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SCIENCE DIRECT
Life Sciences 78 (2006) 2919 – 2922
Life Sciences
www.elsevier.com/locate/lifescie

Endometrium estradiol receptors type I and type II during early pregnancy of rat

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Received 23 May 2005; accepted 15 November 2005

Abstract

By centrifugation in a sucrose density gradient we studied the citosol 17β-estradiol binding sites of blastocyst receptive and non-receptive endometrial zones, as well as uterine horn endometrium whose ovary was extirpated three weeks before pregnancy. The cytosol was prelabelled with {^{3}H}-17β-estradiol 2 and 25 nM. In this work two incubation temperatures were studied. On the other hand, at 4 °C unoccupied receptors were identified as different from the classic receptor 8S type I. At the same time, we found that 25 °C is the optimal temperature for the assay of total receptors to achieve complete exchange of {^{3}H}-17β-estradiol by 17β-estradiol in the binding sites. In these conditions, the major component was the 4S type II receptor, mainly in the endometrium from ovariectomized uteri. Furthermore, 17β-estradiol content was determined in the total homogenized by radioimmunoassay and the results were: 1.42 ± 0.16, 1.22 ± 0.15 and 1.75 ± 0.27 pmol/g wet tissue for receptive, non-receptive and ovariectomized uteri, respectively.

Keywords: Estradiol receptors; Endometrium; Sucrose gradient; Rats

Introduction

Binding properties of two proteins to intracellular specific receptors to 17β-estradiol in different target tissue of rat and humans have been reported (Calzada et al., 1993; Clark et al., 1978; Panko et al., 1981). They are described as union site or receptors type I and type II, respectively. The type I corresponds to the classic receptor and is located in the region of 8S in a sucrose density gradient, characterized by having high affinity and low binding capacity; 4S type II has opposite properties. Furthermore it is not translocated (Lopes et al., 1987) and is identified more easily by exchange analysis, which consists on incubating at 25 °C in presence of 25 nM {^{3}H}-17β-estradiol. At this temperature, the dissociation and reassociation of the receptor esteroid complex is more active and the {^{3}H}-17β-estradiol substitutes the endogenous 17β-estradiol (Sanborn et al., 1971). It has been suggested that this receptor would constitute a reserve mechanism of 17β-estradiol for the receptor type I (Lopes et al., 1987).

In the oestrus cycle and pregnancy, the rat endometrium tissue shows functional changes in short periods of time in relation to ovarian steroid fluctuations. In day five of rat pregnancy, the endometrium presents regularly spaced zones that have undergone a series of biochemical changes (Dey et al., 1991; Heald, 1976), which permit the blastocyst implantation that will be identified easily in the fifth day, showing greater permeability to molecules of great size (trypan blue–albumin complex). These changes are part of the functional differentiation of those zones of the endometrium.

The mechanism that begins and maintains this process is not known, although it is known that increasing levels of progesterone from the beginning of pregnancy and an estrogenic environment are required for implantation, at least from day three (Dickmann et al., 1976; Yoshinaga, 1994). On the other hand, it has been proposed that the endometrium requires additional quantities of estradiol which would be produced by the blastocyst (Dickmann et al., 1977) to complete the functional differentiation of the endometrium.

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doi:10.1016/j.lfs.2005.11.018

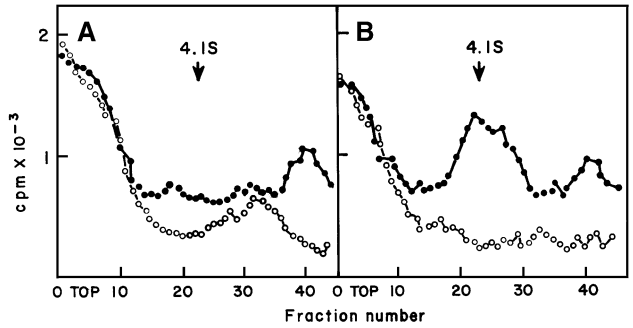

Fig. 1. Analysis by sucrose gradient of receptors type I and type II to 17β-estradiol in endometrium receptive to the blastocyst. The cytosol prelabelled using 2 and 25 nM of {^{3}H}-17β-estradiol (•-----•) or the same concentration of {^{3}H}-17β-estradiol more 200 times the molar concentration of 17β-estradiol not labelled (°-----°), was incubated at 4 and 25 °C, respectively, during 2 h. They are applied on sucrose gradient and the distribution of radioactivity is shown in the figures.

The close vicinity between two functionally different zones [blastocyst receptive (BR) and non-receptive (BNR) endometrial zones] within a same estrogenic environment, possibly requires a receptor complex system to express selectively the target cell function (Psychoyos, 1973).

In this article we propose to study the characteristics of the receptors to 17β-estradiol 8S type I and 4S type II (this last in exchange conditions) in the BR, BNR and endometrium of uterine horn submitted to unilateral oophorectomy (UO) previous to pregnancy, and the content of 17β-estradiol tissue in the same samples were determined.

Material and methods

Female rats of the Long Evans Strain of an average weight of 220±15 g and regular oestrus cycles were used under controlled light–dark conditions. Water and pellet food were supplied ad libitum. The rats were anesthetized with phenobarbital, unilateral oophorectomized and maintained for 21 days prior to study.

The pregnant rats were obtained after housing the mature females in groups of two with one male of the same strain. Vaginal smears were taken daily. Day zero of pregnancy was designated when spermatozoa were found in the smear and then males were removed. On day five between 9 and 11 AM, a group of pregnant rats was injected (i.v.) 1 ml of 1% trypan blue in saline. Thirty minutes later, these rats were sacrificed by cervical dislocation, then the rats were perfused with saline solution and their uterus were obtained.

In this experiment the blue-stained regions represented the implanted sites, whereas the non-stained tissue was considered as non-implanted regions (Heald, 1976; Sanborn et al., 1971).

The samples were frozen in liquid nitrogen and stored. The uteruses were thawed at 4 °C, cut lengthwise and gently agitated with glass pearls to detach the epithelial cells.

The cells were homogenized in a Tris buffer at 4 °C (Tris–HCl 10 mM, EDTA 1.5 mM, glycerol 10%, monothioglycerol 10 mM) in a proportion of 150 mg of wet weight/ml using a glass–glass homogenizer (2 pulses, each for 30 s at 150 revolutions/min with intervals of 60 s). The homogenate was centrifuged for 10 min at 800 *g*. The supernatant was reserved and the pellet was washed two times with the same buffer and recentrifuged. Supernatants were collected and centrifuged for 30 min at 105,000 g to obtain the cytosol, as well as adjusted the protein content to 2–4 mg protein /ml.

Type I estrogen receptors were determined incubating 500 µl of cytosol for 2 h at 4 °C, with 2 nM or 25 nM of ^{3}H-17β-estradiol. For the determination of type II estrogen receptors, identical batches were prepared and incubated for the same period of time at 25 °C. Non-specific binding was assayed using the same radioactive ligand plus a 200-fold excess of no radioactive ligand. Bound and free estradiol was separated using a dextran-coated charcoal separation method (Calzada et al., 1993).

Five hundred microliters of prelabelled [^{3}H-estradiol] cytosol were layered on a linear 5% to 20% sucrose gradient prepared in the same Tris buffer and centrifuged at 45,000 rpm for 15 h. After centrifugation 44 fractions were collected by puncturing the bottom of the tube. The distribution of radioactivity was measured by liquid scintillation counted on a Packard Tri-Carb Model 3380. [^{14}C] bovine serum albumin was used to estimate the location of the 4S region of the gradient in control experiments.

Tissue 17β-estradiol determinations were made in 200 µl of homogenized (approximately 30 mg of wet tissue) extracted with ether and measurements by radioimmunoassay by duplicate with commercial kits (cis Sorin, France).

Results were expressed as femtomoles per milligram protein. Specific saturation data was plotted by the Scatchard method (Scatchard, 1949). Receptor levels were calculated by the graphical method of Rosenthal (Rosenthal, 1967) . Protein values were determined using Lowry method (Lowry et al., 1951).

Results

To establish the optimum conditions for the complex receptor formation analysis, the system was studied in different

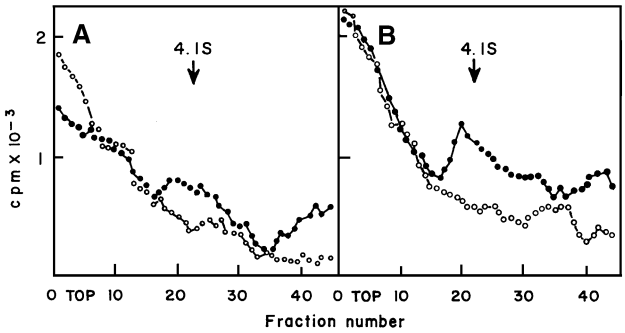

Fig. 2. Analysis by sucrose gradient of receptors, type I and type II to 17β-estradiol in endometrium non-receptive to blastocyst. The cytosol was prelabelled as is described in Fig. 1. Total incorporation of {^{3}H}-17β-estradiol (•-----•), incorporation of {^{3}H}-17β-estradiol in presence of 200 times the concentration molar of 17β-estradiol not labelled (°-----°).