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LOCALIZATION OF ESTRADIOL 17β DEHYDROGENASE IN HUMAN ENDOMETRIUM

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SUMMARY

The localization of estradiol 17β dehydrogenase in human endometrium was studied histochemically, using the technique described by Morse and Heller. This enzymatic activity was exclusively found in the glandular epithelium of secretory endometrium. No activity was detected in proliferative tissue or postmenopausal endometrial adenocarcinoma. Previous biochemical studies had also shown a marked increase in estradiol dehydrogenase activity during the secretory phase of the cycle, under progesterone stimulation. It is now shown that this induction occurs in the endometrial glands.

INTRODUCTION

Biochemical studies previously performed in this laboratory have demonstrated the presence of 17β estradiol dehydrogenase (E_2DH) in human endometrium. This enzyme shows maximum activity during the luteal phase of the menstrual cycle [1] and is inducible by progestins [2]. The potential physiologic importance of the enzyme on the regulation of the intracellular levels of estradiol (E_2) motivated this study of its distribution in endometrium.

Histochemical techniques for the detection of hydroxysteroid dehydrogenases were described by Wattenberg in 1958 [3] and are based on the reduction of tetrazolium salts by reduced cofactors generated during oxidation of the enzyme substrate. An improved procedure, followed in the work reported here, has been published by Morse and Heller [4]. Histochemical methods have been used to demonstrate E_2DH activity in placenta [5, 6] and progesterone 20α dehydrogenase in human endometrium [7]. The authors of this latter article concluded that progesterone 20α dehydrogenase, an enzyme which may be identical to E_2DH, is preferentially localized in the glandular epithelium. Unfortunately, the published photographic evidence is unclear and other authors favor a stromal localization of the enzyme [8].

MATERIALS AND METHODS

Endometrial tissue (3 secretory and 3 proliferative, 1 well-differentiated adenocarcinoma from a postmenopausal patient) was obtained by curettage. The specimens were immediately soaked in a cold solution of 15% dextran T 40 (Pharmacia Fine Chemicals) and 1.5% dimethylsulfoxide (DMSO, Sigma) in Tris–maleate-Tyrode's buffer at pH 7.4 (Solution A), and were frozen in isopentane-dry ice. Sections of 10 μm thickness were cut with a rotary microtome in a cryostat at −20°C. The sections were placed on microscope slides, thawed, and immersed in a solution containing 5% polyvinyl pirrolidone (PVP-40, Sigma), 5% DMSO, and 4.3% dimethylformamide (DMF, Sigma, in Tris–maleate Tyrode's buffer. The slides were placed in Coplin jars filled with 30 ml of the incubation medium and kept for 2 h at 37°C in an N_2 atmosphere. The composition of the medium (ml per 100 ml) was the following:

42.7 ml 6.1% PVP in Tris–maleate-Tyrode's buffer;
1.87 ml 140 mM KCN, pH 7.4;
2.62 ml DMSO;
33.7 ml 2.5 mM nitrobluetetrazolium (NBT, Sigma);
16.5 ml NAD (Sigma);
2.25 ml 17 mM estradiol (Steraloids) in DMF.

The Tris–maleate-Tyrode's buffer contains, per l., 8 g NaCl, 0.2 g KCl, 0.1 g $MgCl_2 \cdot 6\ H_2O$, 0.2 g $CaCl_2$, 0.1 g $NaHCO_3$, 2.1 g Tris (hydroxymethyl) aminomethane (17 mM), and 0.78 g maleic acid, used to adjust the pH to 7.4.

Control sections were treated identically, but DMF was added without substrate to the incubation mixture. In order to ascertain the capability of the reduced cofactor to convert NBT to diformazan, some sections were incubated in medium in which NAD had been replaced by NADH.

The preparations were fixed by immersing the slides for 10 min in a mixture of 9 parts of Solution A and 1 part of 37% formaldehyde, at pH 7.5. The sections were then rinsed with physiologic saline, lightly counterstained by immersion for 30 s in a 1% aq. safranin O solution, mounted with Paragon®, and examined under the microscope.

RESULTS

Figure 1A illustrates the results obtained when secretory endometrium was used. Diformazan

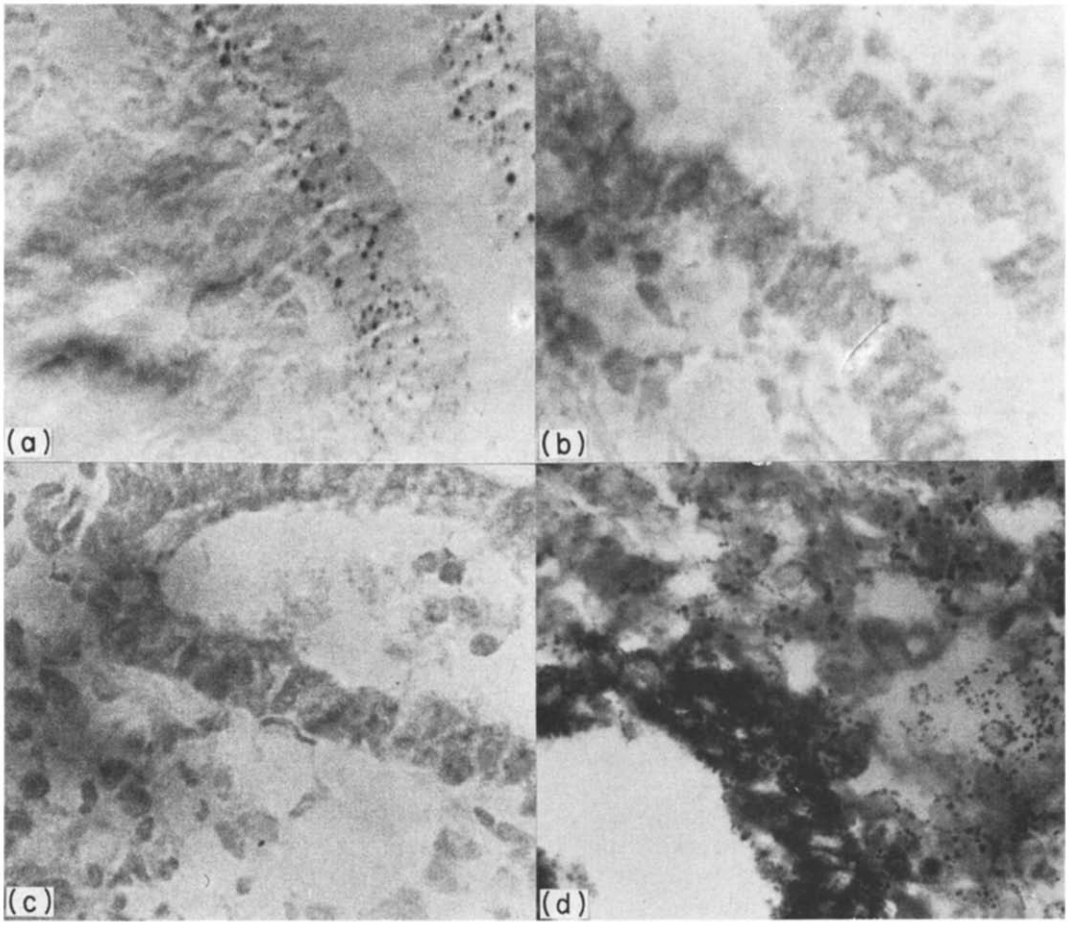

Fig. 1. A. Secretory endometrium. B. Secretory endometrium, control (substrate omitted). C. Proliferative endometrium. D. Proliferative endometrium, NADH control.

granules were present only in the glandular epithelium, indicating a practically exclusive localization of the E_2DH in the glands. The control for this preparation, in which substrate was omitted, showed no granules (Fig. 1B).

Figure 1C corresponds to proliferative endometrium subjected to the same procedure. No granules could be detected either in the glands or the stroma. This preparation was indistinguishable from the control, run without substrate. Parallel incubations of sections of the same specimen using NADH instead of NAD gave heavy diformazan deposits both in glands and stroma (Fig. 1D). Therefore, the negative results obtained with proliferative endometrium indicate that the levels of the endometrial E_2DH activity during the follicular phase of the menstrual cycle are below the sensitivity of the histochemical method used. The marked increase of activity during the luteal phase, which these results show to occur in the glands, are in agreement with previous measurements of enzyme activity in tissue homogenates by isotopic methods [1].

No E_2DH activity was detected in a specimen of well-differentiated endometrial adenocarcinoma taken from a postmenopausal patient. Direct measurements of enzymatic activity have shown very low values in this type of tissue, in agreement with published reports [9].

DISCUSSION

Even though the activity of E_2DH is much lower in human endometrium than in placenta, its physiologic importance in the regulation of E_2 levels in this tissue has been emphasized [2].

The histochemical procedure described here, which produced a negligible background, might therefore be useful in testing for induction of E_2DH by progestins in endometrial tumors, a measurement which could be of considerable interest for the prediction of tumor responsiveness to hormonal therapy.

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