Estrogen derivative relaxes rabbit aorta via the endothelial receptor system

Riccardo Raddino, Giovanna Pelà*, Daniela Uberti, Cinzia Portera, Rubina Ferrari, Tiziano Maria Scarabelli**, Terence Jonathan Cooper**, Carlo Manca*

*Department of Cardiology, University of Brescia, Brescia, *Department of Cardiology, University of Parma, Parma, Italy, **Institute of Child Health, University of College, London, UK*

Key words:
Estrogen; Nitric oxide; Rabbit aorta; Tamoxifen; Vascular estrogen receptors.

Background. **It is well known that sexual hormones, in particular estrogens, may influence the cardiovascular system. Experimental and clinical studies have shown that estrogen directly or indirectly modulates the reactivity of vascular smooth muscle but at present the mechanism of action of this hormone has yet to be clarified. The aim of this study was to evaluate the vascular effects of a synthetic non-steroid estrogen, diethylstilbestrol, and the possible involvement of endothelial function.**

Methods. **We investigated, on aortic strips of a female rabbit, the inhibitory effects of diethylstilbestrol on the contractions induced by different spasmogenic agents, noradrenaline (10^{-6} M), angiotensin II (10^{-6} M), serotonin (10^{-6} M), and KCl (10^{-1} M). Some experiments were performed in high K^+, Ca^{++}-free solution. In some experiments endothelial function was abolished by mechanical ablation. Another series of experiments was incubated (30 min) with N^G-monomethyl-L-arginine, which inhibits nitric oxide synthase or with tamoxifen, a specific antagonist of estrogen receptors.**

Results. **At doses from 10^{-6} M to 10^{-4} M, diethylstilbestrol showed an evident spasmolytic action on contractions induced by noradrenaline, angiotensin II and serotonin but no significant effect was observed on KCl spasm. The inhibitory response of diethylstilbestrol to increased vascular tone induced by noradrenaline disappeared when the endothelial function, validated by the acetylcholine test, was abolished by mechanical ablation. When tested in high K^+, Ca^{++}-free solution, diethylstilbestrol did not significantly shift the cumulative dose-response curve of calcium. In the experiments performed with N^G-monomethyl-L-arginine, diethylstilbestrol failed to induce vasodilation suggesting that its action may be related to synthesis of nitric oxide. Moreover, in the presence of tamoxifen, diethylstilbestrol was unable to induce vasodilation.**

Conclusions. **The early occurrence of vasodilation is in favor of a direct effect and seems to exclude a regulation of gene expression. These results suggest that estrogens may directly regulate vascular tone interacting with its specific endothelial cell receptors through the release of nitric oxide.**

(Ital Heart J 2001; 2 (1): 49-54)

Received July 19, 2000; revision received October 2, 2000; accepted October 5, 2000.

Address:

Dr. Riccardo Raddino

Cattedra di Cardiologia
Università degli Studi
Spedali Civili
Piazzale Spedali Civili, 1
25123 Brescia
E-mail:
r.raddino@usa.net

Introduction

An ever increasing number of clinical and experimental observations show that sexual hormones, in particular estrogens, may interfere with the functions of the cardiovascular system. Premenopausal women have a lower incidence of some cardiovascular diseases, such as hypertension, myocardial ischemia, coronary artery disease; however, the incidence of cardiovascular diseases rapidly increases after cessation of ovarian function[1-3].

These observations supported the hypothesis that this loss of protection was due to a deficit of endogenous estrogens. Furthermore, estrogen replacement therapy in postmenopausal women might have protective effects on cardiovascular morbidity and mortality[4-6].

A number of potential mechanisms for the protective effect of estrogen have been proposed. Estrogen has a beneficial effect on plasma lipoproteins[7], and also appears to inhibit experimentally induced atherosclerosis[8]. Other potential protective mechanisms of estrogen action include calcium antagonism[9,10], hormone-induced release of endothelium-derived relaxing factors, and suppression of contracting factors[11-14]. Estrogen improves endothelium-dependent vasodilation either in female monkeys with dietary atherosclerosis and in postmenopausal women with coronary atherosclerosis[15-17]. Some studies hypothesized that estrogen may exert direct effects on vascular cells through its specific receptors[18,19]. How-

ever, the exact mechanism by which estrogen modulates the vascular tone is not fully understood[20,21]. In this study, isolated rabbit aortic strip preparations were used to evaluate the direct effect of estrogens on vascular reactivity and to verify if this effect is mediated by an estrogen receptor system located on the endothelium.

The mechanism of action of the estrogenic response was assessed by using diethylstilbestrol (DES), a synthetic non-steroid estrogen. Tamoxifen, which derives from DES but presents an antiestrogenic activity on estrogen receptors, was used to study receptor-mediated effects.

Methods

Experimental protocol. Adult New Zealand albino female rabbits weighing approximately 2-2.5 kg were used. Rabbits were killed by a blow on the neck and the thoracic aorta quickly removed and placed in a modified Krebs-Henseleit solution containing in mM: NaCl 113, KCl 4.7, $CaCl_2$ 1.9, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, and glucose 11.5.

Afterwards the media was separated from the connective tissue and adventitia and spirally cut to obtain 2 cm long and 3 mm wide strips according to the Furchgott's technique[22]. These helicoidal strips were placed in isolated organ baths (5 ml) containing Krebs-Henseleit solution at 37°C and gassed with 95% O_2 and 5% CO_2 (pH 7.4).

Preparations were suspended under an isometric passive stretch of 2 g and left to equilibrate for 120 min. Tension was measured by means of an isometric transducer connected to a press writing recorder (Unirecord 7050, Basile, Milan, Italy).

Integrity of the endothelium was validated in every strip with the acetylcholine test[22].

After the period of equilibration, the strips were contracted with noradrenaline (10^{-6} M), angiotensin II (10^{-6} M), serotonin (10^{-6} M) and KCl (10^{-1} M). When contractions reached a plateau (usually 5-10 min after administration), DES (from 10^{-7} M to 10^{-4} M) was added according to the cumulative dose method[23].

In another series of experiments dose-response curves with calcium (from 10^{-4} M to 10^{-1} M) were achieved using high K^+, Ca^{++}-free solution, obtained by replacing 80% NaCl with an equimolar concentration of KCl and by omitting calcium[24]. After the first calcium control curve, a second curve was induced in the presence of DES (10^{-4} M) and allowed to act for 30 min.

In another series of experiments the endothelium was ablated mechanically and the absence of endothelium was assessed pharmacologically using the acetylcholine test[22]. In this experiment, the denuded strip preparations were challenged with a single dose of noradrenaline (10^{-6} M) and the cumulative dose-response curves with DES (from 10^{-7} M to 10^{-4} M) were evaluated.

The next step was to test the effects of L-arginine, a compound involved in the production and release of nitric oxide from endothelial cells, and/or of N^G-monomethyl L-arginine (L-NMMA) which has been reported to inhibit nitric oxide release from endothelial cells, added to the bath 30 min before the contraction induced by noradrenaline (10^{-6} M)[25].

In the last series of experiments tamoxifen (from 10^{-7} M to 10^{-4} M), a specific estrogen receptor antagonist, was added to the bath on the contraction induced by noradrenaline (10^{-6} M) and cumulative curve was obtained. Moreover, the aortic preparations were preincubated with tamoxifen (10^{-6} M), added to the bath 30 min before the contraction induced by noradrenaline (10^{-6} M) and challenged with DES (from 10^{-7} M to 10^{-4} M) according to the cumulative dose method.

Drug solubility. Once dissolved in ethanol, DES was added to the bath solution in order to reach the appropriate concentration. The concentration of ethanol in the bath was kept under 0.1% v/v. This concentration was found not to influence vasodilatory responses. Noradrenaline, angiotensin II, serotonin, KCl, L-arginine, L-NMMA, and tamoxifen were dissolved in water.

Statistical analysis. Data are expressed as mean ± SEM of 5-6 observations. The inhibitory response of DES to aortic strips precontracted with different spasmogenic agents was calculated as percent reduction of the maximal contractile force increase (plateau) taken as 100. Analysis of variance was applied when comparison of data was requested and statistical significance was defined for a probability value < 5% ($p < 0.05$).

Drugs. The following drugs were used: DES, tamoxifen, noradrenaline, serotonin, angiotensin II, acetylcholine hydrochloride, L-arginine, L-NMMA (acquired from Sigma Chemical, St. Louis, MO, USA), KCl, and other reagents for buffer solution (acquired from Bracco, Milan, Italy).

Results

The effects of DES on aortic strips precontracted with four different spasmogenic agents are shown in figure 1. Noradrenaline (10^{-6} M) induced a tonic contraction of 1.8 ± 0.6 g, serotonin (10^{-6} M) of 1.4 ± 0.8 g, angiotensin II (10^{-6} M) of 1.6 ± 0.4 g, and KCl (10^{-1} M) of 2.1 ± 0.8 g.

DES inhibited the contractions induced by noradrenaline, angiotensin II and serotonin starting at 10^{-6} M and reaching the maximum inhibitor effect at 10^{-4} M. The contractions induced by noradrenaline, serotonin and angiotensin II were reduced respectively by 54, 48 and 41% whilst no significant effect was observed on KCl-induced spasm.

When tested in high K^+, Ca^{++}-free Krebs-Henseleit solution, DES (10^{-4} M) did not significantly shift the cu-

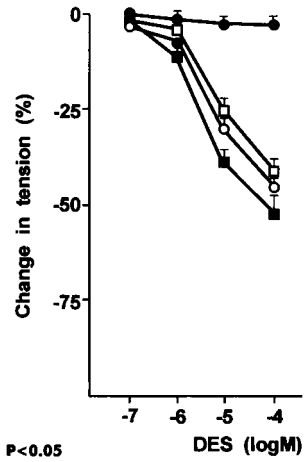

Figure 1. *Dose-dependent relaxing effect of diethylstilbestrol (DES) on rabbit aorta segments precontracted by noradrenaline (■–■), serotonin (○–○), angiotensin II (□–□) and KCl (●–●). Data were calculated as percentages of reduction in maximal contractile force increase. Values represent the mean of 5-6 observations for each spasmogenic agents. Concentrations are expressed as mol/l.*

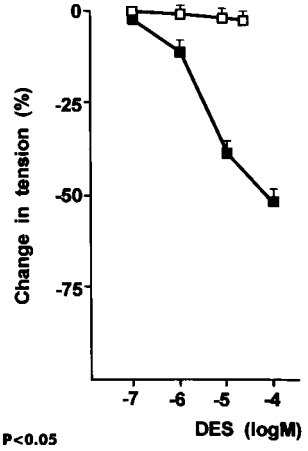

Figure 3. *Dose-dependent relaxing effects of diethylstilbestrol (DES) on contractions induced by noradrenaline (10^{-6} M) on aortic strips with integrity (■–■) of the endothelium and with the endothelium ablated (□–□). Data were calculated as percentages of reduction in maximal contractile force increase. Values represent the mean of 5-6 observations. Concentrations are expressed as mol/l.*

mulative dose-response curve of calcium to the right (Fig. 2). This observation suggests that DES does not interfere with extracellular Ca^{++} fluxes.

When the endothelium was ablated, the relaxant effect of DES on spasm induced by noradrenaline (10^{-6} M) was suppressed (Fig. 3).

Figure 4 shows the effects of DES (10^{-4} M) on contractions induced by noradrenaline on rabbit aortic strips at baseline and in the presence of L-arginine and/or L-NMMA. L-NMMA (0.5 mM), added to the bath 30 min before the contraction induced by noradrenaline (10^{-6} M), abolished the vasorelaxant effect of DES. In the same way, the addition of L-arginine (0.5 mM) alone did not potentiate the endothelium-dependent relaxation of DES. However, L-arginine (1.0 mM) partially reversed the inhibitory effect of L-NMMA (0.3 mM). L-arginine (1.0 mM) and L-NMMA did not exert any significant effect on basal tension (data not shown).

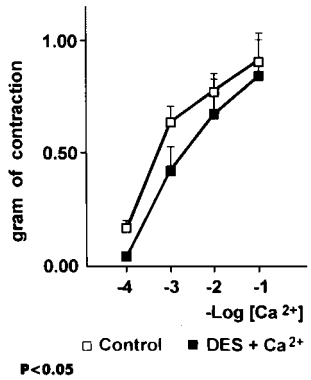

Figure 2. *Rabbit aortic strips: inhibitory effect of diethylstilbestrol (DES) (10^{-4} M) on the Ca^{2+}-induced contraction in high K^+, Ca^{2+}-free solution. Values represent the mean of 5-6 observations. Doses are expressed as mol/l.*

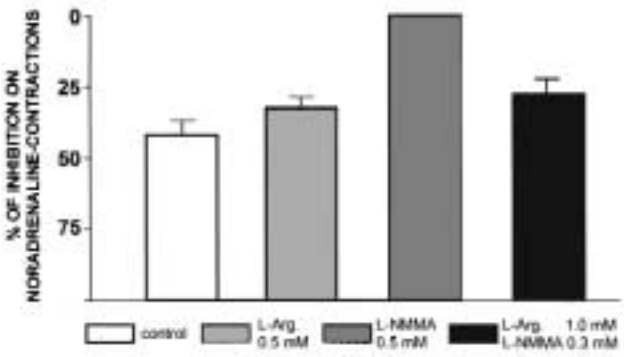

Figure 4. *Percentage of inhibition observed on contractions induced by noradrenaline on aortic rabbit segments in the presence of diethylstilbestrol (10^{-4}M) under control conditions and in the presence of L-arginine (L-Arg), and/or N^G-monomethyl L-arginine (L-NMMA). Data were calculated as percentages of reduction in maximal contractile force increase. Values represent the mean of 5-6 observations. Concentrations are expressed as mmol/l.*

Tamoxifen (from 10^{-7} M to 10^{-4} M) showed no significant effect on the plateau of noradrenaline-induced contraction. Furthermore, tamoxifen added to the bath 30 min before the contraction induced by noradrenaline (10^{-6} M), completely abolished the vasodilatory effect of DES (Fig. 5).

Discussion

In the 1970's DES was used for the treatment of complicated pregnancy; however it has been demonstrated that DES should not be used for any purpose during pregnancy: there is an association between maternal DES therapy and tumor appearance (vaginal and cervical adenocarcinoma and adenosis) as seen in young women exposed during fetal life (Food and Drug Ad-

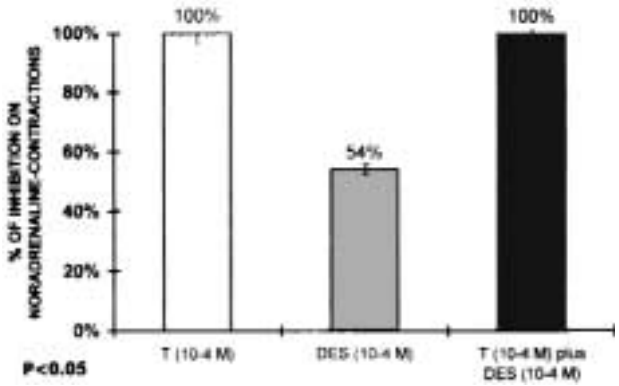

Figure 5. *Percentage of inhibition observed on contractions induced by noradrenaline on aortic rabbit strips in the presence of tamoxifen (T) (10^{-4} M), diethylstilbestrol (DES) (10^{-4} M), and DES (10^{-4} M) plus preincubation with T (10^{-4} M). Data were calculated as percentages of reduction in maximal contractile force increase. Values represent the mean of 5-6 observations. Concentrations are expressed as mol/l.*

ministration Drug Bulletin, 1985). DES has been used as a postcoital contraceptive; however because of the risk of serious adverse effects associated with high dosages of estrogens, DES should only be used rarely or in an emergency (e.g. rape, incest); it should not be used as a routine method of contraception and repeated courses of DES therapy should be avoided.

DES is used for the palliative treatment of advanced, inoperable, metastatic carcinoma of the breast in postmenopausal women and in men; however, because tamoxifen appears to be at least as effective as estrogen therapy in postmenopausal women and causes a lower incidence of severe adverse effects, it is preferred by some clinicians.

In males, DES or DES diphosphate is used for the palliative treatment of advanced carcinoma of the prostate. Hormonal manipulation with estrogens is currently considered a therapy of choice for patients with inoperable prostatic tumors, for patients who refuse orchiectomy and for patients whose disease progresses despite orchiectomy in whom the benefits of estrogen use are considered to outweigh the risk of adverse effects[26].

In the present study we try to state the pharmacological reasons besides the clinical use of the synthetic estrogen DES, considering the structural analogy with natural estrogens.

Numerous clinical and epidemiological studies have shown the protective role of estrogens on the cardiovascular system, but to date the mechanism of this protection is not fully understood[20,21]. A number of potential mechanisms have been proposed[21,27].

Estrogens, as other steroid hormones, act by modulating the transcription for a number of genes: passively spreading through cell membrane they reach the nuclear receptor for estrogen, a protein present in all estrogen-sensitive tissue. After activation, the receptor binds some specific regions of DNA promoting gene transcription.

Recent studies have shown that estrogen is able to induce a rapid improvement in endothelial function, both in patients and in animals affected by atherosclerosis[27-30]. Such effects which can be observed both in the coronary circulation and peripheral district, seem to be mediated by an increased production of nitric oxide[21,27-32]. The molecular mechanism may be dependent on both transcriptional induction of endothelial nitric oxide synthase[33] as well as on acute activation of the enzyme[34].

On the other hand, *in vitro* studies have shown direct effects of estrogens on cardiovascular preparations[10,11,35]. We have previously reported in isolated perfused rabbit hearts an inhibitory effect of 17β-estradiol and DES on coronary basal tone as well as on spasm induced by different spasmogenic agents[36,37]. Other studies have reported direct effects on vascular smooth muscle both through endothelium-dependent[11,13] and endothelium-independent mechanisms[21,27,38,39].

The rapid improvement in endothelial function and the direct effects exerted by estrogens, which cannot be explained with a genomic interaction, seem to be mediated by a receptorial mechanism[21,40]. This supposition might also account for the lack of effect of estrogen on endothelial function, observed in male patients[17].

The role of estrogen receptors is not clear. Nevertheless, specific binding sites for 17β-estradiol have been found in vascular smooth muscle cells including rat aortic smooth muscle cells grown in culture and canine coronary smooth muscle cells[18,19]. Recently the existence of estrogen receptors on the endothelial cells of cultured human umbilical veins and bovine aortas has been shown[41]. These studies have suggested that estrogen may exert a direct effect on vascular cells through its specific receptor whose expression can be up-regulated by the hormone[42].

In order to evaluate the role of estrogen receptors, we used a synthetic non-steroid estrogen, DES, on isolated aortic strips of the rabbit. This study confirms a direct vasorelaxing effect of DES on contractions induced by noradrenaline, serotonin and angiotensin II. On the contrary, DES induced no significant effect on KCl contractions. This finding suggests that this hormone preferentially affects the mobilization of intracellular calcium rather than the influx of calcium from the extracellular milieu, as was documented for KCl[24].

The non-competitive interaction with the calcium curve in the presence of depolarizing solution seems to minimize the supposed interaction of DES with potential-dependent calcium channels[43].

Like acetylcholine, the relaxing effect of DES disappeared when the endothelium was ablated, suggesting that DES, in this district, acts by an endothelium-dependent mechanism.

The finding that the vasodilatory effect of DES was strictly dependent on the presence of endothelial cells justified further investigations. In particular, we verified if the vasodilating effect of DES was related to the synthesis of nitric oxide.

As it is well known, the synthesis of nitric oxide starts from L-arginine. In particular, the activation of the receptorial system implicated in the nitric oxide synthesis pathway leads to a rise in intracellular Ca^{++} which stimulates nitric oxide synthase, resulting in the formation of nitric oxide and citrulline[21,44].

The enzyme is inhibited by an analogue of L-arginine, L-NMMA. Nitric oxide thus formed activates soluble guanylate cyclase, responsible for the cGMP accumulation with subsequent relaxation of vascular smooth muscle[45].

Our experiments showed that the relaxation induced by DES on noradrenaline contractions was abolished by L-NMMA and this abolition was overcome by L-arginine.

The above strongly suggests that endothelial cells can be stimulated by estrogen to synthesize and release nitric oxide. The inhibitory effect induced by tamoxifen, administered 30 min before inducing noradrenaline spasm, seems to suggest that the action of DES on endothelial cells is mediated by specific estrogenic receptors.

Hence, our data show a strict correlation between endothelial function and activity of the estrogen receptor system in the vessels. It is possible to hypothesize that the function of this system is directly influenced by plasma levels of estrogens as observed by other authors who have demonstrated a rapid improvement in endothelial function induced by estrogens[46,47]. These data can improve the knowledge about the pathophysiology of cardiovascular diseases like hypertension and coronary artery disease in postmenopausal women.

The protective effect of replacement therapy with estrogens in postmenopausal women can be explained through an up-regulation of this system[46].

We have observed an increasing incidence of coronary diseases in women after therapy with tamoxifen and this connection needs a more specific epidemiological evaluation.

In conclusion, this work suggests a direct vasorelaxing effect of estrogen on the cardiovascular system. In our experimental condition DES was able to induce vasodilation through a release of nitric oxide from endothelial cells. Our results suggest that this effect is mediated by activation of estrogen receptors probably localized on the endothelium.

The role of the estrogen receptor system has to be elucidated. The activation of such receptors could determine an acute non-genomic activation of nitric oxide synthase as well as the modulation of transcription factors promoting the expression of genes related to endothelial activation.

References

1. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. JAMA 1991; 265: 1861-7.
2. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. N Engl J Med 1987; 316: 1105-10.
3. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease. The Framingham study. Ann Intern Med 1976; 85: 447-52.
4. Stampfer MJ, Colditz GA, Willet WC, et al. Postmenopausal estrogen therapy and cardiovascular disease: ten year follow-up from the Nurses Health Study. N Engl J Med 1991; 325: 756-62.
5. Henderson BE, Paganini-Hill A, Ross RK. Decreased mortality in users of estrogen replacement therapy. Arch Intern Med 1991; 151: 75-8.
6. Grady D, Rubin SM, Petitti DB, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992; 117: 1016-37.
7. Bush TL, Fried LP, Barrett-Connor E. Cholesterol, lipoproteins, and coronary heart disease in women. Clin Chem 1988; 34: B60-B70.
8. Adams MR, Kaplan JR, Manuck SB, et al. Inhibition of coronary artery atherosclerosis by 17-β estradiol in ovariectomized monkeys: lack of an effect of added progesterone. Arteriosclerosis 1990; 10: 1051-7.
9. Han SZ, Karaki H, Ouchi Y, Akishita M, Orimo H. 17β-estradiol inhibits Ca^{2+} influx and Ca^{2+} release induced by thromboxane A_2 in porcine coronary artery. Circulation 1995; 91: 2619-26.
10. Shan J, Resnick LM, Liu QY, Wu X-C, Barbagallo M, Pang PKT. Vascular effects of 17β-estradiol in male Sprague-Dawley rats. Am J Physiol 1994; 266: H967-H973.
11. Collins P, Shay J, Jiang C, Moss J. Nitric oxide accounts for dose-dependent estrogen-mediated coronary relaxation after acute estrogen withdrawal. Circulation 1994; 90: 1964-8.
12. Hayashi T, Fukuto JM, Ignarro LJ, Chaudhuri G. Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits: implications for atherosclerosis. Proc Natl Acad Sci USA 1992; 89: 11259-63.
13. Van Buren G, Yang D, Clark KE. Estrogen-induced uterine vasodilation is antagonized by L-nitroarginine methyl ester, an inhibitor of nitric oxide synthesis. Am J Obstet Gynecol 1992; 16: 828-33.
14. Polderman KH, Stehouwer CDA, van Kamp GJ, Dekker GA, Verheugt FWA, Gooren LJG. Influence of sex hormones on plasma endothelin levels. Ann Intern Med 1993; 118: 429-32.
15. Williams JK, Adams MR, Herrington DM, Clarkson TB. Short-term administration of estrogen and vascular responses of atherosclerotic coronary arteries. J Am Coll Cardiol 1992; 20: 452-7.
16. Herrington DM, Braden GA, Williams JK, Morgan TM. Endothelial-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. Am J Cardiol 1994; 73: 951-2.
17. Collins P, Rosano GMC, Sarrel PM, et al. 17β-estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. Circulation 1995; 95: 24-30.
18. Nakao J, Chang WC, Murota SI, Orimo H. Estradiol-binding sites in rat aortic smooth muscle cells in culture. Atherosclerosis 1981; 38: 75-80.
19. Orimo A, Inoue S, Ikegami A, et al. Vascular smooth muscle cells target for estrogen. Biochem Biophys Res Commun 1993; 195: 730-6.
20. Gerhard M, Ganz P. How do we explain the clinical benefits of estrogen? Circulation 1995; 92: 5-8.
21. Stefano GB, Prevot V, Beauvillain JC, et al. Cell-surface estrogen receptors mediate calcium-dependent nitric oxide release in human endothelia. Circulation 2000; 101: 1594-7.
22. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-6.

23. van Rossum JM. Cumulative dose-response curves. Technique for making the dose-response curves in isolated organs and the evaluation of drugs parameters. Arch Int Pharmacodyn Ther 1963; 143: 299-330.
24. Raddino R, Poli E, Ferrari R, Visioli O. Effects of calcium entry blockers not connected with calcium channel inhibition. Gen Pharmacol 1987; 18: 431-6.
25. Raddino R, Pelà G, Manca C, et al. Mechanism of action of human calcitonin gene-related peptide in rabbit heart and in human mammary arteries. J Cardiovasc Pharmacol 1997; 29: 463-70.
26. Murad F, Kuret JA. Estrogeni e progestinici. In: Goodman Gilman A, ed. Le basi farmacologiche della terapia. Bologna: Zanichelli, 1997: 1371-96.
27. Simoncini T, Genazzani AR, De Caterina R. Towards a molecular understanding of the atheroprotective effects of estrogens: a review of estrogen effects on endothelial activation. Ital Heart J 2000; 1: 104-7.
28. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO III. Acute vascular effects of estrogen in postmenopausal women. Circulation 1994; 90: 786-91.
29. Concina P, Sordello S, Barbacane NA, et al. The mitogenic effect of 17beta-estradiol on in vitro endothelial cell proliferation and on in vivo reendothelialization are both dependent on vascular endothelial growth factor. J Vasc Res 2000; 37: 202-8.
30. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO III. Effects of estrogen replacement therapy on peripheral vasomotor function in postmenopausal women. Am J Cardiol 1995; 75: 264-8.
31. Binko J, Murphy TV, Majewski H. 17 Beta-oestradiol enhances nitric oxide synthase activity in endothelium-denuded rat aorta. Clin Exp Pharmacol Physiol 1998; 25: 120-7.
32. Binko J, Majewski H. 17Beta-estradiol reduces vasoconstriction in endothelium-denuded rat aortas through inducible NOS. Am J Physiol 1998; 274 (Part 2): H853-H859.
33. Mac Ritcjie AN, Jun SS, Chen Z, et al. Estrogen upregulates endothelial nitric oxide synthase gene expression in fetal pulmonary artery endothelium. Circ Res 1997; 81: 355-62.
34. Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PV. Estrogen receptor alpha mediates the non genomic activation of endothelial nitric oxide synthase by estrogen. J Clin Invest 1999; 103: 401-6.
35. White RE, Darkow DJ, Lang JL. Estrogen relaxes coronary arteries by opening BKCa-channels through a cGMP dependent mechanism. Circ Res 1995; 77: 936-42.
36. Raddino R, Manca C, Poli E, Bolognesi R, Visioli O. Effects of 17β-estradiol on the isolated rabbit heart. Arch Int Pharmacodyn Ther 1986; 281: 57-65.
37. Raddino R, Poli E, Pelà G, Manca C. Action of steroid sex hormones on the isolated rabbit heart. Pharmacology 1989; 38: 185-90.
38. Ma L, Robinson CP, Thadani U, Patterson E. Effect of 17-beta estradiol in the rabbit: endothelium-dependent and -independent mechanisms of vascular relaxation. J Cardiovasc Pharmacol 1997; 30: 130-5.
39. Kauser K, Sonnenberg D, Diel P, Rubanyi GM. Effect of 17β-oestradiol on cytokine-induced nitric oxide production in rat isolated aorta. Br J Pharmacol 1998; 123: 1089-96.
40. Joswig M, Hach-Wunderle V, Ziegler R, Nawroth PP. Postmenopausal hormone replacement therapy and the vascular wall: mechanisms of 17 beta-estradiol's effects on vascular biology. Exp Clin Endocrinol Diabetes 1999; 107: 477-87.
41. Hayashi T, Yamada K, Esaki T, et al. Estrogen increases endothelial nitric oxide by a receptor-mediated system. Biochem Biophys Res Commun 1995; 214: 847-55.
42. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. FASEB J 1996; 10: 615-24.
43. Takeda K, Schini V, Stoeckel H. Voltage-activated potassium but not calcium currents in cultured bovine aortic endothelial cells. Pflugers Arch 1987; 410: 385-93.
44. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-6.
45. Murad F, Rapoport RM, Fiscus R. Role of cyclic-GMP in relaxation of vascular smooth muscle. J Cardiovasc Pharmacol 1985; 7: S112-S117.
46. Gerhard M, Walsh BW, Tawacol A, et al. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. Circulation 1998; 98: 1158-63.
47. Virdis A, Ghiadoni L, Pinto S, et al. Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. Circulation 2000; 101: 2258-63.