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Review

# Nongenomic effects of 17β-estradiol—diversity of membrane binding sites

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**Abstract**

The classical model of the action of 17β-estradiol comprises binding of this gonadal steroid hormone to nuclear estrogen receptors leading to the modulation of gene transcription and protein synthesis. However, in the last few years several evidences about the rapid nongenomic action of 17β-estradiol via the stimulation of putative receptors located in the cell membrane have also been reported. These nongenomic responses occur within a few minutes and are insensitive to the inhibitors of transcription and translation; however, no such membrane receptors have been cloned so far. In this review article, we present a survey showing that such membrane binding sites of 17β-estradiol have rather different ligand specificity properties than that of classical genomic estrogen receptors concerning the potential activity of different estrogens and other steroid hormones, supporting the conception of structurally distinct receptors for genomic and nongenomic pathways. The fact that rapid responses to 17β-estradiol could be induced by a wide concentration range from some picomolar to high micromolar doses points to the diversity of these nongenomic membrane binding sites as well as the complexity of their functioning.


*Keywords:* 17β-Estradiol; Rapid nongenomic responses; Membrane binding sites

## 1. Introduction

The effects of sex hormones on mRNA and protein synthesis following binding of steroids to intracellular receptors have been studied for a long time. Such genomic effects are characterised by a specific delay (the latency of onset of 2–8 h) and a sensitivity toward the inhibitors of transcription (actinomycin D) and translation (cycloheximide) [1]. To date, two nuclear estrogen receptor subtypes have been cloned: nERα and nERβ, and a somewhat higher binding affinity of 17β-E2 has been measured for nERα comparing with nERβ (dissociation constants $K_d$ 0.1 nM for in vitro synthesised human nERα protein and 0.4 nM for rat nERβ protein) [2].

However, more than 20 years ago the capture of 17β-E2 by plasma membrane-associated estrogen receptors was also proposed [3]. Still, studies of nuclear localisation of these receptors largely fell in favour [4–7] almost until the end of the previous century when numerous works describing the effects of sex hormones incompatible with the genomic model of steroid action renewed the interest in these sites. Now, such effects are known as nongenomic and characterised by: (1) a short time of responses (usually the effects are measured during seconds or minutes); (2) insensitiveness to the inhibitors of transcription and translation; (3) different pharmacological properties from classical nuclear receptors (typically not blocked by the antagonists of nuclear receptors); and (4) the occurence in highly specialised cells that do not accomplish mRNA and protein synthesis or in cell clones where no nuclear receptors are expressed [1,8–10]. Such rapid effects appear to be indeed membrane receptor mediated as responses can also be elicited by sex steroids coupled to high-molecular weight substances (like BSA) that do not pass across the plasma membrane and do not enter the cell [10].

*Abbreviations:* ATP, adenosine 5′-triphosphate; BSA, bovine serum albumin; BzATP, 2′- and 3′-*O*-(4-benzoylbenzoyl)-ATP; $[Ca^{2+}]_i$, intracellular calcium concentration; DAMGO, Tyr-D-Ala-Gly-MePhe-Gly-ol; DES, diethylstilbestrol; DHT, 5α-dihydrotestosterone; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; 17α-E2, 17α-estradiol; 17β-E2, 17β-estradiol; E1, estrone; E3, estriol; E2:BSA, 17β-E2 coupled to BSA; 5-HT, 5-hydroxytryptamine, serotonin; [$^{125}$I]-his-P, progesterone-11α-hemisuccinate-(2-[$^{125}$I]-iodohistamine); nER, nuclear estrogen receptor; MAPK, mitogen-activated protein kinase; nAChR, nicotinic acetylcholine receptor; NO, nitric oxide; P, progesterone; P2X, ionotropic receptors activated by ATP; *R*(+)-8-OH-DPAT, *R*(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin; [$^{35}$S]GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); T, testosterone

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The stimulation of such nongenomic sites activates conventional second messenger cascades: phospholipase C, phosphoinositide turnover, adenylate cyclase, protein kinases A and C, changes in intracellular pH, the release of intracellular $[Ca^{2+}]_i$, the activation of MAPK which itself can lead to changes in the transcriptional level and thus bring about a cross-talk with sex hormones-mediated nongenomic and genomic responses [10]. Recently, the ability of pertussis toxin to block some of such nongenomic effects has been reported [11] demonstrating that at least some of the nongenomic responses of sex hormones are mediated by the signalling mechanism that involves coupling to G proteins.

The nature and characteristics of such membrane sites are still unclear. Rapid responses can be mediated by the unique membrane receptors which are structurally distinct from intracellular steroid receptors, but no such membrane receptors for 17β-E2 have been isolated or cloned so far. As classical estrogen receptors do not possess either hydrophobic domains for inserting the receptor to the plasma membrane or the potential sites of myristoylation or palmitoylation necessary for anchoring the receptor to the membrane, it is somewhat difficult to conceive a membrane localisation of intact nuclear receptors [12]. However, Razandi et al. [13] presented clear evidences that membrane and nuclear estrogen receptors may arise from a single transcript by transfecting cDNAs for ERα and ERβ into the Chinese hamster ovary (CHO) cells, which lack the endogenous ERs, and describing the expression of estrogen receptors in both nuclear and membrane compartments. Despite an essential progress that this work made in the field of studies of the nongenomic effects of steroid, the protein of membrane receptor still awaits isolation and structural analysis. Moreover, in this study near-identical subnanomolar affinities were measured for 17β-E2 to both nuclear and membrane sites [13]. As rapid responses to 17β-E2 have been reported in a wide concentration range (see below), the post-translational modifications can be different in various cellular systems or the mechanism of functioning of membrane receptors can be even more complex and diverse. For instance, rapid responses to steroids can also occur by the indirect modulation of cell functions by steroids acting as coagonists or by direct nonspecific steroid–membrane interactions that alter membrane physicochemical properties. The latter action can occur, however, predominantly at high steroid concentrations [1,8].

In the present work, we collected available information about the affinity of 17β-estradiol toward binding sites on cell membrane and analysed these data by: (1) quantitative parameters used for the characterisation of biochemical effects and the physiological responses on various signal transduction pathways; (2) the stereospecificity of estradiol action; and (3) the effects of other steroids (various estrogens, androgens, progestins, glucocorticoids, mineralocorticoids) on the same sites as well as the action of antiestrogens (ICI 182780, ICI 164384 and tamoxifen). We also give a survey of species and tissues where these nongenomic responses have been characterised as well as assay methods which have been used.

## 2. Potency of 17β-estradiol in rapid responses—are these effects physiologically important ?

The survey of nongenomic responses to 17β-E2 characterised by quantitative affinity parameters is presented in Table 1 and Fig. 1. It reveals that rapid effects have been measured at the 17β-E2 concentrations of some picomolar to high micromolar range. The differentiation of distinct binding sites is not too apparent being based on the graphical presentation of data, however, distinct site(s) with micromolar affinity (characterised by points 1–17 in Fig. 1) could be considered separately from the site(s) of nanomolar affinity. In this context, it could speculate on the existence of at least two different subtypes of the membrane receptors of 17β-E2. On the other hand, the metabolism of 17β-E2, being somewhat different in various species and tissues, as well as the circulation of sex hormones coupled to plasma proteins make the interpretation of data more complicated.

The most important question in the analysis of the effective concentrations of rapid responses to 17β-E2 is clearly the physiological relevance of these effects. The normal serum concentration of 17β-E2 depends to some extent on species. In premenopausal women, it is $\leq$0.28 nM in the follicular phase and $\leq$1.1 nM in the luteal phase and can rise up to 150 nM in the third trimester of pregnancy [14]. In estrogen replacement therapy in post-menopause, the serum 17β-E2 level can exceed 0.77 nM, being almost 10-fold higher than that seen in untreated post-menopausal women [14]. Therefore, as the effects of 17β-E2 measured at picomolar and low nanomolar concentrations represent the physiological actions of this gonadal steroid, the responses characterised by micromolar effective concentrations seem to be predominantly of pharmacological significance or could become physiologically important only at the ending period of pregnancy.

However, this situation is more complicated. The principal source of circulating estrogens in premenopausal women is ovarian production [14,15]. Beyond menopause, the major source becomes the biosynthesis of estrogens in extragonadal sites. Although the total amount of estrogens synthesised by extragonadal compartments may be small, the local tissue concentration achieved can be high and exert significant biological influence [15]. The predominant source of estrogens in post-menopausal women is the conversion of adrenal androgens. This process is catalysed by aromatase enzyme [15,16] and leads to the formation of relatively weak estrone (E1) which is generally present in serum as the inactive estrone sulfate [14]. The latter compound is hydrolysed to bioactive estrone by sulfatase [17] and estrone can be further converted to the biologically active estradiol by the action of 17β-hydroxysteroid

Table 1
Survey of rapid responses to 17β-estradiol

| Number of site | Biological system | | | Expression of intracellular ERs | Reference |
|---|---|---|---|---|---|
| | Species | Tissue | Gender | | |
| 1 | Human | Medulloblastoma | | | [23] |
| 2 | Human | Neuroblastoma | | | [23] |
| 3 | Bovine | Adrenal chromaffin | | | [24] |
| 4 | Rat | Saphenous artery | Female | | [38] |
| 5 | Human | Medulloblastoma | | | [23] |
| 6 | Rat | Phaeochromocytoma | | | [25] |
| 7 | Human | Neuroblastoma | | | [23] |
| 8 | Rat | Striatal synaptosomes | Female | Paucity of nER in the striatum | [21] |
| 9 | Human | Transfection of genomic DNA into mouse L-M fibroblasts | | | [20] |
| 10 | Human | Spermatozoa | Male | Mature spermatozoa are transcriptionally silent | [33] |
| 11 | Human | Transfection of monocyte $P2X_7$ into COS cells | | No nERs in COS cells | [26] |
| 12 | Mouse | Cerebellum | Male | | [39] |
| 13 | Xenopus | Oocytes | Female | | [40] |
| 14 | Rat | Phaeochromocytoma | | | [25] |
| 15 | Gerbil | Inner ear stria vascularis | Female | Controversial data | [41] |
| 16 | Gerbil | Inner ear stria vascularis | Female | Controversial data | [41] |
| 17 | Xenopus | Oocytes | Female | | [42] |
| 18 | Rat | Hippocampus | Female | ERβ mRNA is higher than ERα mRNA in hippocampus | [27] |
| 19 | Rat | Frontal cortex | Female | ERβ mRNA is higher than ERα mRNA in cerebral cortex | [27] |
| 20 | Rat | Brain | Male | Intracellular binding sites exist | [43] |
| 21 | Rat | Hippocampal neurons | Male and female | | [36] |
| 22 | Guinea pig | Hypothalamus | Female | | [30] |
| 23 | Rat | Liver microsomes | Male | Intracellular ERs have been demonstrated in mammalian livers | [44] |
| 24 | Guinea pig | Hypothalamus | Female | | [28] |
| 25 | Guinea pig | Hypothalamus | Female | | [29] |
| 26 | Rat | Hepatocytes | Female | Intracellular binding sites for estradiol are present in liver | [45] |
| 27 | Croaker | Testicular | Male | Nuclear ERs have been characterised in the testes of Atlantic croaker | [46] |
| 28 | Mouse | ERβ transfected into CHO cells | | CHO cells do not produce ER. Transfection of ERβ yielded expression of ER in both nuclear and membrane fractions | [13] |
| 29 | Human | Spermatozoa | Male | No intracellular ER | [47] |
| 30 | Human | Spermatozoa | Male | Mature spermatozoa are transcriptionally silent | [33] |

Table 1 (*Continued*)

| Number of site | Biological system | | | Expression of intracellular ERs | Reference |
|---|---|---|---|---|---|
| | Species | Tissue | Gender | | |
| 31 | Human | Monocytes | | ERα material exhibited higher density reading than ERβ | [34] |
| 32 | Human | Monocytes | | ERα material exhibited higher density reading than ERβ | [34] |
| 33 | Bovine | Aortic endothelial cells | | | [48] |
| 34 | Rabbit | Uterus | Female | Intracellular ERs are present | [49] |
| 35 | Rat | Hepatocytes | Female | Estrogen binding occur in intracellular fractions of liver | [50] |
| 36 | Mouse | ERα transfected into CHO cells | | CHO cells do not produce ER. Transfection of ERα yielded expression of ER in both nuclear and membrane fractions | [13] |
| 37 | Rat | Median eminence | Male | | [35] |
| 38 | Rat | Pituitary | Female | | [51] |
| 39 | Human | Breast | Female | High levels of nERs | [37] |
| 40 | Rat | Uterine | Female | Lower level of nERs | [37] |
| nERα | Human | In vitro synthesised | | | [2] |
| nERβ | Rat | In vitro synthesised | | | [2] |

| Number of site | Effect | Assay method | Incubation time |
|---|---|---|---|
| 1 | Inhibition of muscle-type nAChR function | $^{86}Rb^{+}$ efflux | Acute |
| 2 | Inhibition of ganglionic nAChR function | $^{86}Rb^{+}$ efflux | Acute |
| 3 | Inhibition of DMPP-induced nAChR-mediated $[Ca^{2+}]_c$ rise | $[Ca^{2+}]_i$ | Some min |
| 4 | Relaxation of norepinephrine precontracted segments | Vasorelaxation | 10 min |
| 5 | Inhibition of muscle-type nAChR function | $^{86}Rb^{+}$ efflux | 48 h |
| 6 | Inhibition of $K^{+}$-induced $[Ca^{2+}]$ rise | $[Ca^{2+}]_i$ | 3 min |
| 7 | Inhibition of ganglionic nAChR function | $^{86}Rb^{+}$ efflux | 48 h |
| 8 | Inhibition of dopamine uptake | $[^{3}H]$dopamine uptake | 13 min |
| 9 | Inhibition of serotonin 5-HT transport | $[^{3}H]$5-HT transport | 10 min |
| 10 | Calcium influx | $[Ca^{2+}]_i$ | Some min |
| 11 | Inhibition of BzATP- or ATP-induced cation current | Patch-clamp technique | Seconds |
| 12 | Inhibition of photoaffinity labelling of $[^{125}I]$-his-P | Inhibition of labelling | 30 min |
| 13 | Inhibition of $I_{KS}$ channel | Voltage-clamp recording | Seconds |

| | | | |
|---|---|---|---|
| 14 | Inhibition of $K^+$-induced norepinephrine secretion | [$^3$H]norepinephrine secretion | 10 min |
| 15 | Inhibition of short circuit current ($I_{SC}$, probe) | Electrophysiology, steady-state measurement | 6 min |
| 16 | Inhibition of short circuit current ($I_{SC}$, probe) | Electrophysiology, initial peak measurement | Seconds |
| 17 | Inhibition of minK protein-induced $K^+$ currents | Electrophysiology | 15 s |
| 18 | Decrease in 5-$HT_{1A}$ receptor function | *R*(+)-8-OH-DPAT-stimulated [$^{35}$S]GTPγS binding | 60 min |
| 19 | Decrease in 5-$HT_{1A}$ receptor function | *R*(+)-8-OH-DPAT-stimulated [$^{35}$S]GTPγS binding | 60 min |
| 20 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 3 h |
| 21 | Potentiation of kainate-induced currents | Whole-cell voltage-clamp recording | 3 min |
| 22 | Decrease in the potency of μ-opioid ligand DAMGO-induced hyperpolarisation | Electrophysiology | 20 min |
| 23 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 20 min |
| 24 | Reduction of potency of μ-opioid agonist DAMGO to activate an inwardly rectifying $K^+$ conductance | Electrophysiology | 20 min |
| 25 | Reduction of potency of μ-opioid agonist DAMGO to activate an inwardly rectifying $K^+$ conductance | Electrophysiology | 20 min |
| 26 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 30 min |
| 27 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 30 min |
| 28 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 45 min |
| 29 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 60 min |
| 30 | Calcium influx | $[Ca^{2+}]_i$ | Some minutes |
| 31 | Calcium transient | $[Ca^{2+}]_i$ | 6 s |
| 32 | NO release | NO determination | 2 min |
| 33 | Translocation of eNOS from membrane to intracellular sites | Immunofluorescence imaging | 15 min |
| 34 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 4 h |
| 35 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 1.5 h |
| 36 | Binding of [$^3$H]17β-estradiol | Radioligand binding | 45 min |
| 37 | NO release | NO determination | 2 min |
| 38 | Binding of [$^3$H]17β-estradiol | Radioligand binding | Overnight |
| 39 | Increase in cAMP | Radioimmunoassay | 60 min |
| 40 | Increase in cAMP | Radioimmunoassay | 60 min |
| nERα | Binding of 16α-[$^{125}$I]iodo-17β-estradiol | Radioligand binding | 16 h |
| nERβ | Binding of 16α-[$^{125}$I]iodo-17β-estradiol | Radioligand binding | 16 h |

Table 1 (*Continued*)

| Number of site | Constant type | Ligand specificity[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Estrogens | | | | | |
| | | 17β-E2 | 17α-E2 | E1 | E3 | DES | E2:BSA |
| 1 | $IC_{50}$ | 56 μM | | | | | |
| 2 | $IC_{50}$ | 43 μM | | | | | |
| 3 | $IC_{50}$ | 40 μM | | | | | |
| 4 | $EC_{50}$ | 31.6 μM | | | | | |
| 5 | $IC_{50}$ | 20 μM | | | | | |
| 6 | $IC_{50}$ | 15 ± 2 μM | | | | | |
| 7 | $IC_{50}$ | 12 μM | | | | | |
| 8 | $IC_{50}$ | 7.2 ± 0.6 μM | | | | | |
| 9 | $IC_{50}$ | 4.4 ± 0.3 μM | | >100 μM | 25.5 ± 2.1 μM | >100 μM | NE at 500 μM[b] |
| 10 | $EC_{50}$ | 3.80 ± 0.26 μM | NE up to 10 μM | | | | Effect at 10 μM |
| 11 | $IC_{50}$ | 3 μM | NE at 10 μM | | | | |
| 12 | $IC_{50}$ | 2.0 μM | 0.3 μM | Small effect at 10 μM | Small effect at 10 μM | Effect at 10 μM | |
| 13 | $IC_{50}$ | 2.2 ± 1.0 μM | | | | | |
| 14 | $IC_{50}$ | 2 ± 1 μM | | | | | |
| 15 | $EC_{50}$ | 1.6 ± 0.6 μM | | | | | |
| 16 | $EC_{50}$ | 1.3 ± 0.7 μM | | | | | |
| 17 | $IC_{50}$ | 0.5 μM | | | | 4.4 ± 0.5 μM | |
| 18 | $EC_{50}$ | 28 ± 13 nM | NE at 50 nM | | NE at 50 nM | Effect at 50 nM | |
| 19 | $EC_{50}$ | 22 ± 8 nM | NE at 50 nM | | NE at 50 nM | Effect at 50 nM | |
| 20 | $K_d$ | 20 nM | | | | | |
| 21 | $EC_{50}$ | 16.370 ± 2.763 nM | NE at 100 nM | | | | NE |
| 22 | $EC_{50}$ | 9 nM | NE at 100 nM | | | | |
| 23 | $K_d$ | 9.94 nM | CE at 136 nM | CE at 136 nM | Small CE at 136 nM | NC at 136 nM | |
| 24 | $EC_{50}$ | 7.5 nM | NE | | | Full antagonist at 100 nM | |
| 25 | $EC_{50}$ | 7.5 nM | NE | | | Full antagonist at 100 nM | NE |
| 26 | $K_d$ | 2 nM | NC at 200 nM | | Small CE at 200 nM | CE at 200 nM | |
| 27 | $K_d$ | 1.6 nM | | $IC_{50}$ 25 nM | $IC_{50}$ 30 nM | $IC_{50}$ 2.8 nM | |
| 28 | $K_d$ | 1.14 ± 0.06 nM | | | | | |
| 29 | $K_d$ | 0.66 nM | NC at 10 μM | | | | |
| 30 | $EC_{50}$ | 0.60 ± 0.12 nM | NE up to 10 μM | | | | Effect at 10 μM |
| 31 | $EC_{50}$ | 0.6 nM | | | | | |
| 32 | $EC_{50}$ | ∼0.5 nM | NE at 1 nM | | | | ∼0.5 nM |
| 33 | $EC_{50}$ | 0.4 nM | | | | | |
| 34 | $K_d$ | 0.36 nM | $IC_{50}$ ∼ 50 nM | | | $IC_{50}$ ∼ 0.4 nM | |
| 35 | $K_d$ | 0.29 nM | NC at 0.4 μM | | CE at 0.4 μM | CE at 0.4 μM | |
| 36 | $K_d$ | 0.287 ± 0.011 nM | | | | | |
| 37 | $EC_{50}$ | 0.1 nM | NE at 10 nM | | | | 0.3 nM |
| 38 | $K_d$ | 0.041 ± 0.014 nM | | CE at 1 μM | CE at 1 μM | CE at 1 μM | |
| 39 | $EC_{50}$ | ∼0.01 nM | NE at 1 nM | | | Effect at 1 nM | |
| 40 | $EC_{50}$ | ∼0.01 nM | NE at 1 nM | | | Effect at 1 nM | |
| nERα | $K_d$ | 0.1 nM | $IC_{50}$ 0.2 nM | $IC_{50}$ 0.3 nM | $IC_{50}$ 1.4 nM | $IC_{50}$ 0.04 nM | |
| nERβ | $K_d$ | 0.4 nM | $IC_{50}$ 1.2 nM | $IC_{50}$ 0.4 nM | $IC_{50}$ 0.7 nM | $IC_{50}$ 0.05 nM | |

| Number of site | Constant type | Ligand specificity[a] | | |
|---|---|---|---|---|
| | | Antiestrogens | | |
| | | ICI 164384 | ICI 182780 | Tamoxifen |
| 1 | $IC_{50}$ | | | |
| 2 | $IC_{50}$ | | | |
| 3 | $IC_{50}$ | | | |
| 4 | $EC_{50}$ | | | |
| 5 | $IC_{50}$ | | | |
| 6 | $IC_{50}$ | | | |
| 7 | $IC_{50}$ | | | |
| 8 | $IC_{50}$ | | | |
| 9 | $IC_{50}$ | | | 16.9 ± 1.4 μM |
| 10 | $EC_{50}$ | | | No antagonistic effect |
| 11 | $IC_{50}$ | | | |
| 12 | $IC_{50}$ | | | Effect at 10 μM |
| 13 | $IC_{50}$ | | | |
| 14 | $IC_{50}$ | | | |
| 15 | $EC_{50}$ | | | NE at 3 μM |
| 16 | $EC_{50}$ | | | NE at 3 μM |
| 17 | $IC_{50}$ | | | Effect at 10 μM |
| 18 | $EC_{50}$ | | Full antagonist $K_B$ 1.3 ± 0.5 nM | |
| 19 | $EC_{50}$ | | Full antagonist $K_B$ 2.3 ± 0.9 nM | |
| 20 | $K_d$ | | | |
| 21 | $EC_{50}$ | | | |
| 22 | $EC_{50}$ | | | |
| 23 | $K_d$ | | | |
| 24 | $EC_{50}$ | Full antagonist $K_e$ ~ 0.3 nM | | |
| 25 | $EC_{50}$ | Full antagonist $K_e$ ~ 0.3 nM | | |
| 26 | $K_d$ | | | |
| 27 | $K_d$ | | $IC_{50}$ 90 nM | $IC_{50}$ 4 μM |
| 28 | $K_d$ | | | |
| 29 | $K_d$ | | | |
| 30 | $EC_{50}$ | | | No antagonistic effect |
| 31 | $EC_{50}$ | | No antagonistic effect at 10 nM | Full antagonist $IC_{50}$ 0.9 nM |
| 32 | $EC_{50}$ | | Full antagonist at > 100 nM | Full antagonist at 1 nM |
| 33 | $EC_{50}$ | | Full antagonist at 10 μM | |
| 34 | $K_d$ | | $IC_{50}$ ~ 20 nM | $IC_{50}$ ~ 2 μM |
| 35 | $K_d$ | | | |
| 36 | $K_d$ | | | |
| 37 | $EC_{50}$ | | | Full antagonist at 10 nM |
| 38 | $K_d$ | | | NC at 1 μM[c] |
| 39 | $EC_{50}$ | ~1 nM | | ~1 nM[d] |
| 40 | $EC_{50}$ | ~1 nM | | ~1 nM[d] |
| nERα | $K_d$ | $IC_{50}$ 0.2 nM | | $IC_{50}$ 3.4 nM; 0.1 nM[c] |
| nERβ | $K_d$ | $IC_{50}$ 0.08 nM | | $IC_{50}$ 2.5 nM; 0.04 nM[c] |

Table 1 (*Continued*)

| Number of site | Ligand specificity[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Androgens | | Progestins | | Glucocorticoids | | | Mineralocorticoids |
| | T | DHT | P | Pregnenolone | Dexamethasone | Cortisol | Corticosterone | Aldosterone |
| 1 | | | 6.1 μM | | 39 μM | | 92 μM | |
| 2 | | | 11 μM | | 45 μM | | 94 μM | |
| 3 | | | | | | | | |
| 4 | | | 29.3 μM | | NE up to 50 μM | | | |
| 5 | | | 1 μM | | >30 μM | | >30 μM | |
| 6 | | | | | | | | |
| 7 | | | 3.3 μM | | >30 μM | | >30 μM | |
| 8 | | | NE up to 30 μM | | | | | |
| 9 | >100 μM | | >100 μM | >100 μM | >100 μM | | >100 μM | |
| 10 | | | | | | | | |
| 11 | | | NE at 10 μM | | | | | |
| 12 | NE | | 20 μM | | | | NE | NE |
| 13 | | | | | | | | |
| 14 | | | | | | | | |
| 15 | | | | | | | | |
| 16 | | | | | | | | |
| 17 | | | Small effect at 10 μM | | | Small effect at 10 μM | | |
| 18 | | | | | | | | |
| 19 | | | | | | | | |
| 20 | NC at 50 μM | | NC at 50 μM | | | | NC at 50 μM | |
| 21 | | | | | | | | |
| 22 | | | | | | | | |
| 23 | NC at 136 nM | | NC at 136 nM | | | | NC at 136 nM | |
| 24 | | | | | | | | |
| 25 | | | | | | | | |
| 26 | NC at 200 nM | | NC at 200 nM | | | NC at 200 nM | | |
| 27 | NC up to 10 μM | | NC up to 10 μM | | | NC up to 10 μM | | |
| 28 | | | | | | | | |
| 29 | Small CE at 10 μM | | NC at 10 μM | | | | | |
| 30 | | | | | | | | |
| 31 | NE at 1 nM | | NE at 1 nM | | | | | |
| 32 | NE up to 100 nM | | NE up to 100 nM | | | | | |
| 33 | NE at 100 nM | | NE at 100 nM | | | | | |
| 34 | | NC up to 5 μM | NC up to 5 μM | | | | | |
| 35 | NC at 0.4 μM | | NC at 0.4 μM | | | NC at 0.4 μM | | |
| 36 | | | | | | | | |
| 37 | NE up to 100 nM | | | | | | | |
| 38 | | NC at 1 μM | NC at 1 μM | | | NC at 1 μM | | |
| 39 | NE at 1 nM | | | | NE at 1 nM | | | |
| 40 | NE at 1 nM | | | | NE at 1 nM | | | |
| nERα | NC up to 20 nM | $IC_{50}$ 221 nM | NC up to 200 nM | | | | NC up to 200 nM | |
| nERβ | NC up to 20 nM | $IC_{50}$ 73 nM | NC up to 200 nM | | | | NC up to 200 nM | |

[a] NE: no effect; CE: competitive effect in radioligand binding assay; NC: no competition in radioligand binding assay; antagonistic effects measured against 17β-E2 in biochemical assays are underlined.
[b] Concentration equivalent to 17β-E2.
[c] 4-Hydroxytamoxifen.
[d] *trans*-Hydroxytamoxifen.

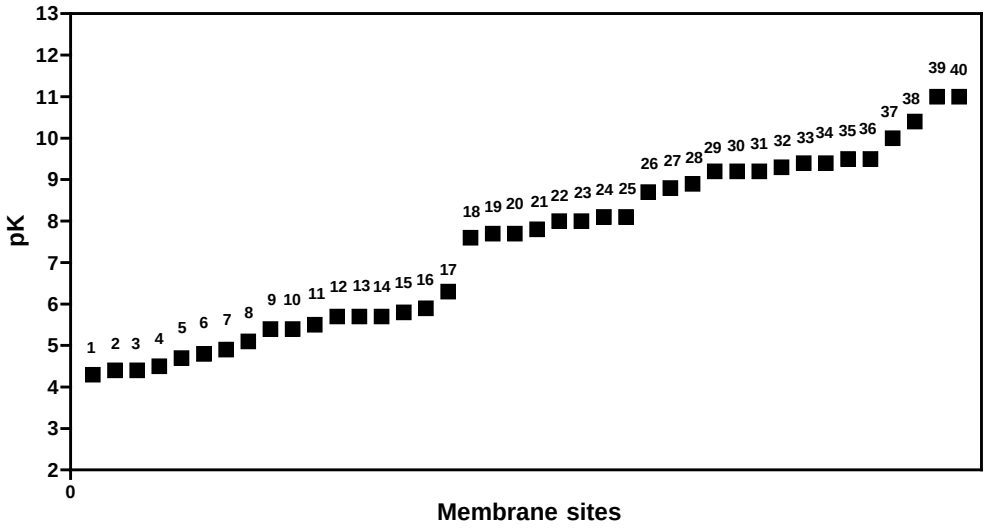


Fig. 1. Graphical presentation of activity constants (p*K*) reported for rapid nongenomic effects to 17β-estradiol. Numbers of points on the graph correspond to the numbers of sites in Table 1.

dehydrogenase (type I) [14], which catalyses the reduction of 17-keto group to 17-hydroxy moiety [15].

Because of its clinical relevance, breast is one of the most well studied tissue concerning estradiol concentration and function. It has been found that mammary tissues can accumulate serum estrogens to the concentrations which are significantly higher than the doses present in serum [14]. The mean concentration of 17β-E2 estimated in normal and benign breast tissues is 0.76 nM, being up to 10-fold higher than its level in serum [14]. However, the human mammary cancer tissue contains all the necessary enzymes for local estrogen biosynthesis leading to a high intratumor estrogen concentration [18]. Although the mean concentration of 17β-E2 in the breast cancer tissue is estimated to be 1.28 nM, the variability of its intratumor level is very high: from the undetectable amount up to more than 5 μM [14]. Other sites where estrogen biosynthesis has been described include the mesenchymal cells of the adipose tissue and skin, osteoblasts and perhaps osteoclasts in bone, possibly vascular endothelial and aortic smooth muscle cells and a number of sites in the brain [19]. The concentration of 17β-E2 determined in the different regions of brain varies indeed to a great extent [20,21]. Also, the level of steroids in the central nervous system has been shown to fluctuate as a function of different phases of the estrous and menstrual cycles but also in response to stress [21,22]. Therefore, the possible involvement of rapid responses to 17β-E2 in cellular functions could not be underestimated even if these effects are in vitro measured at the concentrations higher than those determined in the peripheral circulation. The concentrations of 17β-E2 achieved locally by extragonadal biosynthesis in several target tissues, at or near the estrogen receptors, can be quite high to play a significant biological role.

## 3. Species and tissues where nongenomic effects to 17β-estradiol have been described

Rapid responses to 17β-E2 have been found to occur in the cells derived from several mammalian tissues (Table 1): different cell types from nervous and endocrine systems, reproductive and digestive tracts, blood and blood vessels are able to rapidly respond to 17β-E2 leading to the changes in cellular functions. In addition, nongenomic effects have also been described in some nonmammalian systems: the oocytes derived from *Xenopus* and the testicular cells of croaker (Table 1). Similar to classical responses, acute effects to 17β-E2 are also not gender specific and can occur in the cells of both male and female origin. A rather wide spectrum of the expression of the nongenomic sites which are described so far, despite the relatively short period of studies of rapid responses to sex hormones, let us presume that the nonclassical action of 17β-E2, as well as its physiological relevance, could be more extensive than currently known.

The analysis of data presented in Table 1 and Fig. 1 indicates the existence of 17β-E2 binding sites with different affinities in some cell types: in human spermatozoa both micromolar and low nanomolar effective concentrations have been described (point 10 versus points 29 and 30) demonstrating probably the expression of different subtypes of putative membrane estrogen receptors in these cells. In rat hepatocytes, the difference in published affinity parameters is 10-fold (point 26 versus point 35). It is also remarkable that the cells derived from the various regions of the rat brain respond to 17β-E2 with rather different affinities: in striatal synaptosomes, the effective concentration is in the micromolar range (point 8) whereas in hippocampus and frontal cortex 17β-E2 acts already at nanomolar concentrations (points 18, 19, 21). These data could indicate the existence of the distinct binding sites of 17β-E2 in different

cell types even within the same tissue but could also reflect some essential variations in the reserve of estrogen-specific membrane receptors in various cells. However, the identity and the regulation of such 17β-E2-specific membrane binding sites are still unclear.

In addition, direct interactions between 17β-E2 and other membrane proteins are also possible and can lead to the indirect modulation of cell functions by this gonadal steroid. For instance, nAChR could be a potential target as exposure to 17β-E2 can inhibit the function of both muscle-type and ganglionic nAChR [23,24]; this steroid can nongenomically block also other ligand-gated ion channels: $P2X_2$ [25] and $P2X_7$ receptors [26]; or modulate the affinity of transporters for dopamine [21] and 5-HT [20]. It is interesting that the modulation of cell functions via such interactions seems to occur at micromolar estradiol concentrations (Table 1). At the same time, the nanomolar doses of 17β-E2 can rapidly alter the physiological responses of several G protein-coupled receptors (5-$HT_{1A}$ [27], $GABA_B$, μ-opioid [28–30]) probably via the activation of specific estrogen receptors and interfering in signalling pathways. Such findings extend the number of the cellular events which are regulated by 17β-E2 via nongenomic mechanism(s) and point to the complexity of action of gonadal steroids in physiological processes.

## 4. Assay methods used to characterise rapid responses to 17β-estradiol

Several conventional biochemical methods (radioligand binding, [$^{35}$S]GTPγS binding) and physiological assays (like the measurement of changes in $[Ca^{2+}]_i$, electrophysiological recordings, NO release, vasorelaxation, and norepinephrine secretion) have been used to describe the rapid effects of 17β-E2 on cellular functions (Table 1). As characteristic of the model of the nongenomic action of steroids, mostly incubation periods of seconds or minutes have been applied. Short reaction times also make the question of metabolism of this gonadal steroid less important in order to adequately interpret the data. Still, an interesting feature comes out when analysing the data presented in Table 1: by using the physiological assay methods rapid responses to 17β-E2 with very different effective concentrations (from subnanomolar to a high micromolar range) have been described; however, radioligand binding experiments reveal only the sites characterised by nanomolar affinity constants. This trait could have some essential meaning for the studies focused on the molecular characterisation of membrane estrogen receptors, still being in a putative state so far.

## 5. Ligand specificity of 17β-estradiol membrane sites; stereospecificity, the effects of antiestrogens and other steroids

The potential effects of various estrogenic compounds, as well as other steroids from different structural classes (androgens, progestins, glucocorticoids, mineralocorticoids) to evoke rapid responses, have been tested in various cellular models (Table 1). These published data make it possible to compare the ligand recognition properties of nongenomic sites for the action of 17β-E2 with those reported for classical nERs.

In general, nuclear ERs have been characterised by a low nanomolar binding affinity to 17β-E2 (Table 1). This lipophilic steroid hormone passes across the plasma membrane by simple diffusion, binds to nuclear estrogen receptors, and leads to the modulation of gene transcription and protein synthesis [9]. The ligand binding affinity of other physiological estrogens (17α-E2, E1, E3), as well as synthetic estrogen DES, is also in a low nanomolar range; however, 17α-E2 has been reported to show a somewhat lower affinity than 17β-E2 pointing to the stereospecificity of genomic effects [2] (Table 1). Antiestrogens ICI 164384, tamoxifen and 4-hydroxytamoxifen are the inhibitors acting at small nanomolar doses [2]. Neither testosterone, progesterone, dexamethasone nor corticosterone are efficient at nERs [2,31] (Table 1). The two subtypes of classical estrogen receptors (nERα and nERβ) have been cloned differing to some extent by nucleotide sequences and expression patterns in various tissues; however, the activity profiles of physiological ligands, as well as their quantitative parameters, are on the whole rather similar for both of these nuclear receptors [31,32].

Differently from genomic effects, the receptor(s) mediating rapid responses to 17β-E2 are not yet molecularly identified. At the same time, the activity constants measured for nongenomic effects to 17β-E2 varying from subnanomolar to high micromolar concentration range clearly point to the diversity of these membrane sites as well as to the complexity of their functioning (Table 1, Fig. 1).

The distinct sites with micromolar potency for 17β-E2 (the activity constants in the range of 0.5–50 μM) can be firstly brought forth (points 1–17 in Table 1 and Fig. 1). The stimulation of these sites seems to be strictly regulated by stereospecificity requirements as 17α-E2 has no effect. Similarly, other estrogenic compounds E1, E3 and DES are effective only at very high concentrations or have no activity at all. The only exception seems to be the mouse cerebellum (point 12 in Fig. 1) where 17α-E2 and 17β-E2 are equipotently active (Table 1, Fig. 1). It is also interesting and completely different from classical ERs that antiestrogens are mostly inactive or have the effects of the same direction to 17β-E2 but clearly not inhibitory at these micromolar 17β-E2 membrane sites. At the same time, progesterone is also somewhat active although less potent than 17β-E2 itself. Glucocorticoids are inactive or effective only at high micromolar concentrations, androgens and mineralocorticoids are rather ineffective (Table 1). Taken together, the ligand activity profile, especially no activity of other estrogens and no inhibition by antiestrogens, clearly indicates that these responses cannot be mediated by classical ERs and tempt us to speculate on the existence of distinct membrane estrogen receptor(s)

specifically activated by the micromolar concentrations of 17β-E2. This conclusion is further supported by the paucity of expression of nERs in several cellular systems where such rapid responses to 17β-E2 have been measured (Table 1).

Secondly, many rapid effects to 17β-E2 also occur at nanomolar concentrations (activity constants in the range of 0.01–28 nM) (Table 1). However, relying on the ligand activity profiles, it is difficult to differentiate distinct membrane binding sites. Most of these responses are characterised by high stereospecificity as 17α-E2 is ineffective even at the concentrations several magnitudes higher than the active doses for 17β-E2. Although other estrogens seem to be effective agonists at least at high nanomolar concentrations, DES has been exceptionally reported to behave also as a full antagonist for 17β-E2 effect in the guinea pig hypothalamic slices [28,29]. Also, an interesting point is the action of membrane-impermeable estradiol conjugate E2:BSA. As in several cellular systems (human spermatozoa, human peripheral monocytes, rat median eminence), this conjugate is able to induce similar responses to that of 17β-E2 demonstrating the location of the receptor on the cell surface [33–35], in guinea pig hypothalamic slices and rat hippocampal CA1 neurons this conjugate is not active [29,36]. This can indicate that 17β-E2 might have to cross the membrane to evoke some rapid nongenomic events, hypothetically acting via a subpopulation of the cytoplasmic steroid receptors which are not translocated to the nucleus. Some variability also exists in the behaviour of antiestrogens (Table 1). ICI 164384 acts as a full antagonist with subnanomolar affinity for 17β-E2 in guinea pig hypothalamic slices [28,29] but induces cAMP increase similarly to 17β-E2 in MCF-7 human breast cancer and rat uterine cells [37]. Somewhat variable affinity of ICI 182780 to block 17β-E2 responses (Table 1) could reflect differences in the receptor reserve of various cellular systems. The data published about the action of nuclear estrogen receptor antagonist tamoxifen are also different: no antagonistic effect has been reported in human spermatozoa [33], the low nanomolar blocking action of 17β-E2 responses has been demonstrated in human peripheral monocytes [34] and rat median eminence [35], whereas *trans*-hydroxytamoxifen behaves as a full agonist at nanomolar doses to increase cAMP in MCF-7 human breast cancer and rat uterine cells [37]. All these features point to the high diversity of sites mediating nongenomic responses to 17β-E2. At the same time, it is important to mention that these nanomolar rapid effects to 17β-E2 are strictly estrogen-specific as no effects to testosterone, 5α-dihydrotestosterone, progesterone, dexamethasone, cortisol and corticosterone have been found (Table 1). The interpretation of the nature of the nanomolar affinity sites of nongenomic responses to 17β-E2 is furthermore complicated because of the expression of classical genomic ERs in the most of the cellular systems where rapid effects have been described (Table 1). However, important differences in ligand affinity profiles let us suppose that these rapid responses are mediated by the membrane proteins structurally different from nERs at least considering the domain for ligand recognition.

In summary, 17β-E2 is able to initiate a wide spectrum of rapid effects in an extensive concentration range. For the definite understanding of the nature of these membrane binding sites, it is clear that cloning and the molecular identification of these proteins are indispensable. However, during the accomplishment of this intricate task the design of specific agonists and antagonists selective for membrane binding sites also seems to be a rather promising approach for the further characterisation of these putative receptors.

## 6. Conclusions, further perspectives

It is well known that 17β-E2 is involved in various physiological responses in several tissues besides its important role in the reproductive tract. Over the past years, it has become clear that in addition to action via classical genomic model this gonadal steroid hormone can also initiate rapid nongenomic responses occuring within a few minutes after the agent administration. There are several evidences published in literature supporting the conception of the existence of specific membrane receptors for 17β-E2, which can be structurally different from the classical nuclear receptors. In the present review article, we have shown a clear diversity of such membrane binding sites mediating rapid nongenomic responses to 17β-E2 in various biological systems by analysing the affinity of 17β-E2 toward such putative membrane proteins, the stereospecificity of these effects but also the activity of other estrogens and different steroid hormones.

The molecular nature of 17β-E2 membrane binding sites is still unknown as no such receptors have been cloned so far. However, the wide spectrum of rapid nongenomic effects induced by 17β-E2 clearly demonstrates the physiological significance of these membrane sites in the regulation of several cellular functions. In this context, the molecular identification of membrane receptors for 17β-E2 is highly perspective to better understand the mechanism of the physiological responses where this gonadal steroid hormone participates but it can also open several new possibilities for pharmacological intervention in these processes. The studies with cellular systems, where no nuclear estrogen receptors are expressed, which are transcriptionally silent or have no nucleus, would probably be the best models for the molecular characterisation of such novel receptors. Parallel to this difficult molecular biological task, several new physiological events mediated by the nongenomic action of 17β-E2 via the stimulation of membrane receptors are expected to be described in various native biological systems.

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