International Journal of Multidisciplinary Research and Growth Evaluation.

Pathophysiology of g-protein coupled Oestrogen receptor

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Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 05
September-October 2023
Received: 15-08-2023;
Accepted: 04-09-2023
Page No: 330-335

Abstract

Oestrogens are critical mediators of multiple and diverse physiologic effects throughout the body in both sexes, including reproductive, cardiovascular, endocrine, nervous, and immune systems. Alterations in oestrogen function play important roles in many diseases and pathophysiological conditions. Oestrogens mediate their effects through multiple cellular receptors, the nuclear receptor family (ERα and ERβ) and the G protein–coupled receptor (GPCR) family (GPR30/G protein–coupled oestrogen receptor [GPER]). Although both receptor families can initiate rapid cell signalling and transcriptional regulation, the nuclear receptors are traditionally associated with regulating gene expression, whereas GPCRs are recognized as mediating rapid cellular signalling. The protective and beneficial effects of oestrogen mimicked by selective GPER agonism which were absent or reduced in GPER knockout mice, suggest an essential and a parallel role for GPER in the actions of oestrogen. G protein-coupled oestrogen receptor (GPER) has important transcription dependent outcomes in the regulation of cell growth and programmed cell death secondary to GPER-regulated second-messenger pathways. GPER is expressed ubiquitously and has diverse biological effects such as regulation of endocrine, immune, neuronal and cardiovascular functions. The most important consequences of GPER activation are the regulation of cell growth, migration and apoptotic cell death. These cell growth regulatory effects are important in cancer biology and in the regulation of cardiac and vascular hypertrophy and the response to ischemia, for which GPER is emerging as a novel therapeutic target and prognostic indicator.

Keywords: Oestrogen, GPER

Introduction

The term “Oestrogens” refers to a group of female hormones, oestrone, oestradiol, oestriol and oestetrol [1] belong to the family of organic compounds known as steroids and are primarily synthesized in the ovaries and by other tissues such as the liver, pancreas, adipose tissue, adrenal glands and breast in smaller amounts. In specific physiological conditions, such as pregnancy, oestrogen is also synthesized by the placenta. The word oestrogen is commonly used to refer to oestradiol (or 17β-oestradiol), due to its physiological relevance and predominance during reproductive years. Oestrogen [17 β-oestradiol; E2], is a female sex hormone, which is essential for the development of the female reproductive organs and the secondary sexual characteristics. This hormone also plays a critical role in the development and function of the male reproductive tract [2].

Oestrogen receptors

Oestrogen exerts the physiological effects by interacting with oestrogen receptors (ERs) and subsequently, activating various signalling cascades that extend from seconds to hours [3].

In 1958, Elwood Jensen discovered the oestrogen receptor. More than 20 years later, the first human oestrogen receptor (known today as ERα) was identified. Later the second oestrogen receptor, ERβ, was identified in 1996. Oestrogen receptors are ligand-regulated nuclear transcriptional factors believed to mediate a wide array of biological actions. Oestrogen (E2) is reported to engage in rapid non-genomic signalling events, such as mobilization of intracellular calcium in MCF-7 breast cancer cells, production of cyclic adenosine monophosphate (cAMP) in primary rat uterine cells. The complex mechanisms underlying these cellular actions may involve ERs, the variants of ERα, and unknown receptors [4]. Cellular signal transduction can occur as a result of oestrogen activating G proteins, which then lead to the modulation of downstream cellular pathways. Thus, a potential role for G protein-coupled receptors (GPCR), which utilize E2 as ligand, has been proposed as an important route through which E2 exerts cellular functions [5]. In 1997, a novel seven transmembrane-domain GPCR (G protein-coupled receptor), named GPR30, was first identified and cloned, it was initially speculated that the endogenous ligand activating GPR30 is a chemokine or peptide. In 2007, the physiological role of GPR30 *in vivo* was first examined in rats and the results showed that administration of E2 induced GPR30 expression and attenuated hepatic injury via protein kinase A (PKA)-mediated mechanism in rats. In 2007, GPR30 officially named as GPER by the International Union of Basic and Clinical Pharmacology. GPER is mainly localized in central and peripheral nervous system, uterus, ovary, mammary glands, testes, pancreas, kidney, liver, adrenal, pituitary glands, cardiovascular system and adipose tissue. GPER is expressed ubiquitously and known for various biological effects such as regulation of endocrine, immune, neuronal, and cardiovascular functions [6]. GPER expression pattern is age, species, gender or tissue dependent. Thus, role for GPER as a plasma membrane-based ER is still controversial, and the exact mechanism by which GPER acts in response to E2 remains elusive [7].

GPER-selective ligands

Oestrogens, including 17-β oestradiol, 17-α oestradiol, oestrone, oestriol and their 2-hydroxy and 2-methoxy oestradiol metabolites have shown to bind to GPER based on binding assays, GPER activation aided by the availability of selective, moderately potent GPER agonist (G1) and antagonist (G15, G36) ligands [7]. A wide variety of chemicals, in addition to the physiological and synthetic ligands interacted with GPER such as selective ER modulators, specifically tamoxifen and raloxifene, ER antagonists (such as ICI 182780) and niacin [8]. Phytoestrogens (e.g., genistein, resveratrol, and quercetin) and xenoestrogens or endocrine disruptors atrazine and bisphenol A are also known to interact with GPER. Recently, arsenite and cadmium were shown to interact with GPER [9].

Functional effects of GPER activation

GPER is expressed ubiquitously and its activation has been linked to diverse biological actions.

(*a*) Release of (Luteinising hormone) LH-releasing hormone [10] and insulin [11] in endocrine function.

(b) IL-10 (Interleukin 10) production and secretion [12] in immune function.

(c) Antidepressant effects, implying neural functions [13] and

(d) Cell growth and death as they relate to carcinogenesis [14] and cardiovascular functions [15].

Oestrogen receptor mediated signaling through GPER-coupled pathways (nongenomic pathway)

Classical GPCR are cell membrane proteins which bind their ligands at cell surface, but GPER-1 binding domain exists inside the plasma membranes and the endoplasmic reticulum [16]. GPER, member of the GPCR superfamily, couples to heterotrimeric G proteins, which regulate a multitude of downstream effectors within the cell. GPER coupling includes both Gi/o and Gs proteins. GPER is physically associated with Gαs in its inactive state, and this complex dissociates following oestrogen stimulation and causes GPER-mediated intracellular cAMP (cyclic adenosine monophosphate) production and cAMP-dependent signaling. The ability of GPER to activate adenylyl cyclase was demonstrated as a mechanism involved in the attenuation of ERK1/2 activation [17]. The first activity of GPER to be demonstrated involved the E2-mediated rapid activation of ERK1/2 (Extracellular signal-regulated kinases) in a pertussis toxin-sensitive manner, indicating the involvement of Gi/o proteins [18]. The downstream signaling pathway involved the Src-mediated activation of metalloproteinases, Src (Non-receptor tyrosine kinase) induces matrix metalloproteinase (MMP) production, which cleaves pro-heparin-bound epidermal growth factor (pro-HB-EGF) releasing free HB-EGF. HB-EGF binds to the EGFR (Epidermal growth factor receptor) leading to activation of multiple molecules such as Ras, PI_3K (Phosphoinositide 3-kinase), Akt (Protein kinase B), and (Extracellular signal-regulated kinases) ERk1/2. The downstream signal of PI3K and AKt results in activation of several nuclear receptors which is closely related the proliferation and migration of cancer cell [16]. GPER employs a Gβγ- subunit protein dependent mechanism to promote oestrogen-mediated transactivation of the epidermal growth factor receptor (EGFR)-to-ERk signaling. Free Gβγ-subunit complexes transduce intracellular signals that results in the activation of phosphoinositide-3-kinase, release of calcium and exoproteases stimulation that cleave and release membrane-tethered growth factors [19]. In response to E2, GPER activates the PI_3K /Akt axis and this activation involves EGFR transactivation and with Erα also activates this pathway involving an EGFR-independent mechanism [5]. In addition, GPER activates eNOS (endothelial nitric oxide synthase) to produce nitric oxide within the vasculature [20] and sphingosine kinase to yield sphingosine 1-phosphate in cancer cells. Studies have revealed GPER mediated calcium mobilization and the regulation of potassium channels. These rapid signaling events results in gene expression by GPER, although not to the same extent as ERα. Among the genes whose expression is regulated by GPER are *c-fos* (c-Fos transcription factor), *cyclin A*, connective tissue growth factor, fatty acid synthase. GPER actions also include regulation of cell cycle regulatory proteins such as cyclin D2 and proteins involved in programmed cell death [8].

Physiologic and Pathophysiologic Functions of G Protein–Coupled Oestrogen Receptor

Because of the multiple oestrogen receptors (ERα, ERβ, their many splice variants and GPER) expressed throughout the body, determining the functions of individual receptors in normal physiology and disease has been particularly challenging. The role of GPER have been described in

virtually every physiologic system of the human body. GPER regulatory activity has been shown to ameliorate pathophysiology in a growing number of diseases with implications for neurologic diseases (stroke and traumatic brain and spinal cord injury), cardiovascular diseases (including hypertension, atherosclerosis and myocardial infarct), metabolic diseases (diabetes and obesity), autoimmune diseases (multiple sclerosis, cancer and many more). The widespread involvement of GPER in such a wide array of pathophysiology suggests that GPER-targeted therapies could represent an important new approach to the treatment of these diseases [21].

Cardiovascular system

GPERs are widely expressed in cardiovascular tissues and are readily detectable in the heart, endothelium and smooth muscle cells of the vasculature. GPER regulation appears to be important in blood pressure control, response to ischemia in both the heart and brain, vasodilation seen as primarily endothelial cell dependent and secondary to activation of PI3K/nitric oxide (NO) synthase. Cardiovascular actions mediated by GPER involves increased LDL receptor content, inhibition of atherosclerosis progression, amelioration of cardiac ischemia-reperfusion injury, regulation of cerebrovascular ischemic responses [6]. Endogenous 17β-oestradiol is implicated in sex-specific differences observed in arterial hypertension and cardiovascular disease as the cessation of 17β-oestradiol production following menopause accelerates these conditions. However, the cellular mechanisms and signaling pathways conferring the protective effect of 17β-oestradiol are only partially understood. A protective effect of this hormone is also seen in the absence of both ERα and ERβ which are implicated in cardiovascular protection mediated by 17β-estradiol. These observations provided the initial evidence for the existence of alternative receptors, such as GPER and signaling pathways involved in 17β-oestradiol-mediated regulation of cardiovascular function (Prossnitz and Barton, 2011). Myocardial hypoxia resulting from infarction is an important stimulus that upregulates GPER (mRNA and protein) expression in cardiomyocytes [22]. Activation of GPER by G-1(GPER agonist) resulted in reduced myocardial expression of proinflammatory cytokines (IL-1β, IL-6 and tumor necrosis factor), increased activation of Akt, ERk1/2, increased phosphorylation of endothelial nitric oxide synthase (eNOS) and decreased mitochondrial permeability. Expression of GPER in macrophages contributes to atherogenesis and also suggests a functional role for GPER in atherosclerosis and the associated inflammation. GPER plays a central regulatory role in cardiovascular function and suggest that GPER agonists have potential roles in the treatment of vascular and myocardial disease in both men and women [21]. The relative levels of GPR30 expression are an important determinant in the balance of the deleterious or protective effects of oestradiol in the vasculature. Steroid hormones (oestrogen), G protein-coupled receptor; extracellular signal regulated kinase are important physiological and pathophysiological regulators of cardiovascular function [23]. Acute effects of estradiol on the vasculature, have been suggested to be mediated both by nonclassical receptors as well as classical steroid receptors. GPER activation is the predominant non classical receptor pathway mediating the stimulatory effects of estradiol on ERK activation and regulation of VSMC (vascular smooth muscle cell) apoptosis. For estradiol, both anti and proapoptotic effects have been reported which have been linked to regulation of vascular inflammation in a range of pathological conditions [24]. This balance of the pro and antiapoptotic effects of oestradiol may be a reflection of balances in opposing signaling pathways, determined by the level of expression of GPER. GPER in vascular smooth muscle cell (VMSC) may have an important role in mediating the effects of estradiol on ERK activation and apoptosis. Thus, the effect of oestradiol on vascular smooth muscle cell apoptosis is likely dependent on the balance between ER-mediated PKA activation and GPER mediated PKA inhibition and PI3 kinase activation [23]. Studies using experimental models of disease or human tissue suggest roles for GPER in cardiovascular diseases such as ischemia–reperfusion injury after myocardial infarction, dilated cardiomyopathy, hypertensive cardiomyopathy, arterial hypertension, vascular disease [21].

Reproductive system

Oestrogen and ERα play an eminent role in the development and function of the reproductive system, specifically the uterus, ovary, and the breast. ERα knockout mice displayed several reproductive defects resulting in infertility. On the contrary, GPER knockout mice and ERβ knockout mice have been reported to be fertile [25]. Majority of E2 reproductive functions are mediated by ERα, GPER and G-1(GPER agonist) lack the classic feminizing effects of ERα and E2. G-1 has been demonstrated to increase the frequency and amplitude of rat myometrial contractions [26] and the oxytocin induced contractile response of human myometrium explants [27] suggesting specific functions of GPER in the uterus. GPER role in mammalian primordial follicle formation has been reported [28]. The molecular mechanisms of action of oestrogenic GPER signalling engages several rapid transduction cascades such as MAPKs and PI3K/AKT, which in turn activate the intracellular transcriptional machinery, leading to stimulatory effects in cancer cells, as well as in components of the tumor microenvironment such as Cancer Associated Fibroblasts (CAFs) [29].

In terms of female pathophysiology, increased GPER expression has been associated with endometriosis and is more frequent in malignant versus benign ovarian endometriotic cysts. GPER expression and its activity have also been important in endometrial and ovarian cancers. GPER is expressed in both normal germ cells and somatic cells and is involved in mediating certain actions of E2 in spermatogenesis, regulating both proliferative and apoptotic events [8]. Within the breast tumor microenvironment GPER was included among the hypoxia-regulated genes. In ROS-triggered and hypoxia inducible factors (HIF-1α-) dependent upregulation of GPER may contribute to the adaptive breast cancer cell responses to low oxygen stress. As hypoxia is mainly tackled by the generation of new blood vessels, there are possibilities that GPER may serve as a mediator of tumor angiogenesis. The functional cooperation between HIF-1α and GPER drives the expression of Vascular endothelial growth factor (VEGF) in response to hypoxia and the heavy metal copper, which triggers biological actions as those observed in low oxygen conditions. The HIF-1α/VEGF axis by oestrogen and endothelin activated GPER signaling has been shown to stimulate angiogenesis and tumor growth both *in vitro* and *in vivo*, as evidenced in breast and hepatic cancer models as well as in CAFs [31].

Cancer

Oestrogen receptors are involved in the initiation, migration, and progression of oestrogen related multiorgan cancers, such as breast cancer, ovarian cancer, prostate cancer, testicular cancer, liver cancer and lung cancer. The important consequences of GPER activation are the regulation of cell growth, migration and apoptotic cell death. These cell growth regulatory effects are important in cancer biology [6]. GEPR induced phosphorylation of Src, induces matrix metalloproteinase (MMP) production, which cleaves pro-heparan-bound epidermal growth factor (pro-HB-EGF) releasing free HB-EGF. HB-EGF binds to the EGFR leading to activation of Ras, PI3K, AKt, and ERk1/2. The downstream signal of PI_3K and AKt results in activation of several nuclear receptors which is closely related the proliferation and migration of cancer cell. Studies found that GPER-1 inhibits the growth of ER positive MCF-7 cells, which is probably through G-protein coupled β and γ subunits activating without CAMP signal activation [16]. GPER is also highly expressed in post pubertal testicular germ cell tumors (intratubular germ cell tumors, seminomas, and embryonal carcinomas) with little expression in teratomas [30]. Furthermore, in both androgen dependent and independent prostate cancer cells, G-1 inhibited growth via a sustained activation of ERK leading to G2 cell cycle arrest, resulting in a substantial reduction in tumor xenograft size. Thus, the functions of GPER, like those of E2 in the dysregulated signaling of cancer cell lines are clearly complex, with both growth-promoting and inhibiting actions [8].

GPERin the functional liaison between breast tumor cells and cancer-associated fibroblasts (CAFs)

GPER identified as an alternate oestrogen receptor mediating oestrogen action in normal and malignant tissues, including breast tumor. Oestrogenic GPER signaling has been demonstrated to trigger a network of transduction pathways leading to gene transcription changes, proliferation and migration of breast tumor cells, including triple-negative and tamoxifen-resistant breast cancer cells [31]. The aggressiveness of breast tumors is deeply influenced by the surrounding stroma, hence the functional crosstalk between cancer cells and the tumor microenvironment has received considerable attention in recent years. CAFs actively promote the growth, expansion and dissemination of neoplastic cells, enhance angiogenic responses acting on endothelial cells and pericytes and contribute to tumor [32]. GPER acts as the cross road between cancer cells and these important components of the tumor microenvironment. It was found that in breast CAFs, E2 and the agonists G-1, bisphenol A, niacin and atrazine through GPER signaling induced events like the transactivation of EGFR, phosphorylation of MAPK transduction pathway and subsequent expression of diverse genes as *c-fos*, Early growth response protein 1 (*EGR1)*, connective tissue growth factor (*CTGF*), *VEGF*, cyclin D1, fatty acid synthase (FASN), *β1-integrin* and the *NAD+* (Nicotine adenine dinucleotide) -dependent histone deacetylase silent information regulator 1 (*SIRT1*). Conditioned medium from CAFs treated with GPER agonists induced the migration of breast cancer cells and tubulogenesis in endothelial cells respectively through CTGF and VEGF. Recently, it has been shown that ligand activated GPER generates couples IL1β secretion by CAFs to Interleukin1 receptor type 1 (IL1R1) expression by breast cancer cells. In particular, medium collected from CAFs treated with E2 and G-1 induced the migration and invasive features in breast cancer cells as fibroblastoid cytoarchitecture and F-actin reorganization [33].

Endocrine System

The physiologic effects of E2 are reproduced to varying extents by selective GPER activation and these include actions on the hypothalamus-pituitary gonadal axis, where GPER is expressed in the anterior and posterior pituitary as well as hypothalamus [34]. Selective GPER activation attenuates oxytocin and ACTH responses. GPER is required for E2-mediated desensitization of serotonin signaling in the paraventricular nucleus and is also implicated in the rapid E2-mediated release of luteinizing hormone releasing hormone via enhanced Ca^{2+} oscillations the corresponding neurons as well as negative feedback by E2 on gonadotropin-releasing hormone induced luteinizing hormone secretion. Expression of GPER in multiple organs with endocrine functions including liver, kidney, adipose tissue and muscle suggest multiple additional potential roles for GPER in endocrine function throughout the body [8]. The increased prevalence of obesity, insulin resistance and diabetes after menopause reveals a strong influence of E2 on energy balance and glucose homeostasis [35]. In the brain, G-1 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 [21].

Nervous system

GPER is expressed in the hypothalamic pituitary axis, hippocampus, and brain stem autonomic nuclei [34]. In central and peripheral nervous system the effects of 17β-oestradiol include maintenance of homeostasis, regulation of synaptic plasticity and cognition, neuroprotection and modulation of pain sensation. Recent evidences suggested that acute administration of the oestrogen receptor agonists in a global ischemia model delayed the loss of hippocampal neurons, suggesting that GPER serve a neuroprotective effect. Treatment with 17β-oestradiol enhanced the activation of the pro-survival kinases, Akt and erk-1/2, while knockdown of GPER blocked these signaling events. These signaling effects have been linked to neuroprotective effects in neurodegenerative diseases such as Alzheimer's disease and basal forebrain cholinergic function and cognitive performance and in the reduction of anxiety-altered stress. These results suggest that GPER agonists might represent a new therapeutic approach for stroke and chronic neurodegenerative diseases [36]. Both ERα and GPER activate the ERK 1/2 pathway in trigeminal ganglion neurons and increase allodynia, indicating a role for these two ERs in temporomandibular disorder and migraine [21].

Immune System

17β-oestradiol displays multiple effects in the regulation of immune responses, including the development of T cells, autoimmune disease and inhibition of inflammation. GPER along with ERα, contributes to 17β-oestradiol-induced thymic atrophy. ERα mediates the early blockage of thymocyte development, whereas GPER mediated thymocyte apoptosis [37]. Oestrogens are increasingly receiving attention as potential anti-inflammatory agents for the treatment of auto- immune diseases, particularly multiple sclerosis. In a

mouse model of multiple sclerosis experimental auto-immune encephalomyelitis (EAE)-knockout of GPER interfered with the protective role of 17β-estradiol. 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 $CD4^+$(cluster of differentiation 4), T cells, resulting in upregulation of programmed cell death and inhibition of inflammatory cytokine production by macrophages. These findings suggest that GPER mediates the protective role of 17β-estradiol in multiple sclerosis. Further research showed that the therapeutic effect of ethinyl estradiol in established EAE was mediated via GPER, but not via ERα and possibly involved production of the anti-inflammatory cytokine IL-10. 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 [21].

Conclusion

Oestrogen receptors display distinct expression and activity profiles throughout the body, with both overlapping and unique functions, in which ERα plays a major role in reproductive (proliferative) function, whereas ERβ and GPER are thought of as responsible for more homeostatic roles. The pace of research into the physiological and pathological functions of GPER has been accelerating and potential roles for GPER have now been identified in almost every system of the body. Thus, GPER-selective agents which mimic the beneficial effects of 17β-estradiol without its associated adverse or other feminizing effects could represent an important new family of drugs. Activation of the nongenomic pathways via GPER, can have important genomic effects in the regulation of cell growth and death mediated via second-messenger pathways. The cell growth regulatory effects are relevant to guiding future design of therapeutic interventions in cardiovascular and cancer biology. GPER involvement in such a wide array of pathophysiologies represent an important new approach to the treatment of these diseases

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