CARDIOVASCULAR DISEASE is a leading cause of death, the rate of which is greater in men compared with age-matched premenopausal women (1). However, the long-standing hypothesis that estrogen may contribute to this sex-based difference in disease presentation by providing “vascular protection” has recently come under fire. The controversy that estrogen protects against cardiovascular disease arose because results of randomized clinical trials conducted in postmenopausal women using conjugated equine estrogen in combination with medroxyprogesterone acetate for secondary (Heart and Estrogen/progestin Replacement Study, Ref. 14) and primary (Women’s Health Initiative, Ref. 27) prevention failed to show reductions in cardiovascular endpoints (2, 3, 4, 6, 11, 18). Explanations of why these differences exist between epidemiologic studies and randomized trials need to be explored mechanistically, including the timing for initiation of estrogen treatment and the type and mode of hormonal treatment used (12), as the majority of evidence provided from the basic science literature indicates that estrogen treatments initiated shortly after ovariectomy of experimental animals reduces vascular response to injury, i.e., provides vascular protection (5). Such protection is likely to occur in males as well as females, as in humans genetic variance in one estrogen receptor (ER), ER-α, is associated with increased atherosclerosis and adverse outcomes in men (23, 24).

Receptors for estrogen have been identified in all components of the vascular wall (endothelium, smooth muscle, adventitial cells). In endothelial cells, several mechanisms are identified by which estrogen, through ligation of its receptor or independent of its receptor, modulate gene transcription, mRNA stability, translation, and posttranslational protein function (for review, see Ref. 21). However, less is known about estrogenic regulation in vascular smooth muscle cells. The paper by Kappert et al. in this issue (16a) adds important information to fill this gap in our knowledge by providing evidence that estrogen and its metabolite methoxyestradiol reduce PDGF receptor (PDGFR)-activated migration and proliferation of cultured aortic smooth muscle cells derived from male rats by inhibiting the downstream signaling activity of rac-1 (Fig. 1). This inhibition is signaled through ERs without affecting PDGFR expression/phosphorylation or consecutive binding of receptor-associated Src homology region 2-containing signaling molecules such as Src homology region 2-containing phosphatase-2, PLC-γ, phosphatidylinositol 3-kinase, RasGAP, and p85. Because proliferation and migration of vascular smooth muscle cells are two processes needed for development of myointimal thickening after vascular injury, identification of cellular processes that limit these processes such as inhibition of Rac1 GTPase provides information about how estrogen could limit vascular disease and potential targets for new therapeutic interventions.

PDGFR is a tyrosine kinase that in porcine aortic smooth muscle cells induces migration and proliferation but requires phosphorylation and activation of p38 and p42/44 MAPKs. These effects were inhibited by pretreatment with estrogen (17β-estradiol) through ER-β (10). In mouse aortic smooth muscle cells, estrogen reduced the growth by downregulation of PDGFR and limited activation of ERK (9). Growth factors activate ER-α-mediated gene expression in vascular cells through the AF-1 domain of ER-α but not by activation of the MAPK pathway (17). Therefore, the results of the Kappert study (16a) identify an important, yet perhaps redundant, pathway by which estrogen modulates PDGF-activated cell processes.

rac-1 is a small (21 kDa) member of the Rho family of proteins/GTPase. Estrogen downregulates rac-1 mRNA, protein, and activity in cells derived from female animals including that required for angiotensin II-induced NADPH oxidase activity and reactive oxygen production, ovariectomy-induced increases in expression in smooth muscle of spontaneously hypertensive rats, and expression in mononuclear cells derived from women with ovarian hyperstimulation (19). Because estrogen treatment affected the rac-1 pathway in cells derived from male animals, the study provides important insights into how estrogen affects vascular function in males as well as females (25).

Another exciting observation from the study of Kappert et al. (16a) is that the estrogen metabolite methoxyestradiol was also efficacious in inhibiting rac-1. This observation confirms that metabolites of estrogen also have protective activities and suggests that metabolism of 17β-estradiol within vascular tissue activates molecules that bind ERs, perhaps modulating the threshold, duration, and amplitude of the estrogen signal (8, 10). The results of the Kappert study (16a) add to the growing evidence that estrogen can protect against vascular disease by modulating multiple vascular signaling molecules. The results also suggest that estrogenic protection against vascular disease is likely to be influenced by both the timing of estrogen treatment and the type of hormonal treatment used. The results also suggest that estrogenic protection against vascular disease is likely to be influenced by both the timing of estrogen treatment and the type of hormonal treatment used. The results also suggest that estrogenic protection against vascular disease is likely to be influenced by both the timing of estrogen treatment and the type of hormonal treatment used. The results also suggest that estrogenic protection against vascular disease is likely to be influenced by both the timing of estrogen treatment and the type of hormonal treatment used. The results also suggest that estrogenic protection against vascular disease is likely to be influenced by both the timing of estrogen treatment and the type of hormonal treatment used.

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Fig. 1. Schematic of pathways involved in estrogenic modulation of vascular smooth muscle migration and proliferation. Akt, protein kinase B; COMT, catechol-O-methyl transferase; CYP450, cytochrome P-450; E2, 17β-estradiol; ER, estrogen receptor; HE2, hydroxyestradiol; 2-ME, 2-methoxyestradiol; PDGFR, PDGR receptor; +, activation; − inhibition; ?, unknown mechanism.
20, 22). These data also pose the question as to how genetic variation in enzymes required for estrogen metabolism affects individual response to estrogen or susceptibility for cardiovascular disease, that is, polygenomic (receptors, enzymes) variation in estrogenic responses.

When considering the integrated physiological actions of estrogen, it is important to keep in mind that intact animals, including humans, estrogen could affect the production of PDGF as well as its receptor downstream signaling mechanisms. For example, with ovariectomy content of PDGF in platelets increases, which sustains proliferation of cultured smooth muscle cells in female pigs (3, 16). Therefore, with an estrogen-replete condition, both decrease in production of PDGF and inhibition of PDGFR-ligated signaling cascades would act to reduce and/or limit smooth muscle migration and proliferation in response to injury.

Several key questions arise from the study of Kappert et al. (16a). Importantly, it is not clear that the receptor-ligated effects of estrogen are mediated by ER-β. Although no changes in expression of ER-β were reported under the conditions of their experiments, identification of ER-α and its regulation remain to be determined, as ER-α may be more prominent in smooth muscle of males than females (13). In addition, are the effects of estrogen metabolites mediated through the same receptors? Another interesting issue is whether and how caveolin participates in regulation of ER, PDGFR, and rac-1 (7). Caveolin is a 21- to 24-kDa integral membrane protein that modulates residual signaling molecules in membrane invaginations called caveolae. Estrogen treatment increases caveolin-1 mRNA and protein expression in cultured bovine endothelial cells and rat vascular smooth muscle cells (15, 26). However, modulation of caveolin-1 mRNA and protein by PDGF and subsequent activation of rac-1 expression and activity have yet to be examined.

In summary, the paper by Kappert and colleagues (16a) provides additional mechanistic support to the hypothesis that estrogen provides vascular protection. Importantly, the study identifies the contribution of rac-1, the participation of estrogen receptors (ER, PDGFR, and rac-1) (7). Caveolin is a 21- to 24-kDa integral membrane protein that modulates residual signaling molecules in membrane invaginations called caveolae. Estrogen treatment increases caveolin-1 mRNA and protein expression in cultured bovine endothelial cells and rat vascular smooth muscle cells (15, 26). However, modulation of caveolin-1 mRNA and protein by PDGF and subsequent activation of rac-1 expression and activity have yet to be examined.

REFERENCES