INDUCTION OF ENDOMETRIAL PEROXIDASE SYNTHESIS AND SECRETION BY ESTROGEN AND ESTROGEN ANTAGONIST

ANDREW CHURG and WINSTON A. ANDERSON

From the Laboratory of Cellular and Reproductive Biology, Department of Anatomy, and The Biomedical Center for Population Research, University of Chicago, Chicago, Illinois 60637. Dr. Churg’s present address is the Department of Pathology, University of Chicago, Chicago, Illinois 60637.

ABSTRACT

Synthesis of peroxidase was induced in the uterine epithelium of immature rats by multiple doses over a 24–96-h period of either 17 ß-estradiol, the estrogen-antagonist Parke-Davis CI-628, or a combination of estradiol plus antagonist. Endogenous peroxidase activity first appeared in the cisternae of the rough endoplasmic reticulum of surface epithelial and glandular cells within 24-48 after the initial injection. Uterine peroxidase activity was also visible in the cisternae of the Golgi apparatus, in Golgi-derived secretory granules, and within the uterine and glandular lumen. Some cells of the epithelium produced little or no peroxidase, even after 96 h.

Whereas the antagonist appeared to induce synthesis and secretion of peroxidase, neither the antagonist alone nor the combined treatment (estradiol plus antagonist) reproduced the estradiol-mediated growth in organ size and increased lumen diameter.

INTRODUCTION

Characterization of the proteins produced by the estrogen-stimulated mammalian uterus is presently incomplete. A specific, uterine soluble protein is detectable as early as 45–60 min after administration of estrogen (Barnea and Gorski, 1970; D’Angelo and Gorski, 1970). General protein synthesis increases after 5 h, and synthesis of other specific proteins is masked. Of particular significance is the fact that estrogen induces synthesis of a specific peroxidase in the uterus (Lucas et al., 1955; Martin et al., 1958; Neufeld et al., 1958), which is distributed in the rough endoplasmic reticulum of the uterine epithelial cells (Brökelmann and Fawcett, 1969). Localization of uterine peroxidase allows visualization for the first time of an estrogen-dependent enzyme (Brökelmann and Fawcett, 1969), and this facilitates correlation of biochemical and structural differentiation of uterine epithelial cells at the ultracyto-

Throughout this paper, the 3,3’-diaminobenzidine (DAB) positive reaction product will be referred to as a peroxidase, since uterine peroxidase is a well documented enzyme (see text) and since the histochemical medium employed is designed to demonstrate peroxidase activity. It is of course possible that what is being visualized is either a heme-protein which may possess peroxidase activity, or several substances with peroxidatic activity.
chemical level. Using this system we have examined the time course of synthesis and secretion of peroxidase by uterine epithelial cells after treatment with 17-β-estradiol and the estrogen-antagonist, Parke Davis CI-628.

MATERIALS AND METHODS

Sources of Materials

Uteri from immature female weanling albino Sprague-Dawley rats (Hormone Research Labs, Chicago, Ill.) weighing 40–50 g, and from mature rats (greater than 110 g) in estrous were examined in our experiments.

17 β-estradiol, progesterone, and testosterone were obtained from Sigma Chemical Company, St. Louis, Mo. Parke-Davis CI-628 (l-[2-(P-[α-(p-methoxyphenyl)-β-nitrostyryl] phenoxy) ethyl] pyrrolidine) estrogen antagonist was supplied through the courtesy of Dr. Elwood Jensen.2

Experimental Design

Immature rats were given a subcutaneous dose of 0.1 ml of 17 β-estradiol or inhibitor dissolved in glycerol. Some animals received only a single injection, while others were injected every 24 h (times 0, 24, 48, and 72 h after the beginning of the experimental period). The doses and abbreviations for these immature rats are: EL = physiologic dose 17 β-estradiol: 0.04 µg/24 h; EH = hyperphysiologic dose 17 β-estradiol: 1–4 µg/24 h; IL = 50 µg Parke-Davis CI-628 estrogen antagonist/24 h; IH = 500 µg antagonist/24 h; P = 1 mg progesterone/24 h; T = 1 mg testosterone/24 h; E + I = estradiol plus antagonist (high or low dose as noted). Antagonist was administered 30 min before estradiol, according to Jensen and co-workers (1972). Animals were sacrificed at 0, 12, 24, 36, 48, 60, 72, 84, and 96 h after the first injection. The uteri from anesthetized rats were fixed in situ, dissected out, and minced in a mixture of cold 4% glutaraldehyde + 1% formaldehyde in 0.1 M cacodylate buffer pH 7.2 (Karnovsky, 1965). After 1 h in this fixative, the tissue was washed overnight in the same buffer, and cut into 100-μm slices using an Oxford Vibratome (Oxford Vibratome Company, San Mateo, Calif.). The slices were incubated in Graham and Karnovsky’s (1966) 3’,3’-diaminobenzidine (DAB)-H2O2 medium for the demonstration of peroxidase. After a buffer rinse the tissue was then postfixed in 2% OsO4 in distilled water, dehydrated in ethanol, and embedded in Epon. Sections were examined unstained or stained lightly with lead citrate (Reynolds, 1963) in RCA 4B and Hitachi HU 11C electron microscopes.

Uninjected control females, females injected only with glycerol, and females injected with progesterone or testosterone dissolved in glycerol were sacrificed at each time period as indicated in the experimental plan and the uteri treated as above for the demonstration of endogenous peroxidase activity. The uteri from the mature estrous females were incubated and processed in the same manner.

OBSERVATIONS

Uterotropic Response to Estrogen and Antagonists

A marked uterotrophic response occurred in animals given a single or multiple dose of 17 β-estradiol. Within 24 h after administration of physiologic and hyperphysiologic levels of 17 β-estradiol the following uterotropic changes were observed: (a) hypertrophy and proliferation of the endometrium, (b) thickening of the submucosa and myometrium, and (c) dilatation of the uterine lumen.

Single or multiple injections of the Parke-Davis CI-628 antagonist alone, or of the antagonist plus physiologic or hyperphysiologic doses of 17 β-estradiol, failed to elicit the increase in size and muscle mass characteristic of 17 β-estradiol alone. However, high levels of CI-628 stimulated hypertrophy and secretory activity of the surface and glandular epithelium.

Progesterone alone did not appear to have any effect on the uterus of immature female rats. Testosterone in multiple doses produced an increase in muscle mass and a small increase in lumen diameter without obvious changes in the endometrium.

Stimulation of Endogenous Peroxidase

In the immature control, and in progesterone- and testosterone-treated animals, the columnar cells of the endometrium possessed numerous free ribosomes and polysomes, sparse rough endoplasmic reticulum (RER), and a small Golgi apparatus. The cells were uniform in height, and most contained lipid droplets at the basal ends. When examined for endogenous peroxidase activity, only microbodies and mitochondria were stained by the reaction.

The most prominent change in the epithelium of
Peroxidase Synthesis in Estrous Animals

Peroxidase production was also examined in a number of animals in estrous. The pattern of activity was identical to that in animals given exogenous 17β-estradiol or CI-628. The RER was abundant and highly reactive (Fig. 7), and the Golgi apparatus showed less peroxidase activity in its cisternae (Fig. 8). Condensing vacuoles and secretory granules were, however, more intensely stained. Secretory granules also appeared to be derived from the dilated terminal ends of the RER cisternae (Fig. 7). In contradistinction to the induced animals, mature animals showed peroxidase activity in most cells of the endometrium.

DISCUSSION

Estrogen-induced Synthesis of “Specific Protein” in the Uterus

In the oviduct of the hen and chick, some of the newly synthesized specific proteins evoked by estrogen have been well characterized. These include ovalbumin, conalbumin, lysosyme, and ovo-mucoid (O’Malley and McGuire, 1968; Palmiter and Schimke, 1973). Few well defined estrogen-induced proteins have been reported in the mammalian uterus (Barnea and Gorski, 1970; D’Angelo and Gorski, 1970). Scott and Lisi (1960) demonstrated the appearance of glucose-6-phosphatase activity in the uterus about 12 h after estradiol treatment. Estrogen antagonists, most of which have weak estrogenic activity, have been shown to induce de novo synthesis of alkaline phosphatase (Lerner, 1964).

Early work by Lucas et al. (1955) and Neufeld et al., (1958) demonstrated that the uterus, as well as other tissues, contained high levels of peroxidase. This uterine peroxidase was negligible in spayed animals, but increased more than 200-fold after injection of 17β-estradiol or diethylstilbestrol. The reactions catalyzed by the uterine peroxidase closely resembled those of lactoperoxidase (Martin et al., 1958). The dramatic estrogen-induced increases in activity of uterine peroxidase could be prevented by administration of acetoxycycloheximide, which suggested that protein synthesis was involved. However, most of the early investigators thought that the enzyme was derived from the numerous eosinophils which infiltrated the estrogen-stimulated uterus (Klebanoff and Segal, 1960).

More recent histochemical studies by Brökelmann (1969) and Brökelmann and Fawcett (1969) showed clearly that the peroxidase was localized in the rough endoplasmic reticulum of the endometrial cells in the estrogen-treated, ovariectomized, mature rat. We have confirmed these findings and extended them to show that the endometrium of immature rats can be induced by 17β-estradiol to synthesize, store, and secrete massive amounts of peroxidase. Immature animals receiving physiologic and hyperphysiologic doses of 17β-estradiol commence morphologically detectable synthesis of peroxidase 24-48 h after initial injection of the hormone. Multiple injections maintain and augment the peroxidase level over a 96-h period. This newly synthesized enzyme resides primarily within cisternae of the rough endoplasmic reticulum; it is never present in the groundplasm. In most epithe-
Figs. 1–6 are of uteri treated with 17β-estradiol, estradiol plus CI-628, or CI-628 alone. The tissue has been incubated to show endogenous peroxidase activity. Light micrographs are counterstained with toluidine blue; electron micrographs are lightly stained with lead citrate.

**FIGURES 1 and 2** Cells of the surface epithelium from an animal given combined treatment (E₂ + I₁) for 72 h. The medial (Fig. 1) and basal (Fig. 2) regions of the surface epithelial cells are shown. Stacks of RER containing peroxidase surround the enlarged Golgi complex, which contains dilated cisternae and condensing vacuoles. At the base of the cells the peroxidase reaction product delineates the nuclear envelope and portions of the RER. bl = basement lamina. × 8,000.
FIGURE 3 An endometrial gland from an animal given combined treatment E₂ + LH for 72 h. Most cells contain condensing and secretory granules near the apical membrane and abundant profiles of RER, all staining positively for peroxidase activity. Some cells show little reactivity. × 5,500.
FIGURES 4 and 5  Gland cells from animals receiving CI-628 (Fig. 4) or combined treatment E₁ + I₄ (Fig. 5) for 72 h. The cells lining the gland possess at their apical surfaces vesicular fragments of RER (Fig. 4) and Golgi-derived condensing and secretory granules (Fig. 5). Inset: light micrograph of a gland filled with intensely DAB-positive secretory product (sp). Inset, x 460; Fig. 4, x 5,000; Fig. 5, x 5,700.
FIGURE 6 Portion of a gland from an animal given treatment E\(_2\) + I\(_2\) for 72 h. The lumen contains large amounts of peroxidase-positive secretory product and a number of dense concretions. Product appears to be transferred from gland cells to the lumen via fusion of small peroxidase-positive secretory vesicles with the apical membrane. × 6,200. Inset: from the same animal. A 1-µm thick section shows secretion product in the lumen of the gland and also in the uterine cavity. × 460.
FIGURE 7  Portion of an endometrial gland from a mature animal in estrous. The pattern of reaction is very similar to that in the induced immature animals except that dilatation of the apical RER cisternae is more prominent. All the cells in this gland show activity. X 2,000.
FIGURE 8. This micrograph shows the Golgi regions of several cells of the endometrium of an animal in estrous. Peroxidase-positive staining appears in Golgi cisternae and associated condensing vacuoles and secretory granules. x 5,000.

In uterine cells the Golgi complexes stain for peroxidase activity. The apical cytoplasm of many cells is filled with Golgi-derived condensing and secretory granules, and in such cells the RER is often stacked, an appearance reminiscent of typical secretory cells (Jamieson and Palade, 1971). In some cells, smaller peroxidase-containing vesicles of uncertain origin seem to replace the large
secretory granules, while in other cells dilated terminal cisternae of the RER underlie the plasmalemma.

**Estrogen Antagonists with Estrogenic Potency**

In recent years the development of antiestrogenic agents has contributed to the understanding of the mechanism of estrogen action and proved valuable in distinguishing the binding proteins for estradiol. Although these agents differ in their antiestrogenic potencies (ethamoxytriphetol [MER-25] < clomiphene [MRL-41] < nafoxidine [Upjohn 11,100] < Parke-Davis CI-628), they all inhibit uptake and binding of 17β-estradiol or diethylstilbestrol by rat and mouse uterine tissue (Holtkemp et al., 1960; Duncan et al., 1963; Jensen, 1962; Jensen et al., 1972; Callantine et al., 1966; Callantine, 1967). These antagonists appear to be competitive inhibitors of natural and synthetic estrogens, saturating protein binding sites in the cytoplasm and thus preventing the action of estrogen.

These antagonists are also weak estrogens and induce some of the changes produced by estrogen itself. The Parke-Davis CI-628 inhibitor in our study, evokes massive peroxidase synthesis in the endometrium, particularly by the glandular epithelium. When it is administered along with estrogen, peroxidase synthesis is equally impressive. Some of the cells of the 17β-estradiol- or the CI-628-stimulated uteri did not synthesize peroxidase. It is not clear whether these cells represented an undifferentiated, estrogen-insensitive population of cells or else a different phase in the metabolic cycle of the uterine epithelium. Indeed, uterine peroxidase, like salivary gland peroxidase, could function in the metabolism of halogens and as a bactericidal agent in the uterine fluid.

Distribution and Function of Endogenous Peroxidase

Peroxidases are widely distributed in animal cells. Peroxidase is prominent within the RER of thyroid follicle cells (Strum and Karnovsky, 1970; Strum et al., 1971) of Kupffer cells, peripheral macrophages, and eosinophil leukocytes (Fahimi, 1970; Robbins et al., 1971; Bainton and Farquhar, 1970; Klebanoff and Luebke, 1965). Belding et al. (1970) and Lehrer (1969) have recently shown that myeloperoxidase (also lactoperoxidase) in the presence of H2O2 and a halide exerts strong virucidal, bactericidal, and fungicidal activity. Fahimi (1970) postulated that Kupffer cell peroxidase plays a bactericidal role similar to that played by peroxidases in neutrophils, milk, and saliva (Klebanoff and Luebke, 1965; Morrison and Steele, 1968). Indeed, uterine peroxidase, like salivary gland peroxidase, could function in the metabolism of halogens and as a bactericidal agent in the uterine lumen.

Uterine peroxidase may also play a significant role in estrogen metabolism (Klebanoff, 1965; Klebanoff and Segal, 1960; Brökelmann, 1968). Recently, Lyttle and Jellnick (1972) have shown that estrogen-induced peroxidase from the uterus of immature rats can convert estradiol to water-soluble breakdown products, at least in vitro. They suggest that the peroxidase serves as a mechanism to inactivate the steroid, thus terminating and/or preventing its effects.

The authors thank Drs. H. G. Williams-Ashman and E. DeSombre of the Ben May Laboratory for Cancer Research, the University of Chicago, for reading and offering criticisms of the manuscript. We acknowledge the technical assistance of C. Marcus and S. Paul in this investigation.

The research was supported in part by grant M73.109 from the Population Council, Rockefeller Foundation and in part by United States Public Health Service Grant HD 07 110.

Received for publication 23 August 1973, and in revised form 14 March 1974.

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