Brief Review

Estrogen Signaling in the Adrenal Cortex
Implications for Blood Pressure Sex Differences

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

Currently, more than a quarter of the female adult world population and 3-fold more of postmenopausal women in the United States have arterial hypertension.1,2 Sex differences in blood pressure (BP) and rate of hypertension play an important role in the susceptibility to cardiovascular disease.2–5 Moreover, hypertension is often undiagnosed or inadequately controlled in women, especially after menopause when cardiovascular risk increases.6

Endogenous estrogens are held to contribute to the maintenance of normal BP in premenopausal women, who show lower BP values than age-matched men.7 Estrogens elicit direct vasodilatory effects in vivo, albeit they may affect BP also through other mechanisms, including inhibition of sympathetic tone.8 During the menstrual cycle, BP is inversely related with circulating estrogen concentrations and is lower when 17β-estradiol (E2) levels peak.7 After menopause, both vascular aging, which leads to arterial stiffening, and the cessation of endogenous estrogen production contribute to a raise in BP,2,6 thereby increasing the risk of hypertension, atherosclerotic vascular disease, myocardial infarction, and stroke.2,8,9 Hormone replacement therapy can alleviate the postmenopausal BP increase and can reduce coronary vascular risk and carotid artery subclinical atherosclerosis progression,9–14 but this protective effect is lost in women who are many years after menopause.2,6

The effects of estrogens on other potent pressor mechanisms, and particularly those involving the renin–angiotensin–aldosterone system (RAAS), have received less attention as discussed below. Likely, this can explain why mineralocorticoid receptor antagonists, which entail an effective treatment of resistant/refractory arterial hypertension,9–15 are still underused in postmenopausal women,17 notwithstanding the high rate of resistant hypertension in this population.2

Estrogens and the RAAS

Aldosterone, one of the major effectors of the RAAS, is a major contributor to cardiovascular mortality in patients with heart failure.18–21 Moreover, its inappropriately high levels raise BP in overweight-obese patients with hypertension,22 and in patients with primary aldosteronism,23 the most common form of endocrine hypertension.24 Compared with BP-matched essential hypertensive patients,25,26 these latter patients develop more severe target organ damage27 and are at higher cardiovascular risk. Identification of the molecular mechanisms underpinning the excess production of aldosterone, and the role of estrogens in this context, can thus be of much interest for improving strategies to combat hypertension and the resulting cardiovascular disease.

The RAAS is known to be regulated by estrogens28,29: contraceptive formulations or orally administered postmenopausal hormone therapy increase the renin substrate (angiotensinogen) in plasma through hepatic induction.29 Estrogens also stimulate renin release from the juxtaglomerular cells through estrogen receptor-α (ERα),30 whereas endogenous estrogens exert inhibitory effects on the RAAS.28,29 Experimental studies have shown that circulating levels of aldosterone increase after menopause and that estrogen therapy blunts this increase31–35; however, corresponding data in humans are lacking.

Data on the effects of estrogens on aldosterone synthesis in the adrenocortical zona glomerulosa (ZG) are scarce, but the detection of binding sites for estrogens and progesterone in the adrenal36 suggests a modulatory role of these hormones in aldosterone synthesis, possibly through interactions with secretagogues as angiotensin II, serum K+, adrenocorticotrophic hormone, and other vasoactive factors, including endothelin-1 and urotensin II.37–39

Estrogens can freely diffuse into the nucleus and therefore were initially thought to act exclusively via the nuclear ERα (esr1) and ERβ (esr2).40 This concept changed when, in 1999, membrane subpopulations of ERα and ERβ were identified and also rapid intracellular signaling of estrogens via the 7-transmembrane G-protein–coupled estrogen receptor-1 (GPER-1) was reported.41,42 The subsequent demonstration that E2 affects aldosterone synthesis and release40,42,43 opened a novel exciting chapter of the aldosterone saga. Hence, we will herein review the distribution pattern of ERs in the adrenal cortex, their role in the regulation of aldosterone synthesis, and the implications of the most recent discoveries in this field. For clarity purposes, estradiol,2 the physiologically most relevant estrogen, is referred throughout the text as E2, whereas the term estrogens refer to estrogens and their derivatives.

From the Molecular Internal Medicine, University of Zurich, Switzerland (M.B.); and Department of Medicine-DIMED, University of Padua, Italy (B.C., T.M.S., G.P.R.).

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Correspondence to Gian Paolo Rossi, Clinica dell’Ipertensione, Department of Medicine–DIMED, University Hospital, Via Giustiniani, 2, 35128 Padova, Italy. E-mail gianpaolo.rossi@unipd.it

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ERα and ERβ

Two nuclear ER subtypes, ERα and ERβ, have been identified and cloned in 1986, 1987, and 1996, respectively. Notwithstanding their relatively low (59%) sequence identity in the ligand-binding domains, they exhibit similar binding affinity and specificity for estrogens. After binding E2 in the cytoplasm, and forming homo- or heterodimers, the ERs translocate to the nucleus where, by interacting with estrogen response elements, they modulate expression of peroxisome proliferator activator γ, endothelial nitric oxide synthase, connexin, and heat shock proteins genes (Figure S1 in the online-only Data Supplement). The receptor homo- or heterodimers affect gene expression also indirectly via transcription factor or via ligand-independent mechanisms involving a growth factor that induces phosphorylation of ER. Membrane subpopulations of ERs deriving from the same transcripts yielding nuclear ERs, which anchor to plasma membrane lipids, have been implicated in the rapid (nongenomic) estrogen signaling. ERα induces rapid activation of NO synthase transcription, phosphorylation of serine residues, nitric oxide production, and also endothelial cell angiogenesis and regeneration, whereas ERβ mediates vasodilation via endothelium-dependent hyperpolarization. The nongenomic effects of E2 on endothelial cells involve protein caveolin-1, mitogen-activated protein kinases, and PI3K/Akt leading to increases in intracellular Ca2+ concentrations. For expression and tissue-specific functions of ERs, please refer to Table. Functions of ER-related receptors are discussed in the Data Supplement.

GPER-1

In the 1990s, a novel membrane bound G protein–coupled receptor was independently cloned in 6 laboratories and assigned different names. Initially received the orphan receptor designation GPR30 and was identified as a membrane protein mediating the rapid effects of E2. Because of its high homology with other protein receptors, the natural ligand of GPR30 was suspected to be a peptide, although none among all screened peptides provoked any detectable response in GPR30-transfected cells. In 2000, Filardo et al reported that GPR30 mediates rapid signaling of E2; 5 years later, Thomas, Filardo, et al and Revankar et al independently described that GPR30 intracellularly binds E2 and acts as an ER. Hence, in 2007 the International Union of Basic and Clinical Pharmacology renamed GPR30 as GPER-1. GPER-1 was found to be located at the plasma membrane, the endoplasmic reticulum, and Golgi, at variance with the classic ERs that were detected in the cytoplasm and, on binding E2, in the nucleus. E2 binding to GPER-1, but not to ERα or ERβ, induces synthesis of phosphatidylinositol 3,4,5-triphosphate and intracellular Ca2+ mobilization, which were abolished by the epithelial growth factor receptor inhibitor AG1478, indicating that the estrogen-triggered Ca2+ mobilization involves GPER-1 via epithelial growth factor receptor transactivation. Independent studies were unable to detect any binding of aldosterone to GPER-1–containing membrane fractions; therefore, currently there is no evidence that would support the notion that GPER-1 can also act as an aldosterone receptor. However, the available data suggest that GPER-1 can interact with the nuclear mineralocorticoid receptor by receptor cross talk, (as it does with ERα and ERβ), which is also suggested in aldosterone-dependent renal cancer metastasis. Xenestrogens, phytoestrogens, selective estrogen receptor modulators, selective estrogen receptor downregulators (SERDs), and catecholestrogens can also bind to GPER-1. Whether the same holds true for 7β-hydroxy-epiandrosterone, a metabolite of dehydroxy-epiandrosterone, remains to be confirmed at present. The identification of synthetic small molecules that act as a GPER-1–selective agonist (G1), and antagonist (G15 and G36), has shed some light in this field. Akin to E2, G1 stimulates the rapid Ca2+ currents in a kidney fibroblast COS-7 cell line expressing green fluorescent protein–tagged GPER-1. It is currently unclear whether circulating free concentrations of E2 are sufficient to exert binding to ERs and whether these concentrations exert significant biological effects under nonpregnant conditions. However, the local (tissue) formation of E2 through α-aromatase has been demonstrated in the adrenal and the arterial vasculature, suggesting that the E2 concentrations can be sufficient to act locally. Because aromatase inhibition abrogates the salutary of E2, paracrine E2-mediated actions are likely to play a significant biological role. Measurements of E2 tissue concentrations using microdialysis, however, are still lacking.

Subcellular Localization of GPER-1

Initially localized to the endoplasmic reticulum and found to traffic to the Golgi apparatus, the GPER-1 was, thereafter, found to shuttle dynamically to and from the plasma membrane through vesicular (endosomes) transport. This trafficking forms the basis for GPER-1 desensitization (through endocytosis), resensitization (through recycling to plasma membrane), and down-regulation (through proteolytic degradation). On interaction with its ligand(s), GPER-1 undergoes conformational changes that result in the dissociation of Gα from Gβγ subunit, leading to the intracellular activation of phosphoinositide-3-kinase, with ensuing increase of cAMP and intracellular Ca2+. The latter increases mitochondrial Ca2+, resulting in Ca2+-dependent mitochondrial cAMP production and enhanced aldosterone secretion.

GPER-1–mediated signaling also occurs via recruitment of β-arrestin and change of integrins affinity for extracellular matrix proteins, a process known as inside–out integrin signaling, which could be crucial for cell proliferation, and tumor development. Therefore, ERs interact in several ways differing from those initially conceived, indicating that the picture is far more complex than originally thought.

Expression of ERs in the Normal Adrenal Gland

The first evidence of estrogen binding in the adrenal cortex was found in rodents in 1978, when it was detected in the adrenal cortex of rhesus monkeys, at higher levels in the adult than in the fetal adrenal gland, with no sex differences. In humans, studies of ERs expression in the fetus, which is exposed to high estrogen levels, from the 20th to the 38th gestational week, in the definitive zone of the adrenal cortex showed higher levels of ERβ than ERα mRNA. The predominant ERβ expression was confirmed in
Table. Localization of ERs and Mechanisms by Which They Affect the Vasculature and the Adrenal Cortex

<table>
<thead>
<tr>
<th>Target</th>
<th>Receptor Subtype</th>
<th>Localization</th>
<th>Effect</th>
<th>Mechanism(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculature</td>
<td></td>
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<tr>
<td>Endothelial cells</td>
<td>ERα</td>
<td>Cytoplasm, nucleus, and plasma membrane</td>
<td>NOS activation, proliferation, and migration</td>
<td>↓O₂•− bioactivity, ↑NO bioactivity, and ↑MMP2 and MMP9 activity</td>
<td>Traupe et al60 and Klinge et al61</td>
</tr>
<tr>
<td>Vascular smooth muscle cells</td>
<td>ERα</td>
<td>Cytoplasm, nucleus, and plasma membrane</td>
<td>Vasodilatation, ↓Proliferation, and ↓Migration</td>
<td>↓O₂•− release and ↓MAP kinase</td>
<td>Traupe et al69</td>
</tr>
<tr>
<td>GPER-1</td>
<td></td>
<td>Plasma membrane, endoplasmic reticulum, Golgi apparatus, and endosomes</td>
<td>NOS activation</td>
<td>Intracellular Ca²⁺ mobilization, PIP3 synthesis, ↑NO bioactivity, and ↓O₂•− bioactivity</td>
<td>Revankar et al61</td>
</tr>
<tr>
<td>Vascular smooth muscle cells</td>
<td>ERβ</td>
<td>Cytoplasm, nucleus, and plasma membrane</td>
<td>Vasodilatation</td>
<td>↓O₂•− bioactivity</td>
<td>Traupe et al69</td>
</tr>
<tr>
<td>GPER-1</td>
<td></td>
<td>Plasma membrane, endoplasmic reticulum, Golgi apparatus, and endosomes</td>
<td>Vasodilatation, ↓Proliferation, ↓Migration, and ↓NADPH Oxidase (Nox)</td>
<td>Intracellular Ca²⁺ mobilization, PIP3 synthesis, ↑Endothelin-1 vasoconstriction, and ↓O₂•−bioactivity</td>
<td>Revankar et al61</td>
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<tr>
<td>Adrenal cortex</td>
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<tr>
<td>Zona glomerulosa cells</td>
<td>ERα</td>
<td>Cytoplasm and nucleus</td>
<td>↑Proliferation</td>
<td>↑IGFR activation and expression and ↑Cyclin D1 levels</td>
<td>Sirianni et al68</td>
</tr>
<tr>
<td>GPER-1</td>
<td></td>
<td>Plasma membrane</td>
<td>↑Aldosterone synthesis and Apoptosis</td>
<td>↑FasL expression</td>
<td>Caroccia et al69 and Montanaro et al70</td>
</tr>
<tr>
<td>GPER-1</td>
<td></td>
<td>Plasma membrane</td>
<td>↑Aldosterone synthesis and ↑Proliferation</td>
<td>PKA activation and Cell-cycle arrest</td>
<td>Caroccia et al69 and Chimento et al71</td>
</tr>
</tbody>
</table>

ER indicates estrogen receptor; GPER, G protein–coupled estrogen receptor; IGFR, insulin-like growth factor receptor; MAP, mitogen-activated protein; MMP, matrix metalloproteinase; NOS, NO synthase; and PIP3, phosphatidylinositol (3,4,5)-trisphosphate.

2001, but found to be more abundant in the cortisol-producing definitive zone, and absent in the fetal zone.92

In the postnatal human adrenals, the expression and localization of ERα and ERβ remained unknown until 2007 when in relatively large number of normal adrenal glands the expression of ERβ was reported to change with time from birth to adolescence. Expression decreased at the age of 3 months, when the fetal zone undergoes physiological involution, and increased with adrenarche (at a mean age of 6 years), when the fully developed zona reticularis starts to produce the androgen precursor dehydroepiandrosterone and its sulfate ester, dehydroepiandrosterone sulfate.106 By contrast, ERα expression pattern of ERs in these tumors and in the normal adrenal gland.110 At variance, by systematically examining both GPER-1 and ERα36 expressions in APA, we found markedly different ER mRNA levels between tumors and the normal gland: the GPER-1 prevailed in the former, ERβ in the latter tissue, whereas both ERα and ERα36 were scarcely expressed (Figure S2).69 CD56⁺ aldosterone-producing cells isolated from APAs also exhibited GPER-1 higher expression

Expression Pattern of ERs in Neoplasms of the Adrenal Cortex

In 2008, 3 groups independently reported the expression of ERα and ERβ in adrenocortical tumors with higher levels of ERβ than ERα transcript.92,108,109 In 2009, a pregnant woman with aldosterone-producing adenoma (APA) was described to have a predominant expression of ERβ, suggesting a similar expression pattern of ERs in these tumors and in the normal adrenal gland.110 At variance, by systematically examining both GPER-1 and ERα36 expressions in APA, we found markedly different ER mRNA levels between tumors and the normal gland: the GPER-1 prevailed in the former, ERβ in the latter tissue, whereas both ERα and ERα36 were scarcely expressed (Figure S2).69
than ERβ and ERα, supporting the view that GPER-1 predominates in the aldosterone-producing cells.69 Thus, albeit with profound differences both normal and tumorous adrenocortical tissues express ERα, ERβ, and GPER-1.71,92,108,111 Whether these receptors play any role in cell growth and adrenal neoplasms development and spread remains unknown.73

**Estrogen Modulation of Aldosterone Synthesis**

The adrenocortical carcinoma cell line HAC15 can be used as a model to investigate the effect of E2 on aldosterone production because they express all ER subtypes.69 In these cells, E2 alone, or in conjunction with an ERα antagonist, does not affect CYP11B2 expression. However, when administered in addition to the ERβ antagonist (R,R)-5,11-diethyl-5,6,11,12-tetrahydro-2,8-chrysenediol (THC), it causes a 2.5-fold increase in CYP11B2 gene expression, indicating a tonic inhibitory effect of E2 via ERβ on CYP11B2 transcription. Removal of this inhibition unveils a secretagogue effect of E2 on aldosterone production that occurs via GPER-1 (Figure).69 This was confirmed by experiments with the selective GPER-1 agonist G1, alone or combined with 1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride (MPP), or THC, or both.69 When given in the presence of an ERβ antagonist, G1 markedly increased both CYP11B2 mRNA, and aldosterone levels in the supernatant.69 Moreover, pretreatment with the selective GPER-1 antagonist G-15 abrogated the increase of CYP11B2 gene expression and aldosterone secretion induced by either E2 or G1 during inhibition of ERα and ERβ.69 G-15 also abolished the E2-induced aldosterone secretion that occurred during combined ERα and ERβ blockade.

In HAC15 cells, GPER-1 gene silencing of reduced CYP11B2 mRNA and protein expression by 53% and 72%, respectively, whereas, conversely, ERβ silencing increased CYP11B2 mRNA and protein expression by 62% and 93%, respectively, and aldosterone production by 17%, when compared with mock-transfected cells.69 Neither the ERβ-dependent inhibitory effect nor the GPER-1–mediated stimulatory effect of E2 on aldosterone involve [Ca2+]i cell uptake because exposure of HAC15 cells to E2 alone, or in the presence of the antagonists (THC, or MPP+THC), elicited no changes in [Ca2+]i currents. By contrast, the increase of CYP11B2 expression induced by either E2 or G-1, during combined ERα and ERβ blockade, in HAC15 cells was abolished by a specific PKA inhibitor.69 Hence, collectively these results evidenced that GPER-1–mediated E2-induced aldosterone production occurs via protein kinase A-cAMP signaling. The discovery of the opposing effects of GPER-1 and ERβ on adrenal aldosterone synthesis might explain why estrogens, alone or combined with angiotensin II, elicited no effects on aldosterone secretion in the absence of ERβ antagonists.112 It can, therefore, be concluded that: (1) estrogens physiologically inhibit aldosterone synthesis by activating ERβ receptors; (2) when these receptors are antagonized, or their expression changes (as in APA tissue), E2 becomes a potent aldosterone secretagogue via GPER-1 (Table; Figure).69

![Figure](http://hyper.ahajournals.org/)

**Figure.** Mechanisms of 17β-estradiol (E2)–dependent regulation of the gene transcription of aldosterone synthase (CYP11B2) in the normal adrenal gland and the aldosterone-producing adenoma (APA). A, In the normal adrenal gland, the estrogen receptor β (ERβ), which is the predominant ER in the adrenal cortex, after binding to E2, dimerizes leading to the formation of an active estrogen–ER complex. The complex binds to estrogen response elements (ERE), thereby blunting gene transcriptions of CYP11B2. Given that G protein–coupled estrogen receptor-1 (GPER-1) is expressed at a lower level, GPER-1–mediated effects are negligible. B, In the APA, E2 mostly binds to GPER-1, which is the most abundant receptor subtype in the APA. GPER-1, via cross-talk with ERβ, promotes aldosterone synthesis thereby preventing the tonic inhibitory effect of ERβ on CYP11B2. The mechanisms depicted in A and B may explain why menopause increases circulating aldosterone levels in experimental studies and the inhibitory effect of estrogen therapy on aldosterone levels. These mechanism may also contribute to the high prevalence of hypertension among postmenopausal women and explain how higher estrogen levels in premenopausal women could blunt aldosterone production via the ERβ subtype, whereas the cessation of E2 production after menopause would abolish such inhibition.
ERs and Adrenal Cell Proliferation

A proliferative effect of estrogens was first evidenced on adrenal cells in 2003\(^3\) and confirmed in 2005 by thymidine incorporation studies.\(^3\) Using H295R cells to generate xenograft tumors in athymic (nu/nu) mice in which they silenced the ERs, Sirianni et al.\(^{68}\) showed that E2-induced proliferation involves mainly the ER\(\alpha\) and downstream the activation of cdc2-cyclin D1 expression, although an E2 (ligand)–independent activation was also discovered.\(^{68,70}\) Phosphorylation of serine 118 and 167 of the ER\(\alpha\), or of AKT, or activation of IGF-1 (insulin growth factor-1) receptor also promoted H295R cell proliferation.\(^{68}\) By contrast, GPER-1 was involved in cell apoptosis: G1-induced arrest of H295R cell cycle at the G2 phase leading to DNA damage and death (Table).\(^{71}\) Whether upregulation of GPER-1 underpins inhibition of tumor growth in APA remains to be clarified.

The fetal definitive zone of the adrenal gland contains tightly packed cells with a proliferative phenotype that persists throughout gestation and acquires mineralocorticoid synthesis capacity only in late gestation.\(^{114}\) The subcapsular portion of this zone and the adult ZG exhibits the highest immunostaining for Ki-67, a marker of cell proliferation, which is paralleled by GPER-1 staining.\(^{114}\) These findings, alongside the evidence of ER\(\beta\)-mediated inhibition of tumor growth in APA remains to be clarified.

Adrenocortical ERs and Primary Aldosteronism

The different expression pattern of ERs between APA and normal adrenal cortex,\(^{69,92,106,108}\) along with the capability of E2 to enhance aldosterone production via GPER-1 during ER\(\beta\) blockade,\(^{68}\) suggests crosstalk between GPER-1 and ER\(\beta\) in the development of high BP and primary aldosteronism. In line with this proposition, it was found that E2 decreases aldosterone levels and the response of aldosterone to angiotensin II in ovariecotomized female rats.\(^{33,34}\) Although the expression of ER\(\beta\) and GPER-1 was not investigated, it could be that E2 inhibits the GPER-1–mediated secretagogue effect of E2. Moreover, ovariecotom was associated with increased BP, long-term mortality, and plasma aldosterone; the latter was prevented by E2 or phystostegens.\(^{31}\) Further studies with selective GPER-1 antagonists, or agonists, as opposed to the nonselective agonist E2, are, therefore, necessary to gain further light on the role of 3 ER subtypes in this context.

A Possible Link Between Adrenal ERs and Sex Differences in BP

Compared with age-matched men, premenopausal women are largely protected from high BP and cardiovascular disease, a protection that wanes after menopause along with the cessation of ovarian estrogen production.\(^{2,3,7}\) Evidence exists for a BP-lowering and cardiovascular risk-lowering role of estrogens via their salutary effects on sympathetic activation, salt handling, endothelium-dependent vascular function, and the RAAS.\(^{2,29}\)

The expression of the ERs in the adrenocortical ZG and the functional evidence that the activation of CYP11B2 occurs when the ER\(\beta\) subtype receptor is antagonized, collectively suggest an involvement of aldosterone via ER-related mechanism in the sex differences of hypertension: in premenopausal women high estrogen levels could blunt aldosterone production, and thereby onset of hypertension, via the ER\(\beta\) subtype; conversely, the fall of E2 after menopause could abolish this inhibition. The aforementioned animal studies support this contention.\(^{31,32}\) Whether the density of GPER-1 or its affinity for E2, and the related pathways, change with age and postmenopause remains to be determined, but it is worth noting that E2 itself regulates ER expression.\(^{52,115-117}\) Thus, higher estrogen levels in premenopausal women and loss of estrogen in postmenopausal women can affect the expression and the relative amount of ER\(\alpha\), ER\(\beta\), and GPER-1 in the adrenal cortex.

ER-Related Effects in Women Treated With SERMs

SERMs are approved for the treatment of the most common female cancer worldwide and the second most frequent cause of cancer death in women,\(^{114}\) for example, ER-positive breast cancer, and for ovarian cancers.\(^{114}\) In the United States, the expected number of new cases of these cancers within the current year is about a quarter million accounting for 29% of all cancers.\(^{115}\) Of all breast cancer, =80% express ER and receive SERMs therapy, which means that many women are currently receiving treatment with SERMs because of ER-positive malignancies, although how these drugs affect the regulation of adrenocortical hormone production is unknown. For the reasons discussed above, it is possible that the protective effects of E2-related ER\(\beta\)-mediated inhibition of aldosterone are reduced in women treated with SERMs or SERDs. In fact, SERMs, such as raloxifene and the active metabolite of tamoxifen, 4-hydroxy-tamoxifen, as well as the SERDs faslodex/fulvestrant act as GPER-1 agonists.\(^{4,40}\) Moreover, both SERMs and SERDs bind to ER\(\alpha\) and ER\(\beta\), thus exerting estrogenic effects in some tissue and the opposite in others.\(^{120}\) Hence, when combined with ER\(\beta\) antagonist(s), GPER-1 agonist(s) can increase aldosterone production with an ensuing pressor effect. However, whether SERMs or SERDs adversely affect cardiovascular risk via an enhanced adren al aldosterone production is unknown, but the likelihood might be small because women receiving SERMs or SERDs do not seem to develop hypertension, perhaps because of the increased NO bioactivity and reduced oxidative stress induced by these drugs.\(^{121-123}\) The fact that arterial hypotension, likely because of arterial vasodilation,\(^{63}\) entails a known side effect of faslodex/fulvestrant therapy in women diagnosed with breast cancer supports this contention.\(^{124}\)

Conclusions

The adrenal cortex expresses all ER subtypes: ER\(\alpha\) and its isoform ER\(\alpha\)36, ER\(\beta\), and GPER-1, but ER\(\beta\) shows the highest expression in the ZG.\(^{64,69}\) By contrast, GPER-1 predominates in APAs, which suggests a role for this receptor in primary aldosteronism.\(^{69}\) Estrogens tonically inhibit aldosterone synthesis by acting on ER\(\beta\),\(^{49}\) and, therefore, could physiologically lower its levels in premenopausal women. This could also, in part, explain why hormone replacement therapy reduces BP in postmenopausal women.\(^{2,7}\) The waning of this
ERβ-mediated inhibition, in conjunction with the finding that estrogens potently activate aldosterone production by acting via GPER-1,16 might well contribute to the high prevalence of resistant/salt-sensitive hypertension among postmenopausal women.17 Adrenal estrogen signaling may, thus, substantially account for the sex differences in BP and postmenopausal incidence and prevalence of hypertension.18

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**Disclosures**

M. Barton is an inventor on US patent applications related to GPER-targeting drugs and a consultant to AbbVie, Inc.

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

Brasilina Caroccia, Teresa M. Seccia, Matthias Barton\textsuperscript{1} and Gian Paolo Rossi

\textsuperscript{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 (E₂) 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 E₂ and interact with adaptor proteins (adaptor) and signaling molecules such as c-Src, which mediates rapid signaling via PI3K-Akt and MAPK pathways. E₂ 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, Ca²⁺ 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. E₂-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; E₂, 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.