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ESTRADIOL: MECHANISM OF CARDIORENAL PROTECTION

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Abstract

Premenopausal women have a decreased incidence of cardiovascular disease and a decreased rate of progression of renal disease. With the onset of menopause, however, decreased synthesis of 17β-estradiol (estradiol) is accompanied by an increased incidence of cardiovascular disorders and accelerated progression of renal diseases. The glomerulus and the vascular wall are not static, and components of these structures dynamically increase, decrease, or reorganize in response to physiological and pathological stimuli. Although multiple cellular and biochemical processes are involved in glomerular and vascular remodelling, glomerular mesangial cells (GMCs) in the kidney and smooth muscle cells (VSMC) and endothelial cells in the vasculature are the final common pathway for dynamic changes in glomerular and vascular wall structure. Estradiol may induce protective effects on the renal and cardiovascular system by altering VSMC, GMC, or endothelial cell biology so as to prevent glomerular and vascular remodelling. The purpose of this review is to provide an overview of the participating mechanisms in this regard, as well as the mechanism of estradiol signalling in cardiomyocytes, with an emphasis on mechanisms that might be important in cardioprotection.

Key words: *estrogens, premenopause, vascular wall, cardiomyocytes.*

Introduction

Estradiol (E_2) influences cell growth and differentiation of the male and female reproductive tissues. For example, estradiol regulates the development of mammary glands, uterus, vagina, ovary, testes, epididymis, and prostate and also plays an important role in the vascular system, a system that is essential to reproductive processes (Rosselli et al., 2000). Findings in the last decade indicate that estradiol induces its biological effects via genomic and non-genomic mechanisms and that the effects of estradiol are triggered by estrogen receptor-dependent as well as estrogen receptor-independent mechanisms (Dubay & Jackson, 2001). Estrogen receptors (ER_s) are found in myocardial, vascular smooth muscle cells (VSMC), and endothelial cells in both humans and animals (Bernelot et al., 2012). In VSMC, immunoreactive ER_s have been observed in both cytoplasm and nuclei, especially in the perinuclear region. A significant association between the number of ER_s and normal endothelial cell function has been reported, and suggested that decreased number of endothelial ER_s may represent a risk factor for cardiovascular diseases (Amal et al., 2006). Also, the sex hormone receptors appear to have different subtypes, tissue distribution, and subcellular location and can be modulated by various agonists and antagonists. Two ER_s subtypes have been identified, ER-α and ER-β (Reid, Denger, Kos, & Gannon, 2002). Some studies suggest that ER-α promotes the protective effects of estrogen in response to vascular injury. Differential expression of ER-α and ER-β is observed in some tissues, which suggest different physiological roles for these receptors. In this regard, compared with ER-α, high amounts of ER-β mRNA are in fetal ovaries, testes, adrenals, and spleen of the midgestational human fetus (Brandenberger, Tee, Lee, Chao & Jaffe, 1997). In addition to the classic ERs, another ER, termed type II ER, exists (Markaverich & Gregory, 1991). Some ligands for type II ER, such as bioflavonoids, have no affinity for ER-α or ER-β yet abrogate the effects of estradiol on cell growth, suggesting that, in addition to ER-α and ER-β, the biological effects of estradiol may be modulated via type II ER. The type II ER may also be involved in inducing the effects of estradiol in the vasculature and the kidney. Apart from the cytosolic/nuclear ERs, estradiol also binds with high affinity to membrane fractions prepared from isolated pituitary and uterine cells (Revankar, Cimino, Sklar, Arterburn & Prossnitz, 2005. The functional role of the membrane receptors for estradiol is evident from the findings that estradiol stimulates adenylyl cyclase activity in membranes prepared from secretory human

endometrium Moreover, estradiol induces rapid changes in intracellular calcium levels/flux, K^+ conductance, and cAMP levels.

The effects of estrogens on vascular tone

Although estradiol induces vasoprotective effects by multiple mechanisms, including alterations in plasma concentrations of lipoproteins (decrease in low-density lipoprotein - LDL) levels, decrease in oxidized LDL formation, increase in high-density lipoprotein levels), haemostatic factors, glucose, and insulin, the two most important effects of estradiol in the cardiovascular system are modulation of vascular tone and inhibition of vascular growth. Increased vascular tone is associated with cardiovascular disease, and increased vascular tone is in part due to decreases in endothelium-dependent and endothelium-independent vasodilation. Estradiol induces vasodilator effects on the vasculature via both genomic and nongenomic mechanisms that cause generation of vasodilator agent (such as nitric oxide -NO, cGMP, cAMP, adenosine, and prostacyclin) and alterations in ion channel activity) (Dubay & Jackson, 2001). In contrast to estradiol, little is known regarding the effects of endogenous estradiol metabolites on vascular tone. Generation of vasodilator agents may mediate the vasodilatory effect of 2-hydroxyestradiol. For instance, 2-methoxyestradiol, a methylated product of 2-hydroxyestradiol, induces NO synthesis in cultured bovine carotid artery endothelial cells (Zacharia, Jackson, Gillespi & Dubey, 2000). Because both catecholestradiols and catecholamines share catechol-*O*-methyltransferase (COMT) for t for their metabolism, interactions of these compounds at COMT, may play an important role in determining the effects of these molecules on the cardiovascular system. In this regard, norepinephrine, epinephrine, and isoproterenol inhibit the metabolism of 2-hydroxyestradiol to 2-methoxyestradiol. Moreover, these catecholamines abrogate the antimitogenic effects of estradiol and 2-hydroxyestradiol, but not 2-methoxyestradiol, on VSMC and cardiac fibroblast growth (Zacharia, Jackson, Gillespi & Dubey, 2000a). This suggests that catecholamines block the antimitogenic effects of both estradiol and 2-hydroxyestradiol by inhibiting their metabolism to 2-methoxyestradiol. Estradiol has been shown to induce protective effects on the kidneys. However, whether endogenous metabolites of estradiol are involved remains unclear. 2-hydroxyestradiol and 2-methoxyestradiol inhibit mitogen-induced proliferation and collagen synthesis in human glomerular mesangial cells (Xiao, Gillespie, Baylis, Jackson, & Dubey, 2001) and provide evidence that these antimitogenic effects are ER_s independent. Some of the vasodilatory effects are mediated through indirect actions exerted through the endothelium. The vascular endothelium plays an important role in mediating the gender-related and the estrogen-induced vasodilation. It is known to release relaxing factors such as NO, prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF), as well as contracting factors such as endothelin (ET-1) and thromboxane A_2, and estrogen appear to induce vascular relaxation by modifying the synthesis/release/bioactivity of one or more of these factors. Total NO production is greater in premenopausal women than in men. The cellular origin of the increased NO in women is not entirely clear, but differences in vascular endothelial NO production may underlie the gender differences in vascular tone. Estrogen appears to be responsible for the gender differences in endothelial NO release. Estrogen-stimulated NO production in endothelial cell caveola. Estrogen binds to endothelial surface membrane ER and increases the formation of inositol 1,4,5-trisphosphate (IP_3), which stimulates Ca^{2+} release from the endoplasmic reticulum. Ca^{2+} forms a complex with calmodulin (CAM), which in turn binds to and causes initial activation of eNOS, its dissociation from caveolin-1, and its translocation to intracellular sites. Estrogen may also activate phosphatidylinositol 3-kinase (PI_3K), leading to transformation of phosphatidylinositol- 4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which could activate MAPK. ER-mediated activation of MAPK pathway causes phosphorylation of cytosolic eNOS and its second translocation back to the cell membrane where it undergoes myristoylation and palmitoylation, a process required for its full activation. Activated eNOS promotes the transformation of l-arginine to l-citrulline and the production of NO, which diffuses through the endothelial cell caveola and causes VSMC relaxation (Orshal & Khalil, 2004). In the nongenomic pathway, estrogen binds to endothelial surface membrane ERs, which are coupled to increased Ca^{2+} release from the endoplasmic reticulum and stimulation of MAPK/Akt pathway, leading to activation of eNOS and increased nitric oxide (NO) production. NO diffuses into the VSM cells, binds to guanylate cyclase (GC), and increases cGMP. cGMP causes VSM relaxation by decreasing (Ca^{2+}), and the myofilament sensitivity to Ca^{2+}. ER may also inhibit the production of NADPH, thereby preventing the inactivation of NO and the formation of peroxynitrites ($ONOO^-$). Endothelial ER may also activate cyclooxygenases (COX) and increase PGI_2 production. PGI_2 activates prostacyclin receptors in VSM, activates adenylate cyclase (AC),

and increases the formation of cAMP. cAMP causes VSM relaxation by mechanisms similar to those activated by cGMP. ER may also increase the production of endothelium-derived hyperpolarizing factor (EDHF), which activates K^+ channels and causes hyperpolarization and inhibition of Ca^{2+} influx via Ca^{2+} channels leading to VSM relaxation. Prostacyclin (PGI_2) is also an endothelium-derived relaxing factor that is produced from the metabolism of arachidonic acid by the enzyme cyclooxygenase (COX). COX has two isoforms, COX-1 and COX-2. Estrogen may augment the production of COX products such as PGI_2 (Bobik, 2012). The endothelium may release other relaxing factors even during complete inhibition of the NO-cGMP and the PGI_2-cAMP pathways. Such factors have been shown to activate Ca^{2+}-activated K^+ channels (BK_{Ca}) and to cause hyperpolarization and relaxation of the smooth muscle and thereby designated EDHF. The greater endothelium-mediated relaxation in females compared with males may be related to differences in the endothelium-dependent hyperpolarization of VSMC. Estrogen-deficient states attenuate relaxation transduced by EDHF. The gender differences in vascular tone may be related to differences in the release of or sensitivity to endothelium-derived contracting factors (EDCF) such as ET-1 and thromboxane A_2. ET-1 release from endothelial cells appears to be reduced in females and may explain the decreased vascular tone and blood pressure in female compared with male. The gender difference in ET-1 production by endothelial cells may be related to the plasma levels of estrogen. ET-1 is known to interact with ET_A and ET_B receptors. The interaction of ET-1 with ET□ and ET□ receptors in VSMC activates signaling mechanisms of smooth muscle contraction. In addition to the nongenomic effects of sex hormones on the endothelium, rapid nongenomic effects on VSM have been described.For example, estrogen causes vasodilation in endothelium-denuded vessels, suggesting that the estrogen-induced inhibition of vascular tone has an endothelium-independent component that involves direct action on VSMC. Also, estrogen causes relaxation in endothelium-denuded rabbit, porcine, and human coronary arteries precontracted by ET-1, $PGF_{2\alpha}$, and high KCl depolarizing.The vasodilator effects of estrogen do not appear to be mediated by the classic cytosolic-nuclear ER or stimulation of protein synthesis, but rather through a direct effect of estrogen on plasmalemmal receptors in VSMC. In the genomic pathway, estrogen binds to cytosolic/nuclear ER, leading to inhibition of growth factor (GF)-activated MAPK and gene transcription, and thereby inhibition of VSMC growth and proliferation. In the nongenomic pathway, estrogen binds to plasma membrane ER_s, leading to inhibition of agonist-activated mechanisms of VSMC contraction. An agonist activates a specific receptor, stimulates membrane phospholipase, and increases the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates Ca^{2+} release from the sarcoplasmic reticulum. Also, the agonist stimulates Ca^{2+} entry through Ca^{2+} channels. Ca^{2+} binds CAM, activates myosin light chain (MLC) kinase, causes MLC phosphorylation, and initiates VSMC contraction. DAG causes activation of protein kinase C (PKC). PKC could phosphorylate calponin (CaP) and/or activate a protein kinase cascade, mitogen-activated protein kinase (MAPK), leading to phosphorylation of caldesmon (CaD) and an increase in the myofilament force sensitivity to Ca^{2+}. Possible effects of estrogen include activation of K^+ channels, leading to membrane hyperpolarization, inhibition of Ca^{2+} entry through Ca^{2+} channels, and thereby inhibition of the Ca^{2+}-dependent myosin light chain (MLC) phosphorylation and inhibition of VSMC contraction. Estrogen may also inhibit protein kinase C (PKC) and/or the MAPK pathway and thereby further inhibit VSMC contraction (Orshal & Khalil, 2004).

Cardioprotective effects of estrogens: the role of reactive oxygen species

It is generally agreed that mitochondria are the major source of reactive oxygen species (ROS) generation in mammalian cells. The electron transport chain is an important source of ROS generation. In addition, findings have shown that lipoamide-containing mitochondrial dehydrogenases, such as a-ketoglutarate dehydrogenase(□-KGDH) and pyruvate dehydrogenase (PDH), also are a major source of ROS (Tretter & Adam-Vizi, 2005). Existing data suggest male–female differences in ROS generation (Roy, Cai , Felty & Narayan, 2007). It also should be noted that low levels of ROS also can be involved in estrogen signaling. Borras et al.(2003) reported less H_2O_2 generation and higher levels of antioxidants such as manganese-superoxide dismutase (MnSOD) in mitochondria from female liver and brain. Lagranha et al. (2010) showed that □□KGDH in permeabile female mitochondria generated significantly less ROS than in male mitochondria under conditions of elevated nicotinamide adenine dinucleotide (NADH), which occur during ischemia and early reperfusion. Furthermore, female mitochondria generated less ROS than male mitochondria after anoxia and reoxygenation. Lagranha et al. also found that together with the increase in phosphorylation of aldehyde dehydrogenase ($ALDH_2$) in females, an increase in the activity of $ALDH_2$ also was found in females. A number of studies have suggested a role for nitric oxide in the

cardioprotection experienced by females (Lin, Steenbergen, Murphy, & Sun, 2009). Nitric oxide is generated by several isoforms of the enzyme nitric oxide synthase (NOS). Nitric oxide can signal via activation of guanylyl cyclase or via a posttranslational modification such as S-nitrosylation (SNO), the covalent attachment of an nitric oxide moiety to a protein cysteine group (Sun & Murphy, 2010). Elevated nitric oxide levels via NOS are suggested to enhance mitochondrial biogenesis, which is reported to be higher in females (Stirone, Duckles, Krause, & Procaccio, 2005). Increased NOS and mitochondrial biogenesis are reported to play a role in increased longevity. The mechanisms responsible for the sex differences in ROS and nitric oxide metabolism are yet to be elucidated. In general, protection in females is attributed to estrogen mediated signaling mechanisms. Estrogen can bind to two nuclear receptors (estrogen receptor-alpha and estrogen receptor-beta). Estrogen binding to these estrogen receptors can alter gene expression (via classical nuclear receptormediated mechanisms), or alternatively, estrogen binding to an estrogen receptor localized to the plasma membrane can acutely activate the PI3K pathway. Estrogen also can activate a G-protein-coupled receptor (GPR30), which was recently shown to bind estrogen (Murphy et al., 2011). GPR30 is present in the heart and that a selective GPR30 activator, G1, results in cardioprotection via activation of the PI3K- and ERK signaling pathway. Activation of GPR30 also is reported to reduce hypertension. Thus, two important issues need to be considered regarding estrogen signaling: estrogen can bind to three different receptors, and these associations can lead to alterations in protein levels over the long term, initiate acute signaling pathways, or both. In turn, the estrogen can alter levels of proteins and signaling pathways, leading to posttranslational modifications that can alter protein activity. Estrogen is known to alter the levels of a number of proteins in the heart, including eNOS. This NOS increase in female myocytes is via altered gene expression. Nuedling et al. (2001) reported that estrogen receptor-beta is responsible for the increase in eNOS levels in myocytes. In addition, estrogen via acute signaling pathways can lead to activation of the PI3K pathway as well as phosphorylation and activation of eNOS. Thus estrogen can lead to both an increase in the NOS level and a further increase in the activity of NOS.

Conclusion

It is apparent that there is an array of factors contributing to the greater incidence of cardiovascular disease in postmenopausal women compared with premenopausal women. One contributing factor is the effect of estrogens in the regulation of vascular tone generally and protection of the cardiomyocytes. Numerous studies have suggested both genomic and nongenomic effects of estrogens on the endothelium, VSMC and on the cardiomyocytes but there are yet many unanswered questions.

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