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COMMENTARY

Vascular effects of ovariectomy and chronic oestrogen treatment in rats: controversy or experimental protocol diversity?

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Several clinical studies have indicated that oestrogens have protective properties on the cardiovascular system. Although the beneficial effect has been attributable, at least in part, to their ability to stimulate the endothelial fomation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), the underlying mechanism still remains unclear. In a study from this issue of *British Journal of Pharmacology*, Nawate *et al*. have examined the effects of rat ovariectomy and chronic treatment with 17β-oestradiol on the endothelial function as assessed *ex vivo*. The data indicate that acetylcholine-induced endothelium-dependent relaxations of the isolated mesenteric artery are affected by neither ovariectomy nor chronic hormonal treatment. Despite the maintained endothelium-dependent relaxation, the contribution of the two major endothelial factors NO and EDHF was changed. Indeed, ovariectomy increased the NO-mediated component of the relaxation, most likely as a consequence of the downregulation of the physiological allosteric inhibitor of endothelial NO synthase, caveolin-1. In addition, ovariectomy decreased the EDHF-mediated component of the relaxation and membrane hyperpolarization of the smooth muscle cells, an effect which might be explained by a concomitant decrease of the expression of the gap junction connexin-40 and connexin-43. Furthermore, chronic administration of 17β-estradiol to ovariectomized rats normalized all these effects. This study provides further experimental evidence indicating that the hormonal status plays a determinant role in the control of the endothelial formation of both NO and EDHF.
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Abbreviations: EDHF, endothelium-derived hyperpolarizing factor; hsp 90, heat shock protein 90; NO, nitric oxide

The paper entitled 'Reciprocal changes in endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide (NO) synthase in the mesenteric artery of adult female rats following ovariectomy' (Nawate *et al*., 2005) further investigates the effects of both ovariectomy and chronic treatment with 17β-oestradiol on endothelial function in rats, and in particular on the NO- and the EDHF-dependent component of the relaxation in the mesenteric artery.

The present study indicates that acetylcholine-induced endothelium-dependent relaxations in the mesenteric artery are unaffected by ovariectomy and oestrogen treatment. Despite this absence of effect, the authors performed additional experiments to determine whether the NO component and/or the EDHF component of the endothelium-dependent relaxation were changed. These investigations revealed that ovariectomy increased the NO component of the relaxation as assessed in the presence of charybdotoxin plus apamin and indomethacin to rule out the contribution of EDHF and vasoactive prostanoids, respectively. In contrast to NO, ovariectomy decreased the EDHF component of the relaxation and membrane hyperpolarization of the smooth muscle cells as assessed in the presence of nitro L-arginine and indomethacin to prevent the formation of NO and vasoactive prostanoids, respectively. Both the NO and the EDHF components were restored by chronic administration of 17β-oestradiol to ovariectomized rats.

The present study provides further experimental evidence indicating that the hormonal status plays a determinant role in the control of the endothelial formation of the potent vasoactive factors NO and EDHF, as already observed in the isolated rat mesenteric arterial bed (McCulloch & Randall, 1998), and that this effect appears to be mediated mostly by oestrogens. In addition, this study helps the field to move forward due to the merit of the authors to provide a thorough characterization of the mechanisms controlling the endothelial formation of NO and EDHF in response to changes in the hormonal status. Recent clinical reports on hormone replacement therapy in postmenopausal women have led to serious questioning about the overall benefit of oestrogen–progestin treatments on the cardiovascular system (Grady *et al*., 2002; Writing group for the Women's Health Initiative Investigators, 2002). Therefore, a better understanding of the cardiovascular effects of oestrogens and progestins is urgently warranted. This innovative study identifies novel targets of the action of oestrogens on blood vessels. However, it also provides another piece of controversy of the vascular effects of oestrogen deprivation, at least in animal models.

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The present study shows that acetylcholine-induced NO-mediated relaxation of rat mesenteric artery was enhanced following ovariectomy. Examination of endothelial NO synthase expression in mesenteric arteries has indicated that ovariectomy did not affect its protein level. However, examination of the expression of several allosteric modulators of endothelial NO synthase activity has indicated that the protein level of caveolin-1 was decreased following ovariectomy, whereas those of calmodulin and heat shock protein 90 (hsp90) were unaffected. Thus, downregulation of the physiological inhibitor of endothelial NO synthase, caveolin-1, which leads to an enhanced NO formation in endothelial cells, may account for the increased acetylcholine-induced NO-mediated relaxation in ovariectomized animals. Moreover, the fact that chronic administration of 17β-oestradiol normalized the NO component of the relaxation to acetylcholine and restored the caveolin-1 level suggests that changes in oestrogen levels are major factors controlling the endothelial NO synthase activity and formation of NO *in vivo*. Although the present findings are convincing, they are in contradiction with several previously published studies. Indeed, acetylcholine-induced NO-mediated relaxation in the rat mesenteric artery was unaffected by ovariectomy and chronic treatment with 17β-oestradiol (Liu *et al*., 2001; Sakuma *et al*., 2002; Chataigneau *et al*., 2004) and it was increased or normalized by chronic treatment with 17β-oestradiol in the rat pulmonary artery and aorta (Andersen *et al*., 1999; Squadrito *et al*., 2000; Gonzales *et al*., 2001). Oestrogens also upregulated endothelial NO synthase expression in endothelial cells (for a review, see Chambliss & Shaul, 2002). In addition, ovariectomy was found to cause an increase in caveolin-1 protein level in rat cerebral arterioles (Xu *et al*., 2001).

In addition to the agonist-induced endothelial NO formation, Nawate *et al*. (2005) have also examined the effect of ovariectomy on the basal endothelial formation of NO, which can be estimated indirectly by the potentiation of phenylephrine-induced contractions by a NO synthase inhibitor. The data indicate that ovariectomy markedly attenuated contractions to phenylephrine and that this effect was partially prevented by a specific inhibitor of inducible NO synthase and abolished by a nonspecific inhibitor of NO synthase. The effects of the two NO synthase inhibitors were more pronounced in ovariectomized than in control rats, suggesting a greater basal formation of NO by endothelial and inducible NO synthases. In addition, higher levels of inducible NO synthase protein were observed in mesenteric arteries following ovariectomy. In contrast, previous studies have indicated that ovariectomy caused a marked reduction of basal NO formation in rat and rabbit aorta and rat mesenteric artery (Hayashi *et al*., 1992; Squadrito *et al*., 2000; Liu *et al*., 2001; Chataigneau *et al*., 2004) and that chronic treatment of ovariectomized rats with 17β-oestradiol or 17α-ethinyl oestradiol restored the basal endothelial formation of NO in rat aorta and mesenteric artery (Andersen *et al*., 1999; Squadrito *et al*., 2000; Liu *et al*., 2001; Rahimian *et al*., 2002). Moreover, inducible NO synthase activity was unchanged in lungs from both ovariectomized and 17β-oestradiol-treated ovariectomized rats (Squadrito *et al*., 2000), and 17β-oestradiol induced the expression of inducible NO synthase in the isolated aorta from ovariectomized animals (Binko & Majewski, 1998).

It is now well accepted that gap junctions play a crucial role in EDHF-mediated relaxation and hyperpolarization of vascular smooth muscle cells (Griffith, 2004). Downregulation of the expression of the gap junction connexin-40 and connexin-43 following ovariectomy might contribute to explain the reduced EDHF-mediated hyperpolarization and relaxation observed in the study of Nawate *et al*. (2005). A decreased vascular expression of connexin-43 by ovariectomy has also been observed previously (Liu *et al*., 2002). Altogether, these findings identify connexin-40 and connexin-43 as key elements of the EDHF sensitivity to oestrogens and changes in their expression level might account for the variation of EDHF-mediated responses during the oestrus cycle or after ovariectomy of rats (Liu *et al*., 2001; Sakuma *et al*., 2002). In contrast, previous studies have indicated that ovariectomy did not affect acetylcholine-induced EDHF-mediated relaxation and hyperpolarization in the rat mesenteric artery (Chataigneau *et al*., 2004) and that ovariectomy increased EDHF-mediated relaxation in rat cerebral arteries (Golding & Kepler, 2001). Moreover, a switch from NO to EDHF has been observed in the adenosine diphosphate-induced vasodilation in pial arterioles after ovariectomy in rats (Xu *et al*., 2002).

Altogether, this nonexhaustive review of the literature clearly indicates that ovariectomy is able to cause heterogeneous vascular effects, including either no effect, a stimulatory effect or an inhibitory effect on the basal and stimulated endothelial NO formation and also on EDHF-mediated responses in experimental animals. Such a great diversity of the vascular effects of ovariectomy has already been evoked in a recent review (Félétou & Vanhoutte, 2004) but, up to now, an explanation still remains to be found.

Indeed, it is almost impossible to find a concise explanation for such divergent observations. However, it may be explained, at least in part, by the study of animals at different ages, since aging alters the endothelial function (Van Der Loo *et al*., 2000). It may also be due to the heterogeneity of the vascular bed examined. Indeed, cerebral arteries do not respond to oestrogen deprivation in the same way as peripheral vessels, at least for the EDHF-mediated component of relaxation (Golding & Kepler, 2001; Xu *et al*., 2002; Nawate *et al*., 2005). Alternatively, the use of different experimental procedures and protocols may also contribute. Indeed, different routes of oestrogen administration to the animals (osmotic minipumps, s.c. injections, i.p. injections or gavage), durations of oestrogen treatment (from 1 to 5 weeks) and also doses of oestrogen (from 4 to 100 $\mu g\,kg^{-1}\,day^{-1}$) have been studied.

Despite all these experimental discrepancies, a major and basic question still remains to be answered: are human menopause and hormonal replacement therapy transposable to animal models? In other words, are animal models of ovariectomy and chronic oestrogen substitution suitable for the investigation of the dramatic changes that occur in the blood vessels of menopausal women? Whatever the answer, the findings obtained by the numerous experimental investigations clearly emphasize the importance of the sex hormone status on the control of the vascular endothelial function.

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