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SHORT COMMUNICATION

ESTRADIOL BINDING COMPONENTS IN INTESTINAL MUCOSA OF FEMALE RATS

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Summary—The presence of estrogen binding components (EBC) in intestinal mucosa of female rats was investigated by competitive-binding assay using radiolabelled and nonlabelled estradiol 17β (E_2). EBC were found exclusively in the nuclear fraction and were absent from the cytosolic and from the microsomal fractions. Two types of nuclear EBC with different binding characteristics and capacities were found: $K_{d_1} = 4.8 \pm 0.8$ nM, $n_1 = 18.4 \pm 4.2$ fmol/mg protein ($n_1 = 83.4 \pm 12.5$ fmol/mg DNA) and $K_{d_2} = 31.1 \pm 6.8$ nM, $n_2 = 91.1 \pm 18.5$ fmol/mg protein (412.7 ± 80.0 fmol/mg DNA). Type 1 component showed slightly greater affinity for estrogens as compared to progesterone and dexamethasone whereas type 2 component bound other competitors with even greater affinity than E_2.

INTRODUCTION

It was reported that in the female rat intestinal mucosa the activity of arylhydrocarbon hydroxylases increased after administration of estrogens [1]; this increase in activity was accompanied by an elevation of microsomal cytochrome *P*-450 [2]. Since it is well known that in steroid hormone responsive tissues steroids influence gene expression after binding to specific receptor proteins we were interested to see if estrogens act in the rat intestinal mucosa on the level of microsomal cytochrome *P*-450 according to this mechanism. There are reports showing that specific estrogen binding proteins are present in the gastric mucosa and pancreas of male rats [3]. No data are, however, available on the presence of estrogen binding proteins in the gastrointestinal tract of female rats. With the aim of obtaining some information on the mechanism of action of estrogens on the level of cytochrome *P*-450 in the rat small intestine we searched for estrogen binding components (EBC) in this tissue.

EXPERIMENTAL

Materials

[2,4,6,7-^{3}H] estradiol 17β (E_2) (101 Ci/mmol) was obtained from Amersham International plc (Amersham, England). Nonradioactive steroids (E_2, estrone, estriol, progesterone and dexamethasone) as well as tamoxifen, hydroxylapatite, DNA (calf tymus, type 1), diisopropylfluorophosphate (DFP), trypsin inhibitor (soya bean), dithiothreitol (DTT) were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Norit A was obtained from Serva (Heidelberg, Fed. Rep. Germany), and dextran T-70 from Pharmacia Fine Chemicals (Uppsala, Sweden).

Adult female rats (Wistar) aged from 2 to 3 months were maintained on a commercial diet and water *ad libitum* under constant temperature conditions. Animals were either nontreated or were treated by i.p. injection of E_2 (15 μg E_2 per animal) for 2 days prior to decapitation. The intestinal mucosa was obtained according to Stohs *et al.* [1]. The intestine was slit open and mucosa removed by scrapping with the edge of a glass slide. About 1.5 g of intestinal mucosa was obtained per animal.

Preparation of subcellular fractions

Tissue was homogenized in 9 vol 0.05 M Tris–HCl, 0.15 M KCl buffer, trypsin inhibitor (100 μg/ml), 0.7 mM DTT, 20% glycerol, 1 mM heparin (3 U/ml), 2 mM DFP, pH 7.8 in a Potter glass–teflon homogenizer. Nuclear, microsomal, and cytosolic fractions were obtained by differential centrifugation. In experiments where E_2-treated rats were used the nuclei-free supernatant was treated with dextran-coated charcoal (DCC) to remove free steroid [4].

Binding assay

The presence of EBC was investigated in the nuclear, microsomal, and cytosolic fractions by competitive binding assay using ^{3}H-labelled and nonlabelled E_2.

The crude nuclear pellet was washed three times and resuspended in buffer by careful homogenization in a glass–glass hand homogenizer (1 ml nuclei + 6 ml buffer). An aliquot of the nuclear suspension was added to tubes containing [^{3}H]E_2 (0.2 to 20 nM) with or without a 250-fold excess of unlabelled E_2 and incubated for 2.5 h at 4°C. Nuclei were washed with buffer–hydroxylapatite suspension according to Walters *et al.* [5]; radioactivity was determined after extraction with ethanol. Results were analysed according to Scatchard [6]. Specificity of nuclear EBC was also tested. Samples of nuclear suspensions were incubated with [^{3}H]E_2 at concentrations of 0.5 or 5.0 nM and a 250-fold excess of various nonlabelled competitors as described above; the compounds used were E_2, estrone, estriol, tamoxifen, dexamethasone and progesterone. The inhibition of binding of [^{3}H]E_2 by different competitors was expressed as percent of inhibition found with [^{3}H]E_2.

For EBC binding assay microsomal pellet was rinsed with distilled water and resuspended in buffer to an approximate concentration of 2 mg protein/ml. Microsomal suspension (0.5 ml) was incubated with 5 nM [^{3}H]E_2 ± 250-fold excess

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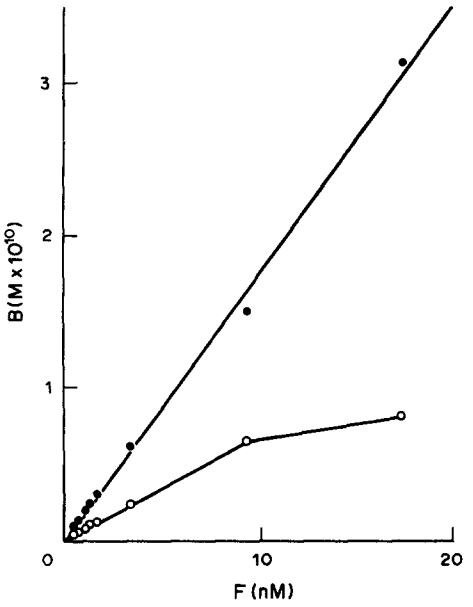

Fig. 1. E_2 binding to the nuclear fraction from female rat intestinal mucosa. Nuclear fraction was incubated with an increasing concentration of [^{3}H]E_2 ± a 500-fold excess of unlabelled E_2 and the bound [^{3}H]E_2 (B) was plotted against the concentration of [^{3}H]E_2 in the incubation medium (F). ●—●, Nonspecific binding; ○—○, specific binding. Each point is the mean of three measurements from the same experiment.

of [^{1}H]E_2 for 60 min in an ice-bath. Samples were applied to Sephadex LH-20 mini-columns to remove unbound steroid [7]; bound radioactivity was counted in the eluates.

When testing for the presence of EBC in the mucosal cytosol samples were labelled with [^{3}H]E_2 (either 0.5 or 5 nM) with or without a 100-fold excess of [^{1}H]E_2. In some experiments a 250- or 500-fold excess of [^{1}H]E_2 was used. Free and bound steroids were separated either by DCC-assay [4] or by Sephadex LH-20 [7]. In some experiments

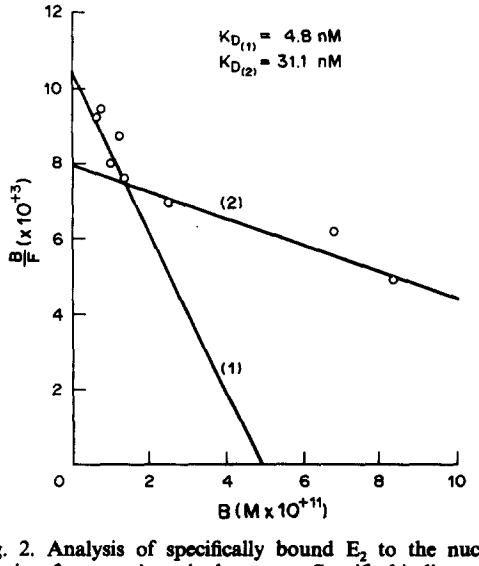

Fig. 2. Analysis of specifically bound E_2 to the nuclear fraction from rat intestinal mucosa. Specific binding of E_2 from Fig. 1 was plotted according to Scatchard [6]; the slope of the line was determined by the method of least squares. (B) Concentration of specifically bound [^{3}H]E_2; (F) concentration of [^{3}H]E_2 in the incubation medium.

Table 1. Displacement of [^{3}H]E_2 from the E_2 binding components in nuclear fraction of rat intestinal mucosa

	Competition ability [^{3}H]E_2 concentration	
Competitor	0.5 nM[a]	5.0 nM[a]
E_2	100[b]	100[b]
Tamoxifen	82 ± 9	120 ± 14
Estrone	74 ± 5	195 ± 22
Estriol	80 ± 8	—
Progesterone	60 ± 9	195 ± 19
Dexamethasone	54 ± 7	185 ± 25

Values are mean ± SE of three experiments. The competition of different ligands for nuclear E_2 binding components was tested as described in Experimental.

[a]A 0.5 nM concentration of [^{3}H]E_2 was chosen to measure the competition for type 1 binding sites; 5.0 nM [^{3}H]E_2 was chosen to measure the competition for type 2 binding sites.

[b]The displacement of [^{3}H]E_2 with unlabelled E_2 was defined as 100%.

receptor assay was performed after selective precipitation of cytosolic proteins with $(NH_4)_2SO_4$ according to Eriksson [8].

Analytical methods

Protein and DNA concentrations were determined according to Lowry *et al.* [9] and Burton [10], respectively.

RESULTS AND DISCUSSION

The subcellular fractions which are known to contain estrogen receptors in different target tissues were examined for the presence of EBC in intestinal mucosa of female rats. Using different experimental approaches we were not able to detect specific EBC in either the cytosolic or microsomal fraction; EBC were, however, found in the nuclear fraction of intestinal mucosa but only when the samples were incubated with [^{3}H]E_2 for shorter times (2.5 h); when the exchange assay was performed overnight no specific binding of E_2 was detected presumably because of the presence of high levels of degradative enzymes in this tissue. Nuclear EBC could be determined in spite of a high level of nonspecific binding; results are shown in Figs 1 and 2. Judging from the kinetic characteristics nuclear fraction contained two different types of EBC; type 1 with $K_{d1} = 4.8 \pm 0.8$ (mean ± SE) nM, $n_1 = 18 \pm 4.2$ (mean ± SE) fmol/mg protein ($n_1 = 83.4 \pm 12.5$ fmol/mg DNA) and type 2 with $K_{d2} = 31.1 \pm 6.8$ nM and $n_2 = 91.1 \pm 18.5$ fmol/mg protein (412.7 ± 80.0 fmol/mg DNA). It has to be emphasized that type 2 EBC were observed in all tested samples in spite of the presence of DTT in the homogenization and incubation buffer; DTT is mainly known to completely inhibit E_2 binding to type 2 nuclear binding sites in rat uterus [11]. The content of type 2 EBC could be slightly increased by pretreating animals with E_2 (in different preparations of mucosal nuclei the increase varied from undetectable to 2.5-fold); no influence of E_2 on type 1 EBC was observed.

The specificity of E_2 binding to EBC in nuclear fraction was also tested at a single concentration of competitors. Neither type 1 nor type 2 EBC were absolutely specific for estrogens (Table 1): type 1 EBC were slightly more specific for estrogens and the antiestrogen tamoxifen as compared to progesterone and dexamethasone; the difference in the displacement ability of [^{3}H]E_2 between estrogens and progestins was, however, not significant enough to satisfy the criteria for estrogen receptors. Type 2 binding sites exhibited a broad specificity concerning steroid binding; they bound other competitors with even greater affinity than E_2 and seemed to be less specific for E_2 if compared to other steroids as competitors.

For both classes of EBC found in female rat intestine the affinity and the specificity for E_2 were relatively low, therefore, their role in cytochrome *P*-450 induction as classical

estrogen receptors is questionable. The previously observed effect of E_2 on the increase of cytochrome *P*-450 level might be the result of direct action of E_2 on *P*-450 mRNA [12].

Although the biological role of the EBC described in the female enterocytes is unknown the EBC with low specificity and higher K_d (type 2) could play a role in the enterohepatic circulation of steroid hormones known to occur in the small intestine [13]. Detection of type 1 EBC could merely be the result of E_2 binding to an Ah receptor, known to induce arylhydroxylase; such a reciprocal relationship between EBC and Ah receptors was reported by Tierney *et al.* [14]. As suggested by Okey *et al.* [15] such components of broad specificity may regulate either the induction of different carcinogen metabolizing enzymes or may play some role in cancerogenesis such as transport of the inducer into the nucleus.

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