Inhibitory Effects of Estradiol on Glycogen Synthesis in Primary Cell Cultures of Human Endometrium

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Abstract

The effect of estradiol on glycogen synthesis was examined in a primary cell culture system of the human endometrium. Estradiol inhibited glycogen synthesis in a dose dependent manner with a minimum effective dose of \(10^{-9}\) M. Progesterone-induced glycogen synthesis was completely abolished by the simultaneous addition of estradiol.

Estradiol did not affect the activity of glycogen phosphorylase in endometrial cells, but the activity of glycogen synthetase was decreased by estradiol irrespective of the presence of progesterone. The inhibitory effect of estradiol on the glycogen synthesis of endometrial cells seemed to be mediated by estradiol receptor, because LY156758, an antiestrogenic drug at receptor site, reversed the inhibitory effect of estradiol.

The human endometrium, the site of nidation is especially important as the nutritional source of the fertilized ovum. It is controlled cyclically by the ovarian steroid hormones such as estrogen and progesterone. The glycogen in the endometrium is one of the most important factors in the development of the blastocyes in the early stages of gestation. The glycogen content of the endometrium in certain infertile women is reported to be greatly reduced (Maeyama et al., 1977).

Progesterone is regarded as playing a key role in glycogen synthesis in the endometrial tissues (Arronet et al., 1957). But the precise regulatory mechanism of glycogen synthesis is not known. To elucidate this mechanism, we established a primary cell culture system of human endometrial cells in which cultured cells are capable of producing glycogen in response to various hormones. Therefore this cell culture system provides a good tool to explore the hormonal regulatory mechanism of glycogen synthesis at the cellular level.

We have already reported the stimulatory action of progesterone in the synthesis of glycogen in this cell culture system (Ishihara et al., 1988). In this study, we further investigated the effect of estradiol on glycogen metabolism in the endometrium.

Materials and Methods

Human endometrial tissue was obtained at hysterectomy under sterile conditions by cur-
ettage immediately after excision of the organ. All specimens were obtained from women under 45 years of age who underwent hysterectomy for benign diseases unrelated to endometrial pathology. The patients had a history of regular menstrual cycles and were not undergoing steroid hormone therapy for at least two cycles prior to the operation. Informed consent was obtained from each subject.

The specimens used in this study were in the early to mid secretory phase as confirmed by histological examination.

Cell cultures of human endometrium

Tissue samples were collected in sterile Medium 199 (pH 7.4) and rinsed three times with Medium 199 to remove red blood cells. The tissue was then cut into small pieces and transferred into Medium 199 which contained 0.1% collagenase (Sigma Chemical Co. St. Louis, MO) and 1% bovine serum albumin (BSA), and was digested for 30 minutes at 37°C under gentle shaking. Digested pieces were washed three times with Medium 199 which contained 1% BSA, and then suspended in Medium 199 supplemented with 10% fetal bovine serum treated with dextran coated charcoal, insulin (Sigma Chemical Co. St. Louis, MO) at 5 µg/ml and sodium penicillin G (30 µg/ml). Growth medium containing dispersed cells were put into a 6-well tissue culture dish (2 ml/35 mm well) manufactured by Becton Dickinson and Company at a density of 4 × 10⁵ viable cells/cm² counted in a Makler Counting Chamber. Cultures were maintained at 37°C, in 5% CO₂, 95% humidified air. After 24 hours of incubation, the culture media were changed and the cell culture was continued for another 48 hours with daily replenishment of 2 ml culture media containing insulin (5 µg/ml), 5% fetal bovine serum and indicated hormones. Estradiol-17β and progesterone were provided by Sigma Chemical Company, St. Louis, Missouri, and LY156758 was synthesized in the Lilly Research Laboratories. Each steroid hormone was dissolved in ethanol, and added to the medium to achieve a final ethanol concentration of 0.5 µl/ml medium.

Measurement of glycogen

Glycogen concentrations in cultured human endometrial cells were analysed by the Montogomery method (Montogomery, 1957). Briefly, cultured cells were harvested with a rubber policeman and transferred into glass test tubes. First of all, the number of cultured cells was counted in a Makler Counting Chamber, and then 2 ml of 25% KOH was added to each tube. After heating at 100°C for 10 minutes, 2 volumes of 95% ethanol was added, then cooled at room temperature for 24 hours and centrifuged. After the supernatant was discarded, the precipitate was dissolved in 2 ml of water, and reacted with 80% phenol and sulfuric acid. The reacted mixture was then transferred to an appropriate calorimeter tube and absorption at 490 nm was measured in a spectrophotometer. The glycogen concentrations were expressed as micrograms of glycogen per 10⁵ of cultured endometrial cells.

Glycogen synthetase (GS) activity

Cultured cells were washed twice with phosphate-buffered saline and, after adding 2 ml of 50 mM Tris HCl buffer (pH 7.4), the cells were then detached with a rubber policeman. The cell suspension was transferred to a teflon-glass homogenizer and homogenized. The homogenates were centrifuged at 9000 g for 10 minutes at 4°C, and the supernatants were used for an enzyme assay.

GS was assayed by a modification of the methods of Thomas et al. (1968), which assessed the activity of the enzyme by counting the radioactivity incorporated into glycogen from UDP-¹⁴C glucose (New England Nuclear Corp. Boston, MA) to give approximately 0.01 µCi/µmole, in the presence of Glucose-6-phosphate (New England Nuclear Corp. Boston, MA). The reaction mixture containing 6.7 mM UDP-¹⁴C glucose, 10 mg/ml glycogen, 6.7 mM G-6-P and 100 µl enzyme solution in a final volume of 160 µl was incubated for 10 minutes at 30°C. Seventy-five µl of the aliquots was spotted on Whatman No. 31 ET filter paper cut into 2 cm squares. The paper was treated with 66% ethanol twice for 30 minutes each time, and allowed to air dry. The radioactivity was counted in a liquid scintillation spectrometer. GS activity was expressed as radioactivity per 100 µg protein. The protein concentration was determined by the method of Lowry et al. (1951).

Glycogen phosphorylase activity

The cell homogenate was prepared by the method described before. Measurement of glycogen phosphorylase activity was carried out by
assaying the incorporation of \([U-^{14}C]\) glucose 1-P into glycogen (Demers et al., 1973). The reaction mixture contained 50 mM \([U-^{14}C]\) glucose 1-P (specific activity: 0.01 \(\mu\)Ci/\(\mu\)mol) (New England Nuclear Corp. Boston, MA), 1% glycogen, 100 mM NaF, 1 mM 5' AMP, 50 mM 2 (N-morpholino) ethanesulfonic acid (MES) buffer (pH 6.1) and 20 \(\mu\)l of the enzyme solution in a final volume of 100 \(\mu\)l. This mixture was incubated at 30°C for 10 minutes. Seventy \(\mu\)l aliquots was spotted on Whatman No. 31 ET filter paper, and ethanol-insoluble radioactivity was counted as described above.

Results were shown as the mean±SD, and statistical evaluation of the results was by \(t\)-test.

**Results**

Fig. 1 shows the effect of various concentrations of estradiol on the accumulation of glycogen. Glycogen in the endometrial cells exposed to estradiol for 48 hours was significantly reduced (\(P<0.05\)) to concentrations of \(10^{-9}\) M (2.1±0.79 \(\mu\)g/10⁵ cells) and \(10^{-8}\) (2.4±0.17 \(\mu\)g/10⁵ cells) in contrast to the control level (4.3±0.29 \(\mu\)g/10⁵ cells). Furthermore, progesterone-induced glycogen accumulation (8.5±0.46 \(\mu\)g/10⁵ cells) was completely abolished by the simultaneous addition of estradiol and progesterone (3.2±0.17 \(\mu\)g/10⁵ cells), as shown in Fig. 2.

LY156758, the antiestrogenic drug at the receptor site, was examined to determine whether it antagonized the action of
Fig. 3. Effect of Antiestrogens (LY 156758) on the glycogen content in cultured human endometrial cells after 48 hours of culture. LY156758 (10⁻⁹M) was added simultaneously in culture media with progesterone and estradiol. Each point represents the mean±SD for six separate culture dishes. *P<0.001 as compared to the control value.

Fig. 4. Effect of progesterone and estradiol on the glycogen synthetase activity in cultured human endometrial cells from four separate determinations. After 24 hours of incubation, the culture medium was changed, and the culture was continued with medium containing hormones as indicated for 12 hours. Glycogen synthetase activities were assayed as described in the “Materials and Methods” section. *P<0.05 as compared to control value.

estradiol in reducing glycogen content (Fig. 3). Glycogen content when stimulated by progesterone was 8.6±0.84 µg/10⁵ cells. The concomitant addition of LY156758 with estradiol completely reversed the inhibitory effect of estradiol on the glycogen accumulation stimulated by progesterone.

Fig. 4 shows the activity of glycogen synthetase in cells cultured with progesterone and estradiol for 12 hours. Progesterone (10⁻⁶M) increased glycogen synthetase activity to 150% of that without the hormone. On the other hand, estradiol (10⁻⁸M) lowered the activity of glycogen synthetase by 50% of the control level. The enzyme activity in cells cultured with both estradiol and progesterone was similar to that in cells cultured with estradiol alone. Fig. 5 shows the activity of glycogen phosphorylase. Glycogen phosphorylase activity without any hormonal effect was 4140±230 dpm/100 µg protein. In contrast to glycogen synthetase, glycogen phosphorylase activity was reduced by progesterone (2680±580/100 µg protein) while estradiol had no effect. The combined addition of progesterone and estradiol also did not have any effect on the activity.
Discussion

Our previous results demonstrated that progesterone increased the content of glycogen in a cell culture system of human endometrium (Ishihara et al., 1988). The mechanism of the stimulatory actions of progesterone was shown to be an increase in the glycogen synthetase activity and a concomitant decrease in the glycogen phosphorylase activity. On the other hand, the present study indicates that estradiol exerts an inhibitory effect on the glycogen irrespective of the presence of progesterone. The inhibitory effect of estradiol on glycogen content appears to be, in part, due to the lowered level of glycogen synthetase. When cycloheximide was added together with estradiol to the medium, the inhibitory action of estradiol was completely abolished (data not shown here), indicating that the decrease in the enzymatic activity is mediated by newly synthesized protein. Glycogen synthetase is known to be present in two interconvertible forms, i.e., active and inactive form. Although estradiol is shown to reduce the total amount of the enzyme, it is to be determined whether or not estradiol changes the ratio of the active form to the inactive form. The effects of estradiol on the glycogen synthesis of endometrial cells seemed to be mediated through its receptor because an antiestrogenic agent by competitive binding to the receptor for estradiol (Kleinberg et al., 1983) blocked the action of estradiol.

The present results may have some physiological relevance in the sense that the inhibitory effect of estradiol is observed at concentration of the hormone available in circulating blood around ovulation. In order to examine whether estradiol actually elicits an inhibitory effect in vivo, estradiol benzoate was given to normally cycling women on day 5 after ovulation. Two days later, glycogen content in the endometrium was found to be reduced to approximately 50% of that in normal women at the same stage of the menstrual cycle (data not described here). It is reasonable to suggest that estradiol directly acts on the endometrium to decrease glycogen content, since a direct effect of estradiol on the corpus luteum is less likely, because, under the experimental conditions, the serum progesterone concentration does not change remarkably. Clinically, inadequate secretion of progesterone is obviously of primary importance as a pathogenic factor in luteal phase defect (Smith et al., 1985), and it is tempting to speculate that abnormally higher levels of estradiol or an increase in the estradiol/progesterone ratio may be
causally related to a decrease in glycogen content in women with a luteal phase defect.

Shapiro et al. (1980) described the stimulatory effect of progesterone on the accumulation of endometrial glycogen with an organ culture system of human proliferative endometrium. However, in their study, the concomitant addition of estradiol with progesterone did not affect the glycogen content in cultured endometrial tissues in contrast with the present results. A reasonable explanation of the discrepancy may be the difference between the culture systems, i.e. one is a cell culture system and the other an organ culture system. In an organ culture system, it is generally difficult to remove hormones, especially hydrophobic hormones, such as steroid hormones, which are present in vivo. Therefore, in vitro experiments using an organ culture system, the effects of the hormones present in vivo are often carried over, even if an organ culture is conducted without the hormones.

One also speculates that the difference may be due to the difference in the phase of the endometrium used, i.e. proliferative phase or secretory phase. Data not presented here clearly demonstrated that estradiol elicits an inhibitory effect on the glycogen content of endometrial cells derived from the proliferative phase as well, thus discounting the difference in the phase as an explanation for disagreements between the results of Shapiro et al. and others.

References


