Editors

Gender, Estrogen, and NOS
Cautions About Generalizations

Virginia M. Miller

Evidence from observational studies as well as prospective randomized trials indicates that the incidence of coronary artery disease is less in premenopausal women compared with age-matched men and in postmenopausal women who are using estrogen replacement therapy.1,2 The mechanisms by which estrogen reduces development of cardiovascular disease are multifactorial and, in addition to alterations in lipid metabolism, include actions on all components of the vascular wall (endothelial, smooth muscle, and adventitial cells), neurons, and blood elements (platelets and leukocytes). Changes in production of nitric oxide (NO) have been implicated as one of the cellular biochemical-related pathways regulated by estrogen that may contribute to gender and hormonal differences in the progression of cardiovascular disease.3–9 NO is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). NOS consists of three isoforms: type I, neuronal; type II, inducible; and type III, endothelial/constitutive. In this issue of Circulation Research, García-Durán et al10 add to the accumulating body of evidence suggesting that estrogen directly modulates expression of NOS, in particular the neuronal isoform (type I) in neutrophils. With use of Western blot analysis of protein isolated from neutrophils, levels of neuronal NOS were greater in neutrophils from premenopausal women during the ovulatory phase of the estrus cycle when estrogen is high compared with the follicular phase when circulating levels of estrogen fall. In addition, expression of neuronal NOS increased in neutrophils of postmenopausal women who were using transdermal estrogen replacement (50 mg/d) for 4 months. The range of circulating estrogen over which these changes in neuronal NOS occurred was physiological, between $5 \times 10^{-10}$ and $2 \times 10^{-9}$ mol/L. These observations suggest that changes in circulating levels of oxidized products of nitric oxide in blood of women during different stages of the estrus cycle as well as with estrogen replacement therapy may be derived from cells other than the those of the endothelium.11–14

García-Durán et al10 examined the gender specificity of estrogen on expression control of neuronal NOS using neutrophils from male subjects. In these cells, there was both a time and dose dependency of induction of neuronal NOS by estrogen. This induction was receptor mediated and showed biphasic stimulation with increases in NOS from $10^{-10}$ to $10^{-8}$ mol/L of estrogen and inhibition at higher concentrations. These concentration ranges are similar to those shown to stimulate neuronal NOS isolated from rabbit cerebellum.15 The observation that estrogen can modulate NOS isoform in male cells is consistent with observations of increases in reactive hyperemia, a vascular response mediated by endothelium-derived NOS, in male to female transsexuals.16

As provocative as these results might be, much remains to be learned regarding effects of sex steroid hormones on leukocyte function and how these effects relate to gender differences in expression of cardiovascular or other diseases. Although the kinetics of the response of induction of neuronal NOS was defined for neutrophils isolated from males, the question arises as to whether the kinetic relationship of induction of neuronal NOS might be altered on a background of concentrations of endogenous hormones such as estrogen itself, progesterone, or testosterone, as has been reported for macrophages.17

The physiological significance of modulation of NOS isoforms by estrogen remains to be tested definitively in different models of cardiovascular disease. Although mechanical vascular injury and high-cholesterol feeding are common manipulations in experimental animals to mimic the pathogenesis of cardiovascular disease in humans, few experimental studies are designed to examine relationships between cardiovascular disease and infections such as Chlamydia, cytomegalovirus, endocarditis, syphilis, or human immunodeficiency virus.18–24 Functionally, increased expression of neuronal NOS in male neutrophils correlated with increased production of NO, reduced expression of CD18 antigen, and reduced adhesion of neutrophils to a plastic surface. Decreased neutrophil adherence may not be “beneficial” when neutrophil infiltration is a first-line defense to limit infective processes. With immunological challenge, NO will be induced in cells other than neutrophils, and cytokines will increase expression of type II inducible NOS.25,26 Because NOS is regulated by NO itself, increased production of NO from other isoforms, especially the inducible isoform, may in fact decrease NO production in other cell types independent of the mechanism by which that isoform of NOS may be upregulated by estrogen. Therefore, effects of modulation of NOS isoforms by estrogen should be considered in the context of integrated physiological function.

Expression control of neuronal NOS in male neutrophils seems to be initiated by receptor activation. However, the specific subtype of the estrogen receptor, that is, estrogen receptor α or β, was not identified. Expression of estrogen

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From the Departments of Surgery and Physiology, The Mayo Clinic and Foundation, Rochester, Minn.

Correspondence to Virginia M. Miller, PhD, The Mayo Clinic and Foundation, Departments of Surgery and Physiology, 200 First St SW, Rochester, MN 55905. E-mail miller.virginia@mayo.edu

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receptor β is upregulated in endothelial cells after injury to carotid arteries in male mice. The induction of specific estrogen receptors after injury or infection in other cell types remains to be determined. Estrogen modulates NO-mediated endothelium-dependent responses in experimental animals and in men lacking estrogen receptor α. These observations suggest that estrogen receptors, other than estrogen receptor α, mediate responses to estrogen. Therefore, future experiments directed toward understanding the differential regulation of estrogen receptors on specific cell types should be important for development of therapeutic selective estrogen receptor modulators (SERMs). For example, the SERM raloxifene, which has been approved for treatment of osteoporosis in women, has yet to be tested in men. Cardiovascular effects of raloxifene are beginning to be defined in experimental animals, and favorable changes in serum biochemical markers of cardiovascular risk in postmenopausal women have been reported. Although raloxifene treatment reduced LDL in ovariectomized cynomolgus monkeys, it failed to reduce atherosclerosis in these animals as in cholesterol-fed rabbits. How this SERM affects primary cardiovascular outcomes in women at increased risk for coronary disease awaits results of the RUTH (Raloxifene Use and The Heart) trial. Future experimental studies should address the specificity of SERMs on activation of NOS isoforms related to estrogen receptor affinity and efficacy in leukocytes from both male and female animals.

In summary, the article by García-Durán et al provides an additional mechanism by which estrogen may affect physiological functions, in particular, function of neutrophils. However, caution is needed in extrapolating findings from studies performed on isolated cells to understanding how a particular action of estrogen may be involved in gender difference in the expression of human disease. Five issues should be considered: (1) genome (G); does the effect of a hormone apply to animals of XX and XY characterization, and/or might polymorphisms in expression of hormone receptors alter responsiveness of cells or animals to changes in hormone concentrations? (2) integrated physiology (I); are responses of individual cells to hormones modulated in the whole animal by endogenous hormones or cytokines that could either synergize or functionally antagonize effects of the hormones of specific cells? (3) receptors (R); how are the various subtypes of estrogen receptors modulated, and are the effects of the hormones initiated by receptor activation, genomic or nongenomic? (4) binding affinities of ligands (L) for the receptors; what are the affinities and efficacy for ligand binding in cells of male and female animals, and is this influenced by the endogenous levels of other hormones? (5) specificity of response (S); are the intracellular pathways activated by the hormone specific to a given type of cell? Individual experiments, for example, the one of García-Durán et al., begin to provide answers to important questions of how estrogen affects neutrophils in men and women. However, it is only when the GIRLS are included in design of experiments that results might begin to be related to how estrogen modulates development of infection-associated cardiovascular diseases in men and women.

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References


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