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Review

Mechanisms of Estrogen Effects on the Endothelium: An Overview

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ABSTRACT

In this review, we aim to provide an overview of the recent advances in understanding estrogen effects on the vascular endothelium. Epidemiological studies suggest the female sex hormone estrogen mediates the relative protection of premenopausal women against cardiovascular disease, compared with age-matched men. However, results from clinical trials of exogenous estrogen supplementation in postmenopausal women have been disappointing, generating much controversy about the role of estrogen and demonstrating the need for further research in this field. Here we have discussed the roles of different estrogen receptors (ERs) such as ERα, ERβ, and G-protein coupled receptor 30; the complex genomic and nongenomic signalling pathways downstream to ER activation and the factors such as age, menopause, pregnancy, and diabetes that might alter estrogen responses. The common themes of this discussion are the complexity and diversity of endothelial estrogen responses and their modulation by 1 or more coexisting factors. Finally, we summarize the emerging therapeutic options including improved targeting of individual ERs and signalling pathways that might maximize the therapeutic potential of estrogenic compounds while minimizing their harmful side effects.

RÉSUMÉ

Dans cette revue, notre voulons donner un aperçu des récents progrès en matière de compréhension des effets de l'œstrogène sur l'endothélium vasculaire. Les études épidémiologiques montrent que l'œstrogène, une hormone sexuelle femelle, intervient dans la protection relative des femmes préménopausées contre la maladie cardiovasculaire comparativement aux hommes appariés selon l'âge. Cependant, les résultats des essais cliniques sur la supplémentation en œstrogènes exogènes chez les femmes postménopausées se sont avérés décevants, suscitant une importante controverse sur le rôle de l'œstrogène et démontrant la nécessité de réaliser d'autres recherches dans ce domaine. Ici, nous avons discuté des rôles des différents récepteurs des œstrogènes (ER : estrogen receptor) comme le ERα, le ERβ et le récepteur 30 couplé aux protéines G; les voies de signalisation génomique et non génomique complexe en aval de l'activation des ER et les facteurs comme l'âge, la ménopause, la grossesse et le diabète qui pourraient modifier les réponses aux œstrogènes. Les thèmes communs de cette discussion sont la complexité et la diversité des réponses de l'endothélium aux œstrogènes et leur modulation par 1 facteur coexistant ou plus. Finalement, nous résumons les options thérapeutiques émergentes incluant l'amélioration du ciblage individuel des RO et des voies de signalisation qui pourraient maximiser le potentiel thérapeutique des composantes œstrogéniques tout en minimisant leurs effets secondaires dangereux.

Inflammatory changes in the vascular endothelium underlie the pathogenesis of atherosclerosis and its complications such as myocardial infarction and stroke, which are the leading causes of death and disability worldwide.[1] Premenopausal women are relatively protected against cardiovascular diseases compared with age-matched men.[2] This sex-specific vasoprotection is often attributed to higher circulating levels of the sex hormone estrogen.[3] Estrogen, especially 17-β-estradiol (E2), its common form in the body, is widely believed to exert protective effects on the vascular endothelium, yet the molecular mechanisms underlying such protection remain incompletely understood.[4] Various cellular and animal studies have suggested a range of potential benefits of estrogen on the endothelium including generation of nitric oxide (NO) and prostacyclins, mediating vasorelaxation, promoting endothelial repair and/or regeneration, and anti-inflammatory and antioxidant effects.[3-8] The clinical evidence, in contrast, has been decidedly mixed. Early evidence from the purely observational Nurses' Health Study suggested beneficial effects of exogenous estradiol supplementation as part of hormone replacement therapy (HRT) in perimenopausal women.[9] In

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contrast, results of the randomized Women's Health Initiative (WHI) trials demonstrated deleterious effects of exogenous estradiol with significantly increased morbidity and mortality because of myocardial infarctions, stroke, and thromboembolism, giving rise to much skepticism about estrogen therapy.[10] However, the participants in the WHI study were postmenopausal women (with a large range of years after menopause) which likely affected the clinical outcome.[11] Indeed, a recent meta-analysis suggests that although most observational studies involve relatively younger women in the perimenopausal period, most randomized clinical trials have enrolled older women giving rise to the 'timing hypothesis' which suggests the loss beneficial HRT effects at a more advanced age.[12]

In the light of such contradictory evidence, it is essential to re-evaluate the roles of estrogen on the vascular endothelium. This review on the mechanism of endothelial estrogen actions will focus on estrogen receptors (ERs), their associated signalling pathways, factors modifying the estrogen responses, and novel therapeutic opportunities.

ERs in the Endothelium

The effects of estrogen, especially E2, the common form in the body, are mediated through its specific receptors, which can exert distinct responses through genomic, nongenomic, and combined pathways depending on the specific subtype and subcellular distribution.

Classical ERs

Although the presence of a specific ER was suggested according to results of estrogen binding studies on animal tissue in the 1960s, the first ER (now known as ERα) was cloned only in 1985.[13] In 1996, another nuclear ER was identified which was then named as ERβ with the previously identified receptor becoming ERα.[14] Until recently, estrogen effects were believed to be entirely dependent on these 2 classical receptors. ERα (NR3A1, 66 kDa) and ERβ (NR3A2, 57 kDa) are nuclear steroid hormone receptors with similarities in their ligand binding and nuclear DNA-binding regions (reviewed in Arnal et al.[8] and Luksha and Kublickiene[15]).

Work from several different research groups, including our own findings, suggest that ERα and ERβ are expressed in the human vascular endothelium.[16,17] However, some studies suggest a greater preponderance of ERβ in a few vascular beds such as internal mammary arteries.[18] Although classical ERs are typically intracellular, there is increasing evidence for the presence of both receptors on the endothelial cell surface.[19] The beneficial vascular effects of estrogen appear to be mostly mediated through ERα rather than ERβ.[15,20]

Although most research on vascular ERs has focused on female subjects, there is evidence of endothelial ER expression in men although its specific role is less clear.[21] Data from several studies suggest a vasoprotective effect of classical ER agonists in men and other studies show a more limited effect, possibly because of higher oxidative stress and lower estradiol levels in men.[22-24] However, a recent study by Villablanca et al. has shown testosterone-stimulated estradiol generation in the male murine endothelium, suggesting a novel pathway involving in situ estrogen synthesis and consequent endothelial ER activation in an autocrine/paracrine manner.[25]

G-protein coupled ER

In addition to the classical ERs, there is increasing evidence for the involvement of a novel G-protein coupled receptor (GPCR) in mediating estrogen functions. G-protein coupled receptor 30 (GPR30) was originally identified as an orphan GPCR in the late 1990s.[26] A seminal study by Revankar et al. identified GPR30 to be an ER in 2005.[27] Since then, there has been an explosion of studies regarding the expression and functional significance of GPR30 in various tissues, *in vivo* and *in vitro*.[28] Several groups have shown the presence of GPR30 protein in rodent endothelium where it appears to exert NO-dependent vasorelaxation and anti-inflammatory effects.[29,30] Similar to classical ERs, GPR30 is also expressed in the vascular endothelium of male and female rodents and exerts vasorelaxant effects albeit to a lesser extent in male animals likely because of lower receptor levels.[31,32] Recent work from our laboratory has identified GPR30 protein in human endothelial cells, which is found in the nucleus and contributes to anti-inflammatory functions.[33]

Estrogen Signalling Pathways

On binding and activating 1 or more of its 3 known receptors, estrogen activates a range of signalling pathways in different tissues, an overview of which is given later in text.

Classical genomic signalling

The most widely studied aspect of estrogen signalling involves classical genomic signalling through ERα and ERβ. In resting cells, both of these classical ERs exist as monomers in the cytoplasm. Estrogen can diffuse through the plasma membrane and reach these ERs, whereupon they bind to estrogen, undergo conformational change, and dimerize into homo- or hetero-dimers. The estrogen-bound receptor dimers then migrate into the nucleus, bind to the estrogen response elements of target genes through their respective DNA-binding domains, and initiate transcription of said genes (reviewed in Smiley and Khalil[20]). ERα and ERβ have similar but not entirely identical effects on gene expression profiles.[34]

Several factors can alter the outcome of classical genomic signalling including additional regulatory domains in the ERs (for example, activation function [AF]-1 and AF-2 regulating ERα transcriptional abilities), the presence of receptor homodimers vs heterodimers and associated epigenetic changes.[15] Interestingly, it has been suggested that a novel 46 kDa splice variant of ERα can antagonize the transcriptional effects of the 66 kDa 'full' form ERα, thereby adding another level of complexity to classical ER signalling.[35]

Nongenomic signalling by classical ERs

Although Szego and Davis had originally suggested rapid nongenomic actions of estrogen in 1969, it is only recently that the significance of these pathways have been understood.[36] Although many such responses are still dependent on the classical ERs, the shorter time frames involved indicate the likelihood of nongenomic signalling pathways rather than classical (gene) transcriptional ones. Indeed, nongenomic signalling can be elicited by ERα and ERβ, affecting various endothelial responses (reviewed in Deschamps et al.,[37] Kim and Bender,[38] Watson et al.,[39] and Wu et al.[40]).