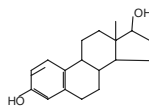
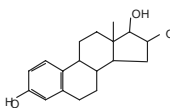


Natural and environmental estrogens

Estrogens:



17- β -estradiol

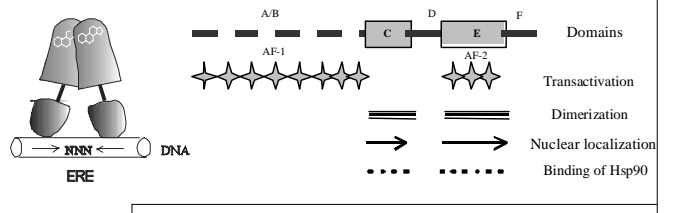
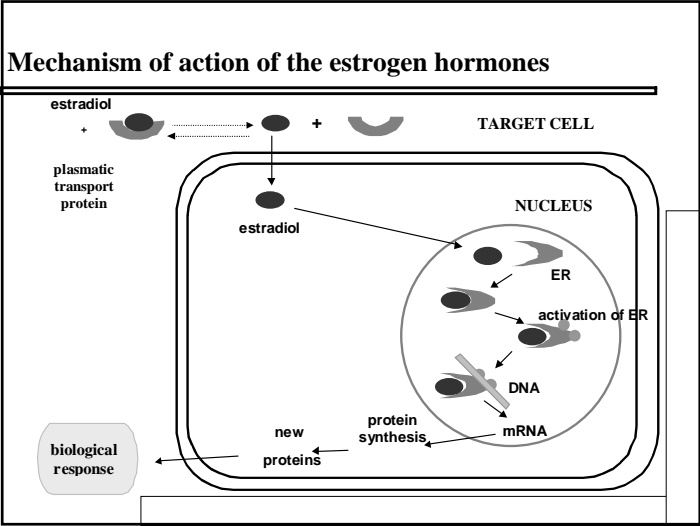


estriol

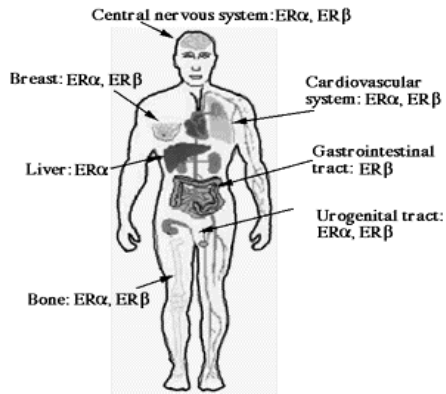
- play a key role in female hormone regulation and signalling
- are responsible for metabolic, behavioural and morphologic changes occurring during stages of reproduction
- are involved in the growth, development and homeostasis of a number of tissues
- control the bone formation, regulation of homeostasis, cardiovascular system and behaviour
- regulate production, transport and concentration of testicular liquid and anabolic activity of androgens in males

Estrogen receptor:

- a member of the nuclear hormone receptor superfamily
- a ligand – inducible transcription factor
- subtype: ER- α (in breast, ovary, brain, liver, bone and cardiovascular system, adrenals, testis and urogenital tract)
- ER- β (in kidneys, prostate and gastrointestinal tract)
- ER- γ (in fish)

ESTROGEN RECEPTORS - ER- α & ER- β :



biosynthesis and release of estrogens

↓ e.g. modulation of CYP11A and/or CYP19 activities

binding to plasmatic transport proteins

↓ e.g. down-regulation of ER protein levels

binding to nuclear estrogenic receptor (ER)

activation of ER

(dissociation of associated heat shock proteins, formation of homodimers)

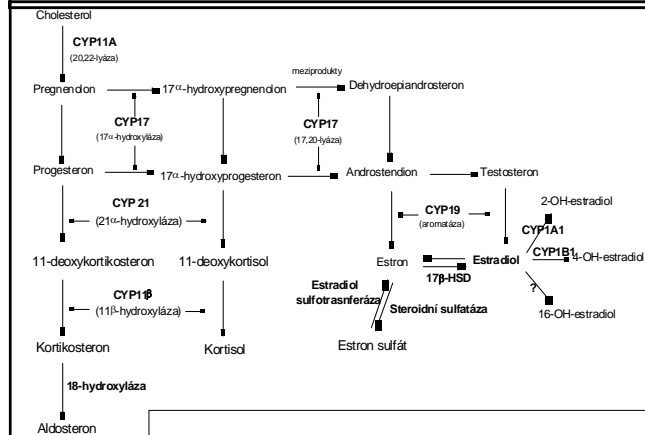
↓ e.g. modulation of other nuclear receptors (PPAR/RXR, RXR/TR)

binding of the activated receptor complex to specific DNA motifs - EREs

chromatin rearrangement and transcription of estrogen-inducible genes

↓ effects at the cellular, tissue, organ, organism, and/or population level

Synthesis and metabolism of estrogens



Cross-talk between estrogen signalling pathways and other receptors

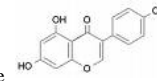
- estrogen signalling pathways and other members of nuclear receptor superfamily
- estrogen signalling pathways and AhR
- estrogen signalling pathways and receptors for EGF and insuline

Environmental estrogens (xenoestrogens, exoestrogens)

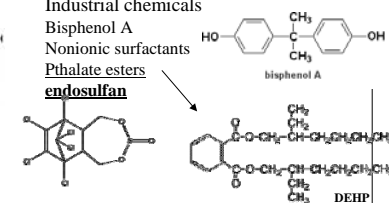
- are a diverse group of substances that do not necessarily share any structural resemblance to the prototypical estrogen (17 β -estradiol) but evoke effects resembling those of estrogen
- **estrogenic substances (estrogen agonist)**
- **estrogen-like substances**
- **ANTI-estrogenic substances**

Exoestrogens - examples (1)

Natural products
genistein
 naringenin
 coumestrol
 zearalenone



Industrial chemicals
 Bisphenol A
 Nonionic surfactants
 Phthalate esters
endosulfan



Environmental pollutant
 DDT
 kepone
 PCBs/OH-PCBs
 PAHs and dioxins

Pharmaceuticals
 Ethinyl estradiol
 Diethylstilbestrol
 gestodene
 norgestrel

Exoestrogens - Relative Potencies to bind to ER α (REPs)

| Chemical group | Substance | REP |
|---------------------|---|------------------------|
| Endogenous hormones | Estradiol | 1 |
| | Estriol | 6.3 · 10 ⁻³ |
| | Testosteron | 9.6 · 10 ⁻⁶ |
| Phytoestrogens | Coumestrol | 6.8 · 10 ⁻³ |
| | Genistein | 4.9 · 10 ⁻⁴ |
| Pesticides | <i>o,p'</i> -DDT | 1.1 · 10 ⁻⁶ |
| PCBs | 2,4,6-trichlorobiphenyl-4'-ol | 1.10 ⁻³ |
| | 2,5-dichlorobiphenyl-4'-ol | 6.2 · 10 ⁻³ |
| | 3,3',5,5' tetrachlorobiphenyl-4,4'-diol | 1.6 · 10 ⁻⁴ |
| alkylphenoles | 4-tert-oktylphenol | 3.6 · 10 ⁻⁶ |
| phthalates | butylbenzylphthalate | 4.10 ⁻⁶ |

REP (Relative Potencies) of selected compounds related to 17- β -estradiol derived from reporter yeast assay

Toxicity assessment - in vivo and in vitro methods

| Assay (ref.) | Exposure type | Detects ER-dependent agonists? | Detects non-ER-dependent agonists? | Distinguishes agonist versus antagonist? | Pharmacokinetics and metabolism included? |
|--|----------------|--------------------------------|------------------------------------|--|---|
| Receptor-based assays | | | | | |
| Receptor binding assay (27) | Cell lysate | Yes | No | No | No |
| Receptor activation assay (32-34) | Cells in vitro | Yes | No | Yes* | No |
| In vitro estrogen-regulated response assays | | | | | |
| MCF-7 cell proliferation assay (41) | Cells in vitro | Yes | Limited | Yes* | No |
| Induction assays (46,48) | Cells in vitro | Yes | Limited | Yes* | No |
| DNA synthesis assays (47) | Cells in vitro | Yes | Limited | Yes* | No |
| In vivo estrogen-regulated response assays | | | | | |
| Uterotrophic response assay (49) | Whole animal | Yes | Limited | Yes* | Yes |
| Vaginal cornification assay (50) | Whole animal | Yes | Limited | Yes* | Yes |
| Vaginal opening (11) | Whole animal | Yes | Limited | Yes* | Yes |
| Uterine fluid imbibition (11) | Whole animal | Yes | Limited | Yes* | Yes |
| Uterine epithelial hypertrophy (51) | Whole animal | Yes | Limited | Yes* | Yes |
| Inhibition of steroid synthesis assays | | | | | |
| In vitro ovarian steroid assay (55) | Minced tissue | No | Yes | Yes | No |
| Ex vivo ovarian steroid assay (56) | Whole animal | No | Yes | Yes | Yes |

*Detection of antagonists requires use of additional groups with test material + estradiol.

In vitro assay

- competitive ligand binding assay
- cell proliferation assay
- endogenous protein expression (or enzyme activity) assay
- reporter gene assay

In vivo assay

- **uterotropic assay**
- **vaginal cornification assay**
- **standard test procedures for reproductive and developmental toxicity (e.g. FETAX)**
- **production estrogen-inducible proteins (e.g. vitellogenin and zona radiata protein)**