Estrogen-induced contraction of coronary arteries is mediated by superoxide generated in vascular smooth muscle

Richard E. White,1 Guichun Han,1 Christiana Dimitropoulou,1 Shu Zhu,1 Katsuya Miyake,3 David Fulton,1,2 Shaylee Dave,1 and Scott A. Barman1

1Department of Pharmacology and Toxicology, 2Vascular Biology Center, and 3Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, Georgia

Submitted 22 November 2004; accepted in final form 21 May 2005

White, Richard E., Guichun Han, Christiana Dimitropoulou, Shu Zhu, Katsuya Miyake, David Fulton, Shaylee Dave, and Scott A. Barman. Estrogen-induced contraction of coronary arteries is mediated by superoxide generated in vascular smooth muscle. Am J Physiol Heart Circ Physiol 289: H1468–H1475, 2005; doi:10.1152/ajpheart.01173.2004.—Although previous studies demonstrated beneficial effects of estrogen on cardiovascular function, the Women’s Health Initiative has reported an increased incidence of coronary heart disease and stroke in postmenopausal women taking hormone replacement therapy. The objective of the present study was to identify a molecular mechanism whereby estrogen, a vasodilatory hormone, could possibly increase the risk of cardiovascular disease. Isometric contractile force recordings were performed on endothelium-denuded porcine coronary arteries, whereas molecular and fluorescence studies identified estrogen signaling molecules in coronary smooth muscle. Estrogen (1–1,000 nM) relaxed arteries in an endothelium-independent fashion; however, when arteries were pretreated with agents to uncouple nitric oxide (NO) production from NO synthase (NOS), estrogen contracted coronary arteries with an EC50 of 7.3 ± 4 nM. Estrogen-induced contraction was attenuated by reducing superoxide (O2•−). Estrogen-stimulated O2•− production was detected in NOS-uncoupled coronary myocytes. Interestingly, only the type 1 neuronal NOS isofrom (nNOS) was detected in myocytes, making this protein a likely target mediating both estrogen-induced relaxation and contraction of endothelium-denuded coronary arteries. Estrogen-induced contraction was completely inhibited by 1 μM nifedipine or 10 μM indomethacin, indicating involvement of dihydropyridine-sensitive calcium channels and contractile prostanoids. We propose that a single molecular mechanism can mediate the dual and opposite effect of estrogen on coronary arteries: by stimulating type 1 nNOS in coronary arteries, estrogen produces either vasodilation via NO or vasoconstriction via O2•−, nitric oxide; coronary circulation.

THE WOMEN’S HEALTH INITIATIVE (WHI) trials indicate that postmenopausal hormone replacement therapy (HRT) bears a significant health risk, including a 29% increase in coronary heart disease and a 41% increase in stroke (30). However, these findings are in stark contrast to prior basic research studies demonstrating clear beneficial effects of estrogen on the cardiovascular and other systems, which had led to HRT becoming the second most prescribed medication in the United States. Despite the fact that estrogen produces favorable effects on plasma lipid profiles, increases fibrinolysis, and acts as a vasodilator to lower systemic blood pressure and increase organ blood flow (21, 33, 37), HRT has been increasingly discontinued in light of recent clinical trials. The obvious paradox is as follows: How could a hormone that produces such an array of salutary effects promote significant pathology and disease? Despite continued investigation, the cellular and molecular basis for these contradictory and confusing responses to estrogen continues to elude explanation.

Many effects of estrogen are mediated by nitric oxide (NO) via stimulation of nitric oxide synthase (NOS). It is generally believed that the primary target of estrogen in the cardiovascular system is type 3 NOS expressed in endothelial cells (eNOS), yet there are also endothelium-independent effects of estrogen on blood vessels or single vascular smooth muscle (VSM) cells mediated by NO (8, 11, 24, 38, 39). Interestingly, VSM cells express aromatase (i.e., estrogen synthase), indicating that estrogen may act acutely in a paracrine or even autocrine manner within the vascular wall (16). Thus direct action of estrogen on VSM might actually be more important in older women because the vascular endothelium becomes increasingly dysfunctional with age (10, 12, 41). Nonetheless, estrogen’s effects on NO production in VSM cells are often ignored. Our present findings now provide evidence for a mechanism of estrogen action that could, for the first time, shed significant light on the controversy of estrogen and HRT. We propose that both beneficial and harmful effects of estrogen can be mediated by the same biological target, type 1 neuronal NOS (nNOS), expressed in VSM cells. Depending on the microenvironment of this enzyme, estrogen exhibits a “yin-yang” influence on cellular function, producing either vasodilation via NO or vasoconstriction via superoxide (O2•−), a powerful oxidant that can further decrease NO bioavailability). Thus, by acting on a single molecular target, estrogen can produce completely opposite effects in vascular and other target tissues and could thereby induce either physiological or pathophysiological responses.

METHODS

Supply of coronary arteries. We obtained fresh porcine hearts from local abattoirs. We generally obtain hearts from castrated male pigs because females are typically conserved for breeding, but we have observed no sexual dimorphism in responses of arteries to estrogen (11, 38). The left anterior descending coronary artery was excised immediately and placed into ice-cold Krebs-Henseleit buffer solution of the following composition (in mM): 122 NaCl, 4.7 KCl, 15.5 NaHCO3, 1.2 KH2PO4, 1.2 MgCl2, 1.8 CaCl2, 1.15 glucose, pH 7.2. Arteries were kept on ice during transport to the laboratory.

Tension studies of intact coronary arteries. Arteries were placed under a dissecting microscope, and excess fat and connective tissue were removed in ice-cold buffer solution. Two-to-four 5-mm rings were obtained from each left anterior descending coronary artery, and...
prepared for isometric contractile force recordings as described previously (38). To control for possible indirect effects of endothelium-derived vasoactive factors, the endothelium was removed physically by rubbing the intimal surface and tested by observing the absence of acetylcholine-induced relaxation. Rings were mounted on two triangular tissue supports, with one support fixed to a stationary glass rod and the other attached to a force-displacement transducer. Isometric contractions or relaxations were recorded on a PC computer using MacLab software. The tissue bathing solution was the modified Krebs-Henseleit buffer described in Supply of coronary arteries. The solution was oxygenated continuously (95% O2-5% CO2) and maintained at 37°C. Coronary ring preparations were equilibrated for 90 min under an optimal resting tension of 2.0 g, and fresh bath solution was added to the tissue chamber every 30 min to prevent accumulation of metabolic end products. After the initial equilibration, preparations were exposed to maximally effective concentrations of a contractile agonist, e.g., PGF2α, to ensure stabilization of the muscles. Pharmacological inhibitors were allowed to equilibrate with the arteries for at least 30 min before measurement of a complete estrogen concentration-response relationship (1–1,000 nM). The effects of 17α-estradiol, an “inactive” estrogen, were determined to control for possible vehicle artifact. Steroid vehicle was usually 50–75% ethanol, and this stock was diluted to a concentration of no more than 0.1% in the vessel chamber. Cell culture. Human coronary artery smooth muscle cells (HCASMC) were purchased from Clonetics/Cambrex and were grown in phenol red-free smooth muscle growth medium with 5% FBS as described previously (39). Steroid hormones and growth factors were removed from FBS by charcoal stripping. Only short-term cultures (passage 3–5) were employed.

Western blot analysis. Arteries were obtained and prepared as described in Tension studies of intact coronary arteries. The endothelium was removed from some of the arteries by gentle rubbing of the luminal surface with a cotton swab. Tissues were stored in liquid nitrogen until immunoblots were to be performed. Frozen tissues were pooled and pulverized (Fisher Scientific). Protein concentrations were determined by Bio-Rad DC protein assay. ADP-sepharose beads were employed to affinity extract NOS proteins from lysates (3). Purified positive control proteins [nNOS and inducible (i)NOS; pituitary extracts] were purchased from Transduction Laboratories. Proteins were separated on SDS-polyacrylamide gels using a Mini Protean II (Bio-Rad) gel kit according to the manufacturer’s instructions. Proteins were then transferred to Hybond-ECL membrane (Amersham Pharmacia Biotech) using a Mini-Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) at 100 V for 1 h. Blots were blocked with 5% nonfat milk overnight at 4°C. The membrane was then rinsed with Tris-buffered saline-TWEEN (TBST) three times for 15 min and two times for 5 min. Blots were then probed with primary antibodies (nNOS, eNOS, or iNOS; 1:1,000; BD Transduction Labs) in TBST containing 1% nonfat milk protein for 1 h. After being washed, the membrane was then incubated with anti-rabbit IgG conjugated to horseradish peroxidase and visualized with an enhanced chemiluminescence system (Amersham).

Fluorescence studies. The cell-permeable form of the O2− fluorescence indicator dihydroethidium (DHE) was employed to examine production of O2− within coronary myocytes. Cells were loaded with the fluorescent indicator during a 45-min incubation with 2 μM DHE (diluted from a 10 mM stock in DMSO) in Krebs solution (see Supply of coronary arteries) at 37°C. After the incubation, cells were washed with Krebs solution and placed on the stage of a Zeiss confocal laser microscope. A perfusion chamber was created by the addition of silicon grease to form in- and outflow chambers at each end of the coverslips inverted onto spacers glued to a slide. Drugs were then added to the cells, and fluorescence was measured. Cell imaging was conducted on a Zeiss 510 NLO laser scanning microscope operating in the confocal mode with a ×40 0.85 numerical aperture objective as described previously (20). Quantitative analysis of confocal images was under the control of Zeiss Physiology software.

Statistical Analysis. All data are means ± SE. Statistical significance between two groups was evaluated by Student’s t-test for paired data. Comparison between multiple groups was made by the One-Way ANOVA test, with a post hoc Tukey test performed for differences among data groups. A probability of <0.05 was considered to indicate a significant difference.

RESULTS

As we and others (8, 24, 38) have demonstrated previously, under normal conditions 17β-estradiol produces an endothelium-independent, concentration-dependent relaxation of precontracted arteries (Fig. 1A). For example, 300 nM 17β-estradiol induced an average relaxation of 41.6 ± 2.5% in endothelium-denuded coronary arteries precontracted with 10 μM PGF2α (n = 4 arteries). But in contrast to this well known effect, when arteries were pretreated (20–30 min) with 100 μM of an inhibitory l-arginine analog, e.g., Nω-monomethyl-l-arginine (l-NMMA), Nω-nitro-l-arginine methyl ester (l-NNAME), or Nω-propyl-l-arginine (l-NPA), to “uncouple” NO production from NOS and reduce NO bioavailability, estrogen now induced a concentration-dependent contraction of endothelium-denuded coronary arteries (Fig. 1B). Treating “NOS-uncoupled” arteries with the same concentrations of 17α-estradiol (a biologically less active steroid) had no effect on tension (n = 3 arteries; Fig. 1B), thus controlling for potential effects of vehicle (0.05% ethanol) or possible nonspecific steroid effects. After estrogen-induced coronary contraction had reached maximum levels, we found that reducing O2− reversed this response. Estrogen-induced contraction was reversed by either 1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol, SOD mimetic, 70.3 ± 9%; n = 4 arteries; Fig. 1B) or 10 mM 4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt (Tiron, O2− scavenger, 70.0 ± 9.5%; n = 8 arteries; data not shown), suggesting that O2− mediated the contractile response of estrogen.

The dependency of estrogen-induced vascular contraction on O2− was further demonstrated by observing no response to estrogen when O2− accumulation was prevented in coronary arteries. As predicted, estrogen had no effect on “NOS-uncoupled” arteries that had been pretreated with either 1 mM Tempol (0% increase in tension, n = 3 arteries, data not shown) or 10 mM Tiron (0% increase in tension, n = 4 arteries; Fig. 2A). In these same arteries, however, further addition of 10 μM PGF2α induced a strong O2−-independent contraction (Fig. 2A). In sum, these experiments indicate that estrogen can either relax or contract coronary arteries depending on whether NOS activity is coupled or uncoupled to NO production, with either NO or O2−, respectively, exerting the predominate influence. A summary concentration-response relationship for estrogen-induced coronary artery contraction is illustrated in Fig. 2B, as is the total lack of a contractile effect when O2− accumulation is prevented (i.e., in the presence of 10 mM Tiron). The calculated EC50 for the contractile effect of estrogen is 7.3 ± 4 nM (n = 10 arteries).

These pharmacological studies on coronary arteries indicate that O2− mediates the ability of estrogen to contract VSM when NO bioavailability is reduced. Additional evidence for this novel mechanism was obtained by observing estrogen-stimulated O2− fluorescence in isolated HCASMC.
Single HCASMC were loaded with the cell-permeable fluorescent probe DHE, which is commonly used to detect generation of O$_2^-$ in VSM (9, 22). The ability of estrogen to stimulate O$_2^-$ production in coronary myocytes is illustrated in Fig. 3. Baseline fluorescence, before and $10\text{ min}$ after addition of 100 μM L-NMMA, is illustrated in Fig. 3A and indicates no spontaneous increases in O$_2^-$ levels in the absence of estrogen over this brief time period (fluorescence intensity: control, 629.5±28; l-NMMA, 691.7±23; n = 6 cell groups; P > 0.12; Fig. 3A). In the absence of l-NMMA, 100 nM estrogen did not affect fluorescence intensity (10–30 min; data not shown). In contrast, in the presence of 100 μM L-NMMA, estrogen increased O$_2^-$ mediated fluorescence significantly after only $10\text{ min}$ (fluorescence intensity: l-NMMA, 688.5±61; l-NMMA + E2, 887.6±68; n = 6 cell groups; P < 0.001; Fig. 3B), with estrogen-stimulated O$_2^-$ production reaching a plateau after $20–30\text{ min}$ (20 min, 985.6±74; 30 min, 1,048±83; n = 6 cell groups). There was no significant increase in O$_2^-$ fluorescence intensity after $20\text{ min}$ (P > 0.05; n = 6 cell groups). These fluorescence studies on single coronary myocytes are entirely consistent with our functional studies on intact coronary arteries, and strongly support our hypothesis that estrogen stimulates O$_2^-$ production in coronary myocytes to contract coronary arteries.

The next series of experiments demonstrated that the biochemical environment of NOS is a critical factor in determining the physiological response of coronary arteries to estrogen. In vitro contractile studies demonstrated that in the presence of only l-NMMA, 300 nM estrogen contracted coronary arteries by an average of 214.2±136% (n = 5 arteries; Fig. 4A). In contrast, when arteries were pretreated with both 100 μM l-NMMA and 1 mM l-arginine (to increase NO availability and attenuate NOS-dependent O$_2^-$ production), 300 nM estrogen now relaxed these non-precontracted coronary arteries by an average of 172.4±48% (n = 6 arteries; P < 0.02 compared with the L-NMMA-only group). In these same vessels, subsequent addition of exogenous O$_2^-$ (i.e., 10 μM pyrogallol, a O$_2^-$ donor) reversed the relaxation effect of estrogen and contracted arteries by an average of 141.5±53% (n = 6 arteries; Fig. 4A). These findings support a critical role for NO activity in mediating effects of estrogen on coronary arteries. Identification of the NOS isoform (i.e., nNOS or eNOS) in coronary arteries was demonstrated by immunoblot studies that detected expression of only the nNOS isoform in coronary artery smooth muscle from either pigs or humans (Fig. 4B). In contrast, both nNOS and eNOS were detected in homogenates (i.e., muscle + endothelium) of porcine coronary arteries (data not shown). Therefore, it is most likely the nNOS isoform that mediates the dual effect of estrogen on coronary smooth muscle.

A final series of experiments was conducted to shed some light on the molecular basis of estrogen-induced coronary artery contraction. The mechanism(s) converting the expected estrogen relaxation response into a O$_2^-$-mediated contractile effect is (are) unknown but would most likely involve an increase in activator calcium in smooth muscle. To test this hypothesis, we placed coronary arteries in a nominally calcium-free solution, uncoupled NOS activity, and then obtained a complete concentration-response relationship for estrogen-induced contraction. In the absence of extracellular calcium, estrogen, at any concentration, had no effect on vessel tension (Fig. 5A; n = 4 arteries), indicating that the contractile response to estrogen did not involve a significant release of calcium from intracellular stores. In contrast, when extracellular calcium was restored to normal (2 mM) levels, vessels...
Estrogen-stimulated NO production from the endothelium and within the vascular wall contributes to beneficial effects of estrogen on the cardiovascular system, as reflected by earlier clinical studies (13, 23, 31) demonstrating that estrogen reduces overall cardiovascular risk by ~50%. In contrast, the more recent WHI trials have reported deleterious effects of postmenopausal estrogen replacement on cardiovascular function (e.g., a nearly 30% increase in coronary heart disease) (30). The reasons for these discrepancies are not apparent. Interestingly, however, the conclusions of the WHI are based solely on findings from older (age range: 50–79; mean age, 63.3) postmenopausal women. One aspect of aging that the WHI did not take into account is the increasing evidence that as a woman ages there is a decline in factors critical for maintaining NOS in the “coupled” (i.e., NO-producing) state. NOS possesses both oxygenase and reductase domains with binding sites for several cofactors, and the disposition of the microenvironment around NOS will determine its primary product. For example, NOS can catalyze an “uncoupled” NADPH oxidation to generate O$_2^-$ rather than NO (1), and O$_2^-$ has been suggested to play a role in age-related increased coronary vascular resistance due to its oxidative potential and ability to further reduce NO bioavailability (10).

There is evidence that the aging process could be associated with increased uncoupling of NOS activity. For example, aging reduces the levels of L-arginine and tetrahydrobiopterin (BH$_4$), both of which are cofactors required to sustain NO production from NOS activity. Serum L-arginine levels and NO production decrease with age (27, 28), which is associated with enhanced oxidative stress on the cardiovascular system (40). Moreover, recent studies (4) indicate that arginase activity is upregulated in the aging vasculature, thus lowering the availability of L-arginine for NOS activity in either endothelium or VSM. Concomitant with age-dependent loss of L-arginine, there is also evidence that BH$_4$ synthesis declines with age (7). BH$_4$, which is essential for generating NO and inhibiting O$_2^-$ production from nNOS (25), is decreased by nearly 30-fold by diet-induced hyperlipidemia (35). Loss of BH$_4$ would increase the tendency to uncouple NOS activity as atherosclerosis progresses to further predispose arteries to oxidative damage. Therefore, it appears increasingly likely that as humans age and atherosclerosis progresses there is reduced NO bioavailability and enhanced oxidative stress. Our findings suggest that a NOS stimulator, such as the estrogen given in HRT, would very likely accelerate the normal age-related decline in cardiovascular function, possibly via enhanced generation of reactive oxygen species (ROS). Not coincidentally, among the three NOS isoforms it is nNOS that has the greatest propensity to increase NO production (18, 34); however, estrogen inhibits NADPH oxidase activity in other

contracted immediately with an average development of 168.4 ± 37 mg tension/mg tissue (n = 4 arteries). As expected, subsequent addition of 10 mM Tiron to scavenge O$_2^-$ reduced calcium-induced contraction significantly (37.0 ± 9.0%; n = 4 arteries; P = 0.002; data not shown). These findings demonstrate that estrogen-induced, O$_2^-$-mediated coronary artery contraction is dependent on influx of extracellular calcium. This mechanism was further substantiated by observing that estrogen-induced contraction (in normal extracellular calcium) was completely attenuated by 1 μM nifedipine, an inhibitor of L-type calcium channels (n = 3 arteries; Fig. 5B), suggesting that estrogen and O$_2^-$ open calcium channels in the plasma membrane of NOS-uncoupled coronary artery myocytes. In addition, the contractile effect of estrogen was prevented by indomethacin, an inhibitor of prostaglandin synthesis. As summarized in Fig. 5B, pretreating coronary arteries with 10 μM indomethacin completely blocked the contractile response to estrogen (n = 3 arteries). These findings suggest that estrogen-generated O$_2^-$ does not act directly on membrane ion channels but instead stimulates production of a vasoconstrictor prosta
glandin.
cell types (32) and decreases NADPH expression in endothelial cells (36). In the present study the response of coronary arteries to estrogen was critically dependent on a L-arginine balance; i.e., in the presence of L-arginine, estrogen relaxed arteries, but the addition of inhibitory L-arginine analogs converted estrogen into a coronary vasoconstrictor (see Figs. 1 and 4A). These findings point strongly to NOS as the source of estrogen-induced production of either $\text{O}_2^\cdot$ or NO in endothelium-damaged arteries. Whereas the present findings certainly cannot rule out other potential sources of estrogen-stimulated $\text{O}_2^\cdot$ production, potential contribution of $\text{O}_2^\cdot$ from NOS-independent sources would seem to play a minor role, if any, in mediating the vascular effects of estrogen in coronary arteries. L-arginine, it appears, is a sine qua non of estrogen-induced coronary relaxation. It seems doubtful that L-arginine would also play such a significant role in preventing $\text{O}_2^\cdot$ generation from NADPH oxidase or xanthine oxidase in the vascular wall. In contrast, L-arginine is highly effective in blunting estrogen-stimulated $\text{O}_2^\cdot$-mediated contraction of coronary arteries. Therefore, we conclude that the most likely molecular target mediating both estrogen-induced relaxation and contraction of endothelium-denuded coronary arteries is NOS expressed in VSM.

It remains unclear as to which NOS isoform could mediate the acute effects of estrogen on VSM. Interestingly, we found L-NPA to be highly effective in converting the “normal” relaxation effect of estrogen into vasoconstriction. Because L-NPA exhibits high (3,000-fold) selectivity for nNOS over iNOS and a 150-fold greater selectivity over eNOS (43), our studies implicate type 1 nNOS as mediating estrogen effects in coronary artery smooth muscle cells. The fact that our studies were performed in either endothelium-denuded vessels or myocytes in culture casts further doubt on eNOS as an estrogen target molecule in our experiments. Furthermore, immunoblot studies detected significant expression of only nNOS protein in coronary artery smooth muscle from either porcine or human vessels (whereas eNOS was detected only in endothelium-intact arteries). Therefore, consistent findings from this combination of functional and molecular approaches strongly suggest that it is the nNOS isoform that functions as novel estrogen target protein in both porcine and human coronary smooth muscle. Previous studies have demonstrated expression

Fig. 3. Estrogen stimulates generation of $\text{O}_2^\cdot$ in NOS-uncoupled, coronary artery myocytes. A: cellular fluorescence images of human coronary artery myocytes loaded with dihydroethidium (DHE, 2 μM; 45 min) to indicate $\text{O}_2^\cdot$ generation. In control (con) experiments fluorescence measurements were taken before and 10 min after treatment with 10 μM Nω-monomethyl-L-arginine (L-NMMA, middle and right, respectively). Mean fluorescence intensity from 6 experiments ± SE is illustrated in bar graph (left). There was no change in fluorescence intensity. B: estrogen-stimulated DHE fluorescence of human coronary artery myocytes. Myocytes were loaded with DHE as above and treated with 10 μM L-NMMA to uncouple NOS activity (middle). Treating same cells with 100 nM 17β-estradiol (E2, 10–30 min) enhanced $\text{O}_2^\cdot$-induced fluorescence. Average effect of estrogen in six experiments ± SE is illustrated in the bar graph (left). *$P<0.001$ indicates a significant increase in fluorescence in estrogen-treated cells. *$P<0.05$ indicates a further significant increase in fluorescence at 20–30 min exposure to estrogen compared with 10-min exposure time. There was no significant change in fluorescence intensity between 20 and 30 min ($n=6$ cell groups).
nary artery dilation (via NO) or constriction (via O_2 molecule, nNOS, can mediate either estrogen-induced coronary artery smooth muscle that maintains flow-induced vasodilation in coronary artery smooth muscle of nNOS in VSM (5, 6, 29), and it is nNOS activity in coronary smooth muscle that maintains flow-induced vasodilation in eNOS-knockout mice (17).

Our findings pose the intriguing possibility that a single molecule, nNOS, can mediate either estrogen-induced coronary artery dilation (via NO) or constriction (via O_2). Fluorescence studies support this hypothesis, as we previously reported estrogen-stimulated NOS activity in HCASMC under normal (i.e., “NOS-coupled”) conditions (39) and now report estrogen-stimulated O_2 generation in NOS-uncoupled HCASMC (Fig. 3B). Therefore, it appears very likely that nNOS expressed in coronary artery smooth muscle influences vascular reactivity, and we speculate that this endothelium-independent mechanism could be especially important in older women with increasingly damaged or dysfunctional endothelium so often seen in aging, atherosclerosis, and/or diabetes (12, 41). In addition, estrogen should also modulate vascular reactivity in men. Aromatase is expressed in VSM (16), thus providing a local mechanism for de novo synthesis of estrogen in the vasculature of both males and females. Our findings now provide a novel molecular mechanism whereby intravascular synthesis of estrogen could impact cardiovascular function in both women and men to produce either physiological or pathophysiological effects.

Estrogen stimulates nNOS activity in hippocampal neurons (15), and early studies suggested beneficial effects of estrogen on brain function; however, WHI findings (26) suggest potentially deleterious consequences of the steroid on global cognitive function in older women. As in the vasculature, it is unclear how estrogen could produce such opposite effects in the brain. Interestingly, Chen et al. (7) have reported a dramatic (81.5%) loss of GTP-cyclohydrolase-I immunoreactivity in brains of aged vs. young humans and other primates. This enzyme is rate-limiting for the synthesis of BH_4, and loss of this co-factor should accelerate NOS uncoupling in the brain and other tissues leading to greater oxidative stress. This model might have important implications on normal cellular function in a variety of systems and explain, at least in part, the controversies concerning the complicated physiology and pharmacology of estrogens, HRT, and aging. Our findings also raise the very intriguing question: If estrogen is indeed a stimulator of oxidative stress in older women, do traditional concepts of menopause need to be reconsidered? In other words, could age-related loss of ovarian function actually be an adaptive mechanism to reduce estrogen-stimulated pathologies due to elevated ROS production as NOS becomes increasingly uncoupled with age?
The fact that estrogen cannot contract arteries in the absence of extracellular calcium or in the presence of nifedipine (with normal extracellular calcium) indicates a dependency on an influx of calcium through dihydropyridine-sensitive calcium channels. In contrast, previous findings (19, 42) suggest that estrogen inhibits calcium channel activity in neurons and A7r5 vascular cells. The present findings, however, indicate a stimulatory effect of estrogen on calcium channels in “NOS-uncoupled” vessels, thereby leading to contraction. Moreover, it is most likely not O₂ that opens calcium channels directly; rather, our results with indomethacin suggest that estrogen and O₂ generate production of a contractile prostaglandin which then induces contraction. Interestingly, we had demonstrated previously that indomethacin also inhibits the contractile effect of another oxidant, H₂O₂, which also can either contract or relax coronary arteries (2). Because O₂ is readily converted to H₂O₂ via SOD, it is possible that the observed contractile effect of estrogen might involve local production of H₂O₂ (or another oxidant species) in coronary artery smooth muscle; however, further studies are needed to identify other ROS and prostaglandins that might play a role in estrogen-induced vascular contraction.

In summary, we propose that estrogen per se is neither “good” nor “bad” in terms of producing physiological effects. Rather, an important action of estrogen is to stimulate NOS activity. It is the disposition of NOS (either coupled or uncoupled) that will then determine which product predominates activity. It is the disposition of NOS (either coupled or uncoupled) that will then determine which product predominates activity. It is the disposition of NOS (either coupled or uncoupled) that will then determine which product predominates activity. It is the disposition of NOS (either coupled or uncoupled) that will then determine which product predominates activity.

ACKNOWLEDGMENTS

We thank Karen Powell and Nancy Godin for technical assistance and Louise Meadows for expertise in performing arterial tension studies. We also recognize the kind cooperation of Thomson Meat Packing, Lanier’s Meat Packing, and Happy Valley Meat Processing companies.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-64779 and HL-07389 (to R. E. White) and HL-68026 (to S. A. Barman) and the American Heart Association Grants 9950179N (to R. E. White) and 0330196N (to D. Fulton).

REFERENCES


