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Alike but not the same: Anatomic Heterogeneity of Estrogen Receptor-Mediated Vasodilation

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Abstract

In view of recent findings on the anatomic heterogeneity of rapid vasodilation via estrogen receptor-dependent mechanisms it is obvious that with regard to human physiology and disease much of it is still unknown, and research in this area is urgently needed. This is also important since chronic drug therapy with estrogens in women systemically affects the circulation and may affect certain arterial beds but not others. It is conceivable that the presence of any vascular disease (as was the case for coronary and carotid atherosclerosis in many of the patients in the large RCTs HERS and WHI) is likely to affect vascular responses to estrogens as well, and that any beneficial effects may be attenuated or even completely lost. Further work is required to decipher the mechanisms of vasodilation brought about by estrogens in humans and experimental animals, whether anatomic heterogeneity exists with regard to vascular beds and individual estrogen receptors, and how vascular disease - atherosclerosis in particular - affects responsiveness. Pharmacological tools for newly identified ERs are now available. The hypothesis that disease may modify or even abrogate estrogen-dependent or ER-selective vasodilation should also be tested. Finally, given that certain clinically approved drugs such as SERM or SERDs (thought only to block or downregulate nuclear ERs) actually cause vasodilation through GPER and have been shown in recent clinical studies to provide cardiovascular protection in postmenopausal women, we may have to re-think our current understanding, concepts, and strategies of how to interfere with the increased risk of vascular disease in women with estrogen deficiency or after menopause.

Keywords

estrogen; vasodilation; vascular tone; artery; estrogen receptor; GPER; heterogeneity; anatomic; atherosclerosis; randomized controlled trial

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It has been 20 years since we first reported acute, endothelium-independent relaxation of precontracted human coronary arteries to 17β-estradiol, the biologicall most relevant sex steroid [1]. The vasodilating effect showed a heterogeneity being more pronounced in arteries obtained from women than those obtained from men [1]. In 1993, only estrogen receptor α (ERα) was known to exist [2], and we thus speculated that this estrogen receptor might be involved in these acute, “non-genomic” responses occurring within minutes. We also suggested it might also play a role in the acute increases in vascular cyclic nucleotide content observed after short-term exposure to 17β-estradiol [1,3], previously observed in uterine tissue [4]. We concluded that the acute vasodilating effects of endogenous estrogen may contribute to the protection from atherosclerosis [1]. Indeed, women with intact ovarian function are largely protected from atherosclerosis in their premenopausal years, and this protection has been closely linked to ovarian estrogen production. Accordingly, ovariectomy

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or ovarian dysfunction markedly increases the risk of coronary artery disease [5]. In agreement with our in vitro findings [1], vasodilator effects after intracoronary application of 17β-estradiol have been demonstrated in atherosclerotic coronary arteries of postmenopausal women [6].

Although acute vasodilating effects of estrogens, particularly those of 17β-estradiol, were first described close to 120 years ago [7] and subsequently have also been demonstrated in men [8], the complexity of the mechanisms through which estrogens mediate vasodilation is not fully understood [9,10]. Vascular effects of estrogen have been observed in many parts of the body, including reproductive organs, kidney, skin, heart, brain, and lung [9], and are particularly evident during pregancy [9]. Aside from the phenolic chemical properties of estrogens, which make them potent antioxidants [11], these sex steroids bind to specific targets in the vascular wall [12,13]. Acute effects of estrogens were first observed in the 1960s by Szego and colleagues, showing rapid increases in cyclic nucleotides in uterine cells [4,14]. In 1977, estrogen binding sites in endometrial cells were reported by Szego and Pietras [14], and estrogen binding sites were first detected in vascular endothelial cells by Colburn and Buonassisi [15]. The same group shortly before reported release of cyclic guanosine monophosphate (cGMP) from endothelial cells in response to acetylcholine [16]. Cyclic GMP would later be shown to be a critical mediator of endothelium-dependent relaxation by NO discovered by Robert Furchgott [17,18], which in turn, is also regulated by estrogen [19].

Today, we know that the vasodilating effects of estrogens and 17β-estradiol in particular are mediated by at least three known estrogen receptors, the “classical” nuclear receptors ERα, ERβ, and a membrane G protein-coupled estrogen receptor (GPER, formerly known as GPR30 which was originally cloned from human endothelial cells [20]. Estrogen receptors have been functionally demonstrated close to fifty years ago [21,22], however ERα was cloned only in the mid-1980s, while ERβ and GPER were cloned in the mid-1990s [23]. Research from the past decade has yielded evidence that membrane subpopulations of both ERα and ERβ exist, and that they are involved in rapid responses to estrogens (Fig. 1) [12,13,24,49]. The mechanisms identified so far mediating acute (“non-genomic”) vasodilatory effects of ER activation include direct effects on vascular smooth muscle [25], endothelial cell-derived release of NO, endothelium-dependent hyperpolarization, and modulation of ion channels [26], among others.

In 2007 we published a comparative analyses of vascular estrogen receptor expression in human arteries and veins, as well as vasodilator responses to 17β-estradiol, also describing mRNA expression of GPER in intact human arteries [27]. Nevertheless, studies on vascular estrogen receptor expression and function are scarce, both with regard to the different estrogen receptors as well as to the different vascular beds in which these receptors have been detected [28]. So far, vasodilating effects of estrogens have been described in arteries from females and males, both in humans and in experimental animals [26], but so far no thorough and systematic investigation has been performed – neither with regard to individual estrogen receptors nor regarding different vascular beds.

In the present issue of the *Journal*, Reslan and colleagues [29] now present results of a comprehensive analysis of the vascular effects of the non-selective estrogen receptor agonist 17β-estradiol and selective estrogen receptor agonists of ERα, ERβ, and GPER, extending and integrating previous studies in rats in which 17β-estradiol or GPER-selective agonists caused vasodilation of precontracted carotid arteries [30], aorta and mesenteric arteries [31,32], and renal arteries [33]. Vasodilating effects of such compounds are also present in murine and human arteries [31]. Using conduit and resistance arteries as well as pulmonary arteries from ovary-intact female rats, the investigators also studied endothelium-dependent,

NO-mediated relaxation as well as α1-adrenoceptor-mediated vascular contraction. Finally, Restan *et al.* also determined protein expression of estrogen receptors in the six different arterial beds, and functionally studied and determined the effects of acute exposure to ER agonists on vascular production of stable metabolites of the endothelial vasodilator NO.

The results presented are remarkable as they allow, for the first time, direct and simultaneous comparison of vascular reactivity as well as acute ER-selective vasodilation between a number of arterial beds, including the pulmonary artery. The data indicate that – except for the mesenteric artery – acetylcholine-mediated relaxation in the ovary-intact rat is strictly NO-dependent in the aorta, carotid artery, renal artery and pulmonary circulation. By contrast, relaxations to 17β-estradiol as a non-selective estrogen receptor agonist at concentrations of or below 1 micromolar are markedly different between these vascular beds causing no relaxation in the carotid and pulmonary artery compared to relaxation in the other four arteries studied. Selective agonists such as 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT, ERα), 2,3-bis(4-hydroxyphenyl) propionitrile (DPN, ERβ), and (±)-1-[(3aR*,4S*,9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]- ethanone (G-1, GPER) were similarly without effect in the pulmonary and carotid artery, while they caused relaxation in the other arteries studied at subnanomolar - i.e. physiological – estrogen concentrations. The findings in the carotid artery contrast data by Broughton *et al.* [30] and Murata *et al.* [34] in male and female rats using G-1, where relaxation in response to activation of GPER was reported. The reason(s) for the different results are currently unclear. With regard to any study investigating acute effects to estrogen receptor agonists, however, it should be noted that the vasodilating effects reported for supra-micromolar concentrations shown here are likely to be only of pharmacological but not physiological relevance. In addition, since high concentrations of ER selective agonists such as PPT, DPN and G-1 may lose their receptor selectivity [35], any data obtained at concentration of 10 μmolar and above are likely not due to selective ER subtype activation. For example, the EC_{50} values of DPN for ERα and ERβ are ~70 nM and 1 nM, respectively, suggesting that at concentrations greater than ~100 nM, selectivity is mostly lost.

This study by Reslan and colleagues confirms our previous observations in porcine epicardial coronary arteries which suggested functional, “inhibitory” ER “cross-talk” [36]. The data presented by Reslan *et al.*, - comparing responses to 17β-estradiol versus the ERα-agonist PPT - , now similarly suggest inhibitory “functional” ER “cross-talk”, since simultaneous activation of all 3 ERs by 17β-estradiol causes less vasodilation than equimolar concentrations of PPT alone - an effect that is particularly evident in the mesenteric and renal arteries. Indeed, cross-talk between estrogen receptors has been demonstrated in non-vascular cells (reviewed in [37]) and is likely to affect molecular and physiological effects of estrogens. Equally surprising was the finding that while 17β-estradiol-mediated vasodilation was fully inhibited by blocking nitric oxide synthase in all 6 vascular beds, individual activation of either ERα, ERβ or GPER alone causes vasodilation that becomes largely NO-independent in the mesenteric and renal artery. this suggests other underlying mechanisms such as endothelium-dependent hyperpolarization or direct, endothelium-independent vasodilator effects on the vascular smooth muscle [1] as well as a possible co-activating effect of all three receptors that involves endothelial nitric oxide synthase.

Why are the data by Reslan *et al.* important? First, in view of the disappointing results of large RCTs in postmenopausal women such as the *Heart Estrogen and Progestin Replacement Study* (HERS) or the *Women's Health Initiative* (WHI) in which hormone mixtures from horse urine with unknown activity on human estrogen receptors were used as “hormone therapy”, the present study again tells us that our understanding of the

mechanisms by which natural *human* estrogens activate estrogen receptors is still scarce - both for human as well as animal physiology. Second, the study by Reslan *et al.* confirms that it is important whether estrogen receptors are activated selectively or simultaneously [36], which appears to determine the activation of rather specific vasodilatory mechanisms. Third, the study provides evidence that at least in resistance arteries, endothelium-dependent hyperpolarization is an important contributor to ER-mediated vasodilation, previously only shown for ERβ in mouse mesenteric [38] and porcine epicardial coronary arteries [36]. Fourth, this study is the first to characterize responses of unselective and selective ER agonists in the pulmonary artery, a vascular bed that is different from all the other arteries investigated because of its high oxygen tension environment. Finally, the data suggest that certain arterial blood vessels – at least in ovary-intact female rats – may be entirely insensitive to ER-mediated vasodilation.

If one extrapolates these findings to human physiology and disease, it will become apparent that much is still unknown and research in this area is urgently needed [39]. This may be important since chronic drug therapy with estrogens in women systemically affects the circulation and may have effects on certain arterial beds but not others. It is conceivable that the presence of any vascular disease (as was the case for coronary and carotid atherosclerosis in many of the patients in the large RCTs Heart Estrogen and Progestin Replacement Study (HERS) and the Women's Health Initiative (WHI)) is likely to affect vascular responses to estrogens as well, and that any beneficial effects may be attenuated or even completely lost [40]. Further work is required to decipher the mechanisms of vasodilation brought about by estrogens in humans and experimental animals, whether anatomic heterogeneity exists with regard to vascular beds and individual estrogen receptors, and how vascular disease, atherosclerosis in particular, affects responsiveness [39,41], and pharmacologcial tools for newly identified ERs are now available [42–48]. The hypothesis that disease may modify or even abrogate estrogen-dependent or ER-selective vasodilation should also be tested [39,41]. Finally, given that certain clinically approved drugs such as SERM or SERDs (thought only to block or downregulate nuclear ERs) actually cause vasodilation through GPER [26,37] (Figure) and have been shown in recent clinical studies to provide cardiovascular protection in postmenopausal women (reviewed in [40]), we may have to re-think our current understanding, concepts, and strategies of how to interfere with vascular disease in women due to estrogen deficiency.

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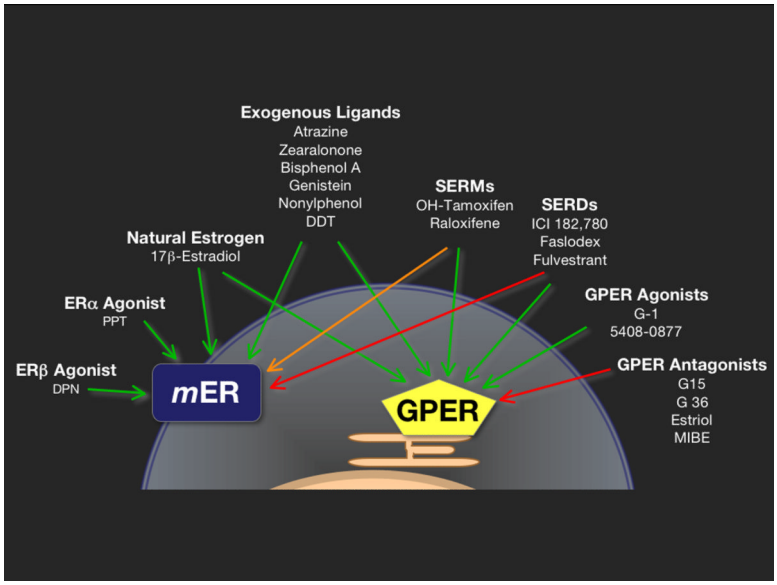

Figure 1.

Agonists and antagonists of membrane-associated subpopulations (*m*ER) of ERα and ERβ, as opposed to GPER with intrinsic activity mediating or inhibiting rapid estrogen signaling. Green arrows: activation, red arrows: inhibition, and the orange arrow: tissue-dependent activation or inhibition. Effects can be achieved by natural (endogenous estrogen such as 17β-estradiol) as well as by synthetic drugs (selective agonists for ERα, ERβ, or GPER), SERMS, SERDs, plant-derived substances (genistein), or highly stable environmental pollutants and xenoestrogens (atrazine, zearalonone, bisphenol A, nonylphenol, or DDT). SERM, selective estrogen receptor modulator; SERD, selective estrogen receptor downregulator. Figure modified from *Steroids* 2012; 77:935-942. M. Barton: Position paper: The membrane estrogen receptor GPER--Clues and questions, with permission of Elsevier Publishers.