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Conventional estrogen receptors are found in the plasma membrane of vaginal epithelial cells of the rat

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Abstract

Estrogens induce rapid (non-genomic) and delayed (genomic) effects on the target cells. The early effects include induction of signal transduction pathway within seconds, whereas the delayed responses require hours and involve transcription and translation. The rapid effects of estradiol (E) on the vaginal epithelial cells (VEC) involved calcium uptake within seconds via the induction of phosphoinositol lipid metabolism as reported in our earlier studies. In this study, we demonstrate the presence of classical estrogen receptors (ER) on the plasma membrane of VEC of the rats. Immunoreactive bands of 67, 56 and 35 kDa are detectable in the membrane fractions (mf) using antibodies recognizing different epitopes of ERα. We have also been able to purify a protein having a mass of 67 kDa from the detergent-soluble fraction of the plasma membrane of VEC, which shows properties identical to the classical receptor purified from the cytosolic fraction of the cells. The membrane receptors get dissociated upon binding to the ligand. Besides a role in signal transduction events induced by estradiol, the membrane estrogen receptors may have an important role to play in translocation of the steroid to the cytosolic compartment.

Keywords: Estradiol; Estradiol receptors; Plasma membrane; Non-genomic effects; Steroid hormones; Rapid responses

1. Introduction

Estradiol (E) exerts pleiotropic effects on its target tissues. Adding to this pleiotropism are the various kinds of receptors, which are being found and the way in which they interact with each other or other factors [1–7]. Of these estrogen receptors (ER), alpha and beta (ERα and ERβ) are known to be cytosolic/nuclear and serve as the transcription factors mediating estrogen-induced changes in the gene expression [8]. The presence of membrane-associated steroid receptors had been quite controversial since first proposed, though target cells had been isolated by columns prepared from ligand-tagged Nylon meshes [9,10] indicative of membrane-associated binding sites. There also have been attempts to show binding of the estradiol to purified membranes [11]. It is only in the last several years that the work on membrane-associated estrogen-binding sites has gained impetus. Estradiol has been shown to initiate second messenger pathway in estrogen responsive cells [12–19], suggesting the presence of membrane-associated binding proteins [20–23]. These sites are being identified using estradiol–BSA (E–BSA) conjugated to FITC directly and elicitation of the second messengers upon exposure to E–BSA [15,16]. Binding of E–BSA to membrane proteins was demonstrated by Zheng and Ramirez [3] who found that the estradiol binds to a large number of polypeptides in the range of 23–40 kDa and subsequently isolated a 23 kDa polypeptide from the mitochondrial membranes as an estradiol-binding protein. Studies from the other groups have demonstrated that E–BSA and estradiol show different modes of action [23], indicating that caution is warranted while using the impeded ligand to demonstrate estradiol-induced effects.

Non-genomic rapid effects have been demonstrated for almost all the steroids, and the cell surface forms of their respective receptors have not only been hypothesized but also characterized in many cases.

Vaginal epithelial cells (VEC) of the rats grow and differentiate under the influence of estradiol. A single intraperitoneal injection of 17β-estradiol (10 μg per 100 g bodyweight) to 30-day-old immature rats initiates a series of events leading to the formation of a cornified envelope in the VEC [24–28]. Among the early responses seen in the rat

Abbreviations: ER, estradiol receptor; E–BSA, estradiol–BSA conjugate; VEC, vaginal epithelial cells; DES, diethyl-stilbestrol

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VEC is calcium uptake via the phosphoinositide-mediated signal transduction pathway resulting in activation of kinases [15,25–27]. These effects are independent of transcription and translation and at the same time blocked by ER antagonist. Such observation suggests the possibility of the existence of plasma membrane-associated ER in VEC.

In this investigation, we characterize these receptors demonstrating the presence of conventional ERα as an integral component of plasma membrane of rat VEC and isolation of ERα by affinity purification. We also show that the binding sites identified by impeded ligand E–BSA are in the low molecular weight range and are not identified by any of the receptor antibodies.

2. Experimental

2.1. Animal model

Immature 30-day-old Wistar rats were maintained in 12-h light-and-dark period with food and water ad libitum.

2.2. Confocal microscopy

VEC collected from 30 rats were cultured in DMEM without serum at 37 °C in 5% CO_2 for a day. Harvested cells were layered on poly-1 lysine-coated coverslips and stained with FITC-tagged E–BSA [17β-estradiol-6-(*O*-carboxymethyl)-oxime–BSA–fluorescein isothiocyanate conjugate (Sigma); molar ratio E:BSA = 10:1]. Cells were pretreated with 1% BSA for 15 min at 4 °C and then incubated with E–BSA–FITC (10 μg/ml) for 30 min at 4 °C [16]. Cells were washed with PBS, fixed in 4% paraformaldehyde for 10 min at 4 °C and scanned in confocal microscope (Meridian). Alternatively, cells were stained for immunofluorescence using mc20 antibodies against ERα (Santa Cruz). After blocking in 1% BSA, cells were incubated in anti-ER antibodies (1 μg/ml) and anti-rabbit antibody conjugated to FITC 1:100 dilution (Sigma) at 4 °C. After washing, cells were fixed with 4% paraformaldehyde for 10 min and examined with confocal microscope (Meridian).

2.3. Membrane isolation

VEC collected from rats, homogenized in buffer A containing 1 mM $NaHCO_3$ and 2 mM $CaCl_2$, 2 mM PMSF, 10 mM leupeptin and 2 μg/ml pepstatin were incubated for 30 min at 4 °C, sonicated and membranes were isolated according to the procedure of Ray [29] with some modifications. The homogenate was centrifuged at 800 × *g* for 30 min at 4 °C. The supernatant was centrifuged at 100 000 × *g* for 1 h and the pellet was suspended in buffer B containing 5 mM HEPES in 0.9% sodium chloride with protease inhibitors (leupeptin 10 mM, pepstatin 2 μg/ml). After centrifuging at 100 000 × *g* for 30 min at 4 °C, the pellet obtained was used as 'membrane fraction' (mf). The membrane fraction was washed with buffer B two to three times before using for subsequent studies. The purity was checked by enzyme assays for Na^+/K^+-dependent ATPase, a marker for plasma membrane [30], succinic dehydrogenase, a marker for mitochondria [31] and glucose-6 phosphatase for microsomal fraction [32]. The assays were carried out on the crude homogenate, supernatant and the membrane fraction.

2.4. Receptor assay

Membrane fractions of VEC from a total of 200 rats were subjected to radio-receptor assay. Aliquots of membrane fraction suspended in buffer B containing molybdate (5 mM) were incubated with ^{3}H-estradiol (specific activity: 151 Ci/mmol, Amersham) in the range of 0.5–12.5 nM overnight at 4 °C. Diethyl-stilbestrol (100-fold excess) was added to assess the specific binding. The samples were filtered through Whatman GF/C filters and washed with excess buffer. The filter discs were counted in a Packard LSA scintillation counter. Data were plotted and analyzed by Scatchard analysis.

2.5. Conjugated ligand and Western blot studies

Iodination was carried out as follows. E–BSA conjugate and BSA were iodinated with iodogen and separated by passing through G50 column, according to the procedure given in laboratory manual [33]. Fractions (0.5 ml) were collected and counted in a γ-scintillation counter (Packard Instruments). The conjugate was recovered generally in four to five fractions. The average counts in different iodination reactions were in the range of 8–1 000 000 cpm/ml which were used for ligand blot assays in 1:1000 dilution.

Membrane fractions were analyzed by SDS–PAGE and proteins were transferred to PVDF membranes. For conjugated ligand studies, the blots were incubated with 1% BSA as a blocking agent, followed by incubation in radio-iodinated E–BSA conjugate (17β-estradiol-6-(*O*-carboxymethyl)-oxime–BSA) overnight at 4 °C. After washing five to six times with phosphate buffer saline (PBS), blots were exposed to X-ray film and developed. Controls were incubated in buffer with radio-iodinated BSA. For Western blot analysis, blots were probed with antibodies against ERα (1–5 μg/ml) in 3% BSA. Antibodies used were mc20 or c311 (Santa Cruz) recognizing the ligand-binding domain and AE 304 (Neomarkers) recognizing the DNA-binding domain of ERα. After washing with PBS, the blots were incubated with appropriate second antibodies conjugated to peroxidase in 1:5000 dilution (Amersham) for 30 min at room temperature and developed with ECL reagent (Amersham).

2.6. Purification of ER

Membrane fraction was incubated with 5 mM CHAPS at 4 °C for 30 min after a brief period of sonication. After centrifugation at 100 000 × *g* for 30 min, supernatant was used to purify receptors. KCl was added to the supernatant to adjust the final concentration to 0.7 M before loading on the affinity column. Affinity columns were prepared by linking BSA to CNBr-activated sepharose 4B and tagged with estradiol-17β hemisuccinate. Receptors were purified from both the solubilized membrane fraction and cytosol using affinity purification method [34]. Recovery was estimated by binding assay in the starting material (cytosolic fraction and solubilized membrane fraction) and elutes. Eluted protein was subjected to SDS–PAGE and Western blot analyses, and gel filtration by high-performance liquid chromatography (HPLC) using IC-125 column.

2.7. Dissociation of receptors from membranes

Membrane fraction of VEC was incubated with different concentrations of estradiol from 10^{-5} to 10^{-10} M, progesterone, various agonists like diethyl-stilbestrol (DES) and environmental estrogens—bisphenol A and *n*-octyl phenol (Aldrich), geneistein and coumestral (Sigma) antagonists of estradiol—tamoxifen (Sigma), ICI 182780 (gift from Dr. Philip W. Shaul) for 30 min at 37 °C (concentrations are mentioned in the legend of Fig. 7). Membranes were pelleted by centrifugation at 100 000 × *g* and the supernatants were analyzed for receptor content by Western blot analysis using mc20 antibodies against ERα (Santa Cruz).

3. Results

3.1. Association of estrogen receptors with membranes

Membrane-associated estrogen-binding sites could be demonstrated by various techniques employed in this paper. Previously, we had shown that impeded ligand in the form of estradiol conjugated to BSA could elicit the calcium spiking in rat VEC [15]. Confocal studies following incubation of the cells from the primary cultures of vaginal epithelium with estradiol–BSA–FITC show the staining on the cell membrane (Fig. 1A and B). Immunofluorescence studies on the impermeabilized cells for estrogen receptors (using estrogen receptor antibody and FITC-conjugated second antibody) demonstrated estrogen receptor staining on the membrane (Fig. 1C and D). CHO and MCF-7 cells were also studied simultaneously as controls, where CHO cells did not display binding to E–BSA or ER antibodies, but the MCF-7 cells were found positive in both the cases (Fig. 1E and H).

The purified membrane fraction consisted of plasma membrane, as the fraction assayed for various marker enzymes did not show significant activity for microsomal enzymes or mitochondria (Table 1).

3.2. Receptor levels

To identify and characterize the estrogen-binding sites in the plasma membrane fraction of VEC, binding studies were carried out on the purified membrane fraction using ^{3}H-estradiol. Incubation of the membrane fraction at a concentration range of 0.5–12.5 nM revealed that the membranes showed specific binding to estradiol with a dissociation constant (K_d) of 0.6 nM. However, the quantity of receptor in the membrane fraction was 2.5 pmol/mg of protein (Fig. 2).

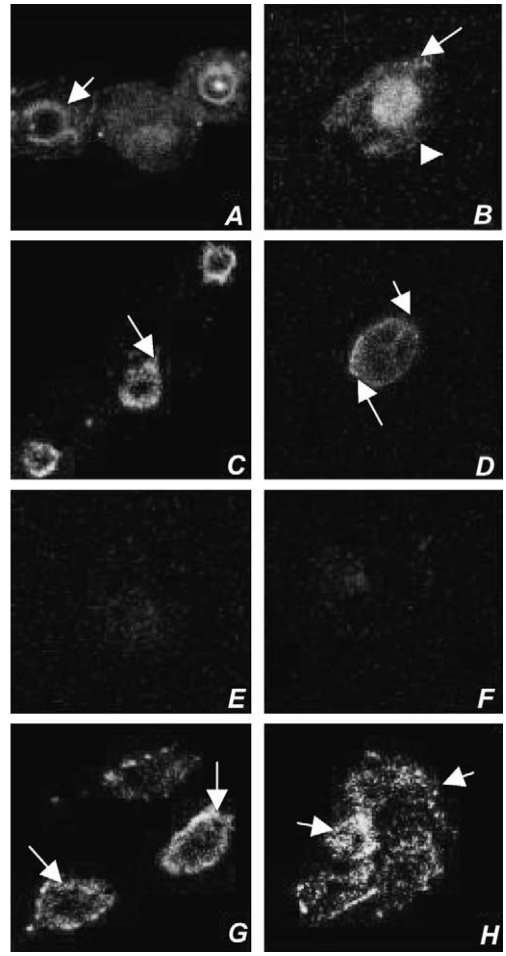

Fig. 1. Labeling of the estrogen-binding sites on live vaginal epithelial cells. (A, B) The cells are labeled with E–BSA–FITC for 15 min. (C, D) The cells are labeled with antibodies against ER (mc20); CHO and MCF-7 cells are used as controls. (E, F) CHO cells labeled with E–BSA–FITC (E) and ER antibodies (F) do not show any staining for ER. (G, H) MCF-7 cells labeled with E–BSA–FITC (G) and ER antibodies (H) are positive for ER receptors. Arrows point to the membrane-associated ER.

3.3. Characterization of binding sites

In order to characterize the membrane-associated estradiol-binding sites, the membrane proteins separated by SDS–PAGE were subjected to conjugated ligand binding using iodinated E–BSA in the presence of 1% BSA. As shown in Fig. 4, a large number of proteins in the range of 20–43 kDa showed specific labeling with 17β-estradiol–BSA (^{125}I). Some blots show very faint staining of 67 and 56 kDa

Table 1
Analysis of subcellular marker enzymes in the membrane fraction

Cell fraction	Markers for			
	Plasma membrane Na^{+}/K^{+} ATPase		Mitochondria SDH[b]	Microsomal glucose-6 phosphatase[a]
	−Ouabain[a]	+Ouabain[a]		
Total homogenate	15.6	13.7	23.1	0.78
Supernatant	13.2	12.4	0.8	0.14
Pellet (membrane fraction)	27.12	12.8	0.01	0.02

[a] Activity expressed as phosphorus released per milligram of protein per reaction.
[b] Activity expressed as dye reduced per milligram of protein per reaction.

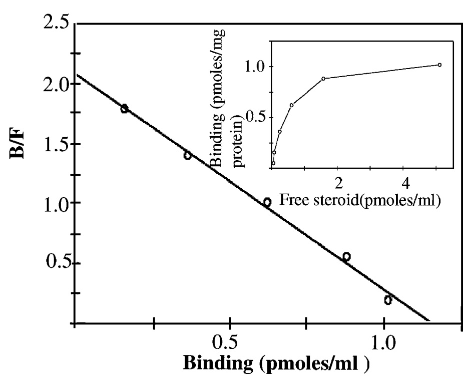

Fig. 2. Scatchard analysis of estradiol-binding protein in purified membrane fraction (mf) of VEC. Estradiol binds to the mf with a K_d of 0.6 nM upon incubation with ^{3}H-estradiol-17β in the range of 0.5–12.5 nM with 100-fold excess of DES. Inset shows the binding curve of the steroid to the mf. Experiments were repeated six times.

proteins. The binding to these proteins (20–43 kDa) could be inhibited by cold E–BSA, as the blots preincubated with this gave negative results. Upon treatment with detergents, some of these proteins could be extracted from membranes (Fig. 3A). Radio-iodinated BSA also did not show any binding in the membrane fraction (Fig. 3B).

Since many polypeptides showed binding to the conjugated ligand, Western blot analysis of the membrane proteins using mc20 antibody against the ligand-binding region and AE 304 against the DNA-binding region of the ERα was carried out. Our results revealed that these antibodies recognized 67, 56 and 35 kDa polypeptides (Fig. 4). None of these polypeptides were labeled consistently in the ligand blot. To rule out contamination from cytosolic fraction, membrane fraction was incubated at 37 °C for 30 min before centrifugation at 100 000 × g, or washed repeatedly (three to four times), but still revealed the presence of 67, 56 and 35 kDa polypeptides. Membrane fractions treated with detergents (1% Triton X-100 or 5 mM CHAPS) showed that these receptors get extracted partially in the detergents and a fraction of these polypeptides is also retained in the residual particulate fraction (Fig. 4).

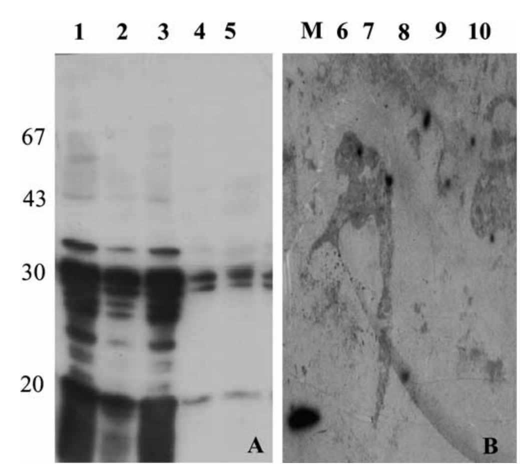

Fig. 3. Ligand blot analysis of the mf of rat VEC using iodinated estradiol BSA. Panel A shows the blot treated with iodinated E–BSA and panel B represents the blot treated with iodinated BSA. Lanes: (1, 6) mf; (2, 7) 1% Triton X-100-treated mf; (3, 8) 5 mM CHAPS-treated mf; (4, 9) supernatant from lanes 2 and 7, respectively; (5, 10) supernatant from lanes 3 and 8, respectively; (M) marker. Experiments were repeated six times.

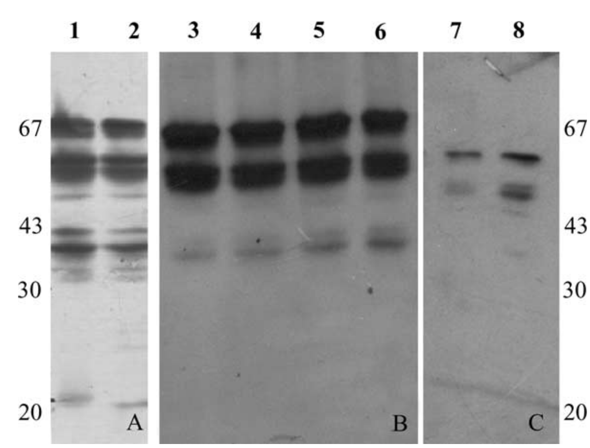

Fig. 4. Western blot analyses of mf of rat VEC separated by SDS–PAGE and treated with anti-estrogen receptor antibodies. Panel A: the blot was treated with ER antibodies (mc20) against LBD of ER. Lanes: (1) purified mf; (2) mf incubated at 37 °C. Panel B: the blot was treated with anti-ER antibody AE 314 against DBD of ER. Lanes: (3) mf; (4) mf incubated at 37 °C; (5) 1% Triton X-100-treated mf; (6) 5 mM CHAPS-treated mf. Panel C: the blot was treated with antibody AE 314. Soluble fraction of membrane after extraction with detergents. Lanes: (7) Triton X-100 (1%); (8) CHAPS (5 mM). Experiment was repeated four times.

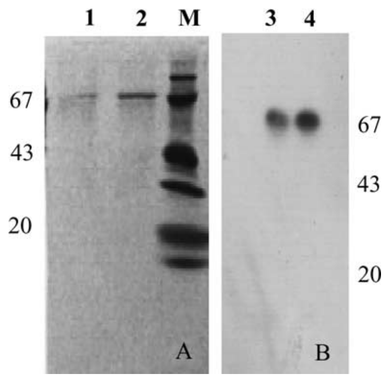

Fig. 5. Analysis of affinity-purified receptor. SDS–PAGE analysis (A) of affinity-purified estrogen receptor from membrane (1) and cytosolic (2) fraction of rat vaginal epithelium. Western blot analysis using estrogen receptor antibody (B) shows presence of identical receptor in membrane (3) and cytosolic (4) fractions. Experiments were repeated six times.

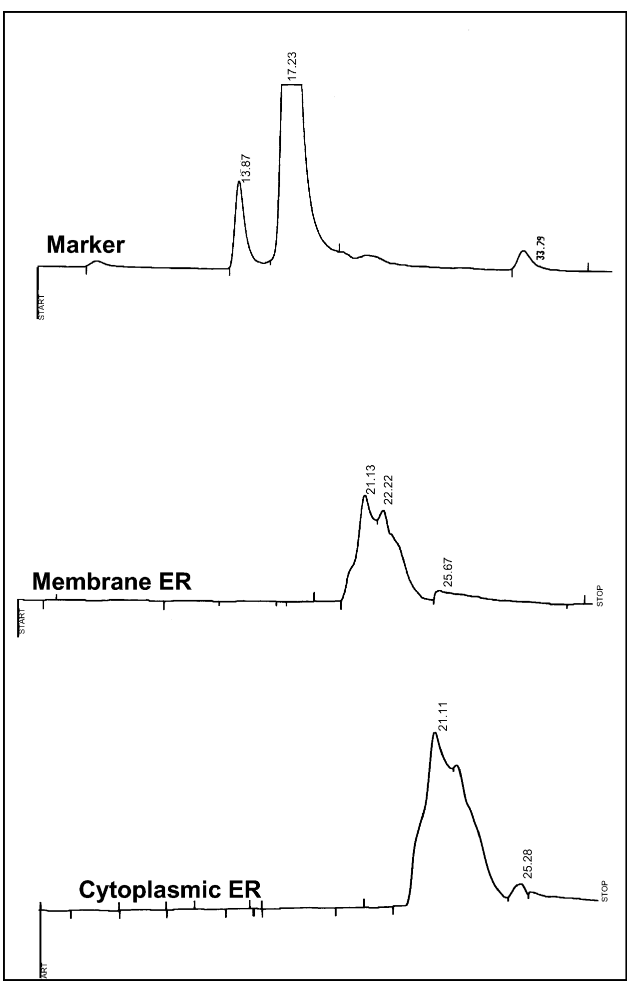

Fig. 6. HPLC analysis of purified receptor from membrane and cytosolic fractions of the rat VEC reveal identical elution patterns of receptors from both the fractions. Experiment was repeated 10 times.

3.4. Purification of the receptor

Purification of ER from the membrane fraction involved extraction of membranes with 5 mM CHAPS. The yield of receptor at the end of purification was about 30%. Nonetheless, using affinity column of sepharose BSA tagged to estradiol-17β hemisuccinate, we could purify a 67 kDa polypeptide from both the cytosolic and the membrane fractions. The purity of the protein was confirmed by SDS–PAGE and Western blot analyses in which a single protein at 67 kDa binds with c311, an ERα antibody (Santa Cruz) (Fig. 5). The purity of the protein was ascertained by gel filtration using IC-125 column in HPLC. The elution profile for both the cytosolic and membrane-associated protein was identical (Fig. 6).

3.5. Dissociation of receptor from membranes upon ligand binding

It was found that upon incubation of the membrane fraction with different analogs/antagonists of estradiol or xenoestrogens with the membrane-bound estrogen-binding sites, receptor gets dissociated from the particulate fraction and gets recovered in the supernatant. Preincubation of the membrane fraction with various concentrations of estradiol (10^{-5} to 10^{-10} M) resulted in the release of the receptor into the supernatant fraction. Western blot analyses of both the pellet and the supernatant fractions show the presence of estrogen receptor. Membrane fraction preincubated with the vehicle, however, did not show any receptor in the supernatant (Fig. 7). Similar studies carried out with

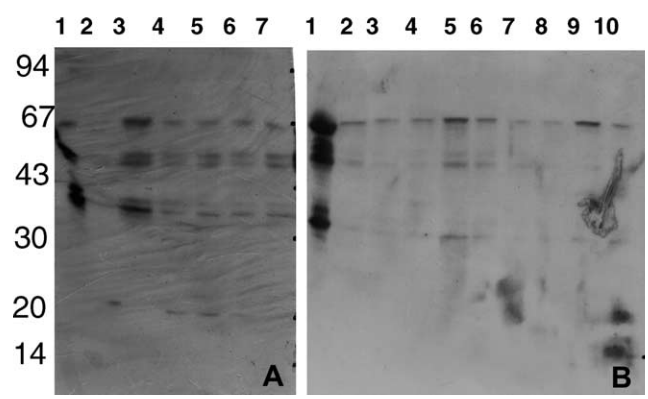

Fig. 7. Dissociation of receptors from the membranes. Membrane fractions were incubated with estradiol, different agonists and antagonists for 30 min at 37 °C, and pelleted down. Immunoblot analysis of the supernatant revealed the presence of receptors. Panel A represents the supernatant of membranes treated with different concentrations of estradiol: (1) mf, (2) mf incubated at 37 °C, mf treated with (3) 1% Triton X, (4) estradiol 10^{-5} M, (5) estradiol 10^{-7} M, (6) estradiol 10^{-9} M, and (7) estradiol 10^{-10} M. Panel B represents the supernatants of the membranes treated with different compounds: (1) mf, mf treated with (2) estradiol 10^{-5} M, (3) estradiol 10^{-7} M, (4) estradiol 10^{-9} M, (5) diethyl-stilbestrol 10^{-5} M, (6) tamoxifen 10^{-5} M, (7) ICI 182780 10^{-7} M, (8) bisphenol A 10^{-7} M, (9) *n*-octyl phenol 10^{-7} M, (10) coumestrol 10^{-7} M. Experiment was repeated five times.

different agonists and antagonists of estradiol (tamoxifen, ICI 182780, DES, bisphenol A, *n*-octyl phenol, geneistein, and coumestral) and Western blot analyses showed the appearance of receptor in the supernatant fraction (Fig. 7).

4. Discussion

The present study demonstrates the presence of conventional ERα in the membrane of vaginal epithelial cells of rats. These results are supported by the data obtained employing various experimental protocols. Previously, we had reported that estradiol induces calcium uptake in VEC that involves phospholipase C activation leading to breakdown of PIP2 and is independent of transcriptional events induced by estradiol [15,27]. Calcium uptake is mediated by impeded ligand and blocked by anti-estrogen tamoxifen, suggesting the involvement of membrane-associated receptors and these would allow estradiol to act across the membrane. Use of impeded ligand tagged to FITC on impermeabilized cells demonstrated staining on membranes, suggesting the presence of estradiol-binding sites. Immunofluorescence studies using antibodies against the DNA-binding domain and the ligand-binding domain of ERα confirmed the presence of receptors on the cell surface.

Further characterization of the receptor involved cell fractionation. Care was thus taken to avoid the presence of contaminants from other cell fractions as well as non-specifically bound proteins. Incubation of the membrane fraction with estradiol demonstrates specific binding of hormone to the membranes with a dissociation constant of 0.6 nM, which is in agreement with the affinity of conventional receptor from VEC [36]. Scatchard analysis of binding data indicates the presence of single estradiol-binding site in the membrane fraction of VEC.

Since the impeded ligand BSA could induce non-genomic effects in rat VEC [15], efforts were made to characterize the estradiol-binding membrane proteins using radio-iodinated E–BSA in a conjugated ligand blot. Our results demonstrate that E–BSA binds to the membrane proteins at low molecular weight range (20–40 kDa) similar to those reported by Zheng and Ramirez [18]. These proteins could be extracted partially by detergents; however, none of these were reactive to antibodies against ERα. Besides these, a faint staining of 67 and 56 kDa proteins could also be seen inconsistently, which show immunoreactivity to ER antibodies. Estradiol and E–BSA have been shown to elicit differential non-genomic responses [23]. Our studies suggest that the proteins bound by E–BSA are not the classical receptors.

Western blot analysis of membrane fractions revealed three major polypeptides, i.e. 67, 56 and 35 kDa. To rule out contamination from the cytosolic fraction due to non-specific attachment of proteins, the membrane fractions were washed three to four times or preincubated at 37 °C for 30 min [35]. Upon extraction using 1% Triton X-100 or 5 mM CHAPS, the residual fraction contained ER protein indicating that ER is an integral component of the membrane and not a contaminant from the cytosol. In the previous studies, affinity purification of conventional ER from the mouse uterine cells yielded three peptides of 66, 54 and 35 kDa and were considered to be alternately spliced products of ER, as all of them cross-reacted with different antibodies against ER [34]. In our studies, antibodies against ER recognized three major protein species in the membrane fraction. On purification using affinity against the ligand, we obtained a single 67 kDa protein from both the cytosolic and the membrane fractions which were recognized by the antibodies recognizing different epitopes of ER. The approach involved in purification and the end product appears identical in molecular mass. They display similar pattern upon tryptic digestion (data not shown) and display similar elution pattern in HPLC (IC-125) column under identical conditions. Both ER from the cytosolic and the membrane fractions elute near 40 kDa in native conditions (Fig. 7), but on denaturation with 25 mM β-mercaptoethanol, they elute at 67 kDa. Amino acid sequence analysis of the conventional ER reveals about 15 cystine residues, which probably account for a compact structure due to extensive cross-linking. In the presence of reducing agents, the structure may open up accounting for less retention in the gels.

These cumulative findings indicate that the major protein in the membrane fraction recognized by the antibodies to ERα is identical to the classical ERα. It is very difficult to explain the presence of this protein in the membrane, but our observations are consistent with the results reported by others [18]. The presence of ER in the membranes of GH3 cells was demonstrated using impeded ligand and immunofluorescence using ER antibodies. The most convincing evidence comes from the treatment with antisense oligo-deoxynucleotides to nuclear α mRNA, which results in the reduction in both the cytosolic and the membrane staining [37]. In another study, transient expression of ERα and ERβ in CHO cells results in the appearance of both membrane and cytosolic staining for ER [38]. These receptors are shown to be coupled to G protein and initiate InsP signaling. The other important outcome of their study was that the ligand-binding domain of ER could be on the membrane to allow effective interaction of the ligand with the receptor. However, the present studies are the first to characterize the membrane-associated ER in non-oncogenic, untransformed cells expressing the receptor at constitutive levels. Further characterization of these receptors, sequencing of this protein, DNA-binding properties and the post-translational modification studies are underway to throw light on the significance of their presence in the membranes.

We also demonstrate that these receptors get partially dissociated from membranes upon binding with ligand/agonist/antagonist. Incubation of the membrane fraction with these compounds resulted in the partial release of the receptor and its recovery in the supernatant. The membrane fractions incubated at 37 °C without any ligand did not have the receptors in the supernatant, whereas incubation with most of the agonists or antagonists resulted in the release of the receptor. Many of the estrogenic compounds are weakly estrogenic and are required in higher concentrations to elicit equivalent response. The concentrations used here are hence (10^{-7} M) much higher than anticipated on exposures except in special cases. This could have implications in receptor-mediated endocytosis of the hormone, though more work is needed to show internalization. Kinetic studies of steroid hormone metabolism, such as those for Vitamin D, suggest that non-specific diffusion across cell membrane cannot fully account for their clearance rate from the serum [39]. Work of Nykjaer et al. [40] shows that Vitamin D is taken up by renal tubular cells via the endocytic receptor megalin and also maintains Vitamin D homeostasis. Similarly, Karthikeyan and Thampan [35] demonstrate the presence of non-activated estrogen receptor in goat uterus plasma membranes which dissociates and translocates upon binding to estradiol and diethyl-stilbestrol and has many properties similar to its cytosolic counterpart.

There are many reports of atypical ER/estradiol-binding protein in the membranes. The β subunit of maxi K channel in blood vessels [4], E–BSA-specific binding proteins [3,18] and a 29 kDa estradiol–peroxidase conjugate-specific protein in the membranes of human sperm [5] are demonstrated to be responsible for rapid actions of steroids. In addition, certain rapid effects of estradiol can be induced through the membrane without the involvement of any kind of receptors but by physical perturbation of lipid environment [41]. Our studies show the presence of classical ER in the membranes of non-transformed cells.

In summary, we have shown that the conventional estradiol receptor is present in the membrane fraction as an integral protein and that it can be purified upon solubilization in detergents by affinity chromatography. These receptors may be critically involved in signaling upon exposure of VEC to estradiol. Furthermore, these receptors get dissociated upon binding to estradiol or its homologs and hence may aid in transportation of these agonists to the cytosol and nucleus.

Acknowledgments

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