Distribution of oestrogen-induced peroxidase in the rat uterus

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Abstract. The activity of oestrogen-induced peroxidase in sections along the uterus of normal and mammary tumour-bearing adult rats was measured. Oestradiol increased the activity of this enzyme in the cervix as well as in other parts of the uterus in ovariectomized or immature rats. Peroxidase activity per mg protein was twice as high in the cervix as in the rest of the uterus where it was evenly distributed along both horns. The concentrations of oestrogen receptors in the cytosol and nucleus in each uterine horn and in the cervix was also determined and found to be lower in the cervix than in other sections of the uterus.

The rapid increase in uterine peroxidase activity after treatment of ovariectomized or immature rats with oestrogen (Lyttle & Jellinck 1972; Jellinck & Lyttle 1973) has led to the proposal (Anderson et al. 1975; Lyttle & DeSombre 1977) that this enzyme may be a useful specific marker for tissues whose growth is regulated by oestrogen. In recent studies, Tsibris et al. (1978a) determined the distribution of oestrogen and progesterone receptors in the cytoplasm of five sections along the length of the human uterine endometrium obtained from non-cancerous pre-menopausal hysterectomy specimen. They also measured the peroxidase activity of these sections (Tsibris et al. 1978b). The concentration of both types of steroid receptors was found to be highest in the fundus and lowest in the cervix while the enzyme was located either exclusively or maximally in the cervix. It was therefore considered of interest to measure peroxidase activity along the length of the rat uterus and also to compare the concentration of nuclear oestrogen receptors in the cervix with that of the rest of the uterus.

Materials and Methods

Determination of uterine peroxidase activity

Mature female Sprague Dawley rats (Canadian Breeding Laboratories, St. Constant, Quebec) weighing 290–320 g were injected sc with oestradiol-17β (10 µg in 0.5 ml saline containing 10% ethanol) 10 h before sacrifice. Six of the animals used were normal rats and 15 cm sections with scissors as illustrated in Fig. 1 and each section weighed and homogenized in 5 ml of Tris-HCl (0.01 M) pH 7.2 in a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 40,000 g for 30 min and peroxidase solubilized by re-homogenizing the sediment in Tris-HCl buffer containing 0.5 M CaCl₂ (Lyttle & DeSombre 1977). It was centrifuged again for 30 min and the supernatant (0.05–0.2 ml) used for determining peroxidase activity by measuring the rate of oxidation of guaiacol at 470 nm (Himmelhoch et al. 1967). The concentration of protein in the uterine extracts was determined by the Bradford dye-binding method using bovine γ-globulin (Cohn fraction II) as standard (Bradford 1976). In some experiments, mature rats ovariectomized under methoxyflurane (Metofane) anaesthesia 14–21 days before sacrifice, and also immature (22–26 day old) animals were used. These groups were injected sc with oestradiol (5 µg in 0.2 ml sesame oil) as described in Table 2 before the assay for uterine peroxidase.

Cytosolic and nuclear oestrogen receptor assay

Mature female rats were given a sc injection of oestradiol (10 µg) 30 min before removal of the uterus. Section 2 (right horn), section 6 (left horn) and section 4 (cervix) of each uterus (Fig. 1) were frozen and pulverized. The
The method of Zava et al. (1976) with minor modifications was used to determine the concentration of oestrogen receptors in the cytosol and nucleus. The tissue sections (50–100 mg wet wt.) were homogenized in 1 ml of TED buffer (0.01 M Tris-HCl, 1.5 mM EDTA, 0.05 mM dithiothreitol, pH 7.4) containing bacitracin (0.5 mM) at 4°C with a Brinkman Polytron PT-10-ST at speed 3.5 for three 10 s intervals before subcellular fractionation. The cytosol (105 000 g supernatant) was treated with dextran-coated charcoal to remove free hormone and the concentration of specific oestrogen receptor sites determined in triplicate by the protamine assay (Chamness et al. 1975) using 15 μM [2,4,6,7-3H]oestradiol (94 Ci/mmol) in the presence and absence of a 100-fold excess of diethylstilboestrol (DES). Bovine serum albumin was the standard for protein determination (Bradford 1976) in these experiments. An extract of the nuclear pellet with TED buffer containing 0.6 mM KCl and 0.5 mM bacitracin was used for the nuclear protamine assay with 30 nM [3H]oestradiol and DES (3 μM) added to correct for non-specific binding.

DNA was measured by the diphenylamine method of Burton (1956) with calf thymus DNA as standard. The results are the mean ± SEM from 3 experiments with pooled uteri from 3–6 rats.

Results

The activity of peroxidase in different sections along the uterus of normal and mammary tumour-bearing adult rats which had been treated with oestradiol (10 μg) 18 h before sacrifice is shown in Fig. 1. Peroxidase activity per mg protein was about twice as high in the cervix (section 4) as in the rest of the uterus where it was distributed evenly along both horns. Although considerable variations in peroxidase activity occurred between individual animals, the 2-fold increase in the cervix over the uterine sections was present without exception and animals bearing DMBA-induced mammary tumours showed the same distribution pattern. The concentration of protein in the CaCl2 extract of all the sections, including the cervix, was similar (29–34 mg protein/g wet wt. of tissue).

Oestrogen receptor levels in the cytosol and nucleus of the uterine horns of normal adult rats were about twice as high as in the corresponding subcellular fractions of the cervix 30 min after injection of oestradiol (10 μg) (Table 1). Receptor concentrations were measured by an exchange assay and in both the cervix and the rest of the uterus, most of the high affinity sites had translocated to the nucleus as a result of treatment with oestradiol.

The evidence that peroxidase is induced in the cervix as well as in the rest of the uterus by oestrogen is provided in Table 2. Very high enzymic activities were observed in both these uterine fractions in immature or ovariectomized rats after treatment with oestradiol. Peroxidase could not be detected in the uter of control (untreated) animals and larger amounts of the enzyme were induced in immature rats primed previously with oestrogen than after only one injection of oestradiol 18 h before sacrifice.
**Table 1.**

Distribution of cytosolic and nuclear oestrogen receptors in different parts of the rat uterus after treatment with oestradiol.

<table>
<thead>
<tr>
<th>Uterine section</th>
<th>Specific binding of oestradiol</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Conc. of cytosol receptors (pmol/mg protein)</td>
</tr>
<tr>
<td>Right horn (sect. 2)</td>
<td>0.025 ± 0.004</td>
</tr>
<tr>
<td>Cervix (sect. 4)</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>Left horn (sect. 6)</td>
<td>0.021 ± 0.007</td>
</tr>
</tbody>
</table>

Mature female rats were given oestradiol (10 μg) by sc injection 30 min before removal of the uteri for oestrogen receptor assay as described in the text. Results are the mean of 3 experiments (3–6 rats/expt.) ± SEM.

**Table 2.**

Induction by oestrogen and distribution of peroxidase in the uteri of ovariectomized and immature rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peroxidase activity</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔE_{470}/min/g tissue</td>
<td>ΔE_{470}/min/mg protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uterine horns</td>
<td>Cervix</td>
<td>Uterine horns</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil (control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>208 ± 96</td>
<td>475 ± 160</td>
<td>5.55 ± 2.58</td>
</tr>
<tr>
<td>Immature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil (control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>91</td>
<td>244</td>
<td>3.75</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>33</td>
<td>87</td>
<td>0.75</td>
</tr>
</tbody>
</table>

(Not primed on day 1)

Ovariectomized adult rats (group of 6) were given oestradiol (5 μg in 0.2 ml oil) sc or the vehicle alone on days 1, 2 and 3, and killed 18 h after the last injection. Peroxidase activity from combined sections 2 and 6 of the uterine horns (Fig. 1) and from the cervix, was determined for each animal and the results expressed as the mean ± SEM. Immature rats (group of 4) were primed with oestradiol (5 μg in oil) on day 1 and given a second sc injection of oestrogen on day 6 before sacrifice on day 7. Values are from pooled sections 2 and 6 and cervix.
Discussion

The results clearly indicate that the activity of peroxidase per mg protein in the cervix of oestrogen-treated rat uteri is higher than in the rest of this organ and that it is induced by oestradiol in all sections of the uterus. In women, Tsibris et al. (1978b) have shown that this enzyme is located exclusively or maximally in the cervix. These workers also measured the concentration of cytosolic oestrogen and progesterone receptors in sections of human endometria at various periods of the menstrual cycle and found the sharpest change in distribution from the fundus to the cervix at the time of ovulation (Tsibris et al. 1978a). The levels of oestradiol and progesterone receptors were very much lower in the cervix than in the rest of the uterus and decreased progressively from the fundus. This confirms earlier studies with oestradiol receptors (Robertson et al. 1971), although others (Brush et al. 1967; Lunan & Green 1975) found more variation in the uptake of [3H]oestradiol in different regions of the human uterus.

In our experiments with the rat, such large variability and differences in receptor occupation in various sections of the uterus was not observed presumably because conditions were standardized by giving the animals a saturating dose of oestradiol before the assay. Most of the translocatable oestrogen receptors would be found in the nucleus of their target-tissue cells 30 min after treatment with oestrogen (Clark et al. 1973; Zava et al. 1976). Bacitracin was added to prevent the proteolytic breakdown of receptors during their isolation (McKelvy et al. 1976; Roy & McEwen 1977).

An inverse relationship between oestrogen receptor content and peroxidase activity in different parts of the uterus was unexpected because oestrogen-induced increases in this enzyme have been shown previously (DeSombre & Lytle 1979) to parallel increases in uterine weight and DNA, while experiments with progesterone and anti-oestrogens also support a receptor-mediated process (DeSombre & Lytle 1979; Jellinck et al. 1979; Lytle et al 1979). However, the results may be explained by the heterogeneous distribution of cells capable of producing peroxidase in the rat uterus (Churg & Anderson 1974) or else, by the enzyme being in a different form (Olsen & Little 1979) in the endometrium and the cervix. In addition, a proportional relationship between nuclear oestrogen receptor concentration and the formation of an oestrogen-induced protein, such as peroxidase, does not have to hold necessarily. The end-product may be processed differently in different tissues.

Treatment of rats with DMBA did not influence the activity or distribution of peroxidase in the uterus even though the animals developed mammary tumours which often contain this enzyme (DeSombre et al. 1975; Jellinck et al. 1979). The variation in the relative activities of peroxidase in different parts of the uterus and differences in distribution of this enzyme in rat and in man may help to explain the relative susceptibility of the cervix and endometrium to toxic and carcinogenic agents. Thus, in the case of diethylstilboestrol (DES), it has been proposed that peroxidase-mediated oxidation and binding to DNA and proteins (Metzler & McLachlan 1978) may be responsible for the harmful effects of this compound (Kurman 1979). Under these circumstances, DES would not only act as substrate but would induce the very enzyme which catalyzes its own metabolic activation (Jellinck & Bowen 1980). Other possible effects of oestrogen-induced uterine peroxidase have been discussed elsewhere (Jellinck et al. 1979).

Acknowledgments

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References


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