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THE G PROTEIN-COUPLED ESTROGEN RECEPTOR GPER/ GPR30 AS A REGULATOR OF CARDIOVASCULAR FUNCTION

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Abstract

Endogenous estrogens are important regulators of cardiovascular homeostasis in premenopausal women and interfere with the development of hypertension and coronary artery disease. These hormones act via three different estrogen receptors affecting both gene transcription and rapid signaling pathways in a complex interplay. In addition to the classical estrogen receptors ER α and ER β , which are known mediators of estrogen-dependent vascular effects, a G protein-coupled estrogen receptor termed GPER that is expressed in the cardiovascular system has recently been identified. Endogenous human 17 β -estradiol, selective estrogen receptor modulators (SERMs) including tamoxifen and raloxifene, and selective estrogen receptor downregulators (SERDs) such as ICI 182,780 are all agonists of GPER, which has been implicated in the regulation of vasomotor tone and protection from myocardial ischemia/reperfusion injury. As a result, understanding the individual role of ER α , ER β , and GPER in cardiovascular function has become increasingly complex. With accumulating evidence that GPER is responsible for a variety of beneficial cardiovascular effects of estrogens, this receptor may represent a novel target to develop effective strategies for the treatment of cardiovascular diseases by tissue-specific, selective activation of estrogen-dependent molecular pathways devoid of side effects seen with conventional hormone therapy.

Keywords

Blood Pressure; Endothelium; Menopause; Nitric Oxide; Vasodilation

1. Estrogen Receptors and Vascular Estrogen Signaling

Hypertension affects one out of four women worldwide, and its prevalence is particularly high among females over 60 years of age (Kearney et al., 2005). Indeed, the first decade after menopause is accompanied by an increase in blood pressure (Burt et al., 1995), pointing to a blood pressure-lowering effect of endogenous estrogens (Barton and Meyer,

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Conflict of Interest

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2009). Importantly, mild elevations in blood pressure can often be found in women shortly after menopause (Barton and Meyer, 2009) and have been associated with a higher risk of cardiovascular diseases such as myocardial infarction, stroke, and congestive heart failure (Vasan et al., 2001). In line with these epidemiological data, a variety of beneficial cardiovascular effects of endogenous estrogens are known based on numerous experimental studies, including vasodilator activity and improvement of ischemia/reperfusion-related myocardial injury (Deschamps et al., 2010; Meyer et al., 2006; Meyer and Barton, 2009). Conversely, randomized clinical trials using conjugated equine estrogens and medroxyprogesterone acetate for postmenopausal hormone therapy could not recapitulate these beneficial effects but reported increased cardiovascular risk instead (Hulley et al., 1998; Rossouw et al., 2002), indicating that this type of is not suitable for primary or secondary prevention of cardiovascular diseases.

The controversial outcome of these trials may partly result from the complexity of vascular estrogen signaling (Figure 1), which involves at least three different estrogen receptors. Estrogen receptor α (ER α) was identified in the 1960's (then termed ER) (Soloff and Szego, 1969). In 1996, the less characterized estrogen receptor β (ER β) was found (Kuiper et al., 1996). ER α and ER β largely function as transcriptional regulators, but a small fraction may also be present at the plasma membrane, where estrogen binding activates intracellular signaling pathways that mediate acute effects of estrogens, such as rapid vasodilation (Meyer et al., 2006; Meyer et al., 2009). In 1997, a third ER, the seven-transmembrane G protein-coupled ER termed GPR30, was cloned from shear-stress exposed human endothelial cells among other sources (Takada et al., 1997). More recently, GPR30 has been shown to activate rapid signaling cascades, such as extracellular signal-related kinase (ERK) and phosphatidylinositol-3-kinase (PI₃K), after estrogen binding (Filardo et al., 2000; Revankar et al., 2005; Thomas et al., 2005). After establishing GPR30 as a *bona fide* estrogen-binding receptor, it was renamed GPER by the International Union of Basic and Clinical Pharmacology (Alexander et al., 2008). Similar to ER α and ER β (Meyer et al., 2006), GPER is expressed throughout the cardiovascular system in humans and animals of both sexes (Bopassa et al., 2010; Broughton et al., 2010; Deschamps and Murphy, 2009; Ding et al., 2009; Filice et al., 2009; Haas et al., 2007; Haas et al., 2009; Isensee et al., 2009; Lindsey et al., 2009; Ma et al., 2010; Takada et al., 1997). This suggests that in addition to ER α and ER β , GPER also plays a physiological role in regulation of vascular and myocardial function. With the identification of three different receptors capable of mediating vascular estrogen signaling, the understanding of the cardiovascular effects of estrogen has become increasingly complex. This review addresses the individual role of the different ERs in cardiovascular function with a particular focus on its newest member, GPER.

2. Ligands of Vascular Estrogen Receptors

Although a variety of natural and synthetic estrogens are able to induce effects on the cardiovascular system, it is important to separate their ability to regulate ER α , ER β , and GPER activity. 17 β -Estradiol, the major human estrogen lost after menopause, is a combined activator of ER α , ER β , and GPER, and mediates numerous beneficial vascular effects. Although 17 β -estradiol is primarily synthesized by the ovaries, the enzyme aromatase converts androgens into 17 β -estradiol and estrone at many sites throughout the body including the vascular wall, where they may have specialized local effects (Harada et al., 1999). Estrone and other endogenous estrogen-based steroids display a low binding affinity for ER α , ER β , and GPER at physiological concentrations, and their physiological actions are less clear (Kuiper et al., 1998; Revankar et al., 2005; Thomas et al., 2005). On the other hand, humans are exposed to a variety of natural and man-made estrogenic compounds at low levels (Jacobs and Lewis, 2002). Natural environmental estrogens are

synthesized by plants (phytoestrogens) and include coumestans and isoflavones, whereas synthetic estrogenic compounds (xenoestrogens, known as endocrine disruptors) comprise chemical detergents, pesticides, and plastic monomers. In fact, the isoflavone genistein is able to induce vasodilation (Martin et al., 2008), and has now been shown to not only activate ER α and ER β , but also GPER (Thomas and Dong, 2006). Some xenoestrogens such as nonylphenol and DDT have also been implicated in regulation of vascular function (Hsieh et al., 2009; Ruehlmann et al., 1998), and these compounds weakly bind ER α /ER β , and are also agonists of GPER (Thomas and Dong, 2006). Taken together, numerous endogenous and exogenous estrogenic compounds implicated in cardiovascular physiology are now known to activate all three known estrogen receptors.

Research to improve successful treatment of estrogen-sensitive tumors, breast cancer in particular, led to the development of drugs that oppose the action of estrogen. Referred to as selective estrogen receptor downregulators (SERDs), compounds such as ICI 182,780 (fulvestrant) abolish ER α /ER β signaling in all tissues (Shanle and Xu, 2010). However, a group of compounds known as selective estrogen receptor modulators (SERMs) do not uniformly act as estrogen antagonists, but are generally regarded as ER agonists in the cardiovascular system, bone, and liver, and as ER antagonists in breast tissue (Katzenellenbogen and Katzenellenbogen, 2002). Importantly, the SERM tamoxifen as well as the SERD ICI 182,780 do not solely interact with ER α and ER β ; they also display significant binding to GPER (Thomas et al., 2005) and induce GPER-dependent signaling in breast cancer cell lines (Filardo et al., 2000; Revankar et al., 2005). Their role as GPER agonists has been confirmed in several other cell types and tissues (Prossnitz and Barton, 2009), indicating that this receptor mediates estrogenic effects even with concomitant blockade of ER α and ER β . This also indicates that older studies investigating cardiovascular effects of SERMs and SERDs may in fact point to GPER-dependent effects.

As GPER is known to bind many of the same ligands as ER α and ER β (Figure 2), selective agonists/antagonists were needed to unravel the functional roles of the individual receptors, particularly GPER. This led to the identification of the first GPER-specific agonist G-1 (Bologa et al., 2006) and the first GPER-selective antagonist G15 (Dennis et al., 2009). Both compounds share a tetrahydro-3H-cyclopenta-[c]quinoline scaffold domain and show an extremely high selectivity for GPER over ER α and ER β . In addition, agonists for ER α (PPT) and ER β (DPN) (Harrington et al., 2003) have been used in the past years. The availability of these compounds has provided the opportunity to better characterize the role of individual ERs, and GPER in particular, in cardiovascular physiology.

3. Mechanisms of Estrogen-Dependent Vasodilation

17 β -Estradiol is a powerful vasodilator of human blood vessels (Mügge et al., 1993; Silva de Sa, Meirelles, 1977), and acute effects on vasomotor tone have been observed in arteries of both women and men (Haas et al., 2007; Mügge et al., 1993). Shortly after the seminal observation that endothelial cells release a relaxing factor in response to acetylcholine (Furchgott and Zawadzki, 1980) and its subsequent identification as nitric oxide (NO) (Furchgott, 1988; Ignarro et al., 1987), enhanced endothelium-dependent responses to acetylcholine were reported in estrogen-treated ovariectomized animals (Gisclard et al., 1988). Similarly, flow-mediated vasodilation and NO production correlate with high estrogen levels during the menstrual cycle and increase after estrogen therapy in postmenopausal women (Miller and Duckles, 2008). These findings support the notion that estrogen acutely and chronically enhances endothelium-/NO-dependent relaxation of human arteries, including human coronary arteries (Barton et al., 1998; Miller, Duckles, 2008). Estrogen-stimulated endothelium-dependent NO release is the result of rapid PI₃K/Akt activation and subsequent phosphorylation of endothelial (type III) NO synthase (eNOS)

(Haynes et al., 2000; Simoncini et al., 2000), although other mechanisms have also been proposed (Chow et al., 2010). This effect is believed to be mediated by subpopulations of ER α and possibly ER β that localize to vesicular invaginations of the plasma membrane termed caveolae (Figure 1) (Chambliss et al., 2000; Chambliss et al., 2002; Simoncini et al., 2000). However, endothelium-dependent relaxation in response to estrogens does not involve NO exclusively, but is also mediated by endothelium-derived hyperpolarizing factors (EDHFs), including epoxyeicosatrienoic acids (EETs), H₂O₂, potassium, and endothelial gap junction communications (Huang et al., 2004; Luksha et al., 2006; Traupe et al., 2007). In addition, estrogens can act directly on vascular smooth muscle cells to regulate vascular function (White, 2002). These endothelium-independent effects include estrogen-dependent regulation of calcium and potassium fluxes (White, 2002), mainly through the activity of large-conductance calcium- and voltage-activated potassium (BK_{Ca}) channels (Figure 1) (Han et al., 2007; White et al., 1995). Indeed, 17 β -estradiol-dependent vasodilator responses in human coronary arteries remain effective after removal of endothelial cells (Mügge et al., 1993). Moreover, estrogen also indirectly reduces vasomotor tone by inhibiting the activity of vasoconstrictors, such as prostanoids, endothelin-1, and angiotensin II (Barton and Meyer, 2009; Mügge et al., 1997).

In addition to 17 β -estradiol, an agonist of ER α , ER β , and GPER, there has been great interest in defining the role of SERMs in the regulation of vascular tone. Tamoxifen and raloxifene, which are agonists of GPER (Prossnitz and Barton, 2009), activate BK_{Ca} channels and evoke acute endothelium-independent vasodilation (Darkow et al., 1997; Hutchison et al., 2001; Leung et al., 2007b). They also induce NO-mediated endothelium-dependent relaxation and increase eNOS phosphorylation in porcine coronary arteries and other vascular beds (Chan et al., 2010; Figtree et al., 1999; Leung et al., 2006; Leung et al., 2007a). Moreover, raloxifene activates eNOS via the PI₃K/Akt-pathway in endothelial cells (Simoncini et al., 2002). Recently, the SERM estrogen-dendrimer conjugate was found to activate eNOS and stimulate growth of endothelial cells (Chambliss et al., 2010). On the other hand, the endogenous SERM and cholesterol metabolite 27-hydroxycholesterol decreases vascular NOS expression and activity (Umetani et al., 2007). Thus, both 17 β -estradiol and several synthetically generated estrogen-like substances affect vascular function in distinct ways, although it is often unclear which ER mediates these responses. Defining the role of individual ERs in response to activation by SERMs may help to identify and selectively target ERs that mediate the beneficial vascular effects of estrogens.

4. Role of ER α and ER β for Vascular Function

Estrogens acutely reduce vascular tone in various arteries of different species, yet the molecular mechanisms and the role of individual estrogen receptors in this response are less clear. Long-term 17 β -estradiol treatment increases basal NO production in thoracic aorta of ER β -, but not of ER α -knockout mice, indicating that ER α mediates NO-dependent vasodilation (Darblade et al., 2002). On the other hand, ER β -knockout mice develop elevated blood pressure as they age, also suggesting a role for ER β (Zhu et al., 2002). Indeed, disruption of ER β abrogates the effect of estrogen to attenuate vasoconstriction (Zhu et al., 2002). Conversely, 17 β -estradiol lacks vasodilatory effects in perfused carotid or femoral arteries of either ER α - or ER β -knockout mice (Guo et al., 2005). In addition, 17 β -estradiol stimulates neither ERK nor PI₃K activity in carotid arteries of ER α - or ER β -deficient animals (Guo et al., 2005). This is not only compatible with the notion that both ER α and ER β are required for estrogen-dependent vasodilation, but also that other mechanisms independent of ER α /ER β may be involved in these responses. Moreover, it is likely that a functional crosstalk between ERs defines the vasodilator effect of estrogens (Matthews and Gustafsson, 2003). In porcine coronary arteries, selective activation of ER α by PPT evokes a rapid, endothelium- and NO-dependent relaxant response within minutes,

followed by a more sustained NO-independent relaxing effect (Traupe et al., 2007). In contrast, non-selective ER activation by 17 β -estradiol and selective activation of ER β by DPN causes a sustained relaxation only (Traupe et al., 2007). This points to an inhibitory role of ER β on ER α -dependent, NO-mediated acute vasodilation. A similar effect has also been suggested in murine femoral arteries, where 17 β -estradiol and PPT induce NO-dependent vasodilation only in animals lacking ER β , but not in wild-type mice (Cruz et al., 2006). Of note, PPT- and DPN-dependent vasodilator effects differ between distinct vascular beds and species (Al Zubair et al., 2005; Bolego et al., 2005). Taken together, functional interactions of both ER α and ER β are likely to regulate vasomotor tone depending on anatomical localization of vascular beds in different species.

Conversely, a number of investigators found that the ER α /ER β -antagonist (but GPER-agonist (Filardo et al., 2000; Thomas et al., 2005)) ICI 182,780 does not block estrogen-dependent vasodilator effects in several vascular beds of different species (Bracamonte et al., 2002; Scott et al., 2007; Shaw et al., 2000; Sudhir et al., 1995; Teoh et al., 1999). Moreover, we recently demonstrated that ICI 182,780 alone causes a rapid dilation in epicardial porcine coronary arteries, which represent a good model of the human coronary vasculature with regard to size and mediators of vascular function (Figure 2B) (Meyer et al., 2010). These findings suggest that this vasodilator response does not depend on the activity of ER α and ER β , but that these responses are mediated by GPER instead (Meyer et al., 2010). In line with these findings, ICI 182,780 causes a rapid, NO-dependent dilation of pressurized carotid (but not femoral) arteries of ovariectomized mice (Guo et al., 2005), and acts as a vasodilator in rat superior mesenteric arteries (Keung et al., 2011). Interestingly, vasodilator effects in response to ICI 182,780 are absent in carotid arteries of mice lacking ER α or ER β (Guo et al., 2005), which would be compatible with crosstalk between GPER, ER α and ER β recently suggested (Albanito et al., 2007; Gao et al., 2011). From a clinical point of view, vasodilator effects of ICI 182,780 (fulvestrant, Faslodex®) via GPER may provide the first mechanistic explanation for symptomatic hypotension, a common side effect of this agent when used as endocrine treatment for advanced breast cancer (Vergote and Abram, 2006).

5. GPER: A Novel Regulator of Vascular Tone and Blood Pressure

GPER is an intracellular transmembrane G protein-coupled receptor that mediates rapid estrogen signaling (Filardo et al., 2000; Revankar et al., 2005; Thomas et al., 2005). The potential relevance of GPER in cardiovascular physiology was perhaps first suggested by one of the approaches used to clone cDNA encoding GPER in human endothelial cells exposed to fluid shear stress (Takada et al., 1997). To better define the role of GPER in estrogen-dependent vasodilation, the selective GPER-agonist G-1 (Bologa et al., 2006) as well as genetically modified animals have been used. G-1 acutely dilates human internal mammary and porcine coronary arteries, rat aorta and mesenteric arteries, as well as rat and murine carotid arteries from males and females, with the effect being less potent in larger conduit arteries (Figure 3) (Broughton et al., 2010; Haas et al., 2009; Lindsey et al., 2009; Lindsey et al., 2011; Meyer et al., 2010). In human internal mammary and murine carotid arteries, the G-1-dependent effect was more pronounced than that of 17 β -estradiol, again suggesting that estrogen-dependent vasodilation involves complex functional crosstalk between GPER, ER α , and ER β (Figure 3A) (Haas et al., 2009). G-1 also inhibits endothelin- (Meyer et al., 2010), angiotensin II- (Lindsey et al., 2009), and serotonin-dependent (Haas et al., 2009) contractions in certain vascular beds. Interestingly, G-1 regulates calcium flux in vascular smooth muscle cells, an effect that is inhibited by GPER siRNA treatment (Haas et al., 2009). In fact, GPER activation by G-1 abrogates calcium flux induced by the vasoconstrictor serotonin indicating calcium-antagonistic or desensitizing effects, with

intracellular application being much more rapid than extracellular application, indicative of the intracellular function of GPER (Haas et al., 2009).

Acute vasodilation in response to G-1 is abolished in animals lacking the GPER gene (Haas et al., 2009) or after pretreatment with the GPER-selective antagonist G15 (Lindsey et al., 2011), providing further evidence for a role of GPER for control of vasomotor tone (Figure 3B). In GPER-deficient mice, development of hypertension in females at 9 months of age has been claimed; however, this assumption was based on blood pressure differences in wild-type mice, and mean blood pressure values were essentially normotensive in all groups investigated (Martensson et al., 2009). On the other hand, intravenous injection of G-1 in normotensive rats acutely reduces mean arterial blood pressure (Figure 3D) (Haas et al., 2009). Moreover, treatment with G-1 for 2 weeks lowers blood pressure only in ovariectomized but not in ovary-intact female or in male mRen2.Lewis rats, an estrogen-sensitive model of hypertension (Lindsey et al., 2009). In these animals, angiotensin II type 1 receptor and angiotensin-converting enzyme gene expression was reduced by G-1 treatment, suggesting that GPER activation lowers blood pressure in part by attenuating vascular angiotensin II signaling (Lindsey et al., 2009). In line with G-1-dependent effects on the renin-angiotensin-aldosterone system is a recent report suggesting that vascular signaling of aldosterone may involve GPER-dependent pathways (Gros et al., 2011). Taken together, these studies clearly indicate a role for GPER activation mediating acute and chronic vasodilation in response to estrogen (Table 1).

6. Mechanisms of GPER-Dependent Vasodilation

While several investigators have reported vasodilator effects of G-1, the mechanisms involved are still scarcely understood. In rat aorta, carotid and mesenteric as well as in porcine coronary arteries, G-1-mediated relaxation depends at least partly on the presence of an intact endothelium and is prevented by the NOS inhibitor L-NAME (Broughton et al., 2010; Lindsey et al., 2011; Meyer et al., 2010). This suggests that release of endothelium-derived NO is involved in GPER-dependent vasodilation in certain vascular beds. However, the exact functional and molecular interactions of GPER with the NO pathway, although suggested in previous studies (Broughton et al., 2010; Filice et al., 2009; Lindsey et al., 2011; Meyer et al., 2010), still need to be substantiated. Of note, although G-1-dependent vasodilation is endothelium-dependent, G-1 treatment of ovariectomized mRen2.Lewis rats alters neither aortic eNOS gene expression nor endothelium- and NO-dependent relaxation to acetylcholine (Lindsey et al., 2009), both of which unexpectedly remain intact despite severe hypertension and ovariectomy in this model. On the other hand, preliminary evidence indicates that GPER activation by G-1 and ICI 182,780 also evokes endothelium-independent coronary vasodilation by regulating potassium efflux via BK_{Ca} channels, possibly mediated by ERK and PI_3K/Akt signaling pathways (Han et al., 2009; Han et al., 2010).

Although there is now evidence that endothelium-derived NO is an important mediator of GPER-dependent vasodilation (Broughton et al., 2010; Lindsey et al., 2011; Meyer et al., 2010), it is unknown whether GPER activation also induces estrogen-dependent vasodilator responses through release of EDHFs, which has previously been shown for $ER\beta$ (Luksha et al., 2006; Traupe et al., 2007). EDHFs are important mediators of endothelium-dependent, NO-independent vasodilation in small resistance arteries, and estrogen therapy as well as a phytoestrogen-rich soy diet improve the EDHF response in mesenteric and uterine arteries of rodents (Burger et al., 2009; Knock et al., 2006). Based on the fact that G-1 dilates resistance arteries (Haas et al., 2009; Lindsey et al., 2011), it is plausible that GPER activation improves EDHF-dependent vasodilation in these small vessels, which may

contribute to the observed blood pressure-lowering effect of G-1 (Haas et al., 2009; Lindsey et al., 2009).

GPER may also reduce vascular tone by interfering with constrictor reactive oxygen species (ROS), since G-1 has antioxidative properties inhibiting ROS production (Broughton et al., 2010). Importantly, an excess amount of ROS impairs endothelial function partly by reducing NO bioavailability (Ushio-Fukai, 2009). Moreover, ROS function as second messengers regulating signaling of G protein-coupled receptors such as the angiotensin II type 1 receptor, resulting in enhanced vasoconstriction (Ushio-Fukai, 2009). This may represent an additional mechanism whereby GPER beneficially affect vascular tone, particularly in the presence of impaired endothelial function due to cardiovascular diseases, such as hypertension or atherosclerosis.

In summary, current evidence suggests that ER α , ER β , and GPER all contribute to estrogen-induced vasodilation, with a role for GPER best described in endothelium-/NO-dependent vasodilation. Selective GPER activation is also likely to induce NO-independent vasodilation and to reduce vascular tone via indirect effects on vasoconstrictors that are yet not fully understood. These responses depend on anatomical localization of vascular beds in different species and the time-course of estrogen administration. In line with its beneficial vasodilator effects, GPER also regulates cell growth and apoptosis of vascular smooth muscle cells (Ding et al., 2009; Haas et al., 2009), pointing towards a possible atheroprotective role of this receptor.

7. Protective Effects of GPER on Myocardial Function

In view of the estrogen-dependent vasodilator effects and low incidence of coronary artery disease in premenopausal women (Meyer et al., 2006), a role for ER in protection from ischemic heart disease has been suggested (Deschamps et al., 2010). Endogenous estrogens convey protection in models of acute myocardial infarction and also have antiarrhythmic activity mimicking ischemic preconditioning, although the exact mechanisms involved are still unclear (Das and Sarkar, 2006; Deschamps et al., 2010). The ability of estrogen to stimulate NO generation has been of particular interest given that NO-dependent vasodilation may increase myocardial perfusion and thus contractility, alongside several other NO-mediated cardioprotective effects (Jones and Bolli, 2006). Of note, NO-mediated cholinergic vasodilation induced by coronary acetylcholine infusion is absent in patients with advanced coronary atherosclerosis, where infusion results in paradoxical vasoconstriction (Ludmer et al., 1986). In line with beneficial effects of estrogen on myocardial function, acute treatment with the non-selective ER agonist 17 β -estradiol before temporary coronary artery occlusion reduces infarct size in female and male animals (Delyani et al., 1996; Hale et al., 1996; Hale et al., 1997). In addition, selective activation of ER β provides cardioprotection that requires the availability of NO (Lin et al., 2009). Acute and chronic treatment of dogs or rodents with the SERM raloxifene also improves ischemia/reperfusion-related myocardial injury, and again partly involves NO-dependent mechanisms mediated by the PI $_3$ K/Akt-pathway (Chung et al., 2010; Nemcsik et al., 2004; Ogita et al., 2002; Ogita et al., 2004). A number of experimental studies on ischemia/reperfusion injury subsequently found beneficial effects mediated by both ER α and ER β , yet some controversies exist whether both receptors provide acute and chronic cardioprotection (Deschamps et al., 2010). Interestingly, functional crosstalk between ER α and ER β determining the estrogen-dependent cardioprotective effects has also been suggested (Babiker et al., 2007).

Although these studies clearly demonstrate a role for both ER α and ER β in cardiac pathophysiology, it is unclear whether effects are mediated by nuclear or membrane

populations of these receptors. Also, several estrogen-dependent effects such as inhibition of cardiomyocyte cell growth and contraction involve mechanisms independent of ER α and ER β (Mercier et al., 2003; Ullrich et al., 2008). More recently, impaired left-ventricular contractility and relaxation capacity consistent with left-ventricular dysfunction were noted in male, but not in female GPER-deficient mice (Delbeck et al., 2011). Moreover, G-1 ameliorates diastolic dysfunction and reduces left-ventricular hypertrophy in a model of salt-induced hypertensive cardiomyopathy independent of blood pressure (Jessup et al., 2010). In addition, involvement of estrogen signaling via GPER in ischemic heart disease has been suggested based on the observation of increased GPER expression mediated by hypoxia-inducible factor HIF-1 α in a murine cardiomyocyte-like cell line in response to mimicking hypoxia (Recchia et al., 2011). These studies clearly indicate a role for GPER in preservation of cardiac structure and function.

Using a model of cardiac ischemia/reperfusion injury, several groups reported additional GPER-dependent cardioprotective effects (Table 1). In rat hearts, G-1 treatment reduces infarct size and postischemic contractile dysfunction independent of sex (Figure 4) (Deschamps and Murphy, 2009; Filice et al., 2009; Patel et al., 2010; Weil et al., 2010), possibly involving a PI $_3$ K kinase/Akt-dependent mechanism (Deschamps and Murphy, 2009). Similarly, the same treatment reduces infarct size and improves functional recovery in male mice by inhibiting the mitochondria permeability transition pore opening (Bopassa et al., 2010). In these animals, blocking of ERK signaling prevents the G-1-induced improvement in heart function and infarct size (Bopassa et al., 2010). In rats, ERK activation by G-1 also mediates negative inotropic effects and induces the phosphorylation of eNOS (Filice et al., 2009), while no involvement of ERK signaling on acute cardioprotection was observed in a second report (Deschamps and Murphy, 2009). Interestingly, improved heart function in response to G-1 has been linked to a reduced production of proinflammatory cytokines including TNF- α , IL-1 β , and IL-6 (Weil et al., 2010).

Taken together and consistent with its dilator effects, GPER activation improves functional recovery and infarct size after myocardial ischemia although the precise mechanisms mediating this protection remain to be characterized. Moreover, functional crosstalk between ER α , ER β , and GPER may also determine the cardiac effects of estrogen as suggested by experimental studies using ICI 182,780 (Filice et al., 2009). In line with its beneficial effects on the heart, GPER may also be protective in scenarios involving acute hypoxic injury of other organs, such as ischemic stroke (Zhang et al., 2010) and liver injury (Hsieh et al., 2007).

8. Clinical Implications and Need for Further Research

Recent research has shown that GPER mediates numerous beneficial effects of estrogens on vascular and myocardial function. With the presence of three different cellular estrogen targets, predicting the cardiovascular response to estrogen has become increasingly complex due to nuclear and extranuclear localization and functional crosstalk between ER α , ER β , and GPER, resulting in activation of multiple genomic and non-genomic signaling pathways (Figure 1) (Meyer et al., 2009). Moreover, the identification of SERMs such as tamoxifen and raloxifene and SERDs such as ICI 182,780 as GPER agonists (Abdelhamid et al., 2011; Filardo et al., 2000; Revankar et al., 2005; Thomas et al., 2005) suggests that many previous studies using these compounds as “pure” agonists/antagonists of ER α and ER β must be reconciled. Evidence for the role of GPER in the cardiovascular system is mainly based on studies using G-1 as a selective agonist. With the availability of GPER-knockout animals (Prossnitz and Barton, 2009) and the GPER antagonist G15 (Dennis et al., 2009), new experimental tools are available for more detailed mechanistic studies to better characterize

the individual roles of GPER, ER α , and ER β in the cardiovascular system (Meyer and Barton, 2009).

The complexity of estrogen signaling might in part also explain why clinical trials using conjugated equine estrogens and medroxyprogesterone acetate for postmenopausal hormone therapy failed to prove a therapeutic benefit on cardiovascular outcomes (Hulley et al., 1998; Rossouw et al., 2002). In addition, there were significant unfavorable effects mainly related to the development of breast cancer and venous thromboembolic events (Hulley et al., 1998; Rossouw et al., 2002). The scientific question therefore remains how to pharmacologically separate the beneficial vascular signaling pathways of estrogens from their harmful actions, which can possibly be achieved by tissue-specific, selective ER targeting devoid of the side effects seen with other estrogens. Interestingly, the SERMs raloxifene and lasofoxifene have recently been reported to reduce the risk of cardiovascular events and breast cancer at least in younger postmenopausal women (Barrett-Connor et al., 2006; Collins et al., 2009; Cummings et al., 2010; Ensrud et al., 2010). On the other hand, raloxifene does not affect blood pressure of normo- and hypertensive postmenopausal women (Cagnacci et al., 2003; Collins et al., 2009; Morgante et al., 2006; Sumino et al., 2010). Compared to other SERMs currently available for postmenopausal women, lasofoxifene appears to have the most favorable cardiovascular benefit-to-risk profile, although it was primarily found to be effective when used for the treatment of osteoporosis and breast cancer (Ensrud et al., 2010). Therefore, randomized clinical trials using lasofoxifene in postmenopausal women who are at increased cardiovascular risk are warranted. This also indicates that the cardiovascular effects of distinct SERMs are likely to differ. Thus, future basic research should further address the question how ER α , ER β and GPER are involved in the beneficial cardiovascular effects observed after treatment with different SERMs. In addition, designing synthetic ligands that selectively mimic the beneficial vascular effects of 17 β -estradiol but lack its deleterious effects at pharmacological doses represents an exciting area of research (Chow et al., 2010). Based on the current evidence, G-1 might be a candidate for such a compound. In fact, with the accumulating evidence that GPER mediates a variety of beneficial cardiovascular effects, this receptor may represent a novel target to develop effective strategies to treat cardiovascular diseases based on the clear-cut evidence of beneficial effects of premenopausal estrogens on the cardiovascular system.

Abbreviations

BK_{Ca} channels	Large-conductance calcium- and voltage-activated potassium channels
EDHF	Endothelium-derived hyperpolarizing factors
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
ERK	Extracellular signal-related kinase
GPER	G protein-coupled estrogen receptor
NO	Nitric oxide
PI₃K	Phosphatidylinositol-3-kinase
ROS	Reactive oxygen species
SERD	Selective estrogen receptor downregulator
SERM	Selective estrogen receptor modulator

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Figure 1.

Proposed estrogen receptor (ER)-activated signaling pathways involved in vasodilation. 17β-Estradiol (E₂) can activate a subpopulation of ER at the plasma membrane (mER) that interacts with adaptor proteins (adaptor) and signaling molecules such as c-Src (1), which is critical for down-stream ER-induced signaling via PI₃K/Akt and MAPK pathways. E₂ also binds to the G protein-coupled estrogen receptor GPER, which is primarily localized to the endoplasmic reticulum (2). GPER activates downstream effectors, such as adenylate cyclase (resulting in cAMP production), and c-Src. c-Src, in turn, activates matrix metalloproteinases (MMP), which cleave pro-heparin-bound-epidermal growth factor receptors (EGFR). EGFR leads to multiple downstream events, including activation of MAPK and PI₃K. Once activated, PI₃K can induce NO generation by NO synthase (NOS) in vascular endothelial (eNOS) or smooth muscle cells (nNOS). Membrane permeant NO, in turn, activates guanylate cyclase (resulting in cGMP production). The NO/cGMP pathway is involved in acute vasodilator mechanisms, such as protein kinase G (PKG)-dependent regulation of myosin light chain phosphorylation, and activation of large-conductance calcium-activated potassium (BK_{Ca}) channels. E₂ also regulates cellular gene expression either via binding of ER dimers in the promotor region of target genes (3), through interaction of ER with other classes of transcription factors (TF) (4), or by regulation of TF phosphorylation (5). Target genes regulating vascular homeostasis include NOS and inflammatory cytokines. Adapted from Mol Cell Endocrinol, Vol. 308, Meyer, M.R., Haas,

E., Prossnitz, E.R., Barton M, Non-genomic regulation of vascular cell function and growth by estrogen, Pages 9-16, ©2009, with permission from Elsevier.

Figure 2.

Agonists and antagonists of GPER and plasma membrane-associated subpopulations of ERα and ERβ involved in rapid vascular estrogen signaling. Green arrows indicate activation, red arrows indicate inhibition, and the orange arrow indicates tissue-dependent activation or inhibition. SERM, selective estrogen receptor modulator; SERD, selective estrogen receptor downregulator.

Figure 3.

GPER-dependent regulation of vascular tone. In precontracted carotid arteries from mice, the selective GPER-agonist G-1 (3 μ M) causes acute dilation, which is even stronger than that of 17 β -estradiol (E2, 3 μ M, A). Dilatory effects of G-1 (3 μ M) in carotid arteries of wild-type mice (GPER +/+) are absent in GPER-knockout animals (GPER -/-, B). Acute exposure to G-1 (3 μ M) and the GPER agonist and ER α /ER β antagonist ICI 182,780 (ICI, 3 μ M) for 60 minutes evokes similar vasodilator responses of porcine epicardial coronary arteries (C). Intravenous injection of G-1 at increasing concentrations (4.12 ng/kg, 41.2 ng/kg, 412 ng/kg, and 20.6 μ g/kg) acutely reduces mean arterial blood pressure (MAP) in normotensive rats. For comparison, the response to acetylcholine (ACh, 30 ng/kg) is shown

(D). CTL, solvent control (ethanol 0.3%). Panels A, B, and D are reproduced from Haas, E., Bhattacharya, I., Brailoiu, E., Damjanovic, M., Brailoiu, G.C., Gao, X., Mueller-Guerre, L., Marjon, N.A., Gut, A., Minotti, R., Meyer, M.R., Amann, K., Ammann, E., Perez-Dominguez, A., Genoni, M., Clegg, D.J., Dun, N.J., Resta, T.C., Prossnitz, E.R., Barton, M., Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity, *Circ Res*, 104(3), 288-291, ©2009 by the American Heart Association, with permission of the publisher. Panel C is adapted from Meyer, M.R., Baretella, O., Prossnitz, E.R., Barton, M., Dilation of epicardial coronary arteries by the G protein-coupled estrogen receptor agonists G-1 and ICI 182,780, *Pharmacology*, 86(1), 58-64, ©2010, with permission from S. Karger AG, Basel, Switzerland.

Figure 4. Sex-independent cardioprotective effects of GPER activation. Selective GPER activation by G-1 (110 nM, black bars) before global cardiac ischemia does not significantly alter heart rate (HR, A) and myocardial contractility (measured as left ventricular developed pressure, B) after 120 minutes of reperfusion in male and female rats compared to untreated controls (open bars). In contrast, G-1 treatment reduces postischemic contractive dysfunction (measured as rate pressure product, RPP, C) and infarct size (measured as percentage of the entire ventricular area, D). * $P < 0.05$ vs. same gender control. Adapted from Deschamps, A.M., Murphy, E, Am J Physiol Heart Circ Physiol, ©2009 by the American Physiological Society, used with permission.

Table 1

GPER-Mediated Effects on Cardiovascular Function.

Function	Organ/Tissue	Species	Reference
Vasodilation	Aorta	Mouse	(Haas et al., 2009)
		Rat	(Lindsey et al., 2009; Lindsey et al., 2011)
	Carotid Artery	Mouse	(Haas et al., 2009)
		Rat	(Broughton et al., 2010)
	Coronary Artery	Pig	(Meyer et al., 2010)
	Mesenteric Artery	Rat	(Haas et al., 2009; Lindsey et al., 2011)
	Internal Mammary Artery	Human	(Haas et al., 2009)
Inhibition of Vasoconstriction	Aorta	Mouse	(Haas et al., 2009)
		Rat (OVX)	(Lindsey et al., 2009)
	Carotid Artery	Mouse	(Haas et al., 2009)
	Coronary Artery	Pig	(Meyer et al., 2010)
Blood Pressure			
Acute Reduction		Rat	(Haas et al., 2009)
Chronic Reduction		Rat (OVX)	(Lindsey et al., 2009)
No Chronic Effect		Rat (Female)	(Lindsey et al., 2009) (Jessup et al., 2010)
Anti-Ischemic Effects	Heart	Mouse	(Bopassa et al., 2010)
		Rat	(Deschamps, Murphy, 2009; Filice et al., 2009; Patel et al., 2010; Weil et al., 2010)
	Cardiomyocytes	Mouse	(Recchia et al., 2011)
Anti-Inflammatory Effects	Heart	Rat	(Weil et al., 2010)
Preservation of LV Structure/Function	Heart	Mouse (Male)	(Delbeck et al., 2011)
		Rat	(Jessup et al., 2010)

LV, left-ventricular; OVX, ovariectomized.