reduction was insignificant (Fig. 1). However, at fullterm it increased again and was significantly higher than that at midterm ($P<0.01$) or that in the nonpregnant animals ($P<0.05$).

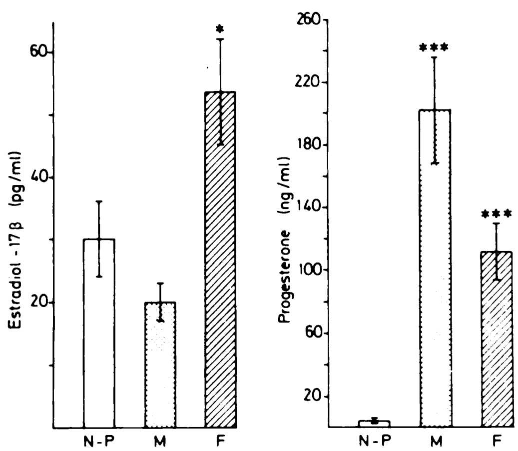

FIG. 1. Estradiol and progesterone concentration in peripheral plasma of nonpregnant (N-P), midterm (M), and fullterm (F) pregnant guinea pigs. Significance of difference from N-P is indicated by *$P<0.05$; ***$P<0.005$.

Uterine Tissue

There was relatively little change in uterine progesterone concentration during pregnancy; the slight increase seen at midterm was not significant. Estradiol concentration nearly doubled ($P<0.05$) at midterm but decreased again at term to a value similar to that in the diestrous animals (Fig. 2).

The uterine/plasma ratio for progesterone was 6, 0.2 and 0.3; for estradiol it was 16, 43 and 7 at diestrous, midterm and fullterm, respectively.

Ovarian Tissue

The data on steroid concentrations in the ovaries is shown in Fig. 3. Progesterone concentration as expected was much higher in ovaries containing corpora lutea (2.1 μg/g) than in those not containing them (0.6 μg/g). However, progesterone concentration even in ovaries not containing corpora lutea was very high (30 times) compared with that in the uterus (Fig. 2). Progesterone concentration in corpus luteum bearing ovaries of the pregnant animals

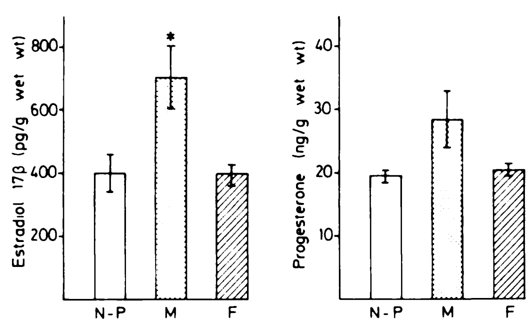

FIG. 2. Estradiol and progesterone concentration in uterine tissue from nonpregnant (N-P), midterm (M) and fullterm (F) pregnant guinea pigs. Significance of difference from N-P is indicated by *$P<0.05$.

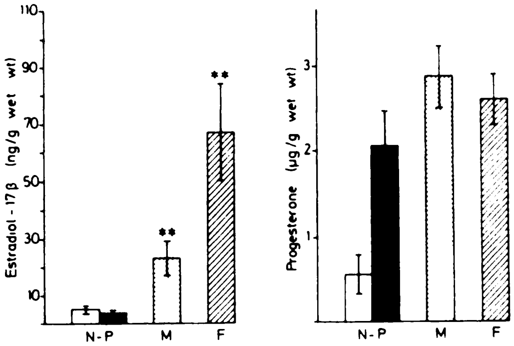

FIG. 3. Estradiol and progesterone concentration in ovaries from nonpregnant (N-P), midterm (M) and fullterm (F) pregnant guinea pigs. Solid columns represent ovaries containing corpora lutea. Significance of difference from N-P is indicated by $^{**}P<0.01$.

was slightly higher than that of the nonpregnant animals but the difference was not significant.

Ovarian estradiol concentration (3.9 ng/g in nonpregnant animals) was ∿10 times that in the uterine tissue (Fig. 3). At midterm it increased by 5-fold and further increased by 3-fold at term. There was no difference in the ovarian estradiol concentration in the diestrous animals regardless of whether corpora lutea were present.

Placental Tissue

The placental progesterone and estradiol concentrations were much lower than the ovarian concentration of these steroids (Fig. 3, 4). The concentrations of progesterone were 2–3 times higher in the placenta than in the uterus, whereas the placental concentration of estradiol was lower than the uterine concentration. When pregnancy advanced from midterm to term, there was a reduction ($P<0.005$) in both progesterone (36%) and estradiol (24%) concentration.

DISCUSSION

Guinea pig reproductive endocrinology is generally thought to correspond more closely to that of the human than to that of other mammalian species (Sisk, 1976; Thorburn et al., 1977). The aim of the present study was to provide information on progesterone and estradiol concentrations in the guinea pig reproductive tissues and to compare these with other mammalian species, particularly man. Table 2 shows a comparison of the present data with those published recently for other species.

Plasma

The plasma progesterone concentration in pregnancy increased by several-fold and showed a maximum around midterm. It decreased by ∿50% at term. This pattern and the values for progesterone in the present study are in agreement with the data published previously (Heap and Deanesly, 1966; Challis et al., 1971; Croix and Franchimont, 1975; Lea et al., 1976). Plasma progesterone levels in the guinea pig at term are similar to those in man (Table 2). However, in the guinea pig this progesterone is virtually all present in the bound form, whereas in the latter ∿10% is free (Batra et al., 1976).

Since the plasma estradiol concentration changed relatively little in pregnancy in the guinea pig, it was extremely low when compared with that occurring in human pregnancy. In this regard the guinea pig is rather similar to the rabbit.

Uterine Tissue

The concentration of uterine progesterone during pregnancy was similar to that in the nonpregnant animal in spite of 30–60-fold increases in the concentration of plasma progesterone. This situation is quite unlike that encountered in man where uterine progesterone keeps up well with the increases in plasma progesterone, at least until midterm (Batra and Bengtsson, 1978). As a result, the uterine

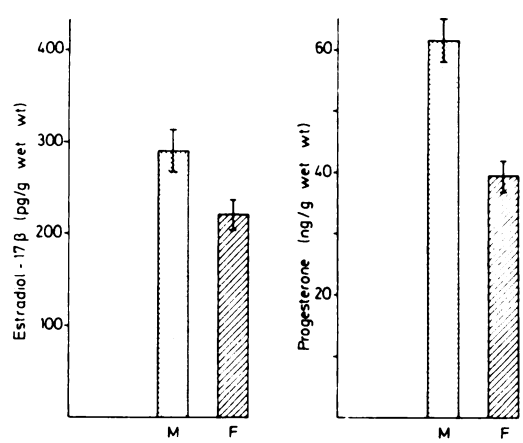

FIG. 4. Estradiol and progesterone concentration in guinea pig placenta at midterm (M) and fullterm (F) of pregnancy.

TABLE 2. Plasma and uterine tissue estradiol and progesterone concentration in guinea pig, man and rabbit in nonpregnant and pregnant (mid- and fullterm) state.[a]

Species	Sample	Estradiol (pg/ml or pg/g wet weight)			Progesterone (ng/ml or ng/g wet weight)		
		Nonpregnant	Midterm	Fullterm	Nonpregnant	Midterm	Fullterm
Guinea pig	Plasma	29.8 ± 6.0	20.5 ± 4.5	59.3 ± 8.9	3.6 ± 0.7	203 ± 37.1	102.0 ± 13.1
	Uterine tissue	399.0 ± 55.1	705.0 ± 97.5	387.0 ± 33.0	19.4 ± 1.0	28.6 ± 4.4	20.6 ± 0.9
Man	Plasma	190.0 ± 56.6 (1)	2900.0 ± 1000.0 (2)	22000.0 ± 1600.0 (2)	10.5 ± 2.3 (1)	27.2 ± 8.4 (2)	175.0 ± 11.3 (2)
	Uterine tissue	607.0 ± 104.0 (1)[b]	1600.0 ± 600.0 (2)	3200.0 ± 310.0 (2)	21.7 ± 4.8 (1)	57.7 ± 16.9 (2)	92.2 ± 6.81 (2)
Rabbit	Plasma	31.8 ± 4.0 (3)	60.0 ± 6.0 (5)	50.0 ± 10.0 (5)	0.3 ± 0.1 (4)	18.0 ± 2.0 (5)	7.0 ± 1.0 (5)
	Uterine tissue	950.0 ± 175.0 (3)	550.0 ± 90.0 (5)	950.0 ± 200.0 (6)	6.1 ± 0.3 (4)	20.0 ± 4.0 (6)	4.0 ± 1.0 (6)

[a]Data in guinea pig are those reported in the present study, whereas in man and rabbit they are those reported in studies indicated by a reference number in parentheses. (1) Batra et al. (1977); (2) Batra and Bengtsson (1978); (3) Batra et al. (1979a); (4) Thorbert et al. (1976); (5) Challis et al. (1973); (6) Challis et al. (1974).

[b]Endometrial tissue, but values are comparable to those recently obtained for the myometrium (Batra et al., unpublished).

progesterone concentrations in the human pregnancy at term are ∿20-fold higher than in the guinea pig in spite of similar plasma progesterone in the 2 species (Table 2). These observations indicate that bound progesterone cannot enter the uterus; and this weakens the proposal that progesterone binding globulin (PBG) might be involved in the transport of progesterone to the uterus (Lea et al., 1976).

The second reason for the very low progesterone concentration in the pregnant guinea pig uterus might be the very potent inactivating effect of progesterone in this species on the uterine progesterone receptors (Milgrom et al., 1973; Freifeld et al., 1974; Baulieu, 1975). Recent data from this laboratory have shown that although there is a reduction in progesterone receptors after progesterone treatment in the rabbit uterus, this reduction was not as dramatic as that reported for the guinea pig (Batra, 1979). Similar evidence seems to exist for the human uterine progesterone receptors (Illingworth et al., 1975; Bayard et al., 1978). One reason for the high inactivating effect of progesterone on its own receptors in the guinea pig uterus might be the very low levels of uterine (and plasma) estradiol required to induce the synthesis of new progestin receptors (Freifeld, 1976). The changes in uterine estradiol concentration during pregnancy were relatively small. This was consistent with the changes observed in plasma estradiol.

Ovarian Tissue

The very high concentrations of both steroids in the ovarian tissue is not surprising in view of the fact that the hormones are synthesized in this tissue. However, this also indicates that ovary, unlike placenta, has a high capacity to retain these hormones (see below). The fact that there was no difference in the concentration of estradiol in contrast to progesterone in ovaries with or without corpora lutea indicates that the corpus luteum in the guinea pig, unlike that in the human, does not synthesize estradiol. It is, however, remarkable that the progesterone concentration in the ovary without corpus luteum was also very high, being 25 times greater than uterine progesterone concentration. Assuming that the corpus luteum is the sole source of progesterone in the ovaries, one would have to conclude that the very high concentrations of progesterone in the ovary not containing corpus luteum reflect an accumulation from the peripheral circulation. If this were the case, the present data would suggest the presence of a very high affinity progesterone binder in the ovarian tissue. The affinity for progesterone of this hypothetical binder would be much greater than that of the uterine tissue, since the uterine tissue was unable to concentrate progesterone owing to the presence of the high affinity PBG in the plasma (see above). Alternatively, unlike the uterine receptors, the ovarian progesterone receptor may not easily be inactivated by progesterone in the presence of relatively high concentrations of estradiol in the ovary. Estradiol receptors in the rabbit corpus luteum similar to the uterine estradiol receptors have been reported (Mills and Osteen, 1977; Miller and Keyes, 1978). The possibility that the difference in the ovary and uterus with respect to progesterone concentration may result from differences in the blood flow cannot be ruled out.

The increase in ovarian estradiol during pregnancy particularly at fullterm is consistent with the increased follicular activity observed in the guinea pig. These follicles are destined to ovulate immediately postpartum (Sisk, 1976). Recently Kalloo and Bhavani (1978) reported that the rate of formation of estradiol in vitro by ovaries of pregnant guinea pigs was about 40 times greater than that of nonpregnant animals.

Placental Tissue

The concentration of either steroid in the placental tissue was surprisingly low compared with the ovarian tissue or human placental tissue (Runnebaum et al., 1975; Batra et al., 1979b). Estradiol concentration in placenta was in fact $\sim$50% of that in the uterine tissue. These data might suggest that the guinea pig placenta does not synthesize estradiol to any great extent, which is in agreement with the previous reports for the absence of aromatase activity in the guinea pig placenta (Ainsworth and Ryan, 1966). This is also supported by the recent finding of Kalloo and Bhavnani (1978) who showed that the major site of estrogen formation in both pregnant and nonpregnant guinea pigs was the ovary. The possibility that the very low concentrations of estradiol in the guinea pig placenta simply reflect contamination from the blood cannot be completely ruled out.

Surprisingly, we also found very low concentration of progesterone in the placental tissue (Heap and Deanesly, 1966). These concentrations were about twice that found in the uterine tissue or human placental tissue (Runnebaum et al., 1975; Batra et al., 1979b). Guinea pig placenta, like that of the human, is known to synthesize substantial amounts of progesterone, because ovariectomy after 20–25 days post-coitum does not result in any significant decrease in the level of peripheral plasma progesterone nor does it affect the course of pregnancy (Heap and Deanesly, 1966). Progesterone concentration in term placentae was lower than in midterm placentae which is also reflected in the plasma progesterone levels. This observation lends further support to the evidence that plasma progesterone is mainly derived from the placenta after midpregnancy. We are, however, unable to provide an explanation for the very low levels of progesterone in the placental tissue. It may be the result of very high binding of progesterone in the plasma, but it is not clear why this does not apply to the concentration in the ovarian tissue (see above) which contained a very high progesterone concentration. Here again, either the differences in blood flow between ovary and placenta or in secretion and metabolism of progesterone by these tissues might account, at least partially, for the differences in their progesterone concentration.

The general conclusion that can be drawn from the present study is that in the guinea pig the concentrations of progesterone and estradiol in the uterine tissue, one of the most important targets for these steroids, as well as in the placental tissue are very low compared with these concentrations in humans. Although estradiol levels in the peripheral plasma are very low compared with the human, the progesterone levels are similar. Although tissue metabolism, blood flow and receptor levels cannot be ruled out as contributing factors, the binding of almost all progesterone by PBG present specifically in the plasma of the pregnant guinea pig (Milgrom et al., 1973; Mc Laughlin and Westphal, 1974), provides a likely explanation for the very low levels of progesterone in the uterine tissue as well as in the placenta. Thus the presence of PBG not only protects this steroid from being metabolized (Challis et al., 1971) but probably also keeps it from accumulating in the target tissues. The biological significance of the latter is not yet clear.

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