Pergamon

0960-0760(94)00177-4

J. Steroid Biochem. Molec. Biol. Vol. 52, No. 3, pp. 277–280, 1995

0960-0760/95 $9.50 + 0.00

Hydrolysis of Estrone Sulfate in Uterine Minces of the 6-Days Pregnant Rat*

Maria Cristina Loza

Unidad de Investigación Médica en Biología de la Reproducción, con sede en el Hospital de Gineco obstetricia "Luis Castelazo Ayala", Subjefatura de Investigación Biomédica, Jefatura de Investigación Médica, IMSS. Apartado Postal 73-032, México, D.F. 03020, México

In view of the possible participation of estrogen 3-sulfoconjugates in reproductive events, such as reproductive cycles, gestation and parturition, an experiment was carried out where the conversion of labeled estrone sulfate ([^{3}H]E_1S) to estrone ([^{3}H]E_1) was measured during *in vitro* incubation with minced uterine tissues representing implantation sites (IS) and non-implanted areas (NIS), from pregnant rats at the time of blastocyst implantation. Significant hydrolysis of the 3-sulfate, by the action of the estrogen sulfatase, was found in both tissues being less in IS than in NIS, when expressed either as pmol of E_1 formed/mg wet tissue/h (238 ± 37 vs 337 ± 15, respectively) or as pmol of E_1 formed/mg protein/h (1278 ± 198 vs 1773 ± 81). Both differences are statistically significant at the 0.001 level. The results obtained here suggest that E_1S present in uterine fluids may be taken up and hydrolyzed by the sulfatase present in both intrauterine tissues of the 6-days pregnant rat. However, the decreased formation of E_1 found in IS suggests that rat blastocyst is able to regulate the local concentration of unconjugated estrogens required at IS by modulating the activity of the estrogen sulfatase.

J. Steroid Biochem. Molec. Biol., Vol. 52, No. 3, pp. 277–280, 1995

INTRODUCTION

In early pregnancy, the hormonal and biochemical composition of embryo and uterine secretions, creates an appropriate environment for blastocyst establishment [1]. Deficiencies in these properties impair implantation and account for embryonic mortality [2] and infertility.

The hormonal requirements for implantation vary among mammals [3]. In the rat, the synergistic action of estradiol (E_2) and progesterone is essential [4]. Ovariectomy before day 4 of pregnancy and daily treatment with 2 mg progesterone results in dormancy of the blastocysts which are maintained unattached in the uterus; implantation can be initiated, however, by a single i.v. dose of E_2. The estrogen dose is critical under these conditions [5]; 20 ng E_2 induces a full number of implantation sites (IS) but this number is reduced by lower doses [5]. With higher doses some protein secretions needed for embryo development are inhibited creating an embryotoxic milieu [6] preventing implantation.

Recent studies [7, 8] show that preimplanted embryos synthesize steroid hormones; among these, estrogens are of special interest because of their importance in the process of implantation. It has been observed that certain parameters which are sensitive to an estrogenic stimulus show a marked change at IS, including increases in the permeability of endometrial capillaries [4], in nuclear receptors for E_2 and progesterone [9], in the translocation of these receptors from cytosol to the nucleus [10] and differences in the activities of several enzymes such as estrogen sulfatase, which hydrolyzes estrogen 3-sulfates, and 17β-hydroxysteroid oxidoreductase, responsible for the reciprocal conversion $E_1 \rightleftarrows E_2$.

Steroid sulfates participate in several biological processes such as reproductive cycles, sperm capacitation and gestation [11]. Estrone sulfate (E_1S) is the predominant estrogen in the plasma of cycling [12], pre and postmenopausal women [13]. During pregnancy, this sulfoconjugate is the major plasma estrogen in the mother as well as in their female fetuses [14]. Previous *in vivo* and *in vitro* studies, have shown that some E_1S

*To avoid further delays in publication this paper has been published without the author's corrections.

Received 21 Mar. 1994; accepted 8 Nov. 1994.

can be hydrolyzed into E_1 by the uterine estrogen sulfatase, the activity of which is inhibited by free estrogens [15, 16] and increased by adenine nucleotides [16], representing a regulatory mechanism of free estrogen levels, since liberated E_1 is quickly converted into E_2 by the oxidoreductase. It has also been found that the conversion of E_1S to E_1, measured in homogenized uterine tissues of the 6-days pregnant rat, can be locally modulated in IS by estrogens, possibly secreted by the embryo [17].

Since it has been postulated that E_1S can be hydrolyzed by the sulfatase in the process of transport across cell membranes, this study employed uterine minces as a model system, in order to acquire more information about the uptake and metabolism of estrogen sulfates in the uterus of the pregnant rat, as well as about the role of the estrogen sulfatase in regulating the local concentration of E_2 at the site of implantation.

EXPERIMENTAL

Chemicals

[6,7-^{3}H]estrone sulfate 53 Ci/mmol, was purchased from New England Nuclear (Boston, MA) and its radiochemical purity was confirmed by TLC in the system toluene–dioxane–acetic acid (20:10:1) before use. Unlabeled E_1S, ADP and ATP were obtained from Sigma (St Louis, MO).

Animals and treatment

Adult Long–Evans rats (200 g average weight) were housed in groups of two with one male of the same strain and were maintained under controlled temperature and light–dark conditions. Daily vaginal smears were taken in order to detect spermatozoa and then it was considered the first day of pregnancy. On day 6, pregnant rats were injected (i.v.) with 1 ml 1% trypan blue in saline, and 30 min later they were killed by cervical dislocation under anesthesia; uteri were immediately removed, cleaned, opened longitudinally and placed at 0°C. The blue stained areas representing the IS [4] were separated from the non-stained tissue considered as non-implanted sites (NIS) and processed separately. Tissues were minced in 10 ml of ice-cold Ringer's medium, filtered through a nylon filter and weighed. Four pregnant rats were used in each experiment.

Incubation

The radioactive substrate (300,000 dpm of [6,7-^{3}H]-estrone sulfate) was placed in the bottom of incubating tubes, adjusting the mass to 40 nmol by the addition of 0.1 ml cold E_1S solution. Minced tissue (20 mg) and 2 ml Krebs–bicarbonate buffer, pH 7.2, were added to the tubes.

A stimulatory effect upon estrogen sulfatase activity was previously found when both ATP and NAD were present in the incubation medium [16]. Therefore, 0.2 ml buffer containing both adenine nucleotides was included, in such an amount that the final medium (2.0 ml) contained 1.6 and 3.0 mM of each cofactor, respectively. A similar effect was previously observed by Dominguez *et al.* [18] with the adrenal steroid sulfatase. In order to avoid transformation of E_1 formed to more polar estrogens, all tubes were placed in a Dubnoff incubator at 37°C for 2 h, under nitrogen atmosphere and without the addition of NADH or NADPH. Blanks without tissue were included in each determination.

The reaction was stopped by heating for 3 min in a boiling water bath and tissues were then homogenized using the same incubation medium. Free [^{3}H]E_1 formed from [^{3}H]E_1S in the experimental and control tubes was extracted as described by Burstein [19], using a scintillating solvent (50 mg POPOP and 4 mg PPO dissolved in 1 l toluene), and then quantified. Lowry's method [20] was used to determine the protein concentration. Statistical comparisons were made using Student's t-test.

RESULTS

On day 6 of pregnancy, the increased permeability of the endometrial blood vessels around the blastocyst could be visualized 30 min after the i.v. administration of 1% trypan blue solution [4]. The blue stained areas which represent IS were distributed along both horns. An average of 5 implanted sites was found when using the Long–Evans strain. Because the incubations were carried out under nitrogen atmosphere and NADPH was omitted in the incubation media, to avoid transformation of E_1 to E_2 and more polar estrogens, E_1 accumulated in all experiments as the major product [17].

Figure 1 shows the effect of substrate concentration on the hydrolysis of E_1S by the action of the uterine estrogen sulfatase present in minced NIS of the 6-days pregnant rat. The comparative degree of hydrolysis of [^{3}H]E_1S by the action of the estrogen sulfatase present in both minced intrauterine tissues, at the time of blastocyst implantation is illustrated in Table 1. One

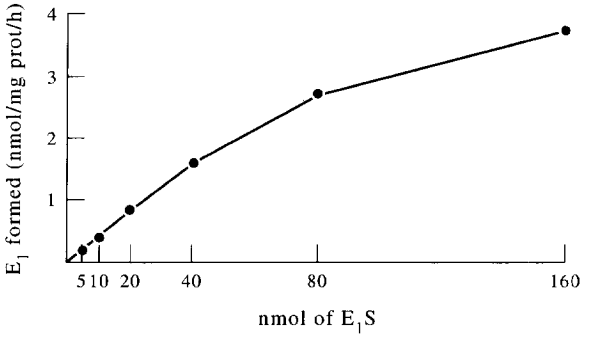

Fig. 1. Effect of substrate concentration on the formation of E_1 by the action of the uterine estrogen sulfate present in NIS at the time of blastocyst implantation points represent the average of 2 individual experiments.

Table 1. Hydrolysis of [^{3}H]E_1S by the action of the estrogen sulfatase in uterine minces of the 6-days pregnant rat

	[^{3}H]E_1 formed pmol/mg tissue/h			[^{3}H]E_1 formed pmol/mg prot./h		
Tissue	$\bar{X}$	SD	Decrease*	$\bar{X}$	SD	Decrease*
NIS	337	15		1773	81	
IS	238	37†	29%	1278	198†	28%

Results are means of 6 assays where IS and NIS obtained from 4 rats were pooled separately.

*Decrease in the activity of the estrogen sulfatase present in IS when compared with that found in NIS.

†$P < 0.001$ when compared with NIS.

can see that the corresponding mean sulfohydrolase activity found in the implanted area was lower than that in NIS, when expressed either as pmol/mg wet tissue/h or as pmol/mg protein/h. These values represent decreases of 29 and 28%, respectively.

DISCUSSION

E_1S has been shown to be an important prehormone and storage form of E_1 and E_2 [21]. In a series of *in vivo* and *in vitro* studies realized in this laboratory, it has been demonstrated that E_1S can be partially hydrolyzed and converted into E_1 by the uterine estrogen sulfatase, the activity of which can be increased by adenine nucleotides [16] and inhibited by free estrogens, mainly E_2 [15–17]. This enzyme appears to be under an estrogen-dependent biological control since, during the estrous cycle of the rat, the highest sulfatase activity is present during diestrus when the ovarian secretion of E_2 is low; while the lowest is found in proestrus when plasma estrogen levels are high [15]. Furthermore, in ovariectomized rats, where most of the circulating estrogens are removed, the enzyme activity is significantly higher than in intact rats [16]. Treatment of ovariectomized animals with E_2 results, on the other hand, in a significant decrease of the estrone sulfatase activity [16].

Based on the above findings, it was considered that uterine tissue of rats contains estrogen sulfatase activity which may be of biological importance at the time of blastocyst implantation, where synthesis and release of estrogens by the embryo can locally modulate E_2 production from sulfated precursors. In a recent study, the conversion of E_1S to E_1 was measured at implantation in crude whole tissue homogenates of IS and NIS from 6-day pregnant rats [17]. The formation of E_1 was noticeably decreased (29.31%) in IS than in NIS. The present study has extended this finding and has employed minced uterine tissues as a model system. The results obtained in these experiments show uptake and hydrolysis of E_1S by both regions of the uterus, being statistically less in IS than in NIS when expressed either as pmol of E_1 formed/mg wet tissue/h (29%) or as pmol of E_1/mg protein/h (28%). In this study, the earlier report on estrogen sulfatase activity at the time of blastocyst implantation has been confirmed. Although it is at present difficult to judge the physiological importance of these findings, evidence is presented to suggest that the embryo is able to modulate the degree of hydrolysis of E_1S by the estrogen sulfatase activity, in order to regulate the local concentration of E_2 needed for its own implantation, possibly through the synthesis and secretion of unconjugated estrogen which has been shown to inhibit the sulfohydrolase activity. Therefore, based on the decreased sulfatase activity found in IS, it is reasonable to consider that part of E_1S that reaches the uterus remains as a sulfoconjugate within the area adjacent to the blastocyst, as a protective mechanism against embryotoxic quantities of E_2 [6].

Acknowledgement—The author wishes to thank Mrs Ana Lidia Canseco Flores for helpful secretarial assistance.

REFERENCES

1. Loza-Arredondo M. C. and Hicks J. J.: Influencia del medio tubario y uterino en el desarrollo embrionario. *Ginec. Obstet. Mex.* 58 (1990) 245–250.
2. Edmonds D. K., Lindsay K. S., Miller J. F., Williamson E. and Wood P. J.: Early embryonic mortality in women. *Fertil. Steril.* 38 (1982) 447–453.
3. Loza A. M. C.: Requerimientos hormonales para la implantación del blastocisto. *Ginec. Obstet. Mex.* 59 (1991) 347–352.
4. Psychoyos A.: Hormonal control of ovoimplantation. *Vitamin Horm.* 31 (1973) 201–256.
5. Johnson D. C. and Dey S. K.: Role of histamine in implantation: dexamethasone inhibits estradiol-induced implantation in the rat. *Biol. Reprod.* 22 (1980) 1136–1141.
6. Thijssen J. H.: Estrogen in tissues: uptake from the peripheral circulation or local production. *Steroids* 50 (1987) 1–3.
7. Loza A. M. C. and Hicks J. J.: Capacidad endócrina del blastocisto de mamífero I. Biosíntesis y metabolismo de progesterona. *Ginec. Obstet. Mex.* 58 (1990) 310–314.
8. Loza A. M. C.: Capacidad endócrina del blastocisto de mamífero II. Biosíntesis y metabolismo estrogénico. *Ginec. Obstet. Mex.* 61 (1993) 86–91.
9. Logeat F., Sartor P., Vuttai M. T. and Milgrom E.: Local effect of the blastocyst on estrogen and progesterone receptors in the rat endometrium. *Science* 207 (1980) 1083–1085.
10. Khan-Dawood F. S. and Dawood M. Y.: Implantation in the rabbit blastocyst: sequential changes in estradiol and progesterone and their receptors. *Am. J. Obstet. Gynec.* 150 (1984) 450–455.
11. Hobkirk R.: Steroid sulfotransferases and steroid sulfate sulfatases: characteristics and biological roles. *Can. J. Biochem. Cell Biol.* 63 (1985) 1127–1144.
12. Platia P. M., Fencl M., Elkind-Hirsch K., Canick J. A. and Tulchinsky D.: Estrone sulfatase activity in the human brain and

estrone sulfate levels in the normal menstrual cycle. *J. Steroid Biochem.* **21** (1984) 237–241.
13. Reed M. J., Cheng R. W., Noel C. T., Dudley H. A. F. and James V. H. T.: Plasma levels of estrone, estrone sulfate and estradiol and the percentage of unbound estradiol in postmenopausal women with and without breast disease. *Cancer Res.* **43** (1983) 3940–3943.
14. Pasqualini J. R., Sumida C., Nguyen B. L., Tardy J. and Gelly C.: Estrogen concentrations and effect of estradiol on progesterone receptors in the fetal and newborn guinea-pigs. *J. Steroid Biochem.* **12** (1980) 65–72.
15. Loza M. C. and Hicks J. J.: Inhibición de la estrógeno sulfatasa uterina por estrógenos libres. *Rev. Iberoam. Reprod. Hum.* **VI** (1989) 129–133.
16. Loza M. C., Valencia A. and Hicks J. J.: Uterine estrogen sulfatase activity. Influence of steroid hormones and adenine nucleotides. *J. Steroid Biochem.* **36** (1990) 301–303.
17. Loza M. C. and Hicks J. J.: Uterine estrogen sulfatase activity at the time of blastocyst implantation in the rat. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 45–49.
18. Domínguez O. V., Valencia S. A. and Loza A. C.: On the role of steroid sulfates in hormone biosynthesis. *J. Steroid Biochem.* **6** (1975) 301–309.
19. Burstein S. and Dorfman R. I.: Determination of mammalian steroid sulfatase with 3-^{3}H-3β-hydroxy-androst-5-en-17-one-sulfate. *J. Biol. Chem.* **238** (1963) 1656–1660.
20. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193** (1951) 265–275.
21. Pasqualini J. R., Lanzone A., Tahri-Joutei A. and Nguyen B. L.: Effect of seven different oestrogen sulphates on uterine growth and on progesterone receptor in the foetal uterus of guinea-pig after administration to the mother. *Acta Endocr. Copenh.* **101** (1982) 630–631.