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Sex Steroids in Reproductive Tract Tissues: Regulation of Estradiol Concentrations by Progesterone[1]

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ABSTRACT

Estradiol-17β (E_2) and progesterone (P) were quantified in the reproductive tract tissues and striated muscle of rhesus monkeys and rats. After homogenates of these tissues had been extracted with ether and defatted, E_2 and P were isolated by chromatography on Sephadex LH-20 columns and quantified by radioimmunoassay. Rhesus monkeys that had been treated with E_2 had significant elevations of this steroid in the reproductive tract tissues (oviduct and uterus) but not in skeletal muscle. When monkeys were treated with a combination of P and E_2 the amount of E_2 in the reproductive tract of this species was significantly reduced. Similar results were obtained in rats, i.e., after combined progesterone and estradiol treatment, the tissue levels of E_2 were significantly lower than those in the uteri of animals not given P. The levels of P were significantly higher in the skeletal muscle of rats treated with E_2 + P. E_2 treatment alone increased the amount of endogenous P found in the uterus of the rat.

INTRODUCTION

Since the introduction of sensitive and specific techniques for measuring steroid hormones by radioimmunoassay (RIA), many hundreds of papers correlating serum or plasma levels of hormone with some physiologic function or experimental protocol have been published. But only a few authors have attempted to accurately measure these steroids in tissues (Wiest, 1973; Contes-Gallagos et al., 1975; Bayard et al., 1975; Senior, 1975). Knowing the blood levels of steroid hormones has enhanced our understanding of endocrine physiology but these levels may not correlate with the amounts present in tissues under different physiological conditions. This was recently suggested by Brenner et al. (1974) who studied the interaction of estradiol-17β and progesterone in the oviduct of rhesus monkeys. Although the blood concentrations of estrogen were maintained at approximately the same level with or without progesterone treatment, the amount of cytoplasmic estrogen receptor diminished with progesterone treatment and the oviduct showed symptoms of estrogen withdrawal. Preliminary data presented in that report indicated that the concentration of estradiol in oviductal tissues was lowered by progesterone treatment.

A similar relation between the symptoms of estrogen withdrawal and an estrogen-progesterone sequence of treatment was observed in studies on the rate of egg transport through the ampulla of the rabbit oviduct (Boling and Blandau, 1971a, b) and on contractions of the rat uterus in vitro (Boling and Conrad, unpublished) and of the rat vagina in vivo (Boling and Job, 1965).

In this report we describe our method for measuring estradiol and progesterone concentrations in tissue samples by RIA, and provide additional data on the levels of these hormones found in the reproductive tract of spayed rhesus monkeys and rats treated with various hormonal regimens.

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MATERIALS AND METHODS

Reproductive tract tissues (oviductal fimbriae and uterus) were removed from spayed rhesus monkeys which had been treated with either estradiol benzoate, (EB), Schering Progynon benzoate, alone or a combination of EB + progesterone, (P), Steraloids Inc. Rectus

abdominis muscle was used as the control tissue. Intramuscular injections of estradiol benzoate alone (10 μg in 0.5 ml of sesame oil) had been administered twice daily to some of the experimental animals (N=10), the treatment regimens ranging from 3 to 34 days. In those that received a combination of EB + P, some (N=4) were injected with both hormones from the start of the treatment period (3–10 days). Others (N=3) were injected with EB alone for 14 days and then with a combination of EB + P for an additional 7 days. The P (0.75 mg in 0.5 ml sesame oil) was injected intramuscularly twice daily. Tissues were removed on the day of the last P injection. The data from the 7 monkeys that received a combination of EB + P has been pooled and contrasted with the data from the 10 monkeys that received EB alone.

Uteri from ovariectomized mature (208–256 g) Sprague Dawley rats were removed after treatment with E_2 or E_2 + P; striated muscle from the anterior thigh was used as the control. Estradiol-17β (E_2, 1.0 μg) and P (0.4 mg) were administered subcutaneously in an aqueous (0.25 ml) suspension and in oil (0.25 ml) respectively. Both hormones were administered every 6 h and the tissues removed according to the following regimens:

Group I: Tissues were removed 50 h after the first E_2 injection and 32 h after the last.

Group II: Tissues were removed 50 h after the first E_2 injection and 2 h from the last.

Group III: Tissues were removed 50 h after the first E_2 injection and 2 h after the last; 26 h after the first P injection and 2 h after the last.

Group IV: Tissues were removed from animals castrated for the same length of time as the groups above, i.e., 16 days.

All tissues were removed and quickly frozen in glass vials suspended in dry ice/ethylene glycol monoethyl ether. They were then stored at −20°C until steroid analyses were performed. Before analysis, the tissues were defrosted, carefully blotted with filter paper, and weighed on a tissue balance. Each sample was homogenized in 2 ml water in a 15-ml ground glass homogenizer and extracted (X2) with 6 ml of ether. Between extractions, the samples were centrifuged and the ether extracts from the two extractions were pooled and washed with 1 ml distilled water. After the ether had been taken to dryness under a stream of air, the lipid residues were removed as follows. To each sample, 5 ml of 70 percent methanol were added and each sample was mixed vigorously for 1 min and placed in a freezer (−20°C) for 16 h. The samples were then centrifuged for 5 min at 1000 rpm in a refrigerated centrifuge (−4°C), the methanol was transferred to a clean test tube, and the precipitate was washed (X2) with 5 ml cold (0°C) 70 percent methanol. The original methanol extract together with the two methanol washes were reduced by evaporation to an aqueous residue. Vigorous shaking in 5 ml of ether (X3) for 1 min extracted the aqueous phase. All samples were centrifuged between extractions. The ether extracts were taken to dryness and the samples were concentrated to the tip of the tube and transferred to Sephadex LH-20 columns in 50 μl of hexane:benzene:methanol (62:20:13). Separation on the Sephadex columns and RIA of P and E_2 have already been described (Resko, 1971; Resko et al., 1974).

Statistical analyses (*t* tests for unequal number of subjects) were performed on a Canon desk computer, Model 167P. To determine the thoroughness of our recovery procedures, different quantities of P and E_2 were homogenized with muscle, allowed to equilibrate for one-half hour, and run through the entire procedure. To determine the minimum sample size, different sizes of uterine tissue from the same uterine horn were analyzed for E_2 and P.

RESULTS

The precision and accuracy of our technique are shown in Table 1. Fifty, 100, and 150 pg of E_2 added to muscle were quantified (mean ± SE) as 50 ± 20 (N=11), 110 ± 40 (N=9), and 130 ± 20 (N=10) pg respectively. Similarly, 500, 1000, and 1500 pg P were estimated as 620 ± 30, 970 ± 80, and 1280 ± 70 (N=8) pg respectively. In 17 separate determinations of E_2, small amounts of tissue, averaging 38.7 ± 2.9 mg in weight, contained 70 ± 20 pg of E_2 (Table 2). A larger amount (approximately double) of tissue (98.2 ± 0.8 mg) from the same uteri contained 100 ± 20 pg E_2. The discrepancy between the two measurements seems to indicate an overestimation of the amount of E_2 in small amounts of tissue. In many small samples, the amount of E_2 found was at or near the lower limit of sensitivity of our assay (10 pg). The assay of progesterone in these same tissues contained 990 ± 60 pg of P whereas the larger, approximately double, amounts contained 2410 ± 1290 pg.

E_2 and P were quantified in the reproductive tract tissues (oviduct and uterus) of spayed rhesus monkeys and rats treated with exogenous E_2 and P. The results for the rat are shown

TABLE 1. Quantification of estradiol-17β and progesterone in rat muscle.

Hormone assayed	Picograms added	N	Picograms found (mean ± SE)
Estradiol-17β	50	11	50 ± 20
	100	9	110 ± 40
	150	10	130 ± 20
Progesterone	500	8	620 ± 30
	1000	8	970 ± 80
	1500	8	1280 ± 70

TABLE 2. Quantification of estradiol-17β and progesterone in rat uteri.

Hormones measured	No. of determinations	Mean (pg) ± SE/100 mg tissue	
		Small[b]	Large[c]
Estradiol-17β	17	70 ± 20	100 ± 20
Progesterone	17	990 ± 60	2410 ± 1290

[a]Small and large piece from the same uterine horn.

[b]Small = 38.7 ± 2.9 (SE) mg, N=17.

[c]Large = 98.2 ± 0.8 (SE) mg, N=17.

in Figs. 1 and 2, for the monkey in Table 3. In the monkeys treated with E_2 alone, the tissue concentrations of E_2 differed markedly in the target (oviduct or uterus) and non-target (striated muscle) tissue [270 ± 30 (SE) pg per 100 mg, N=10 and 70 ± 20 (SE) pg per 100 mg, N=7, respectively]. Second, in the animals treated with estradiol + progesterone, the concentrations of E_2 in the oviduct or uterus were significantly lower than in those treated with E_2 alone (t=3.07, 15 df, P<0.01). On the other hand, P did not affect the tissue concentrations of E_2 in the nontarget tissue but did raise the levels of P in target tissue and, in some cases, in skeletal muscle.

Tissue concentrations of E_2 and P were similarly analyzed by our technique in rat uteri; the quantities of progesterone are shown in Fig. 1. Animals treated with progesterone had significantly more progesterone in uterine tissue than in muscle, t=2.23, 10 df, P<0.05. Likewise, more progesterone was found in the uteri of Group III than of Group II, t=2.64, 10 df, P<0.05. Also, significantly more P was found in the uteri of rats in Group II compared to Group I, though neither of these groups was treated with P. Unlike the estrogen data (Fig. 2), those on progesterone showed that muscle tissue from rats treated with progesterone (Group III) contained more progesterone than muscle from rats treated with estrogen alone (Group II), t=3.69, 10 df, P<0.01. The quantities of E_2 measured in uteri 32 and 2 h after the last estrogen treatment are given in Fig. 2. Uteri removed 2 h after the final estrogen treatment contained 3 times more estrogen than uteri removed 32 h after the last estrogen treatment, t=9.49, 10 df, P<0.001. As in the monkey, P treatment significantly reduced the amount of E_2 in the uterus, t=4.42, 10 df, P<0.01. Tissue from animals spayed two weeks earlier contained little or no E_2. E_2 concentration in skeletal

TABLE 3. The effect of progesterone (P) on estradiol-17β (E_2) concentrations in reproductive tract tissues of the female rhesus monkey.

Tissue	No. of animals	Treatment	Mean (pg) ± SE/amount tissue[a]	
			E_2	P
Reproductive tract[a]	10	E_2[b]	270 ± 30*	120 ± 60**
Skeletal muscle[c]	7	E_2[b]	70 ± 20	220 ± 100
Reproductive tract	7	E_2[b] + pd	130 ± 40*	480 ± 50**
Skeletal muscle	4	E_2[b] + pd	60 ± 20	230 ± 40

[a]Fimbria of the oviduct or uterine endometrium.

[b]Treatment with 2 daily intramuscular injections of 10 μg of estradiol benzoate in sesame oil. Treatment regimens ranged from 3 to 34 days. Tissue samples taken on the day of the last injection.

[c]Rectus abdominis muscle.

[d]Treatment with 2 daily intramuscular injections of 0.75 mg of progesterone in sesame oil. Treatment regimen ranged from 3 to 7 days. Tissue samples taken on the day of the last P injection.

*Differed significantly by a t-test, t=3.07, 15 df, P<0.01.

**Differed significantly by a t-test, t=4.43, 15 df, P<0.001.

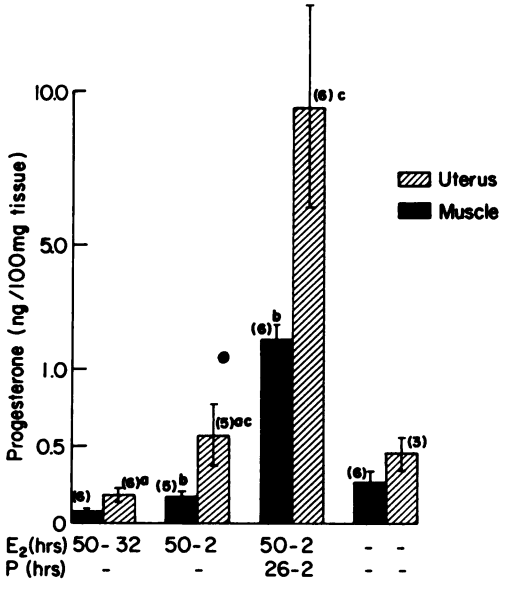

FIG. 1. Concentrations of progesterone (P) in tissues of the ovariectomized rat treated with estradiol-17β (E_2) or E_2 + P. Data are given as means (bars) ± standard error (vertical lines). Numbers in parentheses equal the number of steroid determinations from different uterine horns or pieces of muscle from 3 animals. Bars with same letter are significantly different, a = P<0.001, b = P<0.01, c = P<0.05. See text for treatment shown on abscissa.

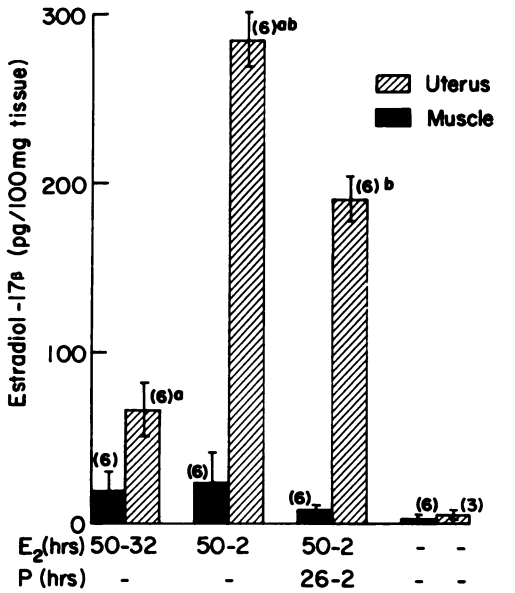

FIG. 2. Concentrations of estradiol-17β (E_2) in tissues of the spayed rat treated with E_2 or E_2 + progesterone (P). Data are presented as means (bars) ± standard error (vertical lines). Numbers in parentheses equal the number of steroid determinations from different uterine horns or pieces of muscle from 3 animals. Bars with same letter are significantly different, a = P<0.001, b = P<0.01. See text for treatments shown on abscissa.

muscle did not differ significantly between the groups.

DISCUSSION

We have described a method for measuring tissue levels of E_2 and P. Under the conditions of our hormone treatments, almost 100 mg of tissue are necessary for an accurate determination, but this amount may change with different hormonal regimens. What tissue constitutes an appropriate blank to run with the assays is difficult to determine. According to our data the concentration of E_2 in striated muscle does not change between treatments, an indication that nonuterine muscle is an appropriate blank for these determinations. The quantities of P, however, do increase in nonuterine muscle after P treatment for reasons that are not completely clear. Therefore, skeletal muscle should not be used as a blank for tissue P determinations. What could be used as a blank for this hormone we have not yet determined.

In the reproductive tract tissue of both rhesus monkeys and rats, we consistently found that progesterone significantly lowers the quantity of E_2 that can be measured by RIA. This agrees with our previous results that P decreases the amount of available cytoplasmic receptors in the monkey oviduct without competing with E_2 for the binding site (Brenner et al., 1974). A similar effect of P on estrogen receptors has been reported in human (Tseng and Gurpide, 1975a) and rat uteri (Mester et al., 1974; Hsueh et al., 1975, 1976). P may also lower the tissue level of E_2 by stimulating the synthesis of the enzyme estradiol dehydrogenase, favoring the conversion of E_2 to E_1 (Tseng and Gurpide, 1975b) or by some other previously unknown mechanism. This function of P corresponds to that observed during egg transport in the rabbit oviduct (Boling and Blandau, 1971a, b) and in uterine (Boling and Conrad, unpublished) and vaginal (Boling and Job, 1965) muscle contractility in the rat, i.e., the action of P induces the same effects on estrogen-primed muscle of the reproductive tract as those associated with estrogen withdrawal (Coutinho and de Mattos, 1968; Boling, 1969; Halbert and Conrad, 1975).

Significantly more P was found in the uteri of rats treated with E_2 alone for 48 h and killed 2 h later than in those treated for 18 h and killed 32 h later. The P that accumulates in the uterus of the E_2-treated ovariectomized rat probably originates in the adrenal gland since the adrenal secretes P in this species (Feder et al., 1968).

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