The G-protein-coupled estrogen receptor GPER in health and disease

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Abstract | Estrogens mediate profound effects throughout the body and regulate physiological and pathological processes in both women and men. The low prevalence of many diseases in premenopausal women is attributed to the presence of 17β-estradiol, the predominant and most potent endogenous estrogen. In addition to endogenous estrogens, several man-made and plant-derived molecules, such as bisphenol A and genistein, also exhibit estrogenic activity. Traditionally, the actions of 17β-estradiol are ascribed to two nuclear estrogen receptors (ERs), ERα and ERβ, which function as ligand-activated transcription factors. However, 17β-estradiol also mediates rapid signaling events via pathways that involve transmembrane ERs, such as G-protein-coupled ER 1 (GPER; formerly known as GPR30). In the past 10 years, GPER has been implicated in both rapid signaling and transcriptional regulation. With the discovery of GPER-selective ligands that can selectively modulate GPER function *in vitro* and in preclinical studies and with the use of *Gper* knockout mice, many more potential roles for GPER are being elucidated. This Review highlights the physiological roles of GPER in the reproductive, nervous, endocrine, immune and cardiovascular systems, as well as its pathological roles in a diverse array of disorders including cancer, for which GPER is emerging as a novel therapeutic target and prognostic indicator.

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Introduction

17β-Estradiol is commonly recognized as the predominant female sex hormone, with a critical role in the development of the female reproductive organs and secondary sex characteristics. However, this hormone is also essential for reproductive development and function in males.[1] In addition to the reproductive system, 17β-estradiol has important physiological roles in almost every other area of the body, including the nervous, immune, vascular, muscular, skeletal and endocrine systems. As expected, disruptions in 17β-estradiol signaling, therefore, contribute to multiple disorders, including cancer, cardiovascular diseases, hypertension, osteoporosis, cognitive and behavioral alterations, neurodegenerative diseases, metabolic disorders (such as obesity and diabetes mellitus) and immune disorders.[2] Our understanding of the widespread physiological effects of 17β-estradiol is complicated by the existence of several types of estrogen receptors (ERs) and multiple modes of cellular signaling mechanisms that span time frames from seconds to hours, or even days.[3,4] The pathophysiological mechanisms involving ERs are further complicated by a diverse array of 17β-estradiol-mimicking compounds, both synthetic and plant-derived, to which humans are increasingly exposed.[5]

In this Review, we provide a brief overview of estrogen signaling and describe the discovery and characterization of its receptors, with particular emphasis on G-protein-coupled estrogen receptor 1 (GPER). We will also discuss studies that have elucidated the functions and importance of GPER in health and disease and those that have revealed the therapeutic potential of small-molecule regulators of GPER activity.

Competing interests
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Estrogen receptors

ERα and ERβ

The first and best described 17β-estradiol receptor, now called ERα, was identified in the rat uterus in the 1960s.[6,7] The second, less well-characterized receptor, ERβ, was identified in the rat prostate in 1996.[8] These highly homologous receptors function as ligand-activated nuclear transcription factors that bind *cis*-acting estrogen response elements in the promoter and enhancer regions of hormonally regulated genes.[9] Both ERα and ERβ, encoded by the genes *ESR1* and *ESR2*, respectively, are soluble receptors that can shuttle between the cytoplasm and the nucleus, but are found predominantly in the nucleus (only ~5% of these receptors are present in the cytoplasm).[4] Highly divergent and sometimes opposing functions for the two receptors have been reported in studies of *Esr1* knockout and *Esr2* knockout mice, which lack the murine ERα and ERβ protein, respectively.[10] In addition to their effects on gene expression (that is, their genomic effects), these ERs are also associated with rapid cellular signaling (termed non-genomic effects) that are thought to be mediated primarily by membrane-associated forms of these receptors.[11]

Although multiple modes of action were suggested for ERs as early as the 1960s,[12–14] not all effects of 17β-estradiol, particularly the rapid and membrane-associated signaling

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Key points

- Estrogen has critical nonreproductive roles in health, including beneficial effects on the skeletal, nervous, endocrine, immune and cardiovascular systems, as well as on many diseases and cancers
- The estrogen receptors (ERs) include ERα, ERβ and G-protein-coupled estrogen receptor 1 (GPER); their expression and signaling mechanisms are complex and potentially exhibit redundant, independent, synergistic and/or antagonistic actions
- Estrogenic compounds (selective ER modulators, ER antagonists, selective ER downregulators, phytoestrogens and xenoestrogens) have multifaceted effects on all types of ERs with receptor-specific pharmacological profiles
- GPER-selective agonists, such as G-1, mediate many salutary effects of estrogen in various tissues and organs with only minor reproductive effects
- GPER represents an important diagnostic, prognostic and therapeutic target; development of GPER-selective agonists and antagonists could contribute to the diagnosis and treatment of many diseases

events, could be attributed to ERα and ERβ.[15] In some cases, antagonists of these receptors could not block certain rapid signaling events, which led to the prediction that alternative membrane-bound ERs also existed.[16] Interestingly, most of the 17β-estradiol-mediated rapid signaling events are associated with G protein signaling or growth factor-mediated pathways.

GPER

A study in 2000 reported that rapid 17β-estradiol-mediated activation of extracellular signal-regulated kinases (ERKs) was dependent on the expression of an orphan G-protein-coupled receptor with seven transmembrane domains.[17] This receptor, then known as GPR30, was cloned by several groups in the late 1990s.[18–23] Following this initial report, other studies described 17β-estradiol-mediated, GPR30-dependent, generation of cAMP[24] and expression of Bcl-2,[25] nerve growth factor[26] and cyclin D2.[27] Furthermore, other researchers described GPR30-mediated expression of c-Fos[28] and an interaction between the effects of progestin and GPR30 expression.[29–31] Two studies published in 2005 described binding of 17β-estradiol to GPR30 in GPR30-transfected COS7 and HEK293 cells, as well as various breast cancer cell lines.[32,33] Together, these results suggested that GPR30 was a 17β-estradiol-binding receptor, which led to its designation as G-protein-coupled estrogen receptor 1 (GPER) in 2007. GPER is now known to be expressed in numerous tissues,[34] and research into its functions has substantially increased.

Estrogen receptor ligands

GPER unselective ligands

Natural endogenous estrogens, predominantly 17β-estradiol, are the primary ligands of ERs. 17β-estradiol is synthesized mainly in the ovaries, although it is also produced at many sites throughout the body, including the breast, brain, adipose tissue and the arterial wall, where it might have specialized local effects.[35] The 17β-estradiol-based steroids estriol (a GPER antagonist at high concentrations[36]), estrone and estrone sulfate can also modulate biological functions, although their specific actions are less clear than those of 17β-estradiol.[37] Plasma concentrations of 17β-estradiol in premenopausal women are ~0.2–1.0 nmol/l, although it increases by many 100-fold during pregnancy. Local concentrations in specific tissues can be much higher than the plasma values, for example in breast tissue (by 10–20-fold)[38] or in the placenta at term (~12 μmol/l).[39] The hydrophobic nature of these steroids allows them to diffuse passively through cell membranes and reach their intracellular targets, the ERs.[40]

A large variety of natural and man-made chemicals also have estrogenic activity (Figure 1).[5] Estrogenic compounds synthesized by plants (phytoestrogens) include flavonoids, such as coumestans and isoflavones.[41] Synthetic estrogenic compounds (known as xenoestrogens, environmental estrogens or endocrine disruptors) include many pesticides, herbicides and plastic monomers.[5] Their widespread use results in chronic low-level exposure to these compounds in humans.[42] Although the majority of phytoestrogens and xenoestrogens are believed to exert their physiological effects through modulation of ERα and ERβ,[43] many of these compounds also activate GPER, including the soy isoflavone genistein, for which serum concentrations up to 500 nmol/l have been measured;[44] nonylphenol; the pesticides dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE); bisphenols,[45] such as bisphenol A (Figure 1), which promotes testicular seminoma cell proliferation;[46] the herbicide atrazine;[47] and possibly equol, a nonsteroidal equine estrogen found in premarin[48] that is formed by human gut bacteria as a metabolite of the isoflavone, daidzein.[49]

Synthetic 17β-estradiol mimetics are also used extensively in clinical and therapeutic applications. For example, 17α-ethynylestradiol is the predominant estrogen used in female contraceptives. Drugs, such as tamoxifen (Figure 1) and raloxifene, which are used in treatments for breast cancer and osteoporosis,[2] act as ER agonists in some tissues and ER antagonists in others, which led to their designation as selective estrogen receptor modulators (SERMs).[50] By contrast, fulvestrant (Figure 1) is a 'pure' ER antagonist that causes ER degradation and/or downregulation, which led to its designation as a selective estrogen receptor downregulator (SERD).[51] However, some members of SERMs and SERDs can also act as GPER agonists,[17,33] which complicates the interpretation of the mechanisms of their action and the receptors involved in both physiological and disease conditions.[52]

GPER-selective ligands

Research into the specific activities of GPER has been aided by the discovery of GPER-selective agents. Since the identification of the first GPER-selective agonist G-1 in 2006, a number of reports have examined the disease-related or health-promoting effects associated with GPER activation. Importantly, studies using G-1 (Figure 1) at concentrations as high as 1–10 μmol/l showed no notable activity of this agent towards ERα in terms of activating or inhibiting rapid signaling events,[33] estrogen response element-mediated transcription[53,54] or ERα downregulation.[53] Furthermore, G-1 had no binding activity on 25 other G-protein-coupled receptors[55] or in *Gper* knockout mice,[56–58] which provided evidence that G-1 is a ligand highly selective for GPER.

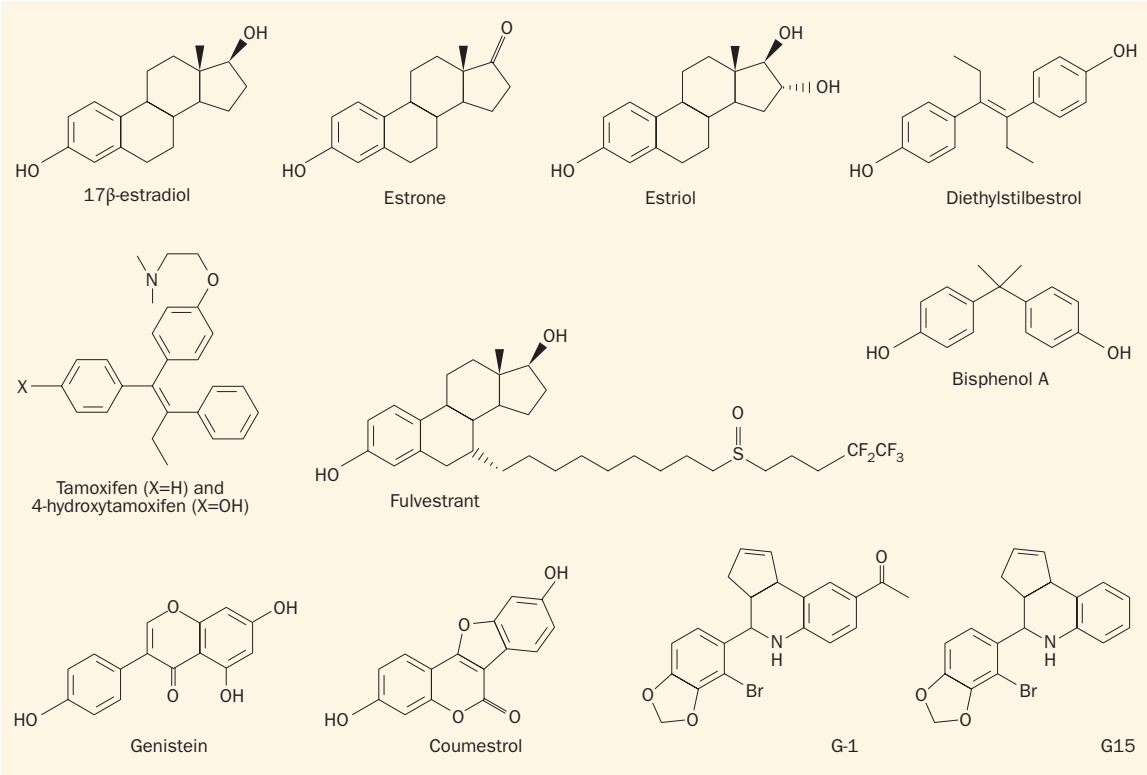

Figure 1 | Structures of selective and nonselective estrogen receptor ligands. Compounds shown include the three major physiological forms of estrogen (17β-estradiol, estrone and estriol); the anticancer agent tamoxifen and its active metabolite 4-hydroxytamoxifen (which is both a selective estrogen receptor modulator and an agonist for GPER); fulvestrant, a selective estrogen receptor downregulator and agonist for GPER; diethylstilbestrol, a nonselective GPER agonist; the phytoestrogens genistein and coumestrol; and the xenoestrogen bisphenol A. Also shown are G-1 (a selective GPER agonist) and G15 (a selective GPER antagonist).

In 2009, the GPER-selective antagonist G15 (Figure 1) was identified,[59] followed by G36, a more selective GPER antagonist than G15, identified in 2011.[54] G15 has a similar structure to G-1,[59] and is effective in inhibiting all G-1-mediated effects tested to date,[59–61] as well as many 17β-estradiol-mediated effects.[59–63] The core structures of G-1, G15 and G36 have been used to generate several radioactively labeled agents that can be used for imaging and potential treatment of GPER-expressing tumors *in vivo*.[64,65]

GPER signaling

Although ERα and ERβ are accepted as the predominant nuclear receptors involved in the genomic effects of estrogen, evidence also indicates that rapid modulation of cell-signaling pathways occurs via a subpopulation of ERs located at the plasma membrane (Figure 2),[4] which has fueled the speculation about a role of GPER.[66] The localization of GPER, however, seems to be predominantly intracellular,[33,67] consistent with reports that describe the constitutive internalization of plasma membrane GPER.[68,69]

Signaling through GPER occurs via transactivation of the epidermal growth factor receptor (EGFR) and involves nonreceptor tyrosine kinases of the Src family.[17] In this mechanism, which is now also accepted for other G-protein-coupled receptors,[70] stimulation of GPER activates metalloproteinases and induces the release of heparin-binding EGF, which binds and activates EGFR,[71] leading to activation of downstream signaling molecules, such as ERK1/2.[72] Moreover, 17β-estradiol-mediated activation of GPER stimulates production of cAMP,[24,32] intracellular calcium mobilization[33,73,74] and PI3K activation (Figure 2).[33] Further research in human breast cancer cells suggests that sphingosine kinase[75] and integrin $\alpha_5\beta_1$[76] are intermediates in 17β-estradiol-mediated EGFR transactivation; the latter study suggesting a role for GPER in fibronectin assembly.[76]

In addition to the above-mentioned rapid signaling events, GPER also regulates transcriptional activity, albeit indirectly, by activating signaling mechanisms that involve cAMP, ERK and PI3K (Figure 2).[77] The genes regulated by GPER include *FOS*, which encodes c-Fos,[28] a protein that forms a heterodimer with various other proteins to form the transcription factor AP-1. In turn, these signaling pathways also activate other transcription factors, such as steroidogenic factor 1,[78] which induce expression of additional genes.[79,80]

GPER in physiology and disease

Reproductive system

The role of 17β-estradiol is best defined in the reproductive system, where this hormone regulates uterine and mammary development and function. Although roles

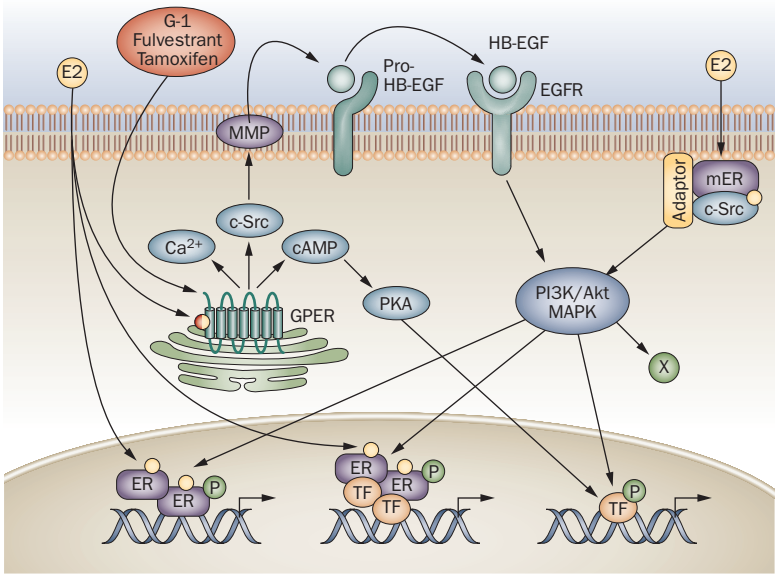

Figure 2 | Nongenomic and genomic estrogen signaling pathways. Endogenous estrogens including 17β-estradiol are nonselective activators of the three known ERs, ERα, ERβ and GPER. 17β-Estradiol activates nuclear ERs, inducing receptor dimerization and binding of receptor dimers to the promoters of target genes. Alternatively, activated ERs modulate the function of other classes of TFs through protein–protein interactions. Subpopulations of ERs at the plasma membrane activated by E2 interact with adaptor proteins (adaptor) and signaling molecules such as c-Src, which mediates rapid signaling via PI3K–Akt and MAPK pathways. E2, or selective agonists such as G-1, or selective estrogen receptor downregulators, such as fulvestrant, or selective estrogen receptor modulators, such as tamoxifen, also activate GPER, which is predominantly localized intracellularly. GPER activation stimulates cAMP production, calcium mobilization and c-Src, which activates MMPs. These MMPs cleave pro-HB-EGF, releasing free HB-EGF that transactivates EGFR, which in turn activates MAPK and PI3K–Akt pathways that can induce additional rapid (nongenomic) effects (X), or genomic effects regulating gene transcription. E2-mediated transcriptional regulation may involve 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: E2, 17β-estradiol; EGFR, epidermal growth factor receptor; ER, estrogen receptor; GPER, G-protein-coupled ER; MMP, matrix metalloproteinase; pro-HB-EGF, pro-heparin-binding-epidermal growth factor; TF, transcription factor.

for GPER are implicated in almost every system of the body (Figure 3), conflicting observations have been published particularly in the reproductive system.[34] No clear developmental or functional defects occur in the reproductive organs of *Gper* knockout mice,[81–84] whereas *Esr1* knockout mice display multiple reproductive defects.[85] Furthermore, in wild-type mice treated with G-1, no change was detected in ductal growth or end bud formation in mammary glands, and no uterine imbibition of water, or proliferative response in the mammary gland or endometrium was observed.[83] However, in another study, G-1 treatment in mice stimulated uterine epithelial proliferation by approximately threefold, compared with a ~15-fold increase in proliferation observed with 17β-estradiol.[59] Importantly, blocking GPER with G15 reduced the 17β-estradiol-mediated proliferative response by ~50%,[59] which suggests that GPER, in part, contributes to this response. Surprisingly, high concentrations of G-1 (1,000-fold greater than those needed to observe a proliferative effect) reduce both 17β-estradiol-mediated uterine imbibition of water, and proliferation, through inhibition of ERK1/2 in the stroma and via phosphorylation of serine 118 in ERα.[86] These data suggest that GPER regulates uterine proliferation, independently of ERα, but via a mechanism that might involve crosstalk with the 17β-estradiol–ERα pathway.

In addition to effects on the mammalian uterus, GPER is also involved in the regulation of meiotic arrest in oocytes of the Atlantic croaker and zebra fish. *In vitro,* 17β-estradiol and G-1 reduced both spontaneous and progestin-induced oocyte maturation in both Atlantic croaker and zebra fish, whereas knockdown of GPER or blockade of GPER with G15 prevented the inhibitory effects of 17β-estradiol, which occur via an EGFR-dependent pathway.[62,87] Furthermore, GPER (mRNA and protein) expression in granulosa and theca cells of the hamster ovary is regulated by gonadotropins and the estrous cycle,[88] and in this tissue, GPER regulates the 17β-estradiol-mediated stimulation of primordial follicle formation.[89] In *ex vivo* studies of human myometrium, GPER enhances contractile responses to oxytocin, which suggests a role for GPER in uterine contractility during labor.[90] Moreover, ERα, ERβ and GPER regulate the proliferative and apoptotic pathways involved in spermatogenesis[91–93] during male reproductive development. Overall, the roles of GPER in the reproductive system are complex and require further investigation, particularly in humans.

Nervous system and neuroendocrinology

The effects of 17β-estradiol in the central and peripheral nervous system include maintenance of homeostasis, regulation of synaptic plasticity and cognition, neuroprotection and modulation of pain sensation. Although many of these effects might involve ERα and ERβ, increasing evidence indicates that GPER has multiple roles in 17β-estradiol-mediated neurological functions. GPER (mRNA and protein) expression have been detected throughout the central (Figure 3) and peripheral nervous system of male and female rodents, including in the hippocampus, hypothalamus and midbrain, as well as the spinal cord and dorsal root ganglia.[74,94,95] However, conflicting results showing *Gper* expression in small arterial surface vessels and pericytes in the brain also exist.[81] Both ERα and GPER activate the ERK1/2 pathway in trigeminal ganglion neurons and increase allodynia, indicating a role for these two ERs in temporomandibular disorder and migraine.[96] Furthermore, in rats, G-1 depolarizes spinal cord neurons,[84] stimulates mechanical hyperalgesia via protein kinase Cξ[97] and mediates visceral hypersensitivity in the absence of inflammation.[98]

17β-Estradiol has many beneficial effects on the brain, including reduction of neuronal loss following stroke, increase in neuronal connectivity and improvement of cognitive performance.[99] GPER has been implicated in 17β-estradiol-mediated effects on cholinergic neurons in the basal forebrain, which suggests that this ER might be an important regulator of cognitive function, particularly in women after menopause.[100] In studies that used immortalized hippocampal cell lines, GPER (along with ERα)

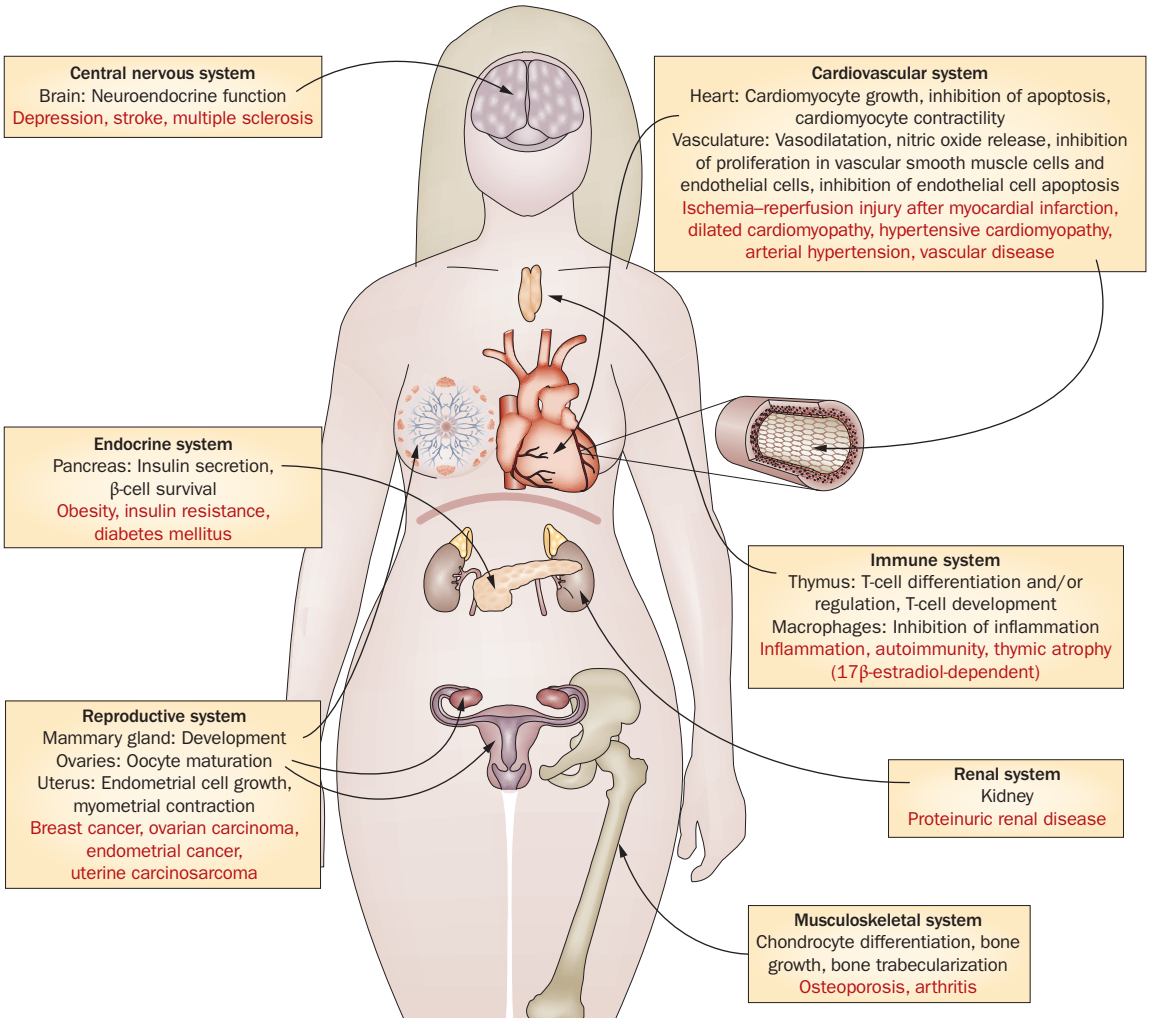

Figure 3 | Involvement of G-protein-coupled estrogen receptor (GPER) action in regulation of physiological responses and disease. GPER is implicated in neuroendocrine and cerebral functions, immune cell function, endocrine regulation and metabolism, cardiovascular and kidney function, and reproductive functions. In addition, studies using experimental models of disease and/or human tissue suggest roles for GPER in diseases (shown in red), such as diabetes mellitus, arterial hypertension, proteinuric renal disease, osteoporosis, arthritis, immune diseases, such as multiple sclerosis, and cancer. Collectively, these studies suggest the therapeutic potential of regulating GPER activity as a novel approach for the treatment of these conditions.

was implicated in the protective effects of 17β-estradiol against glutamate-induced injury,[63] although in cortical neurons G-1 did not have any effect.[101] However, *in vivo* studies showed that G-1 treatment replicates the effects of 17β-estradiol in promoting neuronal survival following global ischemia in the brain.[102,103] Altogether, these results suggest that GPER agonists might represent a new therapeutic approach for stroke and chronic neurodegenerative diseases.[104]

In the brain, G-1 (like 17β-estradiol) attenuates serotonin receptor signaling in the paraventricular nucleus of the hypothalamus and reduces responses to oxytocin and adrenocorticotropic hormone, which suggests that GPER might have a role in mood disorders.[105] Furthermore, G-1 exhibited antidepressant properties in a mouse model of depression, where it reproduced the effects of 17β-estradiol, which were inhibited by the GPER-selective antagonist G15.[59] In primates, GPER contributes to 17β-estradiol-mediated regulation of luteinizing-hormone-releasing hormone neurons, which maintain gonadal function and fertility.[106] This effect probably also involves additional mechanisms.[107] However, whereas GPER activation promoted short-latency prolactin secretion, G-1 did not affect the 17β-estradiol-mediated negative feedback inhibition of either luteinizing hormone secretion or lordosis behavior in rats.[108] Studies with *Esr1* knockout mice showed that ERα is required for 17β-estradiol-regulated positive feedback control of hypothalamic gonadotropin release,[109] which suggests that the actions of GPER are complex and possibly also require the presence of ERα.

Immune system

17β-estradiol displays multiple effects in the regulation of immune responses, including the development of T cells,[110] autoimmune disease[111,112] and inhibition

of inflammation.[111] Studies in *Esr1* knockout and *Gper* knockout mice have shown that GPER, along with ERα, contributes to 17β-estradiol-induced thymic atrophy;[84] ERα mediated the early blockage of thymocyte development, whereas GPER mediated thymocyte apoptosis. Furthermore, in *Gper* knockout mice engineered to express the prokaryotic *lacZ* gene under control of the murine *Gper* promoter, numbers of L-selectin-expressing T cells decreased, consistent with an altered production of these T cells in the thymus.[81] By contrast, other studies using *Gper* knockout mice did not find any difference in 17β-estradiol-induced thymic atrophy,[113] nor in 17β-estradiol-dependent protective effects on arthritis or bone loss in a model of postmenopausal rheumatoid arthritis.[114] These findings suggest complex roles for 17β-estradiol and GPER in the immune system (Figure 3).

Estrogens are increasingly receiving attention as potential anti-inflammatory agents for the treatment of autoimmune diseases, particularly multiple sclerosis.[112] In a mouse model of multiple sclerosis—experimental autoimmune encephalomyelitis (EAE)—knockout of *Gper* interfered with the protective role of 17β-estradiol.[56] In two studies, treatment with G-1 reproduced the ability of 17β-estradiol to protect against the functional and histological manifestations of EAE through enhancing the immunosuppressive activity of $CD4^+Foxp3^+$ T cells, resulting in upregulation of programmed cell death[56] and inhibition of inflammatory cytokine production by macrophages.[55] These findings suggest that GPER mediates the protective role of 17β-estradiol in multiple sclerosis.

Although the protective effects of G-1 against EAE were absent in *Gper* knockout mice, 17β-estradiol-mediated effects were partially retained, suggesting that ERα and GPER can activate independent, yet overlapping, mechanisms. Further research showed that the therapeutic effect of ethynylestradiol in established EAE was mediated via GPER, but not via ERα, and possibly involved production of the anti-inflammatory cytokine Il-10.[115] Another study showed that G-1 treatment elicits *de novo* production of Il-10 in T helper type 17 polarized cells, *in vitro* as well as *in vivo*, via an ERK1/2-dependent pathway.[116] Thus, the immunomodulatory effects of G-1, mediated by activation of GPER, indicate that GPER agonists might have novel clinical applications in chronic inflammatory diseases.

Cardiovascular system

Endogenous 17β-estradiol is implicated in sex-specific differences observed in arterial hypertension and cardiovascular disease,[117,119] as the cessation of 17β-estradiol production following menopause accelerates these conditions.[117] However, the cellular mechanisms and signaling pathways conferring the protective effect of 17β-estradiol are only partially understood.[120] Although ERα and ERβ are implicated in cardiovascular protection mediated by 17β-estradiol, a protective effect of this hormone is also seen in the absence of both receptors.[121–123] These observations provided the initial evidence for the existence of alternative receptors, such as GPER, and signaling pathways involved in 17β-estradiol-mediated regulation of cardiovascular function.

GPER (mRNA and protein) is expressed in murine[82] and human myocardium,[124] as well as in cultured cardiomyocytes.[125] 17β-Estradiol-mediated inhibition of calcium influx and contraction in murine cardiomyocytes is independent of ERα and ERβ,[121] and deletion of *Gper* leads to left ventricular dilatation and elevation of end-diastolic pressure in male, but not female, mice.[126] In patients with myocardial infarction, ischemia–reperfusion injury after reopening of the occluded coronary artery is a critical determinant of outcome and complications, such as arrythmia and heart failure.[127,128] Myocardial hypoxia resulting from infarction[129] is an important stimulus that upregulates GPER (mRNA and protein) expression in cardiomyocytes.[125] Several groups have independently demonstrated that G-1 treatment after myocardial infarction led to reduced reperfusion-related injury and infarct size and improved contractile function in structurally normal hearts from rodents of both sexes.[124,127,130–132] Similar benefits were also obtained for G-1 treatment in cerebrovascular occlusion-related reperfusion injury in animal models of stroke.[102,133] Under these conditions, activation of GPER by G-1 resulted in reduced myocardial expression of proinflammatory cytokines (IL-1β, IL-6 and tumor necrosis factor),[132] increased activation of *Akt*,[134] Erk1/2,[130,134] increased phosphorylation of endothelial nitric oxide synthase (eNOS)[134] and decreased mitochondrial permeability.[131] Some of these cardioprotective effects were blocked by an inhibitor of PI3K.[134]

GPER protein is expressed in human endothelial[23,135] and smooth muscle cells,[58,136] as well as in intact arteries (Figure 3).[136] Expression of GPER in macrophages,[137] which contribute to atherogenesis, also suggests a functional role for GPER in atherosclerosis and the associated inflammation. In human endothelial cells, activation of GPER (but not of ERα)[138] inhibits cell proliferation,[135] indicating an antiangiogenic role for this ER. In human and rat vascular smooth muscle cells, activation of GPER by either G-1[58,139] or raloxifene[140] stimulates the ERK1/2 pathway and inhibits proliferation, similarly to the effect of ERα activation in these cells.[140] These findings are in keeping with the antiproliferative effects of 17β-estradiol on vascular smooth muscle cells from *Esr1* and *Esr2* double-knockout mice.[122] Moreover, the GPER agonists G-1,[58,61,142,143] genistein[144] and fulvestrant[142] (Figure 1) cause vasodilatation in human, porcine and rodent arteries, whereas this effect is blocked by the GPER antagonist G15[61] and is absent in *Gper*-deficient mice.[58]

Elevated vascular resistance is a key feature of arterial hypertension.[119] *Gper*-deficient mice exhibit a normal mean arterial blood pressure that does not change with age.[82] Infusion of the GPER agonist G-1 markedly lowers blood pressure in normotensive[58] and hypertensive rats.[61,145,146] In rats with hypertensive cardiomyopathy, G-1 treatment ameliorates diastolic dysfunction, reduces cardiac hypertrophy and decreases the size of cardiomyocytes.[145] This effect is probably mediated through direct vasodilatory actions of G-1[58,143,147] or 17β-estradiol, as this hormone also has vasodilatory effects (which are derived at least in

part from GPER, as they are blocked by the GPER antagonist G15).[61] Vasodilatory actions of G-1 involve both nitric oxide-dependent and nitric oxide-independent pathways and have been observed in human, pig and rat arteries.[58,61,143,146] Phosphorylation of eNOS as a result of GPER activation might contribute to this response.[49,134] At least some of the vasoprotective effects mediated by GPER are probably the result of interference with endothelial cell dysfunction—a vascular abnormality common to hypertension and coronary artery disease.[118,148]

Altogether, these data indicate a central regulatory role for GPER in cardiovascular function and suggest that GPER agonists have potential roles in the treatment of vascular and myocardial disease in both men and women.

Renal system

Endogenous 17β-estradiol is also implicated in the sex-specific differences in renal disease.[117] GPER is expressed at high levels in renal tubules,[95] as well as in renal epithelial cells (Figure 3).[68] In humans, the *GPER* locus is associated with low-renin hypertension,[149] which leads to kidney injury and vascular dysfunction (the latter abnormality is ameliorated by G-1 treatment).[146] Endothelial cell dysfunction is also present in animals with glomerulosclerosis, which leads to proteinuria due to loss of glomerular filter function. In hypertensive rats, GPER activation reduces proteinuria and improves creatinine clearance despite continued hypertension.[150] These findings suggest a renoprotective role for GPER agonists in hypertensive nephropathy.

Pancreatic function and glucose metabolism

The increased prevalence of obesity, insulin resistance and diabetes mellitus after menopause indicates a protective role for endogenous 17β-estradiol in premenopausal women.[151,152] These protective effects are largely attributed to signaling via nuclear ERα,[153,154] as its deletion results in obesity and insulin resistance.[151,155] However, other forms of ERα signaling are also involved in metabolic diseases;[154,156] for example, insulin secretion mediated by 17β-estradiol occurs through rapid signaling via membrane-bound ERs.[157–159] Although ERα and ERβ individually affect insulin action,[151,155] mice deficient in GPER develop insulin resistance and obesity in a sex-dependent manner.[58,82,160] GPER activation also has anti-inflammatory properties in pancreatic islets through attenuating the effects of proinflammatory cytokines[161] that are important for maintenance of metabolic function (Figure 3).[162] The protective, antidiabetic effects of 17β-estradiol in islet cells seem to involve activation of both membrane-bound ERα and GPER[57,163,164] and might also be induced by GPER agonists, such as genistein.[165]

GPER is expressed in whole adipose tissue in humans and rodents,[58,166] as well as in the human liver,[18–20,22,23] key target organs of insulin resistance.[162] However, the role of GPER in 17β-estradiol-mediated metabolic protection is not clearly defined. GPER is expressed in the pancreatic islets of mice[57,82,161,163,164] and humans,[163] and in female mice it maintains normal metabolic function.[82] GPER deficiency results in a reduction in insulin secretion (stimulated by 17β-estradiol, G-1 and glucose) from the pancreas without affecting the morphology of pancreatic β-cells, which suggests that GPER has a key role in maintaining the metabolic functions of insulin in mice[82,167] and humans.[168] Furthermore, the protective effect of 17β-estradiol on survival of pancreatic β-cells in a mouse model of type 1 diabetes mellitus is absent in GPER-deficient animals.[57] Whether GPER contributes to peripheral insulin resistance is currently not known. However, expression of GPER has been reported in human skeletal muscle,[56,81,161,163] and is unaffected by menopause.[169]

Bone growth and chondrocyte metabolism

Bone and articular cartilage are hormone-sensitive tissues,[170] and serum 17β-estradiol levels inversely correlate with the risk of hip fracture in both women and men.[171] Perhaps the best evidence of a role for endogenous 17β-estradiol in overall bone health and formation of trabecular bone in particular is the postmenopausal onset of osteoporosis. The bone-preserving effects of estrogen therapy, especially with SERMs,[172] which act as GPER agonists, indirectly suggest a role for GPER in bone metabolism (Figure 3). Endogenous 17β-estradiol also plays an important role in bone metabolism in men, since lack of 17β-estradiol owing to aromatase deficiency[173] or mutations in *ESR1*[174] in men lead to osteopenia, enhanced bone remodeling through increased bone resorption and osteoclast activity and suppression of bone growth-plate closure.[175] Although part of this effect is mediated through ERα and ERβ,[172] several avenues of research now suggest a role for GPER in bone and cartilage metabolism. In bone, GPER is expressed in osteocytes, osteoclasts and osteoblasts,[176,177] and is also detected in chondrocytes,[176,178] the differentiation of which is regulated by GPER.[178] In addition, GPER also controls bone growth, as illustrated by several models of GPER deficiency, albeit in a sex-dependent manner (Figure 3). *Gper* deficiency inhibits bone growth in female mice;[82] similar results were reported in ovariectomized, estrogen-treated animals,[113] suggesting a role for GPER in estrogen-induced bone growth and development. By contrast, GPER-deficient male mice show increased femur size, BMD, trabecularization and cortical bone thickness.[160] Tamoxifen, a GPER agonist, decreases tibia length independently from ERα or ERβ.[52] Although *in vitro* studies and clinical trials with SERMs show beneficial effects on bone structure in postmenopausal women,[172] the role of GPER in bone and chondrocyte metabolism in humans is still not clear and warrants further study.

GPER in cancer growth and metastasis

17β-Estradiol is a critical mediator of breast carcinogenesis and is involved in a number of other hormone-sensitive cancers. Normal breast tissue is highly sensitive to 17β-estradiol, which stimulates proliferation of this tissue during puberty, the menstrual cycle and pregnancy; thus, the majority of breast cancers are highly responsive to 17β-estradiol and utilize 17β-estradiol signaling pathways in cancer initiation, progression and metastasis.[179] This understanding has led to the development of various

cancer therapies that target 17β-estradiol signaling, the most widely used of which is tamoxifen.[180] Antiestrogen therapy has been extended to include SERDs (such as fulvestrant), aromatase inhibitors (for postmenopausal women) and other SERMs (such as raloxifene).[50] Many of these agents, particularly tamoxifen and fulvestrant, are also GPER agonists and have complex physiological and therapeutic actions. For example, long-term 17β-estradiol deprivation in the weakly metastatic human breast cancer cell line MCF-7 increased expression of GPER,[181] whereas tamoxifen treatment of these cells stimulated proliferation via GPER-mediated transactivation of EGFR.[182]

GPER protein is expressed in ~50% of all breast cancers (Figure 3), regardless of their ER status,[183] although conflicting results have been reported regarding co-expression of GPER and human epidermal growth factor receptor 2 (HER2).[183–185] Nevertheless, in general, GPER protein expression in breast cancers correlates with clinical and pathological biomarkers of poor outcome. High levels of GPER protein expression in samples of human breast cancers also correlate with increased tumor size and metastasis.[183] Importantly, in patients treated only with tamoxifen, GPER protein expression was increased and survival was markedly reduced in patients with initial GPER-positive tumors, suggesting that patients with breast cancer who have high GPER protein expression should not be treated with tamoxifen alone.[186] In addition, GPER is widely expressed in cancer cell lines and primary tumors of the breast,[17,18,33,187] endometrium,[188–190] ovaries,[47,53,191] thyroid,[190] lung,[192] prostate,[193] testicular germ cells[194] and the brain (E. R. Prossnitz, unpublished work). In cell lines of thyroid, ovarian, endometrial and breast cancers, stimulation of GPER with 17β-estradiol[53,190,195] or other estrogenic compounds, such as atrazine,[47] genistein,[190] bisphenol A[46,196] or tamoxifen[195] activates a signaling mechanism that typically promotes proliferation, although inhibition of proliferation has also been reported.[73] In particular, genistein can stimulate MCF-7 cell growth via induction of acid ceramidase, which occurs through a GPER-dependent mechanism.[197] In endometrial cancer[198] and ovarian cancer,[199] high levels of GPER expression also predict poor survival, whereas among postpubertal testicular germ cell tumors, GPER was highly expressed in intratubular germ cell tumors, seminomas and embryonal carcinomas, with little expression in teratomas.[194]

Importantly, treatment of the ERα-negative human breast cancer cell line SKBr3 with 17β-estradiol or tamoxifen increased the expression of several transcription regulators (including c-Fos) and cytokines (particularly connective tissue growth factor, which promotes cancer cell proliferation and migration).[200] These data indicate that tamoxifen treatment might have a cancer-promoting effect through GPER. In support of this view, endometrial GPER protein expression also correlated with tamoxifen-induced uterine pathology, including bleeding and abnormal endometrial thickening,[201] which correlates with an increased incidence of endometrial cancer.[202]

The overall role of GPER in breast cancer progression is complex. In addition to the effects on epithelial cells, GPER is implicated in 17β-estradiol-mediated activation of cancer-associated fibroblasts, which promote tumor cell proliferation and metastasis through direct association of GPER with chromatin.[203] *GPER* expression was induced in breast cancer cells under hypoxic conditions, which also suggests a cancer-promoting role for this ER, including a role in hypoxia-induced angiogenesis.[125] However, G-1 inhibits endothelial cell proliferation, which indirectly suggests that GPER activity also interferes with angiogenesis.[135] Despite these conflicting data on the role of GPER in cancer, targeting its activity represents an important new approach for cancer therapy.

Conclusions

The salutary effects of estrogens are well-established in many diseases, and selective activation of GPER by G-1, phytoestrogens, SERDS or SERMS can reproduce the beneficial effects of 17β-estradiol. The pace of research into the physiological and pathological functions of GPER has been accelerating over the past 5 years, and potential roles for GPER have now been identified in almost every system of the body. Thus, GPER-selective agents that mimic the beneficial effects of 17β-estradiol without its associated feminizing or other adverse effects could represent an important new family of drugs.

In addition, GPER-specific antagonists could be developed as important additions to the armamentarium of drugs used to treat estrogen-sensitive cancers and other diseases in which estrogen signaling is important. In this regard, the potential contribution of GPER-mediated signaling to the effects of existing clinically approved drugs, such as tamoxifen and fulvestrant, must be considered. GPER-mediated effects should also be taken into account in the future development of SERMs and SERDs. In addition, further research is required to determine to what extent the physiological effects of 17β-estradiol involve GPER signaling and the precise roles of non-selective estrogen receptor ligands in health and disease. The co-dependent, redundant and independent aspects of 17β-estradiol signaling through ERα, ERβ and GPER are likely to be very complex and specific to particular cell types, tissues, ligands and diseases. The data available to date nevertheless pose interesting questions about the therapeutic potential of specifically targeting GPER in disease.

Review criteria

A search for original articles was performed in PubMed. The search terms used included "GPER", "GPR30", "estrogen", "rapid signaling", "SERM", "reproduction", "immune", "vascular", "nervous", "metabolism", "bone" and "cancer" with no restriction on the publication year, language or article type. Additional abstracts were also identified by searching Google Scholar using similar keywords. Reference lists within identified papers were also searched. The authors would like to apologize to their colleagues whose work they could not include due to space restrictions.

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Author contributions

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