Online Supplement

ESTROGEN SIGNALING IN THE ADRENAL CORTEX:
IMPLICATIONS FOR BLOOD PRESSURE SEX DIFFERENCES

Brasilina Caroccia, Teresa M. Seccia, Matthias Barton and Gian Paolo Rossi

1 Molecular Internal Medicine, University of Zurich, Switzerland;
Department of Medicine-DIMED, University of Padua, Italy

Corresponding author:
Gian Paolo Rossi, FAHA, FACC
Clinica dell’Ipertensione
Department of Medicine - DIMED
University Hospital
Via Giustiniani, 2
35128 Padova, Italy
Phone: +39-049-821-7821
Fax: +39-049-821-7873
gianpaolo.rossi@unipd.it
Expression and Functions of nuclear estrogen receptors and estrogen-receptor related receptors

Estrogen receptors
Albeit initially held to have an expression restricted to the mammary gland and reproductive organs, estrogen receptors (ERs) were thereafter detected in other organs, including the liver, adipose tissue, heart, vasculature and adrenal glands.\(^1\) ER\(\alpha\) and ER\(\beta\) show similar, albeit not completely overlapping, tissue distribution with ER\(\alpha\) mostly expressed in the ovarian theca and in Leydig cells, and ER\(\beta\) mainly located in the ovarian granulosa and prostatic stromal cells.\(^2\) ER\(\alpha\) and ER\(\beta\) are also present in CD4\(^+\) and CD8\(^+\) T-lymphocytes, B-lymphocytes, and NK cells,\(^3\) and highly abundant in the cardiovascular system\(^4\) where they are held to play mainly a protective role.\(^4,5\) In endothelial cells, ER\(\alpha\) and ER\(\beta\) mediate nitric oxide release and endothelium-dependent hyperpolarization, respectively, resulting in vasodilatation.\(^6\) In vascular smooth muscle cells activation of either ER receptor subtype inhibits superoxide anion (\(O_2^-\)) generation thus enhancing nitric oxide bioactivity.\(^6\) (Table)

Estrogen receptor-related receptors
Estrogen-related receptor \(\alpha\) (ERR\(\alpha\)) (also known as nuclear receptor subfamily 3, group B, member 1, NR3B1) is an orphan nuclear receptor that was identified based on its high level of sequence identity with ER\(\alpha\).\(^7,8\) ERR\(\alpha\) is expressed in several tissues that require high-energy supply such as the heart, skeletal muscle, and brain.\(^9\) Cholesterol was recently identified as its ligand;\(^10\) moreover, its activity is regulated by prostaglandins.\(^11\) ERR\(\alpha\) immunostaining was found in the three layers of the adult adrenal cortex;\(^12\) moreover, its expression was found to change with development and during aging, being high in the fetus, low after birth, and progressively increasing until adrenarche.\(^13\) ERR\(\alpha\) immunoreactivity is detectable in the nuclei of APA, cortisol-producing adenoma, adrenocortical carcinoma (ACC), and in the adrenocortical carcinoma cell line H295.\(^13\) Of note, ERR\(\alpha\) can activate aldosterone production by acting as a transcriptional activator of CYP11B2, mostly via steroidogenesis factor-1 response element;\(^14\) ERR\(\alpha\) was also reported to enhance cell proliferation of H295 cells via cyclin D1.\(^15\) Besides ERR\(\alpha\) other members of the ERR family, including ERR\(\beta\) (NR3B2) and ERR\(\gamma\) (NR3B3) have been identified.\(^7,9,16\) Although closely related to the classic ER, they do not seem to be activated by natural estrogens, but only (and partially) by synthetic compounds targeting the 'classical' estrogen receptors, such as 4-hydroxytamoxifen, which is also a ligand for GPER-1\(^17,18\) and diethylstilbestrol.\(^12\)

Supplementary References


Figure S1. Non-genomic and genomic estrogen signaling. Endogenous estrogen 17β-estradiol (E$_2$) acts as a nonselective activator of the three known estrogen receptors (ERs), ERα, ERβ, and GPER. For genomic signaling, 17β-Estradiol activates nuclear ERs, resulting in receptor dimerization and binding of receptor dimers to promoters of target genes. Alternatively, activated ERs modulate the function of other classes of transcription factors (TF) through protein–protein interactions. Subpopulations of ERs at the plasma membrane are activated by E$_2$ and interact with adaptor proteins (adaptor) and signaling molecules such as c-Src, which mediates rapid signaling via PI3K-Akt and MAPK pathways. E$_2$ and the selective agonists (G-1), or the selective estrogen receptor down regulators (SERDs, fulvestrant), or selective estrogen receptor modulators (SERMs, tamoxifen) also activate GPER. In the endoplasmic reticulum GPER stimulates cAMP production, Ca$^{2+}$ mobilization and c-Src, which activate MMPs. MMPs cleave pro-HB-EGF, releasing free HB-EGF that trans-activates EGFR that, in turn, activates MAPK and PI3K–Akt pathways with additional rapid (non-genomic) effects (X), or genomic effects. E$_2$-mediated transcriptional regulation involves phosphorylation (P) of ER or other TFs that may directly interact with ER, or bind independently of ER within the promoters of target genes. Abbreviations: Akt, serine/threonine kinase Akt / protein kinase B; E$_2$, 17β-estradiol; EGFR, epidermal growth factor receptor; ER, estrogen receptor; GPER, G protein-coupled ER; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PI3K, phosphoinositide 3-kinase; pro-HB-EGF, pro-heparin-binding-epidermal growth factor; TF, transcription factor. Figure modified from Prossnitz ER, Barton M. The G protein-coupled estrogen receptor GPER in health and disease. Nat Rev Endocrinol. 2011; 7: 715-726.
**Figure S2.** Protein expression of ERα, ERβ and GPER-1 in the normal adrenal cortex and in aldosterone-producing adenoma (APA). Immunohistochemistry shows scant expression of ERα in both tissues, and the predominant GPER-1 expression in APA tissue.