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Research Article

Vasorelaxant Effect of 17α-Ethynylestradiol on Human Saphenous Vein

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Abstract
Purpose: A protective effect for estrogens against cardiovascular problems has long been known. The aim of this study was to investigate the vasorelaxant effect of 17α-Ethynylestradiol (17α-EE) on human saphenous vein.
Methods: The veins were suspended horizontally between two triangular stainless steel hooks for the measurement of isometric tension in individual organ baths containing 10ml Krebs solution, at 37°C and gassed with carbogen under 3gr optimum tension. The effect of different concentrations of 17α-EE (2-40 μM) on vascular tone was investigated in veins precontracted with $PGF_{2\alpha}$. Relaxation was measured after 40min and expressed as the percent decrease of initial contraction. To determine the involvement of potassium channels, endothelium, nitric oxide synthase, guanylylcyclase and prostaglandins in the vasorelaxant effect of estrogen, the veins were incubated with tetraethyl ammonium, N-nitro-L-arginine methyl ester, methylene blue or indomethacin, respectively for 20min prior to experimentation. Responses to 17α-EE were directly compared to those obtained in the same tissues in the absence of the inhibitors.
Results: The mean relaxations induced by 17α-EE with concentrations of 2, 5, 10, 20 and 40μM in tissues precontracted with $PGF_{2\alpha}$ were 19.8 ±5.5%, 26.1±10.8%, 32.2±7.4%, 48.6±10.8%and56±7.6%, respectively. The results of the inhibition of potassium channels, nitric oxide synthase, guanylylcyclase, cyclooxygenase and removing endothelium in relaxation induced by 17α-EE on precontracted veins with $PGF_{2\alpha}$ proved no significant differences.
Conclusion: This study showed that 17α-EE has significant vasorelaxant effect on human saphenous vein in a concentration-dependent manner. This effect is probably independent of potassium channels, nitric oxide synthase, guanylylcyclase, prostaglandin synthesis and endothelium functions.

Introduction

Estrogens are known for their cardiovascular protective properties through direct vasorelaxing mechanisms. Despite extensive research, however, the exact mechanisms underlying estrogen-induced vasorelaxation are unclear.[1] In addition, the underlying mechanism(s) of vasorelaxation by estrogens varies from type to type.[2-4]

Actions of estrogenic compounds on the vascular wall include alteration/ modulation of ion influxes, receptors on smooth muscle cells, and endothelium-derived factors production and activity.[5,6]

Guanylatecyclase enzyme has been proposed as a mediator in estrogen-induced vasodilation. However, their exact role and mechanism is highly controversial.[7,8]

A number of reports indicate that nitric oxide (NO) may play a pivotal role in mediating the effects of estrogens on the vasculature. This potent vasodilator is produced in the vascular endothelial cells.[9-12]

A direct action on the vascular smooth muscles has been also reported as another underlying mechanism of vasorelaxation incited by estrogens. In a previous study, the authors demonstrated that the relaxant effect of 17β-estradiol on human saphenous vein was elicited by calcium-dependent and -independent pathways.[13] The role of K^+ channels was also underlined in another study.[14]

It has been reported that estrogens could also modulate peripheral vascular synthesis of vasodilatory hormones. Prostacyclin is one of these hormones, which its production has been proposed as a pathway in estrogen-mediated vasorelaxation.[15,16]

Altogether, estrogens appear to reduce the risk of cardiovascular disease through a combination of mechanisms including changes in lipid profile, endothelial NO synthesis, cell proliferation and angiogenesis, and the regulation of vascular muscle cell (VSMC) Ca^{2+} and K^+ channels. These effects may be mediated through genomic and/or nongenomic pathways.[17]

Naderali et al.[1] showed that 17α-estradiol lacks any effect on classic estrogen receptors. Accordingly, they

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concluded that the known complications of estrogen-contained oral contraceptive pills, such as endometrial carcinoma and thrombosis, might not be seen with 17α-estradiol.

The mechanisms involved in the rapid vasorelaxant effects of 17α-Ethynylestradiol (17α-EE) are not well understood and are drastically controversial. In addition, there is lack of data in terms of the effects of 17α-EE on veins.

Since it is usually used in coronary artery bypass graft (CABG) surgery, the saphenous vein is of great important to physicians.[18] Furthermore, the incidence of acute closure and accelerated atherosclerosis has been reported high for this vein.[19]

The present study aims to examine, for the first time, the possible relaxant effects of 17α-EE on human saphenous vein in vitro and its possible mechanism(s).

Materials and Methods

Tissue preparation and isometric change recording

After being approved by the Ethics Committee of Tabriz University of Medical Sciences and in conformity with the principles outlined in the Declaration of Helsinki, vein segments were taken from excessive portions of saphenous veins from patients undergoing CABG surgery (n=71), including 58 males (mean age: 57.50±10.32, range: 36-74) and 13 females (mean age: 58.80±2.47, range: 44-71) at Madani Educational Heart Centre, Tabriz University of Medical Sciences, Tabriz, Iran.

Our preliminary experiments showed that there is no significant difference between the contractile or relaxant responses of the saphenous vein rings obtained from male patients and those obtained from female patients. However, to avoid any interference of confounding variables (cardiovascular health, smoking, medications, etc.) in each set of experiments control rings employed from the same male or female patients.

The acquired segments were immediately transferred from operating room to laboratory in ice-cold Krebs solution, trimmed of adjacent tissues and cut into 3-5 mm rings.

The presence and functionality of the endothelium was proven routinely at the beginning of the experiment when there was significant relaxation (more than %50) to acetylcholine (6 μM) in veins precontracted by phenylephrine (0.1μM).[20]

To investigate the role of endothelium in 17α-EE-induced relaxation, the luminal surface of some vein rings was thoroughly scraped off by using cotton thread. The lack of endothelium was confirmed when there was no relaxation in response to acetylcholine (6μM) in the rings precontracted with phenylephrine (0.1μM).[21]

For isometric change recording, two stainless steel triangle hooks introduced through the lumen of the vein rings, one fixed to the bottom of organ bath, while the other connected to a force-displacement transducer (LETICA, Spain). A computer-assisted data acquisition system (ADInstruments, Power Lab/4SP) recorded the changes in isometric tension during the experiments.[13,22]

The employed organ bath (10 ml) consisted of modified Krebs-Ringer bicarbonate solution (NaCl: 118mM, KCl: 4.7mM, KH_2PO_4: 1.2mM, $NaHCO_3$: 25mM, $MgSO_4.7H_2O$: 2.1mM, $CaCl_2$: 2.5mM, glucose: 11.1mM). The solution was aerated with a mixture of 95% O_2 and 5% CO_2, maintaining a pH of 7.3-7.4. The temperature was held constant at 37°C. The optimal tension was adjusted at 3g in all primary tests and throughout the experiments as previously established.[23,24] Each preparation was allowed to equilibrate for at least 60 min prior to initiation of experimental procedures, during which the bath solution was refreshed every 15 min and the tension was adjusted.

Effect of 17α-EE on isolated human saphenous vein rings

Since KCl and $PGF_{2\alpha}$ were shown to produce a stable and long-lasting contraction in human saphenous vein,[22] they were chosen as contractile agents in the present study. In order to obtain the submaximal concentration of these contractile agents, a concentration-response curve to each of them was constructed.

After achieving stable contraction by using either KCl or $PGF_{2\alpha}$, different concentrations of 17α-EE (2, 5, 10, 20 and 40 μM) were added for 40 min as a standard cutoff time in a non-cumulative manner. Each preparation was exposed to only one concentration of 17α-EE. From 17α-EE concentration response curve, 20 μM was chosen as an optimal submaximal concentration and used in all mechanistic experiments. The vehicle (ethanol, at final bath concentration no greater than 0.1%, v/v) alone had no significant relaxant effect on the contractile responses of the vein rings.

Effect of endothelium-removal on 17α-EE -induced vasodilatation

To assess the effect of endothelium, 17α-EE (20μM) was added to both intact and endothelium-denuded vein rings at the plateau of contraction produced by $PGF_{2\alpha}$ (0.8μM). Forty minutes later, the amount of relaxation was assessed and compared with control group.

Effect of guanylatecyclase (GC) inhibition on 17α-EE -induced vasodilatation

Methylene blue inhibits GC by oxidizing its hem iron, and reduces the relaxation elicited by sodium nitroprusside and NO. The effect of methylene blue on 17α-EE-induced relaxation was examined to determine a possible role of soluble GC. For this purpose, vein rings with intact endothelium were incubated with methylene blue (10 μM) for 20 min before induction of contraction with $PGF_{2\alpha}$ (0.8μM). Afterward, the relaxant responses to 17α-EE were documented.

Effect of NO synthesis inhibition on 17α-EE -induced vasodilatation

To assess the role of NO synthesis in 17α-EE-induced relaxation, vein rings with intact endothelium were challenged with N-nitro-L-arginine methyl ester

(L-NAME, 200µM) for 20 min prior to inducing contraction with $PGF_{2\alpha}$ (0.8µM).[25,26] The relaxant responses to 17α-EE were evaluated thereafter.

Effect of cyclooxygenase inhibition on 17α-EE-induced vasodilatation

Indomethacin, an inhibitor of cyclooxygenase, was used for 20 min at a dose of 10µM in some vein rings with intact endothelium.[27,28] After using contractile agents, 17α-EE (20µM) was added for 40 min in these tissues. As indomethacin was dissolved in pure ethanol, a group of vein rings using only pure ethanol (10µL) served as the control.

Effect of potassium channel inhibition on 17α-EE - induced vasodilatation

Tetraethyl ammonium (TEA) is a potassium channel blocker, with nonselective effect at high concentration.[29] In order to examine the effect of potassium channel blockade on 17α-EE-induced relaxation, a group of vein rings with intact endothelium were incubated with TEA (5mM) for 20 min before contraction with $PGF_{2\alpha}$ (0.8µM). Subsequently, the relaxant responses to 17α-EE were investigated.

Chemical Reagents and Drugs

Acetylcholine, 17α-EE, indomethacin, N-nitro-L-arginine methyl ester, methylene blue and tetraethyl ammonium all were purchased from Sigma. Phenylephrine was obtained from SinaDaru Co (Iran) and $PGF_{2\alpha}$ from Abureyhan Co. (Iran). Indomethacin and 17α-EE were dissolved in pure ethanol and the rest in distilled water. Ingredients of Krebs solution obtained from Merck.

Statistical analysis

The SPSS software version 12 (SPSS, USA) was used for statistical comparisons. Data are presented as mean ± standard error of the mean (SEM). The relaxant responses induced by 17α-EE were expressed as percentage of the initial contraction produced by either KCl or $PGF_{2\alpha}$. Linear regressions were performed by least square method for calculation of EC_{50}. The Student's t-test (Independent samples) was used to compare the data and p value less than 0.05 was considered as statistically significant.

Results

Effect of KCl and $PGF_{2\alpha}$ on isolated human saphenous vein rings

Both KCl (2-120 mM) and $PGF_{2\alpha}$ (0.05-2 µM) induced contraction in human saphenous vein tissues in a concentration-dependent manner. These responses reached a plateau after about 15-20 min, remaining stable for 60-70 min. Concentration-response curves to KCl and $PGF_{2\alpha}$ are depicted in Figure 1A and Figure 1B, respectively. Submaximal concentrations of $PGF_{2\alpha}$ and KCl(~ EC_{80}) were chosen for further experiments (0.8 µM and 60 mM, respectively).

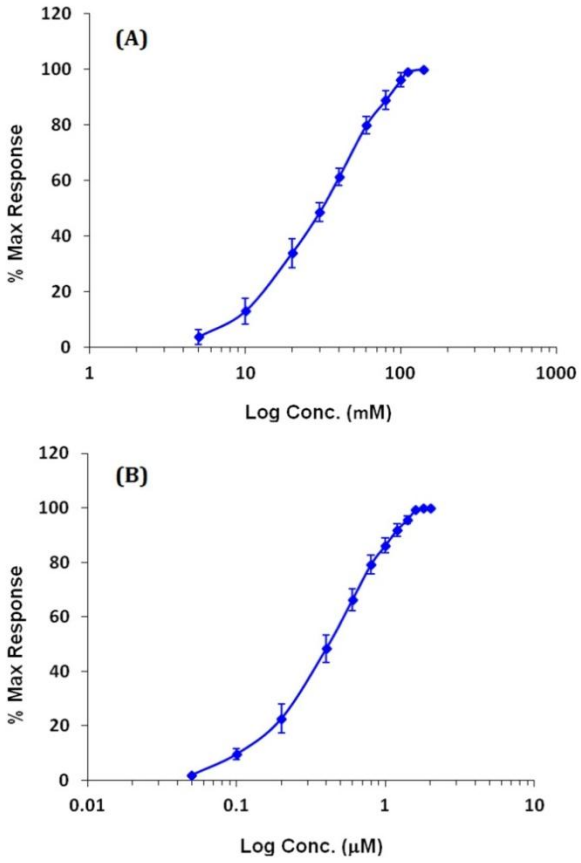

Figure 1. Concentration-response curves to KCl (A) and $PGF_{2\alpha}$ (B) in human saphenous vein.

Effect of 17α-EE on isolated human saphenous vein rings

The relaxant effects of increasing concentrations of 17α-EE (2, 5, 10, 20 and 40 µM) on the employed saphenous vein rings after 40 min are shown in Figure 2. The EC_{50} value for 17α-EE-induced relaxant effect in the specimens contracted by $PGF_{2\alpha}$ was about 20 µM. The relaxant response to 17α-EE was both repeatable and reversible (Figure 3).

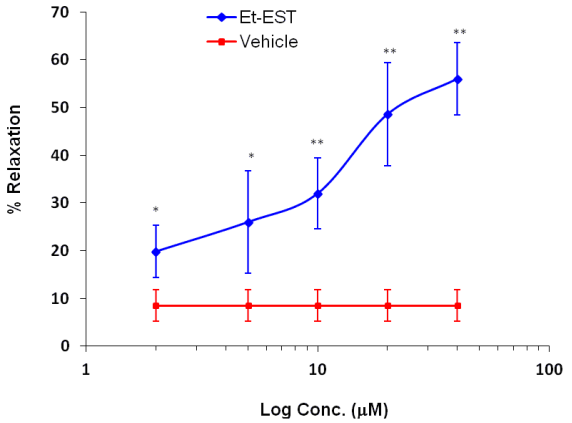

Figure 2. Relaxant effect of different concentrations of 17α-Ethynylestradiol (Et-EST) on human saphenous vein rings precontracted with $PGF_{2\alpha}$ (0.8 µM).
The relaxant responses (mean±SEM) are expressed as the percentage of the initial contraction produced by $PGF_{2\alpha}$. The vehicle volume remained fixed (10 µl) in all experiments (n=8)
*:p<0.05, **: p<0.01.

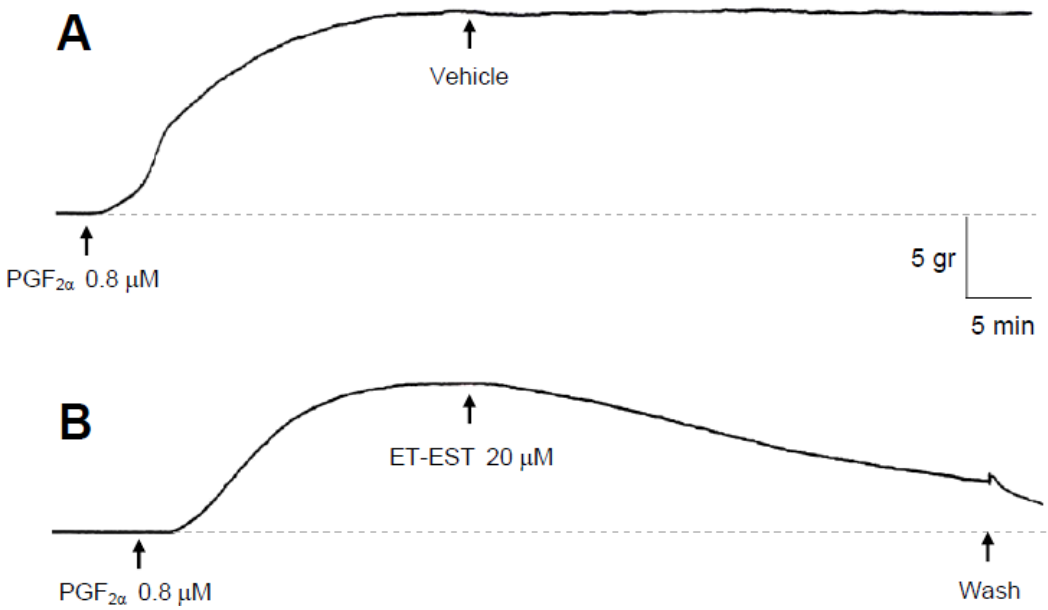

Figure 3. Representative trace showing stable contractile response to $PGF_{2\alpha}$ (0.8 µM) (A) and acute relaxant response to 17α-Ethynylestradiol (ET-EST) (B) in human saphenous vein rings precontracted by $PGF_{2\alpha}$

Effect of endothelium on 17α-EE -induced vasodilatation

The mean percent decrease in contractile response after adding 17α-EE was comparable between the two groups containing endothelium-free and endothelium-intact saphenous veins (60.4±9.6% vs. 48.6±10.8%, respectively (Figure 4). Therefore, denuding endothelium from the human saphenous vein rings did not change the vasorelaxant effect of 17α-EE significantly (p=0.21).

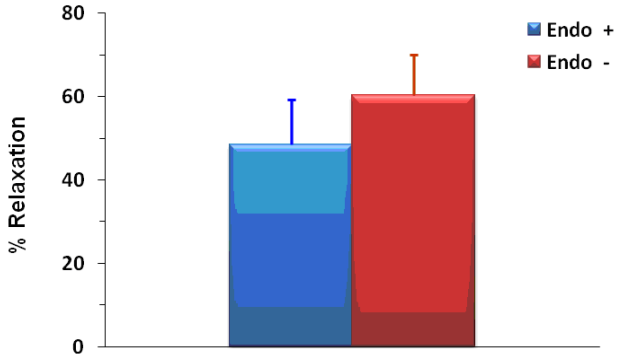

Figure 4. Effect of endothelium denudation on 17α-Ethynylestradiol (17α-EE)-induced vasodilatation in human saphenous vein rings precontracted with $PGF_{2\alpha}$ (0.8µM). Number of experiments=8. The difference was not statistically significant (p =0.21).

Effect of GC inhibition on 17α-EE -induced vasodilatation

The mean 17α-EE -induced relaxation was not significantly different between the samples incubated with methylene blue (GC-inhibitor) comparing with that in the controls (33.7±8.0% vs. 48.6±10.8%; respectively, p=0.14) (Figure 5).

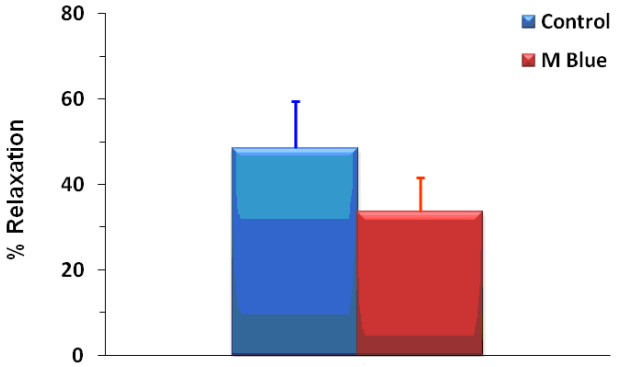

Figure 5. Effect of the inhibition of guanylatecyclase enzyme by using methylene blue (M Blue, 10µM) on 17α-Ethynylestradiol-induced vasodilatation in human saphenous vein rings precontracted with $PGF_{2\alpha}$ (0.8µM). Number of experiments=8. The difference was not statistically significant (p=0.14).

Effect of NO synthesis inhibition on 17α-EE -induced vasodilatation

The mean 17α-EE-induced relaxation was 38.0±9.4% in the tissues incubated with L-NAME (NO synthase inhibitor), and 45.0±8.7% in the cases without L-NAME. There was not a significant difference between the two groups in this regard (p=0.23) (Figure 6).

Effect of cyclooxygenase inhibition on 17α-EE -induced vasodilatation

There was no significant difference between the veins incubated with indomethacin (cyclooxygenase inhibitor) and the control veins in terms of 17α-EE-induced relaxation (38.2±9.3% vs. 32.9±11.0%; p=0.33) (Figure 7).

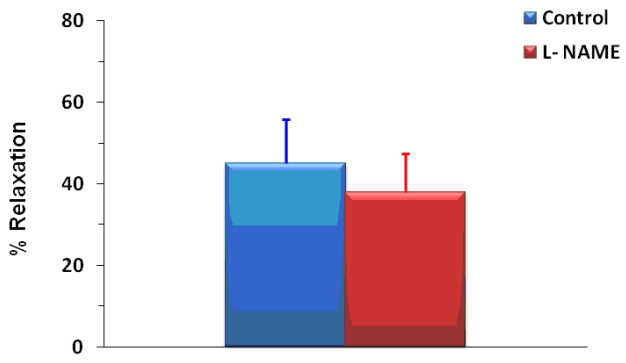

Figure 6. Effect of the inhibition of nitric oxide synthesis by using N-nitro-L-arginine methyl ester (L-NAME, 200μM) on 17α-Ethynylestradiol-induced vasodilatation in human saphenous vein rings precontracted with $PGF_{2\alpha}$ (0.8μM). Number of experiments=8. The difference was not statistically significant (p=0.23).

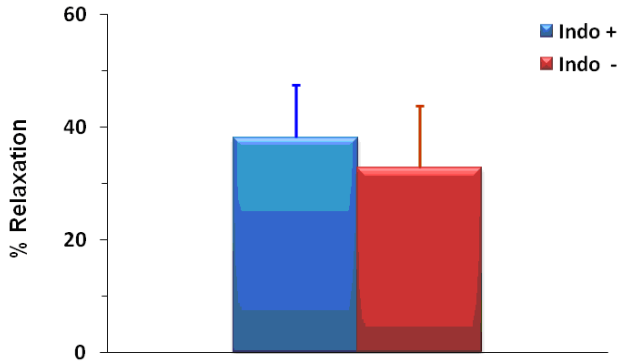

Figure 7. Effect of the inhibition of prostaglandin synthesis by using indomethacin (Indo, 10 μM) on 17α-Ethynylestradiol-induced vasodilatation in human saphenous vein rings precontracted with $PGF_{2\alpha}$ (0.8μM). Number of experiments=8. The difference was not statistically significant (p=0.33).

Effect of potassium channel inhibition on 17α-EE-induced vasodilatation

The mean 17α-EE-induced relaxation did not differ significantly between the veins incubated with TEA (potassium channel-inhibitor) and the control veins (38.6±6.1% vs. 45.2±10.7%, respectively; p=0.22) (Figure 8).

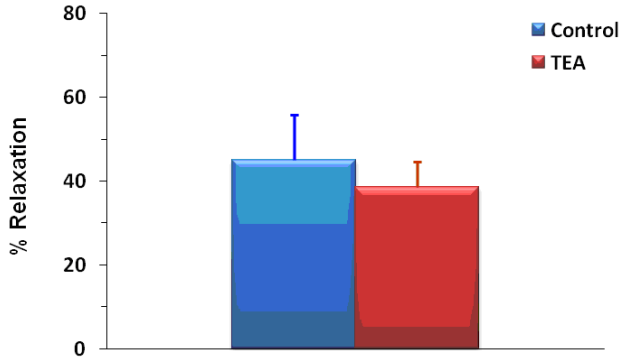

Figure 8. Effect of the inhibition of potassium channels by using tetraethyl ammonium (TEA, 5mM) on 17α-Ethynylestradiol-induced vasodilatation in human saphenous vein rings precontracted with $PGF_{2\alpha}$ (0.8μM). Number of experiments=8. The difference was not statistically significant (p=0.22).

Discussion

Estrogen-induced vasorelaxation is believed to be rendered through interacting with vascular smooth muscle, endothelial cells, and vessel wall.[30-32] Despite extensive research, however, the exact mechanisms underlying estrogen-induced vasorelaxation are unclear.[1] In addition, the underlying mechanism of vasorelaxation by estrogens varies from type to type.[2-4]

In the present study, the acute vasorelaxant effect of 17α-EE on isolated human saphenous vein rings precontracted with KCl or $PGF_{2\alpha}$ was investigated.

A direct effect of estrogens on the endothelium has been proposed as a possible mechanism of vasorelaxation.[33] In the present work, however, there was no significant difference between endothelium-denuded and endothelium-intact vein rings in terms of the mean percent decrease in contractile response after adding 17α-EE. It should be taken into consideration that estrogen-induced vasodilation can be either endothelium-dependent or –independent.[34,35] Likewise, other factors such as gender and hormonal status may play a role in this regard. For example, Martínez et al[36] showed that while gender and hormonal environment had no effect on the estrogen-induced, endothelium-independent component of the relaxation, both of them significantly modulated the estrogen-induced, endothelium-dependent component of the relaxation in rat aorta strips. The role of these possible confounding factors needs to be examined in further studies.

The other factor, which is proposed to have an effect on the vasorelaxation mediated by estrogens, is guanylatecyclase enzyme. In an attempt to clarify the role of this enzyme, methylene blue, an inhibitor of guanylatecyclase, was used.[37,38] In line with some previous reports, adding methylene blue did not modify estrogen-induced relaxation.[8]

Nitric oxide production is another mechanism that has been proposed in estrogen-induced vasorelaxation.[39,40] Thus, in another part of the present work the role of nitric oxide in 17α-EE-induced vasorelaxation was examined. Accordingly, there was no significant difference in the vein rings relaxation produced by 17α-EE in the presence or absence of L-NAME, a potent inhibitor of nitric oxide synthase.[25,26] In conformity with this finding, in a study on human omental artery, Vedernikov et al[41] concluded that the vasorelaxant effect of estrogen (17beta-estradiol) does not involve the products of endothelial nitric oxide. Shaw et al.[42] examined the vasorelaxing effects of 17-beta-estradiol on pre-contracted pressurized (50 mmHg) isolated rat mesenteric and coronary arteries. The vasodilatory responses in both types of artery were unaffected by L-NAME. Similar finding was also reported in another study in rabbit carotid artery.[43]

It is believed that estrogen stimulates nitric oxide production through both endothelium-dependent and independent pathways.[44] Since L-NAME exhibits some selectivity for the inhibition of particular isoforms of nitric oxide synthase,[45] it is possible that other ways of

nitric oxide release remain unblocked even in the presence of L-NAME. To reach a definite conclusion in this regard, however, further clarifying studies are mandatory.

Some studies have suggested that the cardiovascular protection by estrogens may be exerted through the stimulation of prostaglandin synthesis in vessel wall. It has been demonstrated that chronic treatment by estrogen increases in vivo production of prostacyclin and attenuates the level of blood thromboxane in rabbits fed with highly atherogenic diet in comparison with placebo-treated animals.[46] To determine the possible role of prostaglandins in 17α-EE-induced vasorelaxation, we compared indomethacin-incubated tissues with control group. Again, and in conformity with a previous report,[43] no significant difference was detected between the two groups.

A widely discussed probable mechanism by which estrogen may exert its vasorelaxant effect is the alteration in potassium (K^+) channels. To assess this function in the present work, we assessed the 17α-EE-induced relaxation in the presence of TEA, an inhibitor of potassium channels.[47] There was not a significant difference between the two groups in this regard.

As discussed earlier, the results of various studies are widely heterogeneous, and sometimes inconclusive. The type of estrogen and contracturant, the presence or absence of endothelium, and the employed methodology to examine the acute effects may contribute to the variability in the response.[8,48,49] In addition, the vasodilatory effects of different estrogens could widely vary between specific species and/or vascular beds in systemic circulation.[50,51]

Conclusion

This is the first study, which examines the vasorelaxant effect of 17α-EE in human saphenous vein. This concentration-dependent relaxant effect occurs acutely (non-genomic), independent of the mechanisms associated with guanylatecyclase, cyclooxygenase, endothelium, NO and potassium channels.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Naderali EK, Smith SL, Doyle PJ, Williams G. Vasorelaxant effects of oestradiols on guinea pigs: a role for gender differences. *Eur J Clin Invest* 2001;31(3):215-20.
2. Freay AD, Curtis SW, Korach KS, Rubanyi GM. Mechanism of vascular smooth muscle relaxation by estrogen in depolarized rat and mouse aorta. Role of nuclear estrogen receptor and Ca2+ uptake. *Circ Res* 1997;81(2):242-8.
3. Reis SE, Gloth ST, Blumenthal RS, Resar JR, Zacur HA, Gerstenblith G, et al. Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. *Circulation* 1994;89(1):52-60.
4. Bhalla HL, Arora MK, Saxena KK, Surin WR. Chronic use of 17β‑Ethinyl estradiol on cardiovascular hemodynamic profile: "Friend or foe"? *J Pharm Negative Results* 2013;4:54-9.
5. Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. *Endocr Rev* 2003;24(3):313-40.
6. Tostes RC, Nigro D, Fortes ZB, Carvalho MH. Effects of estrogen on the vascular system. *Braz J Med Biol Res* 2003;36(9):1143-58.
7. Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ. Relationship between cyclic guanosine 3':5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and methemoglobin. *J Pharmacol Exp Ther* 1981;219(1):181-6.
8. Martinez C, Sanchez M, Hidalgo A, De Boto MJ. Mechanisms of diethylstilbestrol-induced relaxation in rat aorta smooth muscle. *Vascul Pharmacol* 2003;40(4):197-204.
9. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288(5789):373-6.
10. 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 U S A* 1987;84(24):9265-9.
11. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988;333(6174):664-6.
12. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;327(6122):524-6.
13. Babaei H, Azarmi Y. 17beta-estradiol inhibits calcium-dependent and -independent contractions in isolated human saphenous vein. *Steroids* 2008;73(8):844-50.
14. Babaei H, Azarmi Y. Effect of potassium channels and endothelium derived hyperpolarizing factor on vasorelaxant effect of 17β-estradiol in human saphenous vein. *Pharma Sci* 2008;4:55-66

15. Sherman TS, Chambliss KL, Gibson LL, Pace MC, Mendelsohn ME, Pfister SL, et al. Estrogen acutely activates prostacyclin synthesis in ovine fetal pulmonary artery endothelium. *Am J Respir Cell Mol Biol* 2002;26(5):610-6.
16. Ospina JA, Krause DN, Duckles SP. 17beta-estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. *Stroke* 2002;33(2):600-5.
17. Falkenstein E, Tillmann HC, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones--a focus on rapid, nongenomic effects. *Pharmacol Rev* 2000;52(4):513-56.
18. Tarzamni MK, Eshraghi N, Fouladi RF, Afrasiabi A, Halimi M, Azarvan A. Atherosclerotic changes in common carotid artery, common femoral artery, and ascending aorta/aortic arch in candidates for coronary artery bypass graft surgery. *Angiology* 2012;63(8):622-9.
19. Ouyang P, Tardif JC, Herrington DM, Stewart KJ, Thompson PD, Walsh MN, et al. Randomized trial of hormone therapy in women after coronary bypass surgery. Evidence of differential effect of hormone therapy on angiographic progression of disease in saphenous vein grafts and native coronary arteries. *Atherosclerosis* 2006;189(2):375-86.
20. Azarmi Y, Babaei H, Alizadeh F, Gharebageri A, Fouladi DF, Nikkhah E. Allopurinol Prevents Nitroglycerin-induced Tolerance in Rat Thoracic Aorta. *J Cardiovasc Pharmacol* 2014;63(2):113-9.
21. Babaei H, Gharehbagheri A, Eteraf Oskouei T, Delazar A, Asnaashari S, Bamdad Mogadam S. Role of endothelium on Vasorelaxant effect of Ribes biebersteinii fruit total extract on rat aorta. *Pharm Sci* 2009;15(2):159- 68.
22. Babaei H, Ebrahimi F, Shahbazi Mojarrad J, Azarmi Y, Gharehbagheri A. Vasorelaxant effect of a newly synthesized dihydropyridine ethyl ester (DHPEE) on rat thoracic aorta: Dual Mechanism of action. *Adv Pharm Bull* 2011;1(1):10-7.
23. Azarmi Y, Babaei H. Effect of endothelium and cGMP on vasorelaxant effect of 17b estradiol on human saphenous vein. *Pharm Sci* 2006;11(4):79-85.
24. Babaei H, Azarmi Y. Comparative effect of genistein and 17b-estradiol on human saphenous vein and rat aorta. *Pharm Sci* 2005;10(2):101-10.
25. Bilfinger TV, Vosswinkel JA, Cadet P, Rialas CM, Magazine HI, Stefano GB. Direct assessment and diminished production of morphine stimulated NO by diabetic endothelium from saphenous vein. *Acta Pharmacol Sin* 2002;23(2):97-102.
26. Sahin AS, Atalik KE, Sahin TK, Dogan N. Cooling and response to hydrogen peroxide in human saphenous vein: role of the endothelium. *Fundam Clin Pharmacol* 2005;19(3):341-6.
27. Leung FP, Yao X, Lau CW, Ko WH, Lu L, Huang Y. Raloxifene relaxes rat intrarenal arteries by inhibiting Ca2+ influx. *Am J Physiol Renal Physiol* 2005;289(1):F137-44.
28. Torregrosa G, Burguete MC, Perez-Asensio FJ, Salom JB, Gil JV, Alborch E. Pharmacological profile of phytoestrogens in cerebral vessels: in vitro study with rabbit basilar artery. *Eur J Pharmacol* 2003;482(1-3):227-34.
29. Omae T, Nagaoka T, Tanano I, Yoshida A. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, induces dilation of isolated porcine retinal arterioles: role of nitric oxide and potassium channels. *Invest Ophthalmol Vis Sci* 2011;52(9):6749-56.
30. Diaz M, Ramirez CM, Marin R, Marrero-Alonso J, Gomez T, Alonso R. Acute relaxation of mouse duodenum [correction of duodenun] by estrogens. Evidence for an estrogen receptor-independent modulation of muscle excitability. *Eur J Pharmacol* 2004;501(1-3):161-78.
31. Nakajima T, Kitazawa T, Hamada E, Hazama H, Omata M, Kurachi Y. 17beta-Estradiol inhibits the voltage-dependent L-type Ca2+ currents in aortic smooth muscle cells. *Eur J Pharmacol* 1995;294(2-3):625-35.
32. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med* 1992;117(12):1016-37.
33. Rodriguez J, Garcia De Boto MJ, Hidalgo A. Mechanisms involved in the relaxant effect of estrogens on rat aorta strips. *Life Sci* 1996;58(7):607-15.
34. Andersen HL, Weis JU, Fjalland B, Korsgaard N. Effect of acute and long-term treatment with 17-beta-estradiol on the vasomotor responses in the rat aorta. *Br J Pharmacol* 1999;126(1):159-68.
35. Raddino R, Pela G, Uberti D, Portera C, Ferrari R, Scarabelli TM, et al. Estrogen derivative relaxes rabbit aorta via the endothelial receptor system. *Ital Heart J* 2001;2(1):49-54.
36. Martinez C, Lopez C, Hidalgo A, Sanchez M, Garcia De Boto MJ. Gonadectomy eliminates endothelium-dependent diethylstilbestrol-induced relaxant effect in rat aorta. *Pharmacology* 2003;67(3):136-42.
37. Friebe A, Koesling D. Regulation of nitric oxide-sensitive guanylyl cyclase. *Circ Res* 2003;93(2):96-105.
38. Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. *Biochem Pharmacol* 1993;45(2):367-74.
39. Hekimoglu A, Celik F, Tas T, Ece A, Kavak V. The contribution of nitric oxide on the relaxation effects of diethylstilbestrol. *Saudi Med J* 2008;29(5):662-7.
40. Momoi H, Ikomi F, Ohhashi T. Estrogen-induced augmentation of endothelium-dependent nitric oxide-mediated vasodilation in isolated rat cerebral small arteries. *Jpn J Physiol* 2003;53(3):193-203.
41. Vedernikov YP, Belfort MA, Saade GR, Garfield RE. Inhibition of cyclooxygenase but not nitric oxide synthase influences effects on the human omental artery of the thromboxane A2 mimetic U46619 and

17beta-estradiol. *Am J Obstet Gynecol* 2001;185(1):182-9.
42. Shaw L, Taggart MJ, Austin C. Mechanisms of 17 beta-oestradiol induced vasodilatation in isolated pressurized rat small arteries. *Br J Pharmacol* 2000;129(3):555-65.
43. Salom JB, Burguete MC, Perez-Asensio FJ, Centeno JM, Torregrosa G, Alborch E. Acute relaxant effects of 17-beta-estradiol through non-genomic mechanisms in rabbit carotid artery. *Steroids* 2002;67(5):339-46.
44. White RE. Estrogen and vascular function. *Vascul Pharmacol* 2002;38(2):73-80.
45. Furfine ES, Harmon MF, Paith JE, Garvey EP. Selective inhibition of constitutive nitric oxide synthase by L-NG-nitroarginine. *Biochemistry* 1993;32(33):8512-7.
46. Fogelberg M, Vesterqvist O, Diczfalusy U, Henriksson P. Experimental atherosclerosis: effects of oestrogen and atherosclerosis on thromboxane and prostacyclin formation. *Eur J Clin Invest* 1990;20(1):105-10.
47. Chan W, Yao X, Ko W, Huang Y. Nitric oxide mediated endothelium-dependent relaxation induced by glibenclamide in rat isolated aorta. *Cardiovasc Res* 2000;46(1):180-7.
48. Teoh H, Quan A, Leung SW, Man RY. Vascular effects of estrone and diethylstilbestrol in porcine coronary arteries. *Menopause* 2009;16(1):104-9.
49. Martinez C, Sanchez M, Hidalgo A, Garcia De Boto MJ. Involvement of K(ATP) channels in diethylstilbestrol-induced relaxation in rat aorta. *Eur J Pharmacol* 2001;413(1):109-16.
50. Hilgers RH, Oparil S, Wouters W, Coelingh Bennink HJ. Vasorelaxing effects of estetrol in rat arteries. *J Endocrinol* 2012;215(1):97-106.
51. Reslan OM, Yin Z, Do Nascimento GR, Khalil RA. Subtype-specific Estrogen Receptor-mediated Vasodilator Activity in the Cephalic, Thoracic, and Abdominal Vasculature of Female Rat. *J Cardiovasc Pharmacol* 2013;62(1):26-40.