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REVIEW

G protein-coupled oestrogen receptor 1 (GPER1)/GPR30: a new player in cardiovascular and metabolic oestrogenic signalling

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Oestrogens are important sex hormones central to health and disease in both genders that have protective effects on the cardiovascular and metabolic systems. These hormones act in complex ways via both genomic and non-genomic mechanisms. The genomic mechanisms are relatively well characterized, whereas the non-genomic ones are only beginning to be explored. Two oestrogen receptors (ER), ERα and ERβ, have been described that act as nuclear transcription factors but can also associate with the plasma membrane and influence cytosolic signalling. ERα has been shown to mediate both anti-atherogenic effects and pro-survival effects in pancreatic β-cells. In recent years, a third membrane-bound ER has emerged, G protein-coupled receptor 30 or G protein-coupled oestrogen receptor 1 (GPER1), which mediates oestrogenic responses in cardiovascular and metabolic regulation. Both GPER1 knock-out models and pharmacological agents are now available to study GPER1 function. These tools have revealed that GPER1 activation may have several beneficial effects in the cardiovascular system including vasorelaxation, inhibition of smooth muscle cell proliferation, and protection of the myocardium against ischaemia/reperfusion injury, and in the metabolic system including stimulation of insulin release and protection against pancreatic β-cell apoptosis. Thus, GPER1 is emerging as a candidate therapeutic target in both cardiovascular and metabolic disease.

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Abbreviations

AC, adenylate cyclase; E2, 17β-estradiol; EGF, epidermal growth factor; EGFR, EGF receptor; eNOS, endothelial nitric oxide synthase; ER, oestrogen receptor; ERK, extracellular-regulated protein kinase; GPCR, G protein-coupled receptor; GPER1, G protein-coupled oestrogen receptor 1; GPR30, G protein-coupled receptor 30; GSIS, glucose-stimulated insulin secretion; IGT, impaired glucose tolerance; kDa, kilodaltons; KO, knock-out; MAP, mean arterial blood pressure; MMP, matrix metalloproteinase; NO, nitric oxide; PI3K, phosphoinositide-3-kinase; PKA, protein kinase A; PKB, protein kinase B; PTX, pertussis toxin

Oestrogen effects on cardiovascular and metabolic regulation

Oestrogens exert protective effects on cardiovascular and metabolic systems, which are observed as premenopausal women being less prone to cardiovascular and metabolic disease than age-matched men (Stampfer and Grodstein, 1994; Mendelsohn and Karas, 1999; Ford *et al.*, 2004). Several mechanisms have been suggested for the protective effect on the cardiovascular system including attenuation of vascular

inflammation (Nathan *et al.*, 1999; Nilsson, 2007), stimulation of nitric oxide (NO) formation (Chen *et al.*, 1999) and prevention of vascular smooth muscle cell proliferation (Pare *et al.*, 2002). Oestrogens also reverse the effect of menopause on glucose and insulin metabolism by improving insulin expression and release, improving insulin resistance (Stevenson *et al.*, 1994; Brussaard *et al.*, 1997; Godsland, 2005) and protecting pancreatic β-cells against oxidative damage (Le May *et al.*, 2006).

Nuclear oestrogen receptors (ER) ERα and ERβ

Oestrogens act on specific receptors through both genomic and non-genomic signalling mechanisms. Genomic oestrogen signalling is the most well-described mechanism and involves oestrogen binding to two nuclear ER named ERα and ERβ, which are transcription factors. Following oestrogen binding, ERα and ERβ dissociate from their inhibitory protein complexes to form homo- or hetero-dimers that subsequently interact with target gene promoters to cause either up- or down-regulation of target genes (Hall *et al.*, 2001; Nilsson *et al.*, 2001). Non-genomic oestrogenic signalling is less well understood but generally occurs considerably faster than genomic signalling and proceeds via cytosolic pathways involving classical second messengers. Membrane-associated full-length ERα and two truncated isoforms, ERα 46 kDa and ERα 36 kDa, exist that are at least in part responsible for such signalling (Chen *et al.*, 1999; Figtree *et al.*, 2003; Li *et al.*, 2003; Razandi *et al.*, 2004; Chambliss *et al.*, 2010; Kang *et al.*, 2010). Vascular smooth muscle cells and endothelial cells express ERα and ERβ as demonstrated at both the mRNA and protein levels (Lindner *et al.*, 1998; Mendelsohn and Karas, 1999; Andersson *et al.*, 2001). The ERα mediates a number of the vasculoprotective effects of oestrogen (Mendelsohn and Karas, 1999), including endothelial cell proliferation, vascular re-endothelialization, endothelial NO production, attenuation of vascular inflammation and reduction of vascular smooth muscle cell proliferation (Brouchet *et al.*, 2001; Pare *et al.*, 2002; Vegeto *et al.*, 2003). Furthermore, ERα is partially responsible for the anti-apoptotic effect of oestrogen on pancreatic β-cells (Le May *et al.*, 2006), and regulates skeletal muscle glucose transporters and hepatic insulin sensitivity (Barros *et al.*, 2006). On the other hand, much less information is available regarding the role of ERβ, but it is often presumed to have opposite actions to ERα.

G protein-coupled receptor 30/G protein-coupled oestrogen receptor 1

G protein-coupled receptor 30, now named G protein-coupled oestrogen receptor 1 (GPER1), is a seven transmembrane-domain G protein-coupled receptor (GPCR) that was recently found to bind 17β-estradiol (E2) with high affinity and mediate oestrogenic signals (Revankar *et al.*, 2005; Thomas *et al.*, 2005). Four mouse models lacking the GPER1 gene [GPER1 knock-out (KO)] were reported in the last 2–3 years and used to study the pathophysiological role of this receptor (Wang *et al.*, 2008; Isensee *et al.*, 2009; Mårtensson *et al.*, 2009; Otto *et al.*, 2009). In addition, a GPER1 agonist, G-1, is available with high affinity and high selectivity for GPER1 versus ERα and ERβ (Bologa *et al.*, 2006). This pharmacological agent is currently the most frequently used tool for investigating the role of GPER1 in various systems. In addition, Broughton *et al.* (2010) reported on the use of a GPER1 agonist named 5408-0877 that behaved similarly to G-1. Furthermore, a GPER1 antagonist, G-15, has been reported (Dennis *et al.*, 2009), but so far few studies have employed this agent. Nevertheless, G-1, 5408-0877 and G-15 remain to be fully evaluated for unequivocal specificity for this receptor. Thus, results using GPER1 KO mice and G-1 to study GPER1 physiology are presented separately in this review.

As a GPCR, GPER1 couples to G proteins and modulates second messenger pathways (Figure 1). Cyclic-AMP and Ca^{2+}

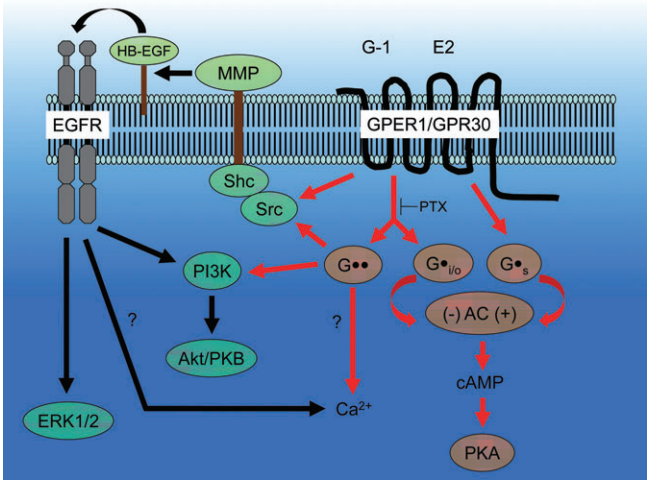

Figure 1
Schematic overview of proposed G protein-coupled oestrogen receptor 1 (GPER1) signalling. GPER1 is stimulated by 17β-estradiol (E2) and the synthetic agonist G-1. Primary signalling (red arrows) by GPER1 involves stimulation of $G\alpha_s$ and a pertussis toxin (PTX)-sensitive $G\alpha_{i/o}$ protein, through which adenylate cyclase (AC) is either positively (+) or negatively (–) regulated to yield an increase or decrease in the cAMP level and subsequent cAMP-dependent protein kinase (PKA) activity. Stimulation of $G\alpha_{i/o}$ also yields the liberation of Gβγ subunits that leads to: (i) activation of phosphoinositide-3 protein kinase (PI3K) and subsequent Akt/PKB protein kinase (Akt/PKB); (ii) activation of Src protein kinase (Src) and subsequent recruitment of the Src-Homology-Containing adaptor protein (SHC), activation of a matrix metalloproteinase (MMP), liberation of heparin-bound epidermal growth factor (HB-EGF) and transactivation of the EGF receptor (EGFR); and (iii) Gβγ-independent activation of the pathway described in (ii). Secondary signalling (black arrows) via transactivation of the EGFR leads to activation of extracellular-regulated protein kinase 1/2 ($ERK_{1/2}$) and PI3K. GPER1 stimulation also leads to elevation of intracellular Ca^{2+} through presently unknown mechanisms (?) that involves either primary signalling through Gβγ or secondary signalling through EGFR transactivation. For more details, see text.

have emerged as the messengers most frequently associated with GPER1 signalling, but several additional mediators have also been suggested, the specific ones in part dependent on the cell-type studied. The GPER1 is somewhat unusual in that it appears to couple to both a $G\alpha_s$ protein yielding stimulation of cAMP production and a pertussis toxin (PTX)-sensitive $G\alpha_{i/o}$ protein in part yielding attenuation of cAMP production. This behaviour was first described by Filardo *et al*. (2002, 2007), who showed that when MDA-MB-231 breast cancer cells, which lack GPER1 expression, were transfected to express GPER1, E2 stimulated both cAMP production and PTX-sensitive of extracellular-regulated protein kinase 1/2 ($ERK_{1/2}$) activation in the cells (Filardo *et al*., 2000). Since then, several investigators have further highlighted this yin-yang type of GPER1 signalling pattern. Indeed, Ding *et al*. (2009) reported that E2-promoted vascular smooth muscle cell apoptosis was dependent on GPER1-mediated protein kinase A (PKA) inhibition and phosphoinositide-3-kinase (PI3K) activation, both of which were found to be PTX sensitive. Furthermore, Maggiolini *et al*. (2004) reported GPER1-mediated and PTX-sensitive c-Fos up-regulation in SkBr3 breast cancer cells, a cell line in which Filardo *et al*. (2002) had previously shown GPER1-dependent stimulation of cAMP production. Although several examples exist of GPCR using different G proteins, coupling of the same receptor to both $G\alpha_s$ and $G\alpha_{i/o}$ is not common (Sidhu *et al*., 1991; Cruciani *et al*., 1993; Mamillapalli *et al*., 2008). On the other hand, dynamic feedback switching from $G\alpha_s$ to $G\alpha_i$ usage upon ligand binding has been described for several receptors including the β_1- and β_2-adrenergic receptors (Daaka *et al*., 1997; Martin *et al*., 2004), the prostacyclin receptor (Lawler *et al*., 2001) and the vasoactive intestinal polypeptide receptor (Luo *et al*., 1999). Whether GPER1 uses such a mechanism or not is still unknown.

The GPER1 also mediates agonist-stimulated increases in the cytosolic Ca^{2+} concentration, but the mechanism of this increase is still poorly understood. This signal has been described as phospholipase C-independent (Revankar *et al*., 2005), PTX-sensitive (Revankar *et al*., 2005; Terasawa *et al*., 2009), and dependent on epidermal growth factor (EGF) receptor activation (Revankar *et al*., 2005). Recently, Ariazi *et al*. (2010) reported that the GPER1-mediated Ca^{2+} response in ERα-positive MCF-7 breast cancer cells is sustained and dependent on the inositol trisphosphate receptor, while the Ca^{2+} response in ERα-negative SkBr3 cells is transient and dependent on the ryanodine receptor.

Stimulation of GPER1 also leads to PI3K and PTX-sensitive Src activation, which in turn yields phosphorylation of Akt and SHC respectively. While Akt phosphorylation has been linked to anti-apoptotic and cardioprotective effects (Weil *et al*., 2010), SHC phosphorylation triggers cleavage of membrane-tethered heparin-bound EGF, presumably through activation of a metalloproteinase, resulting in transactivation of the EGF receptor (Filardo *et al*., 2000). However, there is a debate on whether the PI3K/Akt pathway lies downstream of $G\alpha_{i/o}$ or not. While several groups have shown that the GPER1-coupled Src/SHC/EGF/$ERK_{1/2}$ pathway is PTX sensitive (Filardo *et al*., 2000; Ding *et al*., 2009), and thus dependant on $G\alpha_{i/o}$ signalling, Revankar *et al*. (2005) reported that PI3K/Akt activation is downstream of the Src/SHC/EGF/$ERK_{1/2}$ pathway but insensitive to PTX.

GPER1 expression in cardiovascular and metabolic systems

The GPER1 mRNA has been detected in different vascular segments including mouse mesenteric and carotid artery (Haas *et al*., 2009; Mårtensson *et al*., 2009). Furthermore, Lindsey *et al*. (2009) and Broughton *et al*. (2010) reported GPER1 immunoreactivity in both the intima and media of rat carotid and cerebral arteries and rat aorta, which suggests that GPER1 is expressed at the protein level in both endothelial and vascular smooth muscle cells. On the other hand, Isensee *et al*. (2009) used mice expressing a GPER1-lacZ reporter construct to show that vascular GPER1 expression is restricted to endothelial cells of small arteries in multiple peripheral tissues and to smooth muscle cells in the brain in this species. However, GPER1 transcript has been detected in rat brain microvascular bEnd.3 endothelial cells (Holm *et al*., 2011). Nevertheless, GPER1 seems to be expressed primarily in endothelial cells in small systemic arteries, which suggests that GPER1 regulates endothelial functions directly in such arteries, whereas effects on vascular smooth muscle contractility and vascular tonus are indirect via the endothelium. GPER1 expression was also reported in mouse, rat and human heart at both the mRNA (Mårtensson *et al*., 2009; Patel *et al*., 2010) and protein level (Patel *et al*., 2010). In all, these observations show that GPER1 is expressed in both blood vessels and the heart in several species.

In tissues involved in metabolic regulation, GPER1 mRNA was detected in human pancreas (Owman *et al*., 1996; Carmeci *et al*., 1997; Feng and Gregor, 1997; Takada *et al*., 1997), mouse pancreatic islets (Mårtensson *et al*., 2009), and human (Kvingedal and Smeland, 1996; Owman *et al*., 1996; Carmeci *et al*., 1997; Feng and Gregor, 1997; Takada *et al*., 1997) and mouse skeletal muscle (Mårtensson *et al*., 2009). Human liver displays strong GPER1 expression (Kvingedal and Smeland, 1996; Owman *et al*., 1996; Carmeci *et al*., 1997; Feng and Gregor, 1997; Takada *et al*., 1997; O'Dowd *et al*., 1998), whereas expression in mouse and rat liver is low to nonexistent (Bonini *et al*., 1997; Mårtensson *et al*., 2009; Otto *et al*., 2009), which suggest species variation in this regard. Haas *et al*. (2009) reported GPER1 expression in mouse adipose tissue, which is in contrast to three other studies that reported little or no GPER1 expression in such tissue (Hugo *et al*., 2008; Isensee *et al*., 2009; Mårtensson *et al*., 2009). A major weakness at present in the localization of GPER1 expression is the limited number of studies reporting receptor expression at the protein level, which is most probably due to the limited availability of reliable GPER1 antibodies for such studies.

Cardiovascular phenotype in GPER1 KO mice

Female GPER1 KO mice develop increased mean arterial blood pressure (MAP) at 9 months of age as compared with

age-matched wild-type mice (Mårtensson *et al.*, 2009). This effect was not observed in mice at 6 months of age indicating the developmental nature of this phenotype. Because neither the heart rate nor the relative heart weight was changed in these mice, the increase in MAP is most likely due to an increase in total vascular resistance rather than an increase in cardiac output. The increase in media-to-lumen ratio that was observed in the GPER1 KO mice may explain the increase in blood pressure by causing increased peripheral resistance. On the other hand, other mechanisms at the central nervous system or peripheral level, for example, increased renin-angiotensin system activity, may also explain these phenotypes. Haas *et al*. 2009 showed that the GPER1 agonist G-1 caused a slight reduction in rat MAP and reduced agonist-induced force in isolated rat and mouse arterial rings, the latter that was abolished in the GPER1 KO mouse strain developed by Wang *et al*. (2008), which provides evidence that GPER1 mediates this response. Together, these results suggest that GPER1 plays a role in cardiovascular regulation.

Effects of G-1 on vascular contractility

The GPER1 agonist G-1, at 1 and 10 μM, causes vascular relaxation by reducing agonist-induced contractions in several vascular preparations including rat aorta (Lindsey *et al*., 2009), rat mesenteric arteries (Haas *et al*., 2009), human internal mammary arteries (Haas *et al*., 2009), rat carotid arteries (Broughton *et al*., 2010) and porcine coronary arteries (Meyer *et al*., 2010). The G-1 response was observed in vessels from both ovariectomized and gonadal-intact animals (Lindsey *et al*., 2009) suggesting that it is not influenced by endogenous levels of oestrogens, which are in the range of 0.1–2 nM depending on the phase of the oestrous cycle (Cornil *et al*., 2006). Vascular relaxation by G-1 has been reported in both male and female rats (Broughton *et al*., 2010) indicating that the response is gender independent. A differential effect of G-1 was observed depending on the agonist used for vascular smooth muscle contraction. Indeed, G-1 relaxed arterial rings contracted by endothelin-1, angiotensin II, UTP and prostaglandin F2α, but it had no effect on serotonin-induced contractions (Haas *et al*., 2009; Lindsey *et al*., 2009; Meyer *et al*., 2010). Thus, G-1, in μM concentrations, relaxes isolated arterial rings precontracted by several different agonists. While Haas *et al*. (2009) reported that the G-1 relaxation of prostaglandin F2α-contracted rings was lost upon GPER1 deletion, it remains to be fully determined if GPER1 mediates this G-1 response in general and whether the response is related to the vasorelaxation observed in isolated vascular preparations in response to μM concentrations of E2.

The E2, at physiological concentrations (nM), rapidly increases NO formation in endothelial cells through a mechanism involving the ERα 46 kDa isoform (Chen *et al*., 1999; Figtree *et al*., 2003; Li *et al*., 2003). That this mechanism is responsible for E2-promoted vasorelaxation of isolated vascular segment is unlikely, as the latter requires considerably higher concentrations (μM) of E2. Therefore, E2-promoted NO production most likely has other vascular effects such as anti-inflammatory. Nevertheless, it has been of interest to determine the role of NO in G-1-promoted vasorelaxation. However, such investigations have led to somewhat contradictory results. Lindsey *et al*. (2009) showed that G-1 evoked relaxation of aortic rings from mRen2.Lewis rats precontracted with phenylephrine were blunted by endothelial denudation suggesting that NO contributes to acute G-1-induced vascular relaxation. On the other hand, acetylcholine-evoked relaxation was unaffected by chronic treatment with G-1 for 2 weeks suggesting an NO independent effect. The angiotensin II-induced force response was reduced by a 2 week treatment with G-1 in endothelium-denuded rings, suggesting a direct effect by G-1 on the renin-angiotensin system independent of endothelial NO. This group also showed that the decrease in blood pressure in response to chronic G-1 treatment altered neither the levels of NO metabolites in serum or urine nor vascular eNOS mRNA levels. Thus, Lindsey *et al*. (2009) provided evidence that G-1 affects vascular reactivity both via endothelium-dependent and -independent mechanisms. Broughton *et al*. (2010) showed that endothelium removal abolished G-1-induced relaxation of rat carotid arteries precontracted with prostaglandin H2 agonist U-46619, which led to the conclusion that G-1 causes arterial relaxation via an endothelium- and NO-dependent mechanism. Thus, it is currently premature to draw any firm conclusions about the role endothelial NO in G-1-promoted vascular relaxation.

Effects of G-1 on blood pressure

Haas *et al*. (2009) reported that acute intravenous bolus infusion of increasing concentrations (41.2 $ng \cdot kg^{-1}$–20.6 $\mu g \cdot kg^{-1}$) of G-1 caused a slight (15%) reduction in MAP in normotensive male rats without any clear dose–response relationship of the decrease. Lindsey *et al*. (2009) reported a somewhat more pronounced G-1-promoted decrease (20%) in MAP in hypertensive mRen2.Lewis rats after chronic G-1 infusion via an osmotic minipump over a 2 week period. The latter group showed that the hypotensive effect of G-1 was observed in ovariectomized animals but not in gonadal-intact female or male rats (Lindsey *et al*., 2009). These reports suggest that G-1 is hypotensive in rodents, which is consistent with the hypertensive phenotype of GPER1 KO mice reported by Mårtensson *et al*. (2009). However, the specific relationship of these effects is far from clear.

Effects of G-1 on vascular smooth muscle and endothelial cell proliferation

G-1, at 1 μM, was recently reported to reduce serum-stimulated human vascular smooth muscle cell proliferation (Haas *et al*., 2009). The ER antagonist ICI 182 780, previously reported to act as a GPER1 agonist (Bologa *et al*., 2006), also triggered this response (Haas *et al*., 2009). Thus, GPER1 activation may be protective against unwanted vascular smooth muscle cell proliferation such as that which occurs in atherosclerosis. However, G-1 was also reported to elicit an

antiproliferative effect in endothelial cells including mouse bEnd.3 cells, human microvascular endothelian-1 cells and human umbilicalvein endothelian cells, all which express GPER1 mRNA (Holm *et al*., 2011). The G-1 effect occurred with an IC_{50} value of about 2 μM, was observed both under growth-arrested and growth-stimulated conditions, and involved a block of cell cycle progression from S and G2 phase (Holm *et al*., 2011). Furthermore, the G-1 response was absent in COS-7 cells, which express very low levels of GPER1, and following silencing of GPER1 expression by siRNA treatment, suggesting that it is mediated by GPER1 (Holm *et al*., 2011). Thus, GPER1 activation via this mechanism may antagonize the vasculoprotective mechanism of renewing and healing the endothelium. Therefore, this GPER1 mechanism may be a putative novel avenue to reduce excessive angiogenesis observed under different pathological conditions such as cancer.

Effects of G-1 on cardiac function and ischemic/reperfusion injury

Filice *et al*. (2009) showed that G-1, like E2, induces a negative inotropic effect associated with a slight increase in heart rate in male mice using the isolated Langendorf heart preparation, which suggests a possible role for GPER1 in regulating the excitability of the sinus node cells and/or the cells of the conductive system. However, the G-1 response was inhibited by the ER antagonist ICI 182 780 and mimicked by the ERα and ERβ agonists PPT and DPN indicating that if GPER1 plays a role in this response, it does so through a functional cross talk with ERα and ERβ.

Intriguingly, several recent reports from four different laboratories showed that G-1 also protects the heart from ischaemia/reperfusion injury (Deschamps and Murphy, 2009; Bopassa *et al*., 2010; Patel *et al*., 2010; Weil *et al*., 2010). This is interesting considering that E2 is also cardioprotective following ischaemia/reperfusion injury. Administration of G-1 attenuated post-ischemic contractile dysfunction in both male and female rats through a mechanism involving PI3K. Furthermore, the G-1-induced improvement of cardiac function was associated with a reduction in myocardial inflammation (Deschamps and Murphy, 2009; Weil *et al*., 2010). Bopassa *et al*. (2010) also showed that G-1-promoted cardioprotection is associated with an inhibitory effect on mitochondria permeability transition pore opening. Interestingly, Patel *et al*. (2010) reported that the protective effect of G-1 was abolished by addition of a GPER1 antibody, which provides evidence for the involvement of GPER1 in this response. Therefore, GPER1 may be an interesting new drug target in cardiac ischaemia/reperfusion injuries.

Jessup *et al*. (2010) showed that GPER1 signalling may also be cardioprotective in the hypertensive salt-sensitive mRen2.Lewis rat model. In this model, G-1 stimulation improved myocardial relaxation and cardiac myocyte hypertrophy and wall thickness in rats fed a high-salt diet. Furthermore, these effects were apparently not associated with changes in blood pressure. These results suggest that GPER1 activation may also be therapeutic alternative in the treatment of diastolic dysfunction.

Metabolic phenotype in GPER1 KO mice

High glucose as in diabetes has detrimental effects on both endothelial and vascular smooth muscle cells. Indeed, diabetes is associated with endothelial dysfunction involving an increased inflammatory response, impaired endothelium-dependent relaxation and increased coagulation (Hartge *et al*., 2007). High glucose has also been reported to stimulate production of cytokines and chemokines by vascular smooth muscle cells, and to stimulate expression of genes involved in calcification and bone formation such as osteopontin (Takemoto *et al*., 1999; Dragomir *et al*., 2008). Thus, GPER1-mediated stimulation of insulin release and β-cell survival to promote normalization of blood glucose may have beneficial effects on the cardiovascular system.

Mårtensson *et al*. (2009) reported an impaired glucose tolerance (IGT) in their GPER1 KO mice, which was accompanied by decreased pancreatic islet insulin levels and glucose-stimulated insulin secretion (GSIS) *in vivo* and in isolated islets. On the other hand, β-cell exocytosis and the expression of two early mediators of GSIS in islets, glucose transporter 2 and glucokinase, were not affected (Mårtensson *et al*., 2009). Furthermore, β-cell mass and β-cell area are normal in these mice (N. Wierup, B. Olde and L.M.F. Leeb-Lundberg, pers. comm.). These results are interesting because E2 treatment reverses the IGT, hyperglycaemia and reduced insulin release caused by ovariectomy in rats (Godsland, 2005), and insulin synthesis and plasma insulin levels are increased in pregnant rats (Sutter-Dub, 2002).

Mårtensson *et al*. (2009) also demonstrated that E2-stimulated insulin release both *in vivo* and *in vitro* is completely abolished in their GPER1 KO mice. This is also interesting because this response is triggered by membrane-impermeable E2 analogues and insensitive to ER antagonists (Nadal *et al*., 1998; Ropero *et al*., 2002). These results suggest that E2 stimulates insulin release through a membrane-dependent non-genomic mechanism. Considering that Liu *et al*. (2009) and Balhuizen *et al*. (2010) reported the immunolocalization of GPER1 in pancreatic β-cells, these results strongly favour GPER1 as the mediator of this oestrogenic response.

Recently, it was shown that a membrane-dependent non-genomic mechanism is also involved in the ability of E2 to protect pancreatic β-cell survival in response to oxidative damage (Liu *et al*., 2009). The ERα is known to be an important mediator of this response (Le May *et al*., 2006). However, residual E2 protection remains in ERα and ERβ KO mice, which led Liu *et al*. (2009) to address the role GPER1 in this response. Using GPER1 KO mice developed by Wang *et al*. (2008), they found that following streptozotocin treatment, these mice lost protection of endogenous E2 and were predisposed to insulin-deficient diabetes with loss of β-cells and decreased pancreatic insulin content. Thus, GPER1 also participates together with ERα in this oestrogenic response.

Two observations have been made that the role of GPER1 on β-cell functions is gender specific. Mårtensson *et al*., (2009) reported that the IGT in their GPER1 KO mice and the associated decrease in islet insulin level were observed in females but not in males. While Liu *et al*. (2009) did not observe any IGT or

Table 1
Reported cardiovascular and metabolic functions of GPER1

Tissue/cell	GPER1 regulatory function	Reference
Vascular smooth muscle cell	Antiproliferation	Haas *et al.* (2009)
Vascular smooth muscle	Endothelium-dependent relaxation	Lindsey *et al.* (2009)
Vascular smooth muscle	Endothelium-dependent relaxation	Broughton *et al.* (2010)
Vascular smooth muscle	Relaxation	Haas *et al.* (2009)
Vascular endothelium	Decreased superoxide production	Broughton *et al.* (2010)
Vascular endothelium	No effects on aortic eNOS mRNA and serum nitric oxide levels	Lindsey *et al.* (2009)
Endothelial cell	Antiproliferation	Holm *et al.* (2011)
Pancreatic β-cell	Insulin release	Mårtensson *et al.* (2009); Balhuizen *et al.* (2010)
Pancreatic β-cell	Anti-apoptosis	Balhuizen *et al.* (2010); Liu *et al.* (2009)

GPER1, G protein-coupled oestrogen receptor 1.

decreased islet insulin in the GPER1 KO mice constructed by Wang *et al*. (2008), possibly due to strain differences, only the female mice were predisposed to streptozotocin-induced diabetes. These results suggest that female mice are more dependent on GPER1 in maintaining certain β-cell functions such as insulin production and survival.

Haas *et al*. (2009) reported that the GPER1 KO mouse strain generated by Wang *et al*. (2008) are obese at 10–11 months old. This result differs from that obtained with two other GPER1 KO mouse strains that did not exhibit a change in body fat (Isensee *et al*., 2009; Mårtensson *et al*., 2009) or in serum leptin levels (Mårtensson *et al*., 2009). Furthermore, the high-fat diet-induced weight gain of one of these mouse strains was also unaltered upon GPER1 depletion (Isensee *et al*., 2009). Also, Windahl *et al*. (2009), using ovariectomized GPER1 KO mice generated by Mårtensson *et al*. (2009), reported no significant effect on the ERα-mediated oestrogenic response on total body fat mass and on retroperitoneal and gonadal fat depots. These results suggest that GPER1 does not have major effects on rodent fat metabolism either directly or via ERα-regulated fat metabolism, at least not in mice.

G-1 effects on pancreatic β-cell function

Balhuizen *et al*. (2010) reported recently that G-1 stimulates insulin release from mouse pancreatic islets with an efficacy similar to that of E2. This observation is consistent with the previous observation that E2-stimulated insulin release *in vivo* and *in vitro* is absent in GPER1 KO mice. This group also showed that G-1 protects against cytokine-induced β-cell apoptosis, which is in line with the results by Liu *et al*. (2009) that G-1 also protects against H_2O_2-induced β-cell apoptosis in mouse and human islets. Thus, studies with both GPER1 KO mice and G-1 point towards GPER1 mediating both E2-stimulated insulin release and β-cell survival.

Conclusion

Following a period of initial probing of GPER1 function and some apprehension towards the involvement of this receptor in oestrogen physiology, evidence is slowly but steadily accumulating that GPER1 may participate in oestrogenic responses in some physiological and pathophysiological settings including cardiovascular and metabolic regulation (Table 1). Indeed, several oestrogenic responses that have been known to involve membrane-dependent and non-genomic signalling mechanisms cannot be fully explained by the participation of membrane-bound versions of ERα or ERβ. Furthermore, some oestrogenic responses already described to involve ERα or ERβ have upon further dissection been shown also to involve other putative ER.

Many of the present results on GPER1 function in the cardiovascular system are critically dependent on the specificity of the GPER1 agonist G-1. Some studies respond to the issue of specificity by using, for example, GPER1 antibody or GPER1 siRNA to inhibit the response, thus favouring the view that the G-1 response occurs specifically via GPER1. Further proof of GPER1 involvement will require monitoring the loss of G-1 effects in cells and tissues from GPER1 KO mice. Somewhat surprisingly, data on the reported GPER1 antagonist G-15 is still to a large degree lacking.

Caveats also exist while using receptor KO mouse strains. Four different GPER1 KO strains have been constructed. Unfortunately, the reported phenotypes are somewhat different, which may be due in part to variations in the method used for phenotypic characterization and in part to the mouse strain used. Still, some overlap exists in the vascular and the metabolic phenotypes. The strains made by Wang *et al*. (2008) and Mårtensson *et al*. (2009) both display phenotypes related to vascular function (Olde and Leeb-Lundberg, 2009), an overlap supported by the strong reporter activity in the vasculature noted in the GPR30-lacZ transgenic strain made by Isensee *et al*. (2009). Furthermore, female-specific deficiencies in E2-regulated β-cell function have been identified in both strains (Liu *et al*., 2009; Mårtens-

son *et al*., 2009). Confirming specific phenotypes in future physiological studies and in more refined conditional animal models is an important future goal.

Several promising G-1-promoted cardiovascular responses have been identified including reduction in blood pressure, vasorelaxation and vascular smooth muscle cell proliferation, which suggest that GPER1 activation could have beneficial effects on the vascular system via these mechanisms. However, G-1 also reduces endothelial cell proliferation thus antagonizing the potentially beneficial antiproliferative effect on vascular smooth muscle cells by reducing re-endothelialization. The reported blood pressure decrease in response to a bolus intravenous injection of G-1 is relatively small arguing that GPER1 activation may have limited importance in this regard. On the other hand, the protective effect of G-1 on the heart myocardium following ischaemia/reperfusion could be further exploited for therapeutic benefits. In terms of metabolic regulation, there is now increasing evidence using GPER1 KO mouse models and pharmacological approaches with G-1 that GPER1 has insulinotropic effects by both protecting pancreatic β-cell survival and stimulating insulin release. Thus, GPER1 could be an interesting therapeutic target in type 2 diabetes and/or pancreatic islet transplantation. In summary, GPER1 represents a promising new drug-target for the prevention/treatment of both cardiovascular and metabolic disease. However, the precise mechanisms of function of this receptor are only beginning to be identified, thus urging for further mechanistic studies.

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Conflict of interest

The authors declare no conflict of interest.

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