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## Editorial

## Sex and NO — beyond regulation of vasomotor tone

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*See article by Fraser et al. [43] (pages 111–118) in this issue.*

The mechanisms contributing to sex differences in susceptibility to and mortality from cardiovascular and renal disease are still unknown. Sexual dimorphisms of coronary artery disease were first suggested by Heberden in 1802 [1], and researchers have described vascular actions of estrogens since the late 19th century [2–4]. Subsequently, several studies provided indirect and direct evidence for an involvement of estrogens in the regulation of vascular tone [5–9]. Most of the mechanisms were only discovered in the past two decades (reviewed in Refs. [10,11]), following the observation by Furchgott and Zawadzki that vascular smooth muscle tone is regulated by an endothelium-derived relaxing factor [12], later identified as the free radical nitric oxide (NO) [13]. A number of studies suggested protective effects of estrogen on atherosclerosis and related diseases [14,15], and multiple actions of estrogens on vascular homeostasis including modulation of vascular enzymes [16], vasoactive proteins and NO were subsequently identified (reviewed in Refs. [10,11,17]).

NO is a labile gas formed by multi-enzyme complexes, the NO synthases (NOSs) [18–20]. However, these enzymes not only catalyze the generation of NO from L-arginine, but also contribute to the formation of superoxide anion (O<sub>2</sub><sup>-</sup>), which reacts with NO at a diffusion-limited rate to form the potent oxidant peroxynitrite (ONOO<sup>-</sup>) [21–24]. Accordingly, NOSs have been implicated in cardiovascular diseases such as hypertension, atherosclerosis, and heart failure [25]. Three major NOS isoforms have been identified [20]. Initially, NOS isoform expression was thought to be cell-/organ-specific and isoforms were termed accordingly. Meanwhile, it has been

demonstrated that ‘neuronal NOS’ (NOS1) [18], ‘macrophage NOS’ (NOS2) [19], and ‘endothelial cell NOS’ (NOS3) [20] or splice variants of the respective enzymes are expressed in a variety of tissues [26–29], including macrophages [19], kidney, [27,30] skeletal muscle [28], and myocardium [31,32]. The terminology formerly used to indicate calcium-dependency has also been abandoned as NOS3 also exhibits calcium-independent activity under certain conditions [33–35]. In addition, induction of ‘constitutive’ isoforms has been observed (i.e. induction of NOS3 by estrogen) [20,36], and the ‘inducible’ isoform NOS2 is constitutively expressed in certain organs (i.e. the kidney) [27,30]. Interestingly, inhibition of NO synthases may exert detrimental as well as beneficial effects on tissue and organ injury [25,37], which may be related to differential generation of NOS-derived free radicals [21–24].

Reperfusion injury is a complex event altering intracellular homeostasis, cardiomyocyte function, and electromechanical properties of the ischemic and post-ischemic myocardium. Increased oxidative stress as a consequence of ischemia–reperfusion is considered an important factor contributing to these abnormalities, as administration of free-radical scavengers attenuates reperfusion injury [37,38]. Calcium-independent NOS2 contributes to myocardial injury in vitro [39] and has been recently implicated in myocardial dysfunction, reperfusion injury and apoptosis in vivo [40,41]. On the other hand, NOS2 participates in the protective effects of ischemic preconditioning [31,42]. The relative contribution of NOS2-derived NO, O<sub>2</sub><sup>-</sup>, ONOO<sup>-</sup>, or nitroxyl anion (NO<sup>-</sup>) [38] to these differential effects remains to be determined.

Using the Langendorff model, Fraser et al. now report that 17β-estradiol, a natural estrogen with vasodilating properties [10], increases the activity of calcium-independent but not calcium-dependent NO synthase in left ventricular myocardium after ischemia and reperfusion in ovariectomized rats as measured by the citrulline assay [43]. These effects were independent of NOS2 or NOS3

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protein expression and accompanied by increased myocardial cyclic guanosine monophosphate content and improved recovery of left ventricular function after ischemia as compared to untreated controls. These observations are important and point to novel modes of action by which estrogens may improve myocardial function in post-ischemic myocardium.

The findings of Fraser et al. are in contrast with previous studies reporting no effects of 17 $\beta$ -estradiol on calcium-independent NOS activity, but increased calcium-dependent NOS activity as well as expression of NOS1 and NOS3 during pregnancy, a state of high estrogen levels [44–46]. Similar results were obtained using cultured endothelial cells [36,47]. One of the questions yet to be answered is whether the beneficial effects observed by Fraser et al. are due to increased activity of NOS2 or whether they also involve other NOS isoforms expressed in the myocardium [26,31,32]. Interestingly, the promoter region of the NOS2 gene contains a sequence partly resembling an estrogen responsive element [48,49] and several studies indicate that estrogen modulates NOS2 expression and/or activity. Indeed, female but not male NOS2-deficient mice are more susceptible to death due to endotoxemia than their wild-type counterparts, suggesting that estrogen modulates NOS2-mediated protection [50]. Furthermore, it has been demonstrated that 17 $\beta$ -estradiol increases expression of NOS2 through an estrogen-receptor mediated mechanism in rat aortic vascular smooth muscle [49]. In contrast, 17 $\beta$ -estradiol treatment suppresses the expression of NOS2 but elevates endothelial NOS3 expression in transplanted arteries [51], suggesting different mechanisms of action of 17 $\beta$ -estradiol in different disease states.

Another unanswered question is whether the improvement of myocardial function after 17 $\beta$ -estradiol therapy is related to the actions of NO itself such as vasodilation [13], improvement of myocardial perfusion [32], and increased myocardial contractility [52], or whether the beneficial effects are secondary to other mechanisms. As indicated by the authors, increases of myocardial cyclic guanosine monophosphate content may not solely derive from NOS, since other intracellular sources of intracellular cyclic guanosine monophosphate exist. Increases of cGMP may be due to scavenging of  $\cdot\text{O}_2^-$  by 17 $\beta$ -estradiol, which is a potent antioxidant [10,53]. Moreover, NO and/or estrogen may directly interfere with mechanisms downstream of NO generation such as apoptosis [54–57] or protein kinase C activation [37,58], which contribute to ventricular contractility, have been implicated in reperfusion injury and modulation of which may attenuate post-ischemic myocardial injury [57,59].

The observations made by Fraser et al. may have clinical implications. Estrogen replacement therapy in postmenopausal women is associated with a reduction in cardiovascular mortality [11] and increased levels of plasma nitrate/nitrite, an indirect marker of NO pro-

duction [60]. Whether, and by what mechanisms, estrogen replacement therapy also contributes to myocardial protection in postmenopausal women as suggested by the initial observation that women have less angina pectoris than similarly aged men made by Heberden 200 years ago [1] and whether these effects are antagonized by certain progestogens [53,61] requires further study.

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## References

- [1] Bush TL, Miller VM. Effect of pharmacologic agents used during menopause: impact on lipids and lipoproteins. In: Mishell DR, editor, Menopause: physiology and pharmacology, Chicago: Year Book Medical Publishers, 1987, pp. 187–208.
- [2] MacKenzie JN. Irritation of the sexual apparatus. *Am J Med Sci* 1884;87:360.
- [3] Markee JD. Rhythmic vascular uterine changes. *Am J Physiol* 1932;100:32–39.
- [4] Reynolds SRM, Foster FI. Peripheral vascular action of estrogen, observed in the ear of the rabbit. *J Pharmacol Exp Ther* 1940;68:173–184.
- [5] Silva de Sa MF, Meirelles RS. Vasodilating effect of estrogen on the human umbilical artery. *Gynecol Invest* 1977;8:307–313.
- [6] Barton M, Cremer J, Mügge A. 17 $\beta$ -Estradiol acutely improves endothelium-dependent relaxation to bradykinin in human coronary arteries. *Eur J Pharmacol* 1998;362:73–76.
- [7] Gisclard V, Miller VM, Vanhoutte PM. Effect of 17 $\beta$ -estradiol on endothelium-dependent responses in the rabbit. *J Pharmacol Exp Ther* 1988;244:19–22.
- [8] Lieberman EH, Gerhard MD, Uehata A et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med* 1994;121:936–941.
- [9] Mügge A, Riedel M, Barton M, Kuhn M, Lichtlen M. Endothelium-independent relaxation by 17 $\beta$ -oestradiol of human coronary arteries in vitro. *Cardiovasc Res* 1993;27:1939–1942.
- [10] Barton M. Vascular effects of estrogens. Rapid actions, novel mechanisms, and potential therapeutic implications. *Acta Pharmacol Sin* 1999;20:682–690.
- [11] Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *New Engl J Med* 1999;340:1801–1811.
- [12] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;299:373–376.
- [13] Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987;84:9265–9269.
- [14] Barr DP, Russ EM, Eder HA. Influence of estrogens on lipoproteins in atherosclerosis. *Trans Assoc Am Phys* 1952;65:102–113.
- [15] Oliver MF, Boyd GS. Influence of reduction of serum lipids on

- prognosis of coronary heart disease A five year study using oestrogen. *Lancet* 1961;xx:499–505.
- [16] Proudler AJ, Ahmed AI, Crook D, Fogelman I, Rymer JM, Stevenson JC. Hormone replacement therapy and serum angiotensin-converting-enzyme activity in postmenopausal women. *Lancet* 1995;346:89–90.
- [17] Barton M, Lüscher TF. Estrogen and vascular resistance. *Curr Opin Endocrin Diabet* 1999;6:218–224.
- [18] Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991;351:714–718.
- [19] Xie QW, Cho HJ, Calaycay J et al. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* 1992;256:225–228.
- [20] Förstermann U, Boissel JP, Kleinert H. Expressional control of the 'constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). *FASEB J* 1998;12:773–790.
- [21] Xia Y, Dawson VL, Dawson TM, Snyder SH, Zweier JL. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. *Proc Natl Acad Sci USA* 1996;93:6770–6774.
- [22] Xia Y, Zweier JL. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. *Proc Natl Acad Sci USA* 1997;94:6954–6958.
- [23] Vásquez-Vivar J, Hogg N, Pritchard KAJ, Martasek P, Kalyanaraman B. Superoxide anion formation from lucigenin: an electron spin resonance spin-trapping study. *FEBS Lett* 1997;403:127–130.
- [24] Wever RMF, van Dam T, van Rijn HJM, de Groot PG, Rabelink TJ. Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. *Biochem Biochem Res Commun* 1997;237:240–244.
- [25] Beckmann JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. *Am J Physiol* 1996;271:C1424–1437.
- [26] Balligand JL, Kobzik L, Han X, Kaye DM, Belhassen L, O'Hara DS, Kelly RA, Smith TW, Michel T. Nitric oxide-dependent parasympathetic signaling is due to activation of constitutive endothelial (type III) nitric oxide synthase in cardiac myocytes. *J Biol Chem* 1995;270:14582–14586.
- [27] Mohaupt MG, Elzie JL, Ahn KY, Clapp WL, Wilcox CS, Kone BC. Differential expression and induction of mRNAs encoding two inducible nitric oxide synthases in rat kidney. *Kidney Int* 1994;46:653–665.
- [28] Gath I, Closs EI, Gödtel-Armbrust U, Schmitt S, Nakane M, Wessler I, Förstermann U. Inducible NO synthase II and neuronal NO synthase I are constitutively expressed in different structures of guinea pig skeletal muscle: implications for contractile function. *FASEB J* 1996;10:1614–1620.
- [29] Schwarz PM, Kleinert H, Förstermann U. Potential functional significance of brain-type and muscle-type nitric oxide synthase I expressed in adventitia and media of rat aorta. *Arterioscler Thromb Vasc Biol* 1999;19:2584–2590.
- [30] Barton M, Vos I, Shaw S et al. Dysfunctional nitric oxide synthase as a determinant of salt-sensitive hypertension. Mechanisms of renal artery dysfunction and role of endothelin for vascular hypertrophy and glomerulosclerosis. *J Am Soc Nephrol* 2000, in press.
- [31] Dawn B, Xuan YT, Qiu Y et al. Bifunctional role of protein tyrosine kinases in late preconditioning against myocardial stunning in conscious rabbits. *Circ Res* 1999;85:1154–1163.
- [32] Bolli R, Manchikalapudi S, Tang XL et al. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase: evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res* 1997;81:1094–1107.
- [33] Fleming I, Bauersachs J, Fisslthaler B, Busse R. Ca<sup>2+</sup>-independent activation of the endothelial nitric oxide synthase in response to tyrosine phosphatase inhibitors and fluid shear stress. *Circ Res* 1998;82:686–695.
- [34] Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 1999;399:601–605.
- [35] Igarashi J, Thatte HS, Prabhakar P, Golan DE, Michel T. Calcium-independent activation of endothelial nitric oxide synthase by ceramide. *Proc Natl Acad Sci USA* 1999;96:12583–12588.
- [36] Hishikawa K, Nakaki T, Marumo T, Suzuki H, Kato R, Saruta T. Up-regulation of nitric oxide synthase by estradiol in human aortic endothelial cells. *FEBS Lett* 1995;360:291–293.
- [37] Bolli R, Dawn B, Tang XL et al. The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol* 1998;93:325–338.
- [38] Ma XL, Gao F, Liu GL et al. Opposite effects of nitric oxide and nitroxyl on postischemic myocardial injury. *Proc Natl Acad Sci USA* 1999;96:14617–14622.
- [39] Igarashi J, Nishida M, Hoshida S et al. Inducible nitric oxide synthase augments injury elicited by oxidative stress in rat cardiac myocytes. *Am J Physiol* 1998;274:C245–252.
- [40] Oyama J, Shimokawa H, Momii H et al. Role of nitric oxide and peroxynitrite in the cytokine-induced sustained myocardial dysfunction in dogs in vivo. *J Clin Invest* 1998;101:2207–2214.
- [41] Koglin J, Grnaville DJ, Glysing-Jensen T et al. Attenuated acute cardiac rejection in NOS2 -/- recipients correlates with reduced apoptosis. *Circulation* 1999;99:836–844.
- [42] Guo Y, Jones WK, Xuan YT et al. The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene. *Proc Natl Acad Sci USA* 1999;96:11507–11512.
- [43] Fraser H, Davidge ST, Clanachan AS. Activation of Ca<sup>2+</sup>-independent nitric oxide synthase by 17 $\beta$ -estradiol in post-ischemic rat heart. *Cardiovasc Res* 2000;46:111–118.
- [44] Weiner P, Lozaoin I, Baylis SA, Knowles RG, Charles IG, Moncada S. Induction of calcium-dependent nitric oxide synthases by sex hormones. *Proc Natl Acad Sci USA* 1994;91:5212–5216.
- [45] Weiner CP, Knowles RG, Moncada S. Induction of nitric oxide synthases early in pregnancy. *Am J Obstet Gynecol* 1994;171:838–843.
- [46] Goetz RM, Morano I, Calovini T, Studer R, Holtz J. Increased expression of endothelial constitutive nitric oxide synthase in rat aorta during pregnancy. *Biochem Biophys Res Commun* 1994;205:905–910.
- [47] Goetz RM, Thatte HS, Prabhakar P, Cho MR, Michel T, Golan DE. Estradiol induces the calcium-dependent translocation of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 1999;96:2788–2793.
- [48] Chartrain NA, Geller DA, Koty PP et al. Molecular cloning, structure, and chromosomal localization of the human inducible nitric oxide synthase gene. *J Biol Chem* 1994;269:6765–6772.
- [49] Binko J, Murphy TV, Majewski H. 17 $\beta$ -estradiol enhances nitric oxide synthase activity in endothelium-denuded rat aorta. *Clin Exp Pharmacol Physiol* 1998;25:120–127.
- [50] Laubach VE, Foley PL, Shockey KS, Tribble CG, Kron IL. Protective roles of nitric oxide and testosterone in endotoxemia: evidence from NOS-2-deficient mice. *Am J Physiol* 1998;275:H2211–2218.
- [51] Saito S, Aras RS, Lou H, Ramwell PW, Foegh ML. Effects of estrogen on nitric oxide synthase expression in rat aorta allograft and smooth muscle cells. *J Heart Lung Transplant* 1999;18:937–945.
- [52] Kojda G, Kottenberg K, Stasch JP, Schör K, Noack E. Positive inotropic effect of exogenous and endogenous NO in hypertrophic rat hearts. *Br J Pharmacol* 1997;122:813–820.
- [53] Zhu X, Bonet B, Gillenwater H, Knopp RH. Opposing effects of estrogen and progestins on LDL oxidation and vascular wall cytotoxicity: implications for atherogenesis. *Proc Soc Exp Biol Med* 1999;222:214–221.
- [54] Rossig L, Fischlerer B, Breitschopf K et al. Nitric oxide inhibits caspase3 by S-nitrosation in vivo. *J Biol Chem* 1999;274:6823–6826.

- [55] Pike CJ. Estrogen modulates neuronal Bcl-xL expression and beta-amyloid-induced apoptosis: relevance to Alzheimer's disease. *J Neurochem* 1999;72:1552–1563.
- [56] Alvarez RJ, Gips SJ, Moldovan N, Goldschmidt-Clermont PJ et al. 17 $\beta$ -Estradiol inhibits apoptosis of endothelial cells. *Biochem Biophys Res Commun* 1997;237:372–381.
- [57] Holly TA, Drincic A, Byun Y et al. Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. *J Mol Cell Cardiol* 1999;31:1709–1715.
- [58] Studer RK, DeRubertis FR, Craven PA. Nitric oxide suppresses increases in mesangial cell protein kinase C transforming growth factor beta, and fibronectin synthesis induced by thromboxane. *J Am Soc Nephrol* 1996;7:999–1005.
- [59] Tian R, Miao W, Spindler M et al. Long-term expression of protein kinase C in adult mouse hearts improves postischemic recovery. *Proc Natl Acad Sci USA* 1999;96:13536–13541.
- [60] Best PJ, Berger PB, Miller VM, Lerman A. The effect of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. *Ann Intern Med* 1998;128:285–288.
- [61] Heart and Estrogen/progestin Replacement Study (HERS) Research Group, Hulley S, Grady D, Bush T et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *J Am Med Assoc* 1998;280:605–613.