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GPER/GPR30 and Regulation of Vascular Tone and Blood Pressure

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Abstract

Natural estrogens such as 17β-estradiol are endogenous vasodilators and have been implicated in the gender differences of hypertension. These hormones activate estrogen receptors ERα and ERβ, which mediate part of estrogen-dependent vasodilation. In addition, a novel G protein-coupled estrogen-binding receptor termed GPER/GPR30 has been identified that is expressed in the cardiovascular system. Using knock-out animals or drugs selectively targeting GPER/GPR30, a significant role for this receptor as a mediator of acute estrogen-dependent vasodilation involving nitric oxide (NO) and blood pressure-lowering activity has been demonstrated. The accumulating evidence that GPER/GPR30 is responsible for control of vascular tone indicates that this receptor may represent a novel drug target for pharmacologic treatment of hypertension in postmenopausal women and possibly also men.

Keywords

Blood Pressure; Endothelium; Hormone Therapy; Hypertension; Menopause; Nitric Oxide; Vasodilation

1. Endogenous Estrogens and Their Receptors in the Cardiovascular System

Globally, more than 25% of women have hypertension, with the prevalence being particularly high in women more than 60 years of age [1]. Whereas blood pressure levels are lower in premenopausal women compared to age-matched men, they markedly increase during the first decade following menopause [2]. In fact, the prevalence of hypertension is higher in women than in men more than 70 years of age [2], which translates into a higher cardiovascular risk [3]. Similarly, whereas the prevalence of coronary artery disease is lower in premenopausal women compared to age-matched men, these gender-based differences

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Conflict of Interest
None.

narrow after menopause [4]. As a result, cardiovascular disease represents the leading cause of death in men and women alike [5]. These data also suggest that endogenous estrogens confer a protective effect on the development of hypertension and atherosclerotic vascular disease [4, 6].

In line with these epidemiological data, experimental studies have demonstrated a variety of beneficial effects of endogenous estrogens on the cardiovascular system often independent of sex, which include acute and chronic vasodilator activity ultimately lowering blood pressure [4, 6, 7]. However, the underlying mechanisms are still incompletely understood. Estrogens are traditionally referred to as ligands of the classical estrogen receptors α (ERα) and β (ERβ) [4, 7, 8]. These receptors primarily function as ligand-activated nuclear transcription factors modulating expression of hormonally regulated genes. ERα and ERβ also activate rapid intracellular signaling pathways in response to estrogen, which are presumably mediated by plasma membrane-associated subpopulations of the receptors [8, 9]. Via such rapid or “non-genomic” mechanisms, both ERα and ERβ mediate vasodilation that occurs within only a few minutes [10]. In 1997, a seven-transmembrane G protein-coupled receptor (GPR30) was cloned from shear stress-exposed human endothelial cells [11] among other sources, and in 2000 it was demonstrated that this receptor is estrogen-responsive [12]. More recently, binding of estrogen to GPR30 has been shown [13, 14] that results in activation of rapid signaling cascades [12-14]. After establishing GPR30 as a *bona fide* estrogen-binding receptor, it was renamed GPER by the International Union of Basic and Clinical Pharmacology [15]. Similar to ERα and ERβ [4, 7], GPER/GPR30 is expressed throughout the vascular system in humans and animals of both sexes [11, 16-26]. These findings suggest that in addition to ERα and ERβ, GPER/GPR30 is likely to be involved in the vascular effects of estrogen and to play a physiological role in the control of vascular homeostasis in females and males.

2. Vascular Effects of Non-Selective GPER/GPR30 Agonists

The predominant endogenous human estrogen 17β-estradiol is synthesized primarily in the ovaries, and binds ERα, ERβ, and GPER/GPR30 with high affinity (dissociation constant K_d 0.05-0.09 nM for ERα and ERβ [27], K_d 2.7-6.6 nM for GPER/GPR30 [13, 14]). 17β-Estradiol is a powerful vasodilator of human blood vessels from males and females [16, 28, 29]. In addition, the estrogen-based steroids estrone and estriol have vasodilator properties in certain vascular beds [30, 31], although their binding affinity for GPER/GPR30 is low at physiological concentrations [14]. In addition to gonadal steroid synthesis, estrogens are produced locally at many sites throughout the body including the vascular wall, where the androgens testosterone and androstenedione are converted into 17β-estradiol and estrone, respectively, by the enzyme aromatase [32]. Interestingly, healthy young men taking the aromatase inhibitor anastrozole display impaired flow-mediated vasodilation and reduced plasma 17β-estradiol levels [33]. In line with these findings, short-term exposure to 17β-estradiol improves endothelium-dependent vasodilation in male patients [34], and male mice lacking ERβ develop hypertension with aging [35]. These data indicate a physiologically relevant role of endogenous estrogens as vasodilators even at low concentrations as seen in men and postmenopausal women. Importantly, short-term treatment with 17β-estradiol improves endothelium-dependent vasomotion in early postmenopausal women, whereas in

aged menopausal women, hormone therapy abrogates vasodilation yielding vasoconstriction instead [36, 37].

Estrogenic compounds are also synthesized by soy and other plants (phytoestrogens), and mediate numerous vascular effects similar to 17β-estradiol, including vasodilation [38]. One of the most widely studied phytoestrogens, the isoflavone genistein, activates ERs including GPER/GPR30 [39]. Other man-made estrogens (xenoestrogens) and GPER/GPR30 agonists comprise chemical detergents and pesticides such as nonylphenol and DDT [39], and limited experimental data also suggests a role for these compounds in regulation of vascular function [40, 41]. Despite the widespread use of certain xenoestrogens and the subsequent chronic low-level exposure to humans [42], the potential impact of these agents on vascular homeostasis has not been investigated.

Based on their clinical use, the role of selective estrogen receptor modulators (SERMs) for regulation of vascular tone has been evaluated in a variety of studies. These drugs generally act as ER agonists in the cardiovascular system, bone, and liver, and as ER antagonists in breast tissue [43]. Moreover, SERMs such as tamoxifen and raloxifene are also agonists of GPER/GPR30 [44, 45]. These compounds evoke acute endothelium-dependent as well as endothelium-independent vasodilation in porcine coronary arteries and other vascular beds [46-51]. Raloxifene also activates endothelial nitric oxide synthase (eNOS) via ERα-dependent activation of the PI_3K/Akt-pathway [52] as has previously been shown for 17β-estradiol [53].

In addition to SERMs, selective estrogen receptor downregulators (SERDs) such as ICI 182,780, which abolish ERα/ERβ signaling regardless of the type of tissue, have been used experimentally and therapeutically [54]. Importantly, ICI 182,780 displays significant binding affinity to GPER/GPR30 [14] and acts as a GPER/GPR30 agonist in breast cancer cells and several other cell lines and tissues [12, 13, 44]. These findings suggest GPER/GPR30 can mediate estrogenic effects even when ERα and ERβ are concomitantly blocked.

In summary, several natural and synthetically generated estrogens that have been implicated in the regulation of vascular function not only activate ERα and ERβ, but also the recently discovered GPER/GPR30. Whereas these substances (including the major human estrogen 17β-estradiol) are nonspecific activators of ERα, ERβ, and GPER/GPR30, selective agonists (G-1 [55]) and antagonists (G15 [56]) of GPER/GPR30 as well as genetically modified animals have been introduced, which aid in the delineation of GPER/GPR30's specific vascular effects.

3. GPER/GPR30-Dependent Vasodilation

Human endothelial cells exposed to fluid shear stress were used as one of the first experimental approaches to identify and clone a cDNA encoding GPER/GPR30 [11]. Although these experiments suggested a potential role for GPER/GPR30 in vascular regulation, this possibility has been strengthened by recent research using the selective GPER/GPR30 agonist G-1 [55] in several vascular beds. In fact, G-1 acutely dilates human internal mammary and porcine coronary arteries, as well as rodent aorta, carotid, and mesenteric arteries independent of sex, a response that is less potent in the conduit arteries

(Figure 1A-1C) [17, 19, 23, 26, 57]. G-1 also indirectly inhibits endothelin- [57], angiotensin II- [19], serotonin- [17], and thromboxane A_2 receptor-dependent [23] contractions in certain vascular beds. In GPER/GPR30 knock-out animals as well as after pretreatment with the GPER/GPR30 antagonist G15, the vasodilator effect of G-1 is lacking [17, 26], which further underscores a role for GPER/GPR30 in the control of vasomotor tone (Figure 1A). Interestingly, G-1-dependent relaxation in human internal mammary and murine carotid arteries is even more pronounced than that of the non-selective ER agonist 17β-estradiol (Figure 1B and 1C) [17]. This points to a potential crosstalk between GPER/GPR30, ERα and ERβ, which are all involved in regulation of estrogen-dependent vasodilation.

On the other hand, the GPER/GPR30-dependent vasodilator response does not necessarily depend on the activity of ERα and ERβ as pointed out by studies using the ERα/ERβ-antagonist but GPER/GPR30-agonist ICI 182,780 [12, 14]. In porcine coronary arteries, ICI 182,780 alone evokes rapid relaxation [57]. In line with these findings, ICI 182,780 causes a rapid, nitric oxide (NO)-dependent dilation of pressurized carotid (but not femoral) arteries from ovariectomized mice [58]. Moreover, ICI 182,780 does not block vasodilation in response to 17β-estradiol in several vascular beds of different species [59-63]. Conversely, NO-dependent vasodilatory effects of ICI 182,780 in these arteries are also abolished in animals lacking ERα or ERβ [58], again suggesting potentially complex crosstalk between GPER/GPR30, ERα and ERβ. Further studies using genetically modified animals or selective agonists/antagonists of specific ERs are needed to clarify their individual role in this context.

While several independent investigators have reported vasodilator effects in response to G-1, the mechanisms involved in GPER/GPR30-dependent regulation of vasomotor tone are still scarcely understood. Interestingly, GPER/GPR30 activation abrogates calcium flux induced by the vasoconstrictor serotonin indicating calcium-antagonistic or desensitizing effects [17]. Moreover, G-1-induced relaxation in rat aorta, common carotid, and mesenteric arteries as well as in porcine coronary arteries depends at least partly on the presence of an intact endothelium and is inhibited by the NOS inhibitor L-NAME [23, 26, 57]. This suggests that GPER/GPR30-dependent vasodilation is elicited via release of endothelium-derived NO in these vascular beds. In line with these findings, vasodilation of murine carotid arteries in response of the ERα/ERβ-antagonist but GPER/GPR30-agonist ICI 182,780 is absent in the presence of L-NAME [58]. This suggests that the potential beneficial vascular effects of GPER/GPR30 activation are at least partly mediated by ameliorating endothelial cell dysfunction, a vascular abnormality common to hypertension and atherosclerosis that is characterized by impaired endothelial NO production [64]. Conversely, chronic G-1 treatment of surgically postmenopausal (ovariectomized) mRen2.Lewis rats, a model of postmenopausal hypertension, has no effect on aortic eNOS gene expression [19]. In addition, endothelium-dependent relaxation was not affected by hypertension in these animals [19], although findings from hypertensive animals have some limitations and thus should not be generalized. However, the potential molecular pathways whereby GPER/GPR30 interacts with the NO pathway remain to be determined. Furthermore, preliminary evidence indicates that GPER/GPR30 activation by G-1 and ICI 182,780 also evokes endothelium- / NO-independent coronary vasodilation via BK_{Ca}

channel-mediated membrane hyperpolarization.[1], [2] In particular, endothelium-independent vasodilator effects of G-1 are likely to be present in resistance arteries [26].

In summary, current evidence suggests that GPER/GPR30 is a mediator of estrogen-induced vasodilation, involving both endothelium-dependent and -independent mechanisms. The GPER/GPR30-dependent responses depend on distinct vascular beds in different species and the time-course of estrogen administration, which may be reflected by functional ER crosstalk between GPER/GPR30, ERα and ERβ.

4. Effects of GPER/GPR30 Activation on Blood Pressure

Estrogens have been implicated in the gender differences of hypertension, since blood pressure is lower in premenopausal women compared to age-matched men [6]. In line with their acute vasodilatory effects, the loss of endogenous estrogens following menopause is associated with a pronounced increase in blood pressure levels [6]. Increased vascular resistance is a key feature of arterial hypertension in women and men alike [6], and is likely to be modulated by GPER/GPR30 activation [26]. Intravenous injection of G-1 into normotensive male rats acutely reduces mean arterial blood pressure (Figure 1D) [17]. Moreover, in hypertensive ovariectomized mRen2.Lewis rats, treatment with G-1 for 2 weeks lowers blood pressure and reduces gene expression of angiotensin II type 1 receptor and angiotensin-converting enzyme, although G-1 has no effect in estrogen-intact female or in male littermates [19, 65]. G-1 also inhibits angiotensin II receptor binding and angiotensin II-induced intracellular calcium increase in mesenteric smooth muscle cells of female mRen2.Lewis rats,[3] suggesting that G-1 lowers blood pressure by attenuating vascular angiotensin II signaling. Interestingly, genetic linkage studies have indicated that the locus of the GPER/GPR30 gene is associated with low-renin hypertension. Indeed, in a model of GPER/GPR30-deficient adult female mice, higher mean arterial blood pressure has been reported than in age-matched controls, although absolute values were similar compared to younger GPER/GPR30-knockout and wildtype animals [66]. Moreover, G-1 reduces left-ventricular hypertrophy and myocyte size, and ameliorates diastolic dysfunction in an estrogen-intact animal model of salt-induced hypertensive cardiomyopathy [65]. Thus, GPER/GPR30 is likely involved in the estrogen-mediated beneficial effects on blood pressure as well as subsequent cardiac hypertrophy and remodeling. This is important in view of the high prevalence of hypertension and heart failure with normal ejection fraction (i.e. diastolic heart failure) in postmenopausal women [6, 67].

5. Implications for Research and Possible Therapeutic Application

Recent studies have shown that GPER/GPR30 mediates both acute and chronic vasodilatory effects in males and females with similar efficacy compared to ERα and ERβ. Thus, the beneficial blood pressure-lowering effects that protect premenopausal women from

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[2]Han, G.; Ma, H.; Barman, S.A.; Sellers, M.; Yu, X.; Stallone, J.N.; White, R.E. Rapid estrogen signaling via GPER in human coronary artery smooth muscle. *Faseb J*, **2010**, *24 (Meeting Abstract Supplement), abstract 957.1*.
[3]Lindsey, S.H.; Bhat, M.; Aileru, A.; Chappell, M.C. GPR30 attenuates functional AT1 receptor expression in rat mesenteric smooth muscle cells. *Faseb J*, **2011**, *25 (Meeting Abstract Supplement), abstract 1088.8*.

developing hypertension [6] likely result from activation of (at least) three different estrogen-binding receptors. This also implicates that predicting the cellular response to non-selective ER activation becomes increasingly complex due to functional crosstalk between ERα, ERβ, and GPER/GPR30, which ultimately affects multiple rapid signaling pathways as well as gene transcription [8]. With the availability of knockout animals and selective agonists/antagonists of the different ERs, future studies should aim to better characterize the individual role of GPER/GPR30, ERα, and ERβ for the control of vascular tone and blood pressure. Interestingly, estrogen-independent activation of these receptors by antihypertensive drugs such as olmesartan and nebivolol may also play a role in the regulation of vascular homeostasis [68-70].

Contrary to the clear-cut experimental and epidemiological evidence, many clinical trials using conjugated equine estrogens and medroxyprogesterone acetate for postmenopausal hormone therapy failed to prove a therapeutic benefit on cardiovascular outcomes and were associated with significant adverse effects [71, 72]. Although the design of these studies has been widely criticized for issues such as timing and type of treatment [37, 73], the unfavorable outcome may also have resulted from concomitant activation of multiple beneficial and harmful estrogen signaling pathways and the use of the toxic medroxyprogesterone acetate [37, 73]. Indeed, a very recent analysis of the Women's Health Initiative Estrogen-Alone Trial suggests that in younger hysterectomized postmenopausal women monotherapy with equine estrogens reduces cardiovascular risk compared to placebo (HR 0.59, 95% CI 0.38-0.90) [74]. Moreover, additional recent reports point to a reduction in the risk of cardiovascular events in younger postmenopausal women treated with the SERM and GPER/GPR30 agonist raloxifene, whereas lasofoxifene demonstrated even greater effects [75-78]. How much of this risk reduction is due to GPR30/GPER activation remains unclear at this point, but future therapeutic approaches should include strategies selectively targeting ERs that mediate beneficial vascular activity. The fact that a GPER/GPR30-selective agonist such as G-1 largely recapitulates the beneficial cardiovascular effects of estrogen(s) without the latter's feminizing effects suggests that activation of this receptor may evolve as new therapeutic strategy in the treatment of vascular disease, possibly in a gender-independent fashion.

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List of Abbreviations

BK_{Ca} channel	Large-conductance Ca^{2+}- and voltage-activated K^+ channel
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
GPER	G protein-coupled estrogen receptor
GPR30	G protein-coupled receptor 30

NO	Nitric oxide
PI_3K	Phosphatidylinositol 3-kinase
SERD	Selective estrogen receptor downregulator
SERM	Selective estrogen receptor modulator

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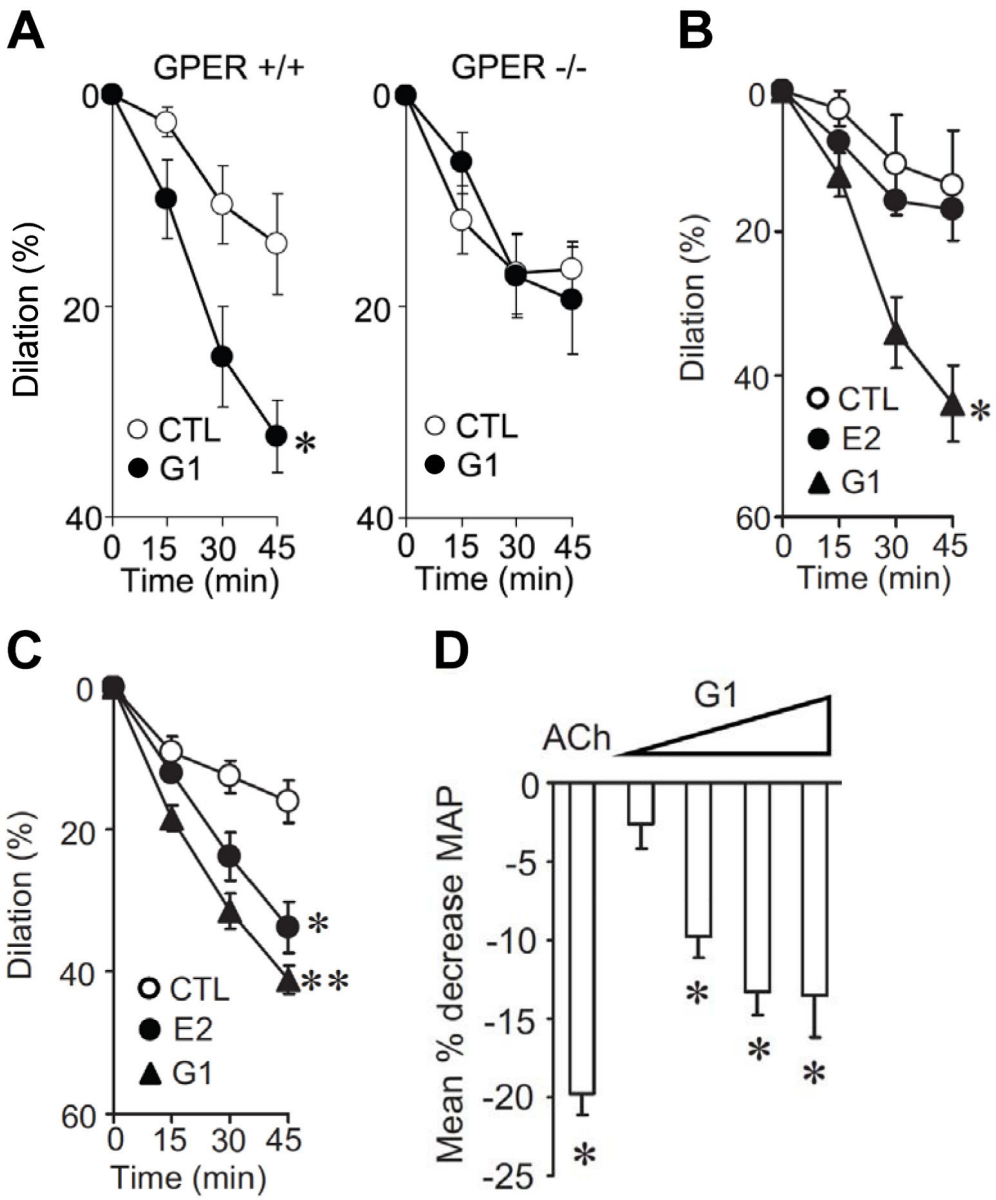

Figure 1.

GPER/GPR30-dependent regulation of vascular tone. In carotid arteries of wild-type mice (GPER +/+), the selective GPER/GPR30-agonist G-1 causes time-dependent acute dilation, which is absent in GPER/GPR30-knockout animals (GPER −/−, A). In murine carotid (B) and human internal mammary arteries (C), the dilator effect of G-1 is even stronger than that of 17β-estradiol (E2). Injection of G-1 at increasing doses (4.12 ng/kg, 41.2 ng/kg, 412 ng/kg, and 20.6 μg/kg) acutely reduces mean arterial blood pressure (MAP, calculated as 1/3 *Max* + 2/3 *Min*, where *Max* is the systolic pressure and *Min* the diastolic pressure) in normotensive male rats. For comparison, the response to achetylcholine (*ACh*, 30 ng/kg) is shown (D). 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.,

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