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UTERINE ESTROGEN SULFATASE ACTIVITY AT THE TIME OF BLASTOCYST IMPLANTATION IN THE RAT

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Summary—The conversion of estrone sulfate (E_1S) to estrone (E_1) was measured during the *in vitro* incubation of the labeled sulfoconjugate with implantation sites (IS) and nonimplanted regions (NIS) of uterine horns from 6-day pregnant rats. Extensive metabolism of E_1S occurred in both tissues, being noticeably less (29.31%) in IS than in NIS. Estrogen sulfatase activity present in the uterus of ovariectomized virgin rats was found to be higher than in both uterine regions of the pregnant rats. We suggest that E_1S present in uterine fluids may be accessible to be metabolized into unconjugated estrogens by both intrauterine tissues of 6-day pregnant rats. This metabolism could be locally modulated in IS through the participation of the estrogen sulfatase, the activity of which is in turn controlled by the presence of free estrogens, possibly synthesized and/or secreted by the embryo, which has been shown to inhibit the sulfohydrolase activity.

INTRODUCTION

In order for blastocyst implantation to occur in the rat, the endometrium must be adequately differentiated, a state which results from the sequential interaction of progesterone and estrogens [1]. Ovariectomy before the presumed estrogen surge on day 4 of pregnancy results in dormacy of the blastocyst and delayed implantation [2]. Implantation can be initiated, however, if the animals are pretreated with progesterone followed by a single injection of estradiol-17β (E_2) [2]. This model has been shown to be very sensitive for testing the estrogenic importance in the process. The biochemical composition of the oviductal and endometrial fluid also has an eminent role [3].

On the other hand, it is now well acknowledged that the embryo participates actively in the early events associated with its own implantation [4]. There is data that shows that the presence of the embryo locally influences protein synthesis [5], membrane properties [6], electrical conductivity, cAMP and cGMP concentrations [7] and adenylate cyclase activity [8] at the implantation sites (IS). Nuclear receptors for E_2 and progesterone are present in 2-fold concentrations in these regions [9].

Steroid sulfates participate in several metabolic processes. They are considered as storage forms in steroid biosynthesis and metabolism in endocrine [10] and hepatic tissues [11].

During pregnancy, estrogen sulfates are the major circulating estrogens in the mother as well as in their female fetuses [12]. On the other hand, it has been proven that estrone sulfate (E_1S) is an important prehormone and storage form of estrone (E_1) and E_2 [13, 14]. Its presence was reported several years ago in plasma and endocrine tissues including the uterus.

A specific effect of E_1S on the endometrial cell surface related to variations in its secretory capacities has been reported [15]. However, the possible role of this sulfoconjugate in the free estrogen production at IS and the mechanisms controlling its local levels have not been considered to date.

In earlier *in vivo* and *in vitro* studies, we have shown that E_1S can be partially hydrolyzed into E_1 by the uterine estrogen sulfatase, the activity of which can be inhibited mainly by free estrogens [16, 17] and increased by adenine nucleotides [17], representing a regulatory mechanism of free E_2 levels within the uterine tissues, since liberated E_1 can be transformed into E_2 by the 17β-hydroxysteroid oxidoreductase.

Recent studies, reviewed by the present authors [18], point out that blastocysts of several species have the endocrine capacity to synthesize and secrete steroids including E_2.

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This suggested to us that such local steroid production may have some influence upon estrogen sulfate metabolism in the adjacent endometrium.

In this study, it is our purpose to determine the possible influence of the rat blastocyst upon the uterine estrogen sulfatase activity in order to regulate the local concentration of E_2 required at its IS.

EXPERIMENTAL

Chemical

[6,7-^{3}H(N)]E_1S, ammonium salt (53 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Unlabeled E_1, E_1S, ADP and ATP were obtained from Sigma (St Louis, MO). The purity of the sulfoconjugates was confirmed by TLC in the system toluene–dioxane–acetic acid (20:10:1) prior to use.

Animals and treatment

Virgin adult rats of the Long–Evans strain with an average weight of 200 g and having regular estrous cycles, determined by daily vaginal smears, were used under controlled light–dark conditions; water and pellet food were supplied *ad libitum*.

The animals were divided into two groups. The rats in group A were anesthetized with phenobarbital and subjected to bilateral ovariectomy; 20 days after surgery, they were used as a control. Group B consisted of pregnant rats. These were obtained after housing the females in groups of 2, with 1 male of the same strain. They were kept under the same conditions as group A. Daily vaginal smears were taken; the first day of pregnancy was arbitrarily designated when spermatozoa were found in the frotis and males were then removed. On day 6, pregnant rats were injected (i.v.), between 9 and 11 a.m., with 1 ml of 1% trypan blue in saline [1]; 30 min later, they were sacrificed by cervical dislocation and uteri were immediately obtained, cleaned, slit longitudinally and placed at 0°C. The blue stained regions representing the IS [1] were separated from the nonstained tissue considered as nonimplanted regions (NIS). They were pooled, weighed and processed separately. Pooled tissue obtained from 4 rats was considered as one experiment.

Incubation

Different tissues were homogenized in a Krebs–bicarbonate buffer, pH 7.2, containing 30 mM nicotinamide, in such a way that 0.8 ml contained 10 mg of uterine tissue; 0.8 ml aliquots were added to the tubes containing 300,000 dpm of [^{3}H]E_1S adjusting their specific activities, according to the experiment, by the addition of 0.1 ml unlabeled E_1S solutions.

In a previous report [17], we found that a stimulatory effect upon uterine estrogen sulfatase activity was obtained when the combination of ATP and NAD was present in the incubation medium. Therefore, 0.2 ml of the buffer containing both adenine nucleotides were included in all determinations in such an amount that the final media (2 ml) contained 1.6 and 3.0 mM of each cofactor, respectively. Dominguez *et al.* [19] reported a similar effect when measuring the adrenal steroid sulfatase. In order to avoid further transformation of E_1 to more polar estrogens all incubations were left at 37°C for 2 h without the addition of exogenous NADH or NADPH. Blanks without enzyme or with boiled enzyme were included in each determination.

At the end of the incubation period, the reaction was stopped by heating for 3 min in a boiling water bath. The [^{3}H]E_1 liberated was extracted with a scintillating solvent (50 mg POPOP, 4 g PPO dissolved in 1 l toluene) as described by Burstein [20]; Lowry's method [21] was used to determine the protein concentration. The statistical difference between means was assessed by the two-tailed nonpaired *t*-test.

RESULTS

Preliminary experiments demonstrated that the uterine estrogen sulfatase activity, measured as the amount of [^{3}H]E_1 liberated from 40 nmol of [^{3}H]E_1S at the experimental conditions described above, was directly proportional to the incubation time, up to 120 min. Since the incubations were performed without the addition of NADPH, to avoid further transformation of E_1 to E_2 and more polar estrogens, E_1 was accumulated as the major product formed.

Table 1, shows the results obtained when the uterine estrogen sulfatase activity was measured at the time of blastocyst implantation; the amount of [^{3}H]E_1 formed from all concentrations of the labeled sulfoconjugate used in the experiment was significantly higher in NIS than in IS. These values are plotted against the various concentrations of [^{3}H]E_1S used as substrate, from 5 to 160 nmol (Fig. 1).

Table 1. Uterine estrogen sulfatase activity at the time of blastocyst implantation

Cold E_1S added (nmol)	E_1 formed (pmol/mg tissue/h)		Decrease[a] (%)
	IS	NIS	
5	27.6	40.2	31.3
10	54.6	76.7	28.8
20	105.3	151.1	30.3
40	200.8	290.7	30.9
80	385.8	529.7	27.2
160	611.3	807.0	24.3

Results are means of 2 assays where IS and NIS obtained from 4 rats were pooled separately.
[a]Values represent the decrease in the sulfatase activity found in IS when compared with NIS.

The results presented in Table 1 were obtained from the experiments where tissue homogenates of the control group (20 days ovariectomized rats) as well as those of IS and NIS were incubated in parallel with 300,000 dpm of $[^3H]E_1S$ equivalents to 40 nmol. Extensive metabolism was found in all tissues; however, both intrauterine tissues of the pregnant animals showed an inhibitory effect on the estrogen sulfatase activity when compared with that of the ovariectomized group (control), 31.28 and 51.33% in NIS and IS, respectively. On the other hand, the corresponding mean enzyme activity found in the implanted areas of the uterus showed a decrease of $29.31 \pm 5.71\%$ compared with that found in NIS.

DISCUSSION

For blastocyst implantation to be initiated, preparation of the endometrium and embryo development must be concerted in a very close manner. In this process, where steroid hormones have an important role, the biochemical composition of the uterine secretions and the passage of diffusible substances, operating in both directions, let the embryo establish in an embryotrophic environment, different from that of serum [3]. Deficiencies in the biochemical properties of this nutrient pool impair implantation and account for embryonic mortality [22].

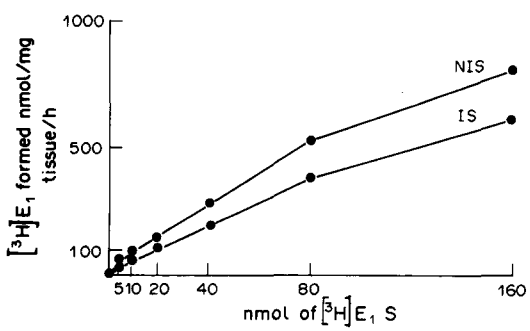

Fig. 1. Comparative degree of desulfation of $[^3H]E_1S$ by the action of the uterine estrogen sulfatase present in IS and NIS of six-days pregnant rats.

In the rat, maternal estrogens are essential for embryo implantation in a progesterone primed uterus [1]. We have known for some time that blastocysts of hypophysectomized and/or ovariectomized rats are maintained unattached in the uterus, if 2 mg daily injections of progesterone are administered; implantation only occurs following a minute dose of E_2. At this point, the estrogen concentration is critical [23, 24]. An i.v. pulse (20 ng) of E_2 induces a full number of IS 18 h later; using lower doses, the number of sites is reduced [24]. On the other hand, high doses of E_2 are shown to be embryotoxic [25] and to inhibit progesterone induction of protein secretions needed by the blastocyst.

It has been postulated that the endometrial functions are influenced by blastocyst synthesis and/or release of estrogens. This estrogen is of great importance in the establishment of pregnancy in mammals; however, its precise role in this process is still debated. Various histological, ultrastructural and biochemical modifications occur at the IS, including the decreased capability to bind E_2 [26] and the increase of nuclear receptors for both progesterone and E_2 [9]. The translocation of these receptors from the cytosol to the nucleus is thought to be mediated by the steroids from embryonic origin [27].

E_1S has been considered to be a prehormone in several mammalian tissues including the uterus which releases unconjugated estrogens after hydrolysis by the estrogen sulfatase [13, 14]. In this paper we demonstrate that the rat blastocyst produces an inhibition of the estrogen sulfatase activity (Fig. 1). At the various substrate concentrations used in the experiment, the amount of E_1 liberated from E_1S was significantly less in IS than NIS (Table 1).

We recently found a similar effect in *in vitro* [16] and *in vivo* [17] studies. When using different doses of E_2 administered to ovariectomized rats, the estrogen sulfatase activity was noticeably reduced, suggesting that this sulfatase is a hormone-dependent enzyme which may play a role in the control mechanisms of free estrogen levels within the uterus. Whenever most of the circulating estrogens were removed, by means of ovariectomy, a higher sulfohydrolase activity was obtained when compared with both intrauterine tissues during blastocyst implantation (Table 2).

Table 2. Estrogen sulfatase activity present in the uterus of ovariectomized virgin and pregnant rats on day 6

Number of experiments	Group	E_1 formed (nmol/mg protein/h) $\bar{X}$	SD	Decrease[a] (%)
12	Control[b]	19.50	0.98	—
6	NIS	13.40	1.00[c]	31.28
6	IS	9.49	1.26[cd]	51.33

[a]Decrease in the activity of the estrogen sulfatase when compared with the control group. The values found in IS represent a decrease of 29.31% ± 5.71 of those found in NIS.
[b]Ovariectomized virgin rats, 20 days before the experiment.
[c]< 0.001 when compared with control.
[d]< 0.005 when compared with NIS.

According to our results (Table 2) it is feasible to consider that free estrogens may locally regulate the ratio of free to sulfated estrogens through the control of the estrogen sulfatase activity. These estrogens could possibly come from the blastocyst and from those crossing the capillaries and diffuse in the intercellular space, due to the increased permeability of this area. Therefore, we have considered that if part of the E_1S that reaches the uterus remains as sulfoconjugate within the IS, due to the decreased activity found in this tissue, it may represent a protective mechanism against large quantities of E_2. The latter is important because E_2 has been shown to be embryotoxic [25].

Another hypothesis is that the decreased enzymatic activity found in IS allows a higher tissular concentration of E_1S, which may be related with its local effects. *In vivo*, this sulfoconjugate is an active uterotrophic agent which induces an increase in progesterone receptors [28] and protein synthesis [29]. E_1S also has a specific effect on the surface of the endometrial cells, different from that of E_2, which resembles the secretory process [15].

A very recent study provided the first description of the effect of E_1S on the sulfation of endometrial proteins [30]. This sulfoconjugate, as well as E_2, increases the sulfation of endometrial-associated proteins as well as proteins released into the uterine secretions.

The sulfate incorporation induced by E_1S is carried out toward tyrosine and carbohydrates residues of glycoproteins and differs from that observed with E_2. This finding constitutes a significative fact since sperm migration and blastocyst final differentiation take place in the uterine fluid. The local effects of E_1S on the characteristics of the uterine secretions related with blastocyst metabolism and implantation must, however, be demonstrated.

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