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PERSPECTIVE

Estrogen: A Mitochrondrial Energizer That Keeps on Going

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ABSTRACT

Estrogens demonstrate vasoprotective activity in many experimental models. These effects have been attributed to beneficial activity of these steroids on lipid metabolism as well as direct effects on the vasculature via modulation of nitric-oxide synthase and phosphatidylinositol-3 kinase/Akt signaling pathways. In this issue of *Molecular Pharmacology*, Stirone et al. (p. 959) present evidence suggesting that 17β-estradiol may also exert vasoprotective effects in cerebral blood vessels via stimulation of mitochondrial energy production capacity and inhibition of reactive oxygen species production. These data indicate not only yet another potential mechanism underlying the vasoprotective effects of estrogens but also that the estrogen receptor may coordinate gene expression in both the nuclear and mitochondrial genomes.

The estrogen steroid hormones are commonly recognized for their well characterized role in regulation of female reproductive function. 17β-Estradiol and estrone, the predominant estrogens in humans, along with progesterone, are essential in control of the menstrual cycle and maintenance of pregnancy. In addition to the classic reproductive actions of estrogens, these ovarian steroids modulate physiological functions in diverse systems, such as the musculoskeletal, gastrointestinal, immune, neural, and cardiovascular systems. The varied effects of estrogens are mediated by estrogen receptors expressed in these tissues. The two estrogen receptors, ERα and ERβ, are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. These intracellular receptors contain DNA-binding domains that recognize specific DNA sequences, known as estrogen response elements, within the promoters of target genes. Docking of the steroid to the carboxyl-terminal ligand-binding domain induces a conformational change within the protein that leads to its activation. This conformational change produces novel surfaces on the ligand-binding domain allowing for recruitment of various transcriptional cofactors, resulting in activation of target gene transcription and hence altered cellular and physiological function.

The average life expectancy of women is significantly longer than that of men, a fact that has been attributed to the decreased risk of vascular disease women have during their reproductive years. As ovarian steroid levels decrease during menopause, the risk of vascular disease increases to the point that the "protective" effect is completely lost a decade after the onset of menopause. A variety of experimental models established estrogens as critical mediators of this protection, and the effect seems to be multifaceted. Estrogens have been demonstrated to inhibit atherosclerosis, an effect that is correlated with their well characterized ability to decrease low-density lipoprotein cholesterol and increase high-density lipoprotein cholesterol. In addition, estrogen has direct effects on the vessel wall decreasing vascular resistance via reduced vascular tone, an effect that is mediated through targeting both the endothelial and vascular smooth muscle cells (White, 2002).

Several pathways suggesting vascular effects of estrogen have been extensively studied and include ER regulation of 1) the nitric-oxide synthase (NOS) pathway, 2) norepinephrine (NE) synthesis in the hypothalamus, 3) the Akt pathway via nongenomic action mediated by its interactions with the p85 subunit of phosphatidyl inositol-3 kinase (PI-3 kinase)

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ABBREVIATIONS: NOS, nitric-oxide synthase; NE, norepinephrine; E2, 17β-estradiol; ER, estrogen receptor; PI-3, phosphatidyl inositol-3; TNF, tumor necrosis factor; eNOS, endothelial NOS; ROS, reactive oxygen species; COX, cytochrome oxidase; ICI 182,780, faslodex; ET, estrogen therapy; HT, hormone therapy; CEE, conjugated equine estrogen; MPA, medroxyprogesterone acetate.

(Martin et al., 2000; Simoncini et al., 2000; Nuedling et al., 2001; Peng et al., 2003). The link between estrogen signaling and nitric oxide levels can be approached by several direct and indirect mechanisms. The best-characterized direct regulation was reported on the *NOS III* gene, wherein an Sp1 element was shown to mediate the estrogen-mediated increase in *NOS III* transcription (Kleinert et al., 1998). In addition, it has been shown that estrogen can block the activity of the TNF-α promoter (An et al., 1999) and that prolonged TNF-α presence, a hallmark of the postmenopausal status in women, leads to decreased endothelial NOS activity, which is reversed by the TNF-α blocker Ethanercept (Arenas et al., 2005). Peng et al., (2003) have reported elegant studies evaluating the role of norepinephrine synthesis as measured by the levels of NE metabolite 3-methoxy,4-hydroxyphenylglycol in the hypothalamus of the spontaneously hypertensive rat model. In an estrogen-depleted state, they demonstrated an increase in arterial pressure in female spontaneously hypertensive rats that was coincident with a decrease in 3-methoxy,4-hydroxyphenylglycol levels. Consistent with this hypothesis, forearm vasoconstrictor responses to NE were attenuated after estrogen supplementation (Sudhir et al., 1996). Finally, Simoncini et al. (2000) have shown that mice treated with estrogen show increased endothelial NOS (eNOS) activity and decreased vascular leukocyte accumulation, after ischemia, in a reperfusion injury model. This vascular protective effect of estrogen was abolished in the presence of PI-3 kinase or eNOS inhibitors, implicating a nongenomic pathway involving direct interaction between the p85 subunit of PI-3 kinase and ERα in the cytoplasm of the endothelial cells, ultimately leading to increased eNOS activity.

In this issue of *Molecular Pharmacology*, Stirone et al. (2005b) describe yet another potential mechanism underlying the observed vasoprotective effects of estrogen. This group previously demonstrated expression of ERα and regulation of the PI-3 kinase / Akt and eNOS pathways by E2 in cerebral blood vessels (Stirone et al., 2002, 2005a). Recent reports indicating expression of ER in mitochondria (Monje and Boland, 2002; Chen et al., 2004a,b; Yang et al., 2004) and evidence implicating mitochondrial dysfunction and reactive oxygen species (ROS) in the etiology of vascular disease (Ballinger et al., 2000; Lesnefsky et al., 2001; Ramachandran et al., 2002; Ballinger, 2005; Madamanchi et al., 2005) led these investigators to examine the role of E2 on mitochondrial function in cerebral blood vessels. Using ovariectomized female rats, it was demonstrated that E2 replacement led to increased expression of key mitochondrial proteins, including cytochrome *c*, and subunits I and IV of cytochrome oxidase (COX). In addition, functional assays of mitochondrial citrate synthase and COX activity indicated that E2 treatment results in significantly increased mitochondrial function. With increased mitochondrial energy capacity comes concern of an increased ROS production; however, Stirone et al. (2005b) show that E2 treatment induces expression of manganese superoxide dismutase and decreases hydrogen peroxide production from isolated cerebral blood vessel mitochondria. Thus, these data suggest that vasoprotection by E2 is mediated, at least in part, by modulation of mitochondrial function, specifically increased capacity for energy production and decreased ROS production.

Many questions remain as to the mechanism by which E2 regulates mitochondrial function. Stirone et al. (2005b) show that ERα is localized to both the nucleus and mitochondria and coordinates the expression of both nuclear and mitochondrial genes. These effects were clearly ER-mediated, in that the induction of cytochrome *c* was inhibited by the ER antagonist ICI 182,780, but were not affected by PI-3 kinase or NOS inhibitors, suggesting that the effects were completely independent of E2 modulation of these signaling pathways. One hint of how E2 may be controlling expression of the nuclear-encoded mitochondrial genes is the observation of induction of nuclear respiratory factor-1 (NRF-1), a key transcription factor responsible for regulating the expression of an array of nuclear encoded mitochondrial genes. However, it remains unclear whether ER regulates NRF-1 or any of the nuclear-encoded mitochondrial genes expression directly. The possibility of regulation of mitochondrial encoded genes by ER is more intriguing. The fact that ERα was found in the mitochondria suggests it may be playing some direct role in regulation of gene expression within the organelle. Limited data indicating the presence of estrogen response elements within the regulatory regions of genes in mitochondrial DNA (Demonacos et al., 1996) and induction of mitochondrial genes by E2 in addition to subunit I of COX described in the current study supports this possibility (Chen et al., 2004b). However, the role of ER in the mitochondria has been met with considerable controversy and additional studies will be required to resolve its role within this organelle.

The identification of an additional mechanism potentially mediating vasoprotective effects of estrogen is particularly timely given relatively recent clinical evidence questioning the validity of the protective effect in women. Estrogen therapy (ET) or hormone therapy (HT; combinations of an estrogen(s) and a progestin) are commonly prescribed to peri- and postmenopausal women for a variety of sequelae associated with estrogen deficiency, including vasomotor flushes (hot flashes), urogenital atrophy, and decreased bone mineral density. The assumed reduction in vascular disease, including coronary heart disease and stroke, was generally considered to be an additional benefit of this therapy. However, recent clinical trials have provided results that question the benefits of ET/HT on vascular disease. The Women's Estrogen for Stroke Trial (WEST) demonstrated that ET offers no protection against cerebrovascular disease in women with a history of stroke (Viscoli et al., 2001). A considerably larger study, the Women's Health Initiative (WHI), was principally designed to assess the effect of ET and HT on coronary heart disease in postmenopausal women. In 2002, the HT (combined conjugated equine estrogens plus medroxyprogesterone) versus placebo component of the trial was unexpectedly discontinued after it was determined that there was an increased incidence of vascular events and breast cancer in the HT group (Rossouw et al., 2002). The overall health risks surpassed the observed benefits noted in the HT group, which included reduced risk of fracture (Rossouw et al., 2002). Later, in 2004, the ET component of the WHI trial was also interrupted based on data indicating increased risk for cerebrovascular events associated with treatment (Anderson et al., 2004). Based on the outcome of these trials, it has been recommended that HT not be used to prevent vascular disease and should only be used for short durations for treatment of menopausal symptoms (http://www.nhlbi.nih.gov/whi). However, recent clinical data contradicting ex-

perimental data supporting estrogen's vasoprotective role creates a conundrum.

Why are the overwhelming experimental animal data indicating a vasoprotective role for estrogen at odds with recent clinical data indicating either no beneficial effect or even a detrimental effect? One obvious issue is the identity of the estrogen(s) used. Whereas the most commonly used estrogen in animal studies is E2, the WHI study used conjugated equine estrogen (CEE), which is the most commonly prescribed estrogen HT component in the United States. CEE contains a number of estrogenic steroids, many of which are sulfated esters. Several of the estrogens within CEE are not well characterized in terms of their pharmacology and thus cannot be directly compared with a single estrogenic steroid such as E2. Distinct estrogens as well as combinations of estrogens within the CEE mixture may exhibit a unique array of activities. Another essential pharmacological difference that may confound the comparison is the type of progestin used in the HT combination. Although progesterone is the primary physiological progestin in humans, the WHI used medroxyprogesterone acetate (MPA). Like CEE, MPA was chosen because it is the most prescribed replacement. However, MPA displays unique pharmacological properties, including significantly greater activity at the glucocorticoid receptor than progesterone (Bamberger et al., 1999) and the ability to antagonize the beneficial effects of E2 in models of coronary vasospasm and neuroprotection in which progesterone exhibited beneficial effects (Miyagawa et al., 1997; Nilsen and Brinton, 2003). Thus, the distinct pharmacological profiles of the various hormone replacement regimens are a plausible explanation for the mystery; however, considerable basic and clinical research is required to resolve this issue.

Even for experimental models in which the vasoprotective effects of estrogen are accepted, the precise mechanism is still unclear and is likely to involve multiple pathways. The observation that mitochondrial function is regulated by E2 adds additional complexity to this field and leaves several fascinating questions to be resolved. The relative contribution of the mitochondrial effects of estrogen to vasoprotection as well as the mechanism by which ER coordinates the regulation of both nuclear and mitochondrial genome are two essential areas that will certainly be explored in the future.

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