Unconjugated Estradiol in the Myometrium of Pregnancy

SATISH BATRA

Department of Obstetrics and Gynecology, University Hospital, Lund, Sweden

ABSTRACT. By chemically digesting myometrium in a mixture of NaOH and sodium dodecyl sulphate, estradiol could be recovered almost completely by extraction with ethyl acetate. The concentration of estradiol-17β (E_2) in the extracted samples could reliably be determined by radioimmunoassay. Compared to its concentration in the plasma, E_2 in the pregnant human myometrium was very low, and as a result, the tissue/plasma estradiol concentration ratio was less than 0.5. In the pseudopregnant rabbit, this ratio ranged between 16 and 20. (*Endocrinology* 99: 1178, 1976)

A LARGE number of studies, in recent years, have shown binding of estrogens to target tissue (such as the uterus) proteins. There is also a vast amount of quantitative data available on the levels of estrogens in plasma of various animal species including the human. However, information about the concentration of estrogen in the myometrium and endometrium is very limited. To the best of my knowledge, no report on the level of estrogen in the human myometrium (but see (1)), and only a single recent report on the level of estradiol-17β (E_2) in the endometrium has been published (2). The lack of this important information is probably due to the fact that the estrogen concentration of the tissues is very low and until recently, before the advent of radioimmunoassays, methods were not sensitive enough to determine quantitatively the levels of estrogen in small samples of the material available.

Recently, a relatively simple method for the extraction of progesterone from the myometrium and its subsequent determination by radioimmunoassay has been described (3). By making minor modifications in that method, we were able to determine E_2 in small samples of human and rabbit myometria.

Materials and Methods

Tissues

Uterine pieces from human subjects were removed during deliveries by cesarean section or during hysterotomy operations and collected in ice-cold Krebs-Ringer bicarbonate solution (4). There was no significant loss in tissue E_2 when the tissue was left for as long as 1 h in this medium at 4 C.

After dissecting away the endometrium immediately after the collection of tissues, myometrial tissue samples weighing 200–400 mg were obtained and stored frozen at −20 C unitl used.

Tissue digestion and extraction of estradiol

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 as described previously (3). The digested material was extracted 3 times with 3 volumes of ethyl acetate. The combined extracts were evaporated to dryness under air at 40 C. Removal of SDS from the extracted samples, before radioimmunoassay, was accomplished by Sephadex LH-20 gel chromatography as described previously (3), except that 6 ml (Fig. 1) of the eluate was collected.

Radioimmunoassay

After evaporation of the collected (6 ml) eluate, the residue was dissolved in 1 ml of ethyl acetate and a suitable amount, depending on the predicted concentration of E_2, was taken for the radioimmunoassay. The procedure for radioimmunoassay was that described by Lindberg *et al.* (5), using the same antiserum except that a 1:100,000 dilution was used for tissue E_2 and rabbit plasma E_2 assays.

Uterine pieces obtained from pseudopregnant rabbits (hCG-induced) were processed in the same manner. When desired, an aliquot of the redissolved eluate can be used directly for the radioimmunoassay of progesterone since this

Received December 16, 1975.

Supported by the Ford Foundation and the Swedish Medical Research Council (project No. 4781).

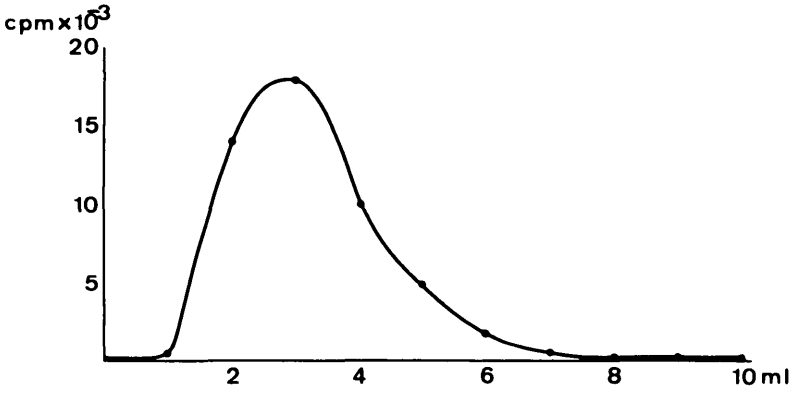

FIG. 1. Elution pattern of [^{3}H]-estradiol-17β from a Sephadex LH-20 microcolumn (Pasteur pipette, id 5 mm, 7.5 cm column height). After the introduction of 0.3 ml of the sample, which was contaminated with SDS, the column was eluted with ethyl acetate and the eluate was collected in 1 ml aliquots.

steroid is completely recovered in the first 3.5 ml of the eluate (3).

Chemicals

Sodium lauryl sulphate (SDS) and estradiol-17β were purchased from Sigma Chemical Company. Radioactive [2,4,6,7-^{3}H]estradiol-17β (114 Ci/mmol) was obtained from New England Nuclear Corporation. Human chorionic gonadotropin (Gonadex) was a gift of LEO, Sweden.

Results

The data of the recovery experiments were obtained by adding a known amount of authentic unlabeled E_2 and the recovery was calculated at the end of the completed assay procedure (3). There was almost complete recovery of E_2 added to the SDS (digestion mixture) alone or to rabbit myometrial samples digested in SDS (Table 1). The recovery of E_2 added to human myometrial samples was slightly lower (88.5%).

The precision was measured by replicate analyses of three different pools of tissues digested in SDS (Table 2). The concentrations of E_2 in these tissue pools ranged from 77 to 186 pg/ml, and the precision both for human and rabbit tissues was satisfactory.

The concentrations of E_2 in the myometrium and plasma of pregnant women and pseudopregnant rabbits are shown in Table 3. Mid-term myometrial tissues were those taken at abortions, performed after 18–20 weeks of pregnancy. E_2 concentration in the pregnant human myometrium at mid-term and at term was extremely low as compared to the respective levels in the plasma (Table 3). The ratio of tissue E_2 (per g) to plasma E_2 (per ml) was 0.48 and 0.13 at mid-term and term pregnancy, respectively. This ratio in the case of pseudopregnant rabbit varied between 16 and 20 (Table 3).

Discussion

The digestion of tissue with a mixture of NaOH and a cationic detergent (SDS) before extraction by ethyl acetate gave excellent results for recovery of uterine estradiol, as was found recently for uterine progesterone (3). In the two recently published methods for the determination of tissue estradiol in non-pregnant human endometrium (2) and

TABLE 1. Recoveries of estradiol-17β added in SDS or in tissue digested in SDS*

E_2 added to	n	Amount added (pg)	Amount recovered (pg)	Per cent recovery
SDS	6	200	189 ± 14.4†	94.5
Rabbit myometrium in SDS	7	200	199 ± 21.0	99.5
Human myometrium in SDS	6	200	177 ± 10.9	88.5

* SDS refers to the digestion mixture consisting of 5% sodium dodecyl sulfate and 0.5 N NaOH (see *Materials and Methods*).

† Mean ± SD.

TABLE 2. Replicate analyses of estradiol-17β in different pools of tissues

Tissue pool	n	Mean conc. in pool (pg/ml)	C.V.* (%)
Rabbit myometrium	8	135 ± 13.3†	9.9
Rabbit myometrium	6	186 ± 8.6	4.6
Human myometrium	5	77 ± 7.7	10.0

* Coefficient of variation.
† Mean ± SD.

pregnant rabbit myometrium (1), relatively low recoveries (about 65%) of added radiolabelled E_2 were reported. We also obtained not only low but variable recoveries of radiolabelled E_2 when tissue was homogenized either in water or in strong alkali instead of the alkali-detergent mixture used in the present method. With the present method, there was consistently a complete recovery of radiolabelled E_2. Since the endogenous E_2 can almost completely be recovered in the extract, the sensitivity of the assay depends entirely on the method used for the determination of the steroid in the extracted sample.

Relative to the plasma concentration, estradiol in the human myometrium was very low and as a result the tissue/plasma estradiol ratio was less than 0.5. There are no other data on the estradiol concentration in the human myometrium available in the literature. It has been shown that, for progesterone, the myometrium/plasma (M/P) ratio in mid-term pregnancy is about 2 and decreases to slightly less than 1 at term (3,6–8). Interestingly, in non-pregnant human endometrium the concentrations of a number of steroids measured (2) were significantly higher than those in the plasma. The endometrium/plasma estradiol ratio was 10 and 4 in proliferative and secretory phases, respectively (2).

Several reasonable explanations can be given for the low M/P ratio in the pregnant human. To begin with, a very low concentration of free (not protein-bound) E_2 in the plasma would lead to a low M/P ratio. Some evidence in support of this is available in the literature (9,11). Not only the per cent free E_2 in plasma (1 to 2% of total) is reported to be much lower than the corresponding fraction of free progesterone (6,9,10) but, as shown by Tulchinsky's data (9), it significantly decreased in pregnancy.

The second possibility for which some evidence also exists is that the binding of estradiol in the myometrium during pregnancy is insignificant or very much reduced. In fact, Henderson and Schalch (12) were unable to detect estrogen receptors in the pregnant human myometrium and endometrium in contrast to the non-pregnant tissues. It can be calculated from their data that even the non-specific binding of E_2 in the pregnant myometrium was considerably lower than in the non-pregnant tissue. It has also been shown recently that E_2 receptor levels in both myometrium (13) and endometrium (14) are significantly lower in the secretory phase than those in the proliferative phase. Furthermore, progesterone seems to be directly responsible for this effect (14). A combination of the above two possibilities could easily account for the low M/P ratio observed in pregnancy.

TABLE 3. Estradiol-17β concentration in the myometrium and plasma of pregnant women and pseudopregnant rabbits; values are means ± SD

Tissue and source	n	E_2 concentration		Ratio
		Tissue (ng/g)	Plasma (ng/ml)	Tissue/plasma
Myometrium, human (pregnant, term)	5	4.0 ± 0.53	31.1 ± 2.7	0.13 ± 0.01
Myometrium, human (pregnant, mid-term)	3	2.2 ± 0.70	4.4 ± 0.69	0.48 ± 0.10
Uterus, rabbit (pseudopregnant, 12th day)	4	0.5 ± 0.02	0.03 ± 0.003	16.2 ± 1.5
Uterus, rabbit (pseudopregnant, 18th day)	4	1.0 ± 0.09	0.05 ± 0.001	20.4 ± 1.5

Pseudopregnancy in rabbits was induced by an iv injection of 70 IU of hCG.

Finally, the entry of E_2 in the myometrial cell, in spite of high plasma concentrations, as a limiting step (15) together with the changes in the metabolism of E_2 occuring in pregnancy, may also contribute to the relatively low myometrial E_2 concentration.

Values for rabbit uterine E_2 concentration in pseudopregnancy shown in the present results (Table 3) are comparable to those recently reported for pregnant rabbit myometrium (1). It is also of interest that in the pseudopregnant rabbit (Table 3), as in the pregnant rabbit (1), the tissue/plasma estradiol ratio was very high, at least greater than 10 (1).

The striking difference in the M/P ratio between rabbit (>15) and human (0.1–0.4) may be due to a difference in the above-mentioned aspects of E_2 dynamics in the two species, although the metabolic aspect may be of greater importance in this respect. There is substantial evidence showing that the rabbit uterus possesses a very high activity for estradiol-estrone interconversion (16–18). Furthermore, the activity of 17β oxidoreductase, the enzyme responsible for this interconversion, increases many times during pregnancy (16) and the rabbit appears to be rather exceptional, among other laboratory animals, in this respect. Conflicting data on the activity of this enzyme in the human myometrium, particularly with respect to the influence of progesterone, have recently been reported (19–22). Krishnan *et al.* (19) reported that there was a higher activity in tissues in the proliferative phase which, is contradictory to the extensive data published in previous studies (20–22).

Acknowledgments

I am greatly indebted to Professor Lars Philip Bengtsson for his support and encouragement. Mrs. Kristina Källstrand, Mrs. Bodil Nilsson and Mrs. Iréne Larsson provided excellent technical assistance. Antiserum for radioimmunoassay was kindly supplied by Dr. Kjell Martinsson.

References

1. Challis, J. R. G., I. J. Davies, and K. T. Ryan, *Endocrinology* **95:** 160, 1974.
2. Guerrero, R., B. -M. Landgren, R. Mortiel, Z. Cekan, and E. Diczfalusy, *Contraception* **11:** 169, 1975.
3. Batra, S., and L. Ph. Bengtsson, *J Steroid Biochem* (In Press).
4. Batra, S., and E. E. Daniel, *Can J Physiol Pharmacol* **48:** 768, 1970.
5. Lindberg, B. S., P. Lindberg, K. Martinsson, and E. D. B. Johansson, *Acta Obstet Gynecol Scand* [*Suppl*] **32:** 5, 1974.
6. Batra, S., L. Ph. Bengtsson, H. Grundsell, and N. -O. Sjöberg, *J Clin Endocrinol Metab* **42:** 993, 1976.
7. Runnebaum, B., and J. Zander, *Acta Endocrinol* [*Suppl*] (*Kbh*) **150:** 5, 1971.
8. Brummer, H. C., and W. P. Collins, *J Obstet Gynaecol Br Commonw* **79:** 985, 1972.
9. Tulchinsky, D., *J Clin Endocrinol Metab* **36:** 1079, 1973.
10. Tulchinsky, D., and D. M. Okada, *Am J Obstet Gynecol* **121:** 293, 1975.
11. Fisher, R. A., and D. C. Anderson, *Steroids* **24:** 809, 1974.
12. Henderson, S. R., and D. S. Schalch, *Am J Obstet Gynecol* **112:** 762, 1972.
13. Illingworth, D. V., G. P. Wood, G. L. Flickinger, and G. Mikhail, *J Clin Endocrinol Metab* **40:** 1001, 1975.
14. Tseng, P., and E. Gurpide, *J Clin Endocrinol Metab* **41:** 402, 1975.
15. Milgrom, E., M. Atger, and E. E. Baulieu, *Biochim Biophys Acta* **320:** 267, 1973.
16. Jütting, G., *Acta Endocrinol* [*Suppl*] (*Kbh*) **145:** 64, 1970.
17. Dennis, P. M., A. Hughes, and G. H. Thomas, *J Endocrinol* **40:** 257, 1968.
18. Macartney, J. C., and G. H. Thomas, *J Endocrinol* **43:** 247, 1969.
19. Krishnan, A. R., B. K. Bajaj, V. Hingoroni, and K. R. Laumas, *Acta Endocrinol* (*Kbh*) **80:** 719, 1975.
20. Gabb, R. G., and G. M. Stone, *J Endocrinol* **62:** 109, 1974.
21. Tseng, L., and E. Gurpide, *Endoncrinology* **94:** 419, 1974.
22. Pollow, K., H. Lübbert, E. Boguoi, G. Kreutzer, R. Jeske, and B. Pollow, *Acta Endocrinol* (*Kbh*) **79:** 134, 1975.