INFLUENCE OF ESTROGEN, PROGESTERONE AND ESTROUS CYCLE ON γ-GLUTAMYLTRANSPEPTIDASE OF RAT ENDOMETRIUM

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1. Introduction

The endometrium of rat uterus undergoes a variety of histophysiological changes during estrous cycle, pregnancy and lactation. These alterations, governed by the actions of endogenous steroids, exhibit characteristic biochemical features. Of the metabolic changes, altered syntheses of enzymes are more pronounced in the endometrium [1–4]. Gamma-glutamyltranspeptidase (GGT; EC 2.3.2.2), a membrane-bound enzyme catalysing the transfer of γ-glutamyl moiety of glutathione to amino acid, is detectable in a variety of mammalian tissues [5]. Biochemical and histological changes related to implantation are more pronounced in the endometrium and our earlier investigation noted the rise of GGT activity in the endometrium during deciduoma progression and higher activity in the implantation areas of uteri from gravid rats [4]. Since endogenous hormones related to pregnancy have a major influence on the heterogeneous population of endometrial cells, it was interesting to study the influence of exogenous estrogen and progesterone on endometrial GGT of ovariectomized rats. We report here that administration of estrogen or progesterone to rats 15 days after ovariectomy enhances GGT activity in the endometrium. Response to estrogen is noticeable earlier than that to progesterone. In addition, data on endometrial enzyme activity of normal cycling rats are also presented.

2. Materials and methods

2.1. *Animals*

Six-week-old Wistar rats were bilaterally ovariectomized and maintained under laboratory conditions for 15 days prior to hormone treatment. Groups of ovariectomized rats were administered subcutaneously either estrogen, dissolved in 10% alcohol (v/v in normal saline), at a dose of 10 μg/kg body wt or progesterone, dissolved in 70% alcohol (v/v in normal saline), at a dose of 15 mg/kg body wt every 24 h up to 4 days. Ovariectomized control animals received the respective vehicles only. For studies on the influence of estrous cycle on endometrial GGT, 8-week-old intact rats were used.

2.2. *Chemicals*

L-γ-Glutamyl-*p*-nitroanilide, estrogen (β-estradiol) and progesterone (4-pregnene-3,20-dione) were purchased from Sigma, USA. Other chemicals were obtained from BDH, England.

2.3. *Enzyme assay*

Animals were killed by cervical fracture and uteri were excised and chilled immediately. Further operations were carried out at 0–4°C. Uterine horns were slit with a razor blade and endometrium was scraped out, free from myometrium. Endometrial mass was homogenized in a small volume (0.5 ml) of 0.05 M Tris–HCl buffer (pH 7.5) and the enzyme was assayed by incubating an aliquot of the homogenate at 37°C for 5 min with buffered substrate containing 4.4 mM L-γ-glutamyl-*p*-nitroanilide, 22 mM glycylglycine and 11 mM $MgCl_2$ in 0.05 M Tris–HCl (pH 8.5). The reaction was terminated by the addition of 1.5 N acetic acid and the nitroaniline released was measured at 405 nm against a reagent-tissue blank [4,6]. Protein was estimated by the Lowry method [7].

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3. Results and discussion

The results of the present investigation demonstrate the influence of exogenous hormones on GGT activity of rat endometrium. GGT is barely detectable in the myometrium [4] and hence, in the present investigation, enzyme activity has been measured only in the endometrium. Enhanced GGT accompanies the characteristic growth reactions of the uterus following estrogen treatment. Table 1 presents data on the levels of endometrial enzyme activity in estrogen treated rats. As early as 24 h post administration of estrogen, GGT activity is enhanced in the endometrium of ovariectomized rats. A further increase is noticeable on receipt of multiple doses of estrogen. GGT activity in the endometrium of cycling rats shows a peak at estrous (table 2). The enzyme activity at other stages of the cycle, however, does not show a significant difference. Luminal epithelium, glandular epithelium and stroma, constituting the heterogeneous population of endometrial cells, undergo cellular alterations following exposure to estrogen [8] and the enhanced enzyme activity at estrous can be attributed to the action of endogenous estrogen. A similar increase in endometrial lactate dehydrogenase activity at estrous has been attributed to the action of estrogen [1]. This enhancement has been demonstrated in rats receiving exogenous estrogen too [3,9]. Administration of estrogen, however, does not elicit a similar response with other endometrial enzymes. Enzymes such as isocitrate dehydrogenase, acid phosphatase, alkaline phosphatase and glutamate dehydrogenase have been reported to decrease in the endometrium of ovariectomized rats treated with ethinylestradiol [3].

Table 2
Gamma-glutamyltranspeptidase activity in the endometrium of cycling intact rats

Stage of cycle	Wet wt of uterus (mg)	Enzyme activity (nmol . mg protein^{-1} . min^{-1})
Proestrous	426.50 ± 36.42	6.08 ± 0.98
Estrous	363.83 ± 27.62	18.92 ± 2.43[a]
Metestrous	244.00 ± 25.86	8.59 ± 1.54
Diestrous	219.17 ± 4.73	7.57 ± 1.41

Values represent mean ± SEM of six animals

[a] $P < 0.01$ when compared with other stages of cycle

Unlike the estrogen treated rats, the progesterone treated ones show enhanced enzyme activity only at 48 h post steroid treatment (table 3). Receipt of multiple doses, however, results in a comparatively higher level of GGT at 96 h post treatment. Relationship of GGT activity and hormone influence has been demonstrated in a variety of tissues. While GGT activity of rat seminal vesicle has been shown to be testosterone-dependent [10], administration of estrogen and progesterone to ovariectomized rats elicits a response similar to pregnant state in the mammary gland [11]. The enhancement of mammary gland GGT activity, observed at the onset of lactation, has also been reported to involve prolactin [12]. It is interesting to

Table 1
Effect of exogenous estrogen on endometrial gamma-glutamyltranspeptidase of ovariectomized rats

Status	No. hormone doses	Wet wt of uterus (mg)	Enzyme activity (nmol . mg protein^{-1} . min^{-1})
Control	–	55.50 ± 6.13	6.01 ± 0.56
Estrogen treated:			
24 h post administration	1	148.00 ± 6.37	13.10 ± 1.23[a]
48 h post administration	2	206.00 ± 8.04	18.43 ± 0.99[a]
72 h post administration	3	209.17 ± 8.07	23.86 ± 1.00[a]
96 h post administration	4	223.83 ± 17.49	27.69 ± 0.72[a]

Values represent mean ± SEM of six animals

[a] $P < 0.001$ when compared with control

Table 3
Effect of exogenous progesterone on endometrial gamma-glutamyltranspeptidase of ovariectomized rats

Status	No. hormone doses	Wet wt of uterus (mg)	Enzyme activity (nmol . mg protein^{-1} . min^{-1})
Control	–	57.50 ± 1.52	6.99 ± 0.60
Progesterone treated:			
24 h post administration	1	87.67 ± 3.29	7.67 ± 1.14
48 h post administration	2	104.17 ± 6.04	12.25 ± 2.01[a]
72 h post administration	3	102.00 ± 5.30	19.44 ± 4.47[a]
96 h post administration	4	104.33 ± 6.72	38.66 ± 3.81[b]

Values represent mean ± SEM of six animals

[a] $P < 0.05$, [b] $P < 0.001$ when compared with control

note from our observations that progesterone per se induces synthesis of GGT in the endometrium unlike the lack of effect on mammary gland [11]. This may be explained on the basis of tissue specificity since maintaining rats under a regimen of estrogen and progesterone does not elicit similar alterations in GGT activity of different tissues [11]. The data from the present study, in addition to providing information on induction of endometrial GGT by estrogen or progesterone, suggest the usefulness of GGT assay as a marker for steroid action on the endometrium.

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