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Short communication

17β-Estradiol acutely improves endothelium-dependent relaxation to bradykinin in isolated human coronary arteries

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Abstract

Improvement of endothelial function has been implicated in the cardioprotective effects of estrogens in women. In isolated human coronary arteries, we investigated whether 17β-estradiol affects endothelium-dependent responses to bradykinin, an endothelium-derived vasodilator locally produced by endothelial cells. Concentration-response curves to bradykinin (0.03–300 nM) or nitroglycerine (0.01–1 μM) were obtained before and after 30 min of incubation with 17β-estradiol (3 μM) or solvent control (ethanol 0.2% vol/vol). Incubation with 17β-estradiol enhanced relaxations to bradykinin (from 43 ± 6 to $83 \pm 3\%$, $P < 0.0001$) but not those to nitroglycerine (n.s.). Improvement of bradykinin-mediated endothelium-dependent relaxation may represent a novel mechanism contributing to the cardioprotective effects of estrogen in women.

Keywords: Bradykinin; Endothelium; 17β-Estradiol; (Human); Coronary Artery; Nitroglycerine

1. Introduction

Cardiovascular disease is a major cause of morbidity and mortality in postmenopausal women and is reduced by estrogen replacement therapy (Stampfer et al., 1991). Favorable changes in lipid metabolism (Fåhreus, 1988) and direct actions of estrogens on the vascular wall have been demonstrated (Mügge et al., 1993; Sudhir et al., 1995). Acute administration of estrogen improves exercise capacity in postmenopausal women with coronary artery disease (Rosano et al., 1993) and potentiates endothelium-dependent relaxation in coronary arteries using acetylcholine as a pharmacological stimulus (Reis et al., 1994). It has been recently demonstrated that chronic estrogen treatment inhibits angiotensin converting enzyme activity in animals and patients (Proudler et al., 1995; Tanaka et al., 1997). Angiotensin converting enzyme is identical with kininase II and is involved in the breakdown of bradykinin (Deddish et al., 1996). Thus, estrogen increases local levels of bradykinin, a potent endothelium-dependent vasodilator produced by vascular endothelial cells (Lüscher and Vanhoutte, 1990). Whether estrogen also affects bradykinin-mediated vascular function is unknown. We therefore investigated the effects of 17β-estradiol on bradykinin-mediated endothelium-dependent relaxation in human coronary arteries.

2. Methods

2.1. Patient characteristics and vascular tissues

Human coronary arteries were obtained during cardiac transplantation in accordance with the institutional ethic's committee guidelines. All patients received orthotopic heart transplantation due to idiopathic dilated cardiomyopathy, the age range was 18–57 years (mean age: 40.9 years). Seven patients were men and two of the patients were women, 48 and 57 years of age. The left main coronary artery was isolated from the recipient's heart and was immediately placed in cold (4°C) Krebs Ringer bicarbonate solution (4°C, composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, edetate calcium disodium 0.026, glucose 11.1), dissected free from surrounding connective tissue and fat and cut in to rings

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(4–5 mm in length). All arteries used for the experiments were free of macroscopic atherosclerotic disease.

2.2. Organ chamber set-up

Arterial rings were suspended in organ chambers containing 10 ml of Krebs–bicarbonate solution, connected to force transducers (K 30, Hugo Sachs Elektronik, March-Hugstetten, Germany) and maintained at 37°C, oxygenated with 95% O_2 and 5% CO_2 (pH 7.4) as previously described (Mügge et al., 1993). In some preparations, the endothelium was removed using a soft wooden probe and its absence was verified by lack of response to bradykinin (0.3 μM). Arterial rings were allowed to equilibrate for 30 min. Resting tension was gradually increased and the arterial rings were repeatedly exposed to 80 mM KCl until the optimal tension for generating force during isometric contraction was reached (Mügge et al., 1993). Arterial rings were left at this resting tension throughout the remainder of the study. After equilibration for 30 min, rings were randomly assigned to different protocols.

2.3. Experimental protocols

Coronary artery rings were precontracted with prostaglandin $F_{2\alpha}$ (1–3 μM, Förstermann et al., 1988; Mügge et al., 1993) to approximately 80% of contraction of KCl (80 mM). After a stable contraction plateau was reached, arterial rings were exposed to cumulative concentrations of bradykinin (0.03–300 nM) or nitroglycerine (0.1 nM–1 μM). After the experiments, arterial rings were washed several times with fresh Krebs bicarbonate solution and equilibrated for 1 h. Arterial rings were then preincubated with 17β-estradiol (3 μM) or solvent control for 30 min. This concentration of 17β-estradiol was shown to be maximally effective to dilate precontracted human coronary arteries without affecting basal tone of quiescent rings (Mügge et al., 1993). After preincubation for 30 min, rings were again precontracted and exposed to the same vasodilator as before.

2.4. Drugs

Bradykinin, EDTA, prostaglandin $F_{2\alpha}$ and potassium chloride were purchased from Sigma Chemicals Nitroglycerine (Perlinganit®) was from Schwarz Pharma (Monheim, Germany). A stock solution of 17β-estradiol (0.3 M) was dissolved in ethanol (96% vol/vol) and subsequently diluted with distilled water, the final concentration of ethanol was 0.2% (vol/vol). All other substances were prepared fresh daily and dissolved in distilled water immediately before use. 17β-estradiol was a gift by Schering (Berlin, Germany). All concentrations given represent the actual concentration of drugs in the organ chamber.

2.5. Calculations and statistical analysis

Data are given as mean ± S.E.M., *n* refers to the number of rings used per experiment. Vasodilator responses were calculated as percent relaxation of maximal precontraction induced by prostaglandin $F_{2\alpha}$. Contractions to prostaglandin $F_{2\alpha}$ were normalized to contraction evoked by potassium chloride (80 mM) and expressed as mN/mm. Data were analyzed using the *t*-test for paired observations

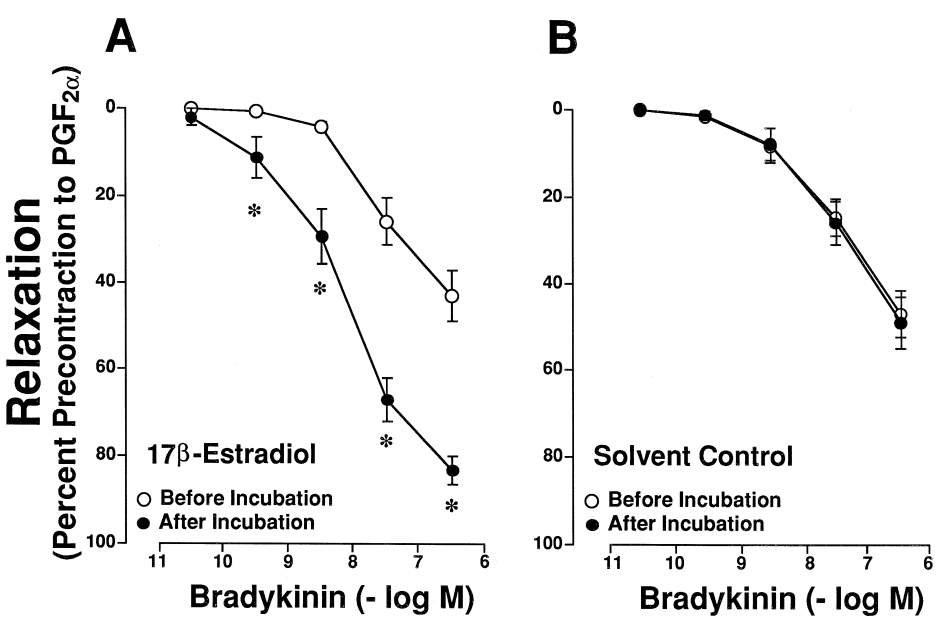

Fig. 1. Effect of 17β-estradiol on endothelium-dependent relaxations to bradykinin in isolated human coronary arteries. Incubation with 17β-estradiol for 30 min markedly potentiated relaxation to bradykinin (A). Incubation with the solvent control (0.2% vol/vol ethanol) had no effect on relaxations to bradykinin (B). Data are means ± S.E.M., *: $P < 0.05$.

and ANOVA, followed by Bonferroni's correction. A P value of < 0.05 was considered significant.

3. Results

Prior to incubation, the average contraction induced by prostaglandin $F_{2\alpha}$ averaged 6.2 ± 0.5 mN/mm in the 17β-estradiol group and 6.08 ± 0.38 mN/mm in the solvent control group (n.s). Incubation with either 17β-estradiol or solvent had no effect on precontraction with prostaglandin $F_{2\alpha}$ (6.04 ± 0.37 mN/mm and 5.99 ± 0.42 mN/mm, respectively, n.s. vs. pre-incubation).

In rings precontracted with prostaglandin $F_{2\alpha}$, bradykinin induced a concentration-dependent relaxation with a maximum response of $43 \pm 6\%$ of precontraction (Fig. 1A) which was abolished by endothelium removal (data not shown). Incubation with 17β-estradiol (3×10^{-6} mol/l) markedly enhanced endothelium-dependent relaxations to bradykinin from $43 \pm 6\%$ to $83 \pm 3\%$ of precontraction ($n = 20$ rings from nine patients, $P < 0.0001$, Fig. 1A). The solvent control had no significant effect on the responses to bradykinin ($n = 16$ rings from eight patients, n.s., Fig. 1B).

Prior to incubation, nitroglycerine-induced relaxations averaged $93 \pm 1\%$ in control group ($n = 16$ from eight patients) and $94 \pm 1\%$ in the 17β-estradiol group ($n = 15$ from nine patients) and were unaffected by either treatment ($93 \pm 1\%$ and $95 \pm 1\%$, respectively, Fig. 2A and B). Precontraction induced by prostaglandin $F_{2\alpha}$ was not different between groups and unaffected by treatments (data not shown).

4. Discussion

This study demonstrates for the first time that in human coronary arteries 17β-estradiol potentiates endothelium-dependent responses to bradykinin without affecting endothelium-independent relaxation to nitroglycerine. Bradykinin is an important vasodilator substance which—in contrast to acetylcholine—is released by the vascular endothelium (Lüscher and Vanhoutte, 1990). Bradykinin mediates relaxation through the release of nitric oxide (NO) and endothelium-derived hyperpolarizing factor in animals (Lüscher and Vanhoutte, 1990) and humans (Nakashima et al., 1993; Stork and Cocks, 1994) and local bradykinin concentrations are likely to be modulated by the chronic effects of estrogen (Proudler et al., 1995; Tanaka et al., 1997).

In line with previous findings, bradykinin evoked endothelium-dependent relaxations in human coronary arteries with a maximal response of about 50% of precontraction (Nakashima et al., 1993; Stork and Cocks, 1994). 17β-estradiol markedly enhanced the relaxant response to bradykinin but not to nitroglycerine indicating a rapid effect on the endothelium. This observation supports the concept that bradykinin acts on the endothelium to indirectly alter vascular smooth muscle tone. Alternatively, 17β-estradiol could act on the vascular smooth muscle to potentiate the latter effect of bradykinin, however, in this study endothelium-dependent relaxations to nitroglycerine were unchanged by 17β-estradiol. The findings of the present study are in line with previous studies demonstrating that estrogen acutely improves endothelium-mediated vasodilation in vivo after pharmacological stimulation with

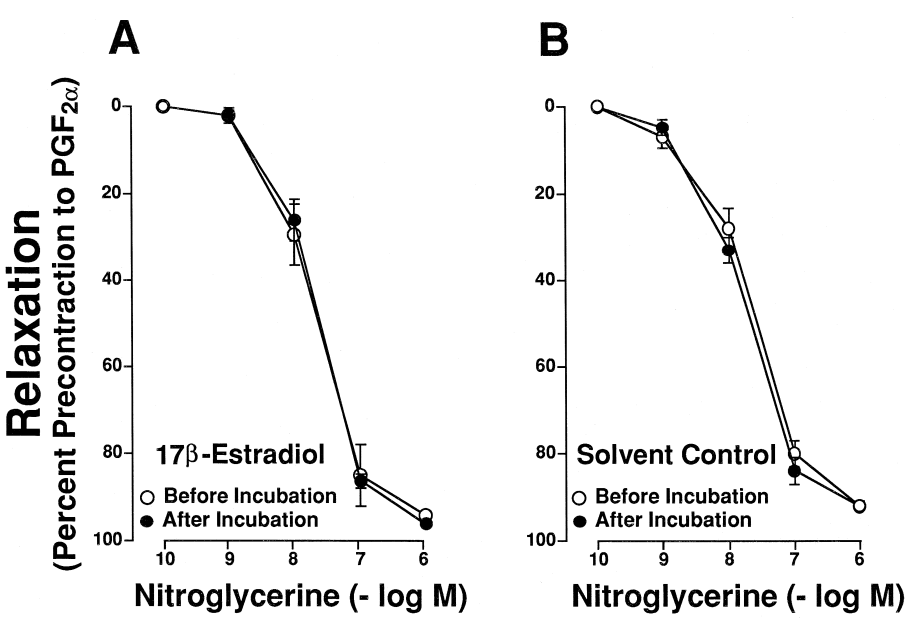

Fig. 2. Effect of 17β-estradiol on endothelium-independent relaxations to nitroglycerine in isolated human coronary arteries. Incubation with 17β-estradiol (A) or solvent control (B) for 30 min had no effect on the relaxations to nitroglycerine. Data are means ± S.E.M.

acetylcholine in coronary arteries of primates (Williams et al., 1992) and postmenopausal women (Reis et al., 1994).

The mechanisms underlying these rapid effects remain to be determined and may involve several mechanisms. First, estrogens are antioxidants due to their phenolic structure (Sugioka et al., 1987) and may scavenge free oxygen radicals such as superoxide anion (Arnal et al., 1996) thereby increasing the bioavailability of NO. Thus, the potentiation of endothelium-dependent relaxation observed in the present study could, at least in part, be due to prolongation of the half-life of NO. Second, the potentiating effect of 17β-estradiol may involve NO-independent relaxation through endothelium-dependent hyperpolarization in response to bradykinin (Nakashima et al., 1993). Indeed, 17β-estradiol rapidly activates endothelial cell potassium channels (Rusko et al., 1995) and may thereby promote endothelium-dependent hyperpolarization of smooth muscle cells (Nakashima et al., 1993). In contrast, upregulation of endothelial NO synthase (Hishikawa et al., 1995) or bradykinin receptors (Madeddu et al., 1997)—which has been observed only after long-term treatment with estrogen—are unlikely mechanisms explaining the acute potentiation of bradykinin-induced relaxation observed in the present study.

In postmenopausal women receiving estrogen replacement therapy, 17β-estradiol plasma concentrations in the range of 30 nM have been measured, and concentrations of 17β-estradiol are even higher in pregnancy (White et al., 1995). However, it remains to be determined whether the effect on bradykinin-induced relaxation observed in the present study is also operative in vivo.

In summary, we have demonstrated that in non-atherosclerotic human coronary arteries short-term administration of 17β-estradiol rapidly potentiates bradykinin-mediated endothelium-dependent relaxation. This effect may contribute to the cardioprotective effects of estrogens.

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