ELSEVIER

European Journal of Pharmacology 308 (1996) 305–309

ejp

Short communication

Relaxant effects of 17ß-estradiol in the rat tail artery are greater in females than males

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Received 25 January 1996; revised 8 May 1996; accepted 14 May 1996

Abstract

To investigate whether sex differences contribute to the variability reported for acute effects of 17ß-estradiol on vascular reactivity, the response to 17ß-estradiol was compared in male and female isolated perfused rat tail arteries. 17ß-Estradiol (10^{-7}–10^{-5} M) attenuated the contractile response to norepinephrine in female, but not male, arteries, but had no effect when the endothelium was removed. Relaxation to 17ß-estradiol reached a steady state within approximately 15 min. This hormone appears to acutely relax pre-contracted arteries from females but not males by a non-genomic effect requiring an intact endothelium.

Keywords: Estrogen; Endothelium, vascular; Norepinephrine

1. Introduction

Premenopausal women have a lower prevalence of cardiovascular disease than men of the same age. Estrogen-replacement therapy in postmenopausal and ovariectomized women reduces the incidence of atherosclerotic disease (Nabulsi et al., 1993). Thus, a 'protective' role of estrogen in the prevention of cardiovascular disease has been established in women. Estrogen-replacement therapy in postmenopausal women has been shown to inhibit atherosclerotic plaque formation (Sarrel, 1990) and protect against coronary occlusion (Gruchow et al., 1988); however in postmenopausal women taking estrogen-replacement therapy, analyses of lipid changes cannot fully account for the cardiovascular protective effects (Adams et al., 1990). Thus it is necessary to investigate other mechanisms by which estrogen may provide cardiovascular benefits in order to fully understand and exploit the protective role of estrogen against cardiovascular disease. One of these mechanisms may be acute, non-genomically mediated actions of estrogen on the vasculature (White et al., 1995).

Most of the well-established effects of estrogen are thought to be genomically mediated by estrogen diffusing through the plasma membrane and forming a complex with a cytosolic or nuclear receptor that binds DNA and stimulates the expression of a set of genes with specific steroid-response elements (Morley et al., 1992). However, such a mechanism does not explain all of the actions of estrogen. For instance, estrogen greatly increases intracellular calcium concentrations in chicken and pig ovarian granulosa cells in less than 5 s, an effect which is not blocked by inhibitors of RNA and protein synthesis or the conventional estrogen receptor antagonist tamoxifen (Morley et al., 1992). Estrogen receptors have been detected on rat pituitary cell surface membranes (Bression et al., 1986) and breast cancer cell membranes (Nenci et al., 1981). Clearly, estrogen can have additional actions other than those mediated by its cytosolic or nuclear receptor.

A non-genomically mediated action of estrogen may contribute to its effects on blood vessels. Recently, in vitro, acute effects of 17ß-estradiol on the vasculature have been reported (Vargas et al., 1995; Jiang et al., 1991; Paredes-Carbajal et al., 1995; Shan et al., 1994). However, there is considerable variation in the nature of the effects of estrogen in these studies, which may reflect differences in the animal model, vascular bed, and/or contractile agents used. In the present study, possible sex differences in the acute effect of estrogen on contractile responses were investigated using the perfused rat tail artery model. We have determined the time course and endothelium dependence of the response to estrogen in arteries contracted with norepinephrine and compared responses in arteries from age-matched male and female rats.

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PII S0014-2999(96)00374-3

2. Materials and methods

2.1. Tissue isolation and preparation

Male and female Fischer-344 rats aged 4 months (Harlan Sprague-Dawley, Indianapolis, IN, USA) were decapitated and tail arteries removed. Two segments (1.5 cm each) from each artery were cannulated at both ends and placed in a perfusion-superfusion system. In some experiments, one segment was denuded by mechanically rubbing the lumen of the artery with wire. Segments were perfused with oxygenated Krebs' buffer (mM: NaCl, 118; KCl, 4.8; $CaCl_2$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 25; $MgSO_4$ 1.2; ascorbic acid, 0.3; and glucose, 11.5) at a rate of 2 ml/min, and the entire assembly was kept at 37°C. The perfusion pressure was monitored by a pressure transducer, and resulting signals were digitized by a MacLab analog/digital converter and recorded by a Macintosh computer. Tissues were allowed to equilibrate for 1 h before starting the experimental protocol.

2.2. Experimental protocol

In order to investigate the effect of 17ß-estradiol, the vascular contraction to exogenous norepinephrine was recorded before and after the addition of 17ß-estradiol (10^{-7}, 10^{-5} M) to the buffer solution. Tissues (denuded and endothelium-intact) were allowed to reach a steady level of contraction (12–15 min) in the presence of a single concentration of norepinephrine (3×10^{-7} M). Norepinephrine was then washed out with Krebs' buffer (10 min), and the tissue was brought to a second contraction with the same concentration of norepinephrine in order to ensure a consistent response. Tissues were then perfused with 17ß-estradiol (10^{-7}, 10^{-5} M) or vehicle, dimethyl sulfoxide (DMSO), for 30 min. Two additional contractions to the same concentration of norepinephrine were then performed in the presence of a single concentration of 17ß-estradiol. The acute effect of estradiol on the contraction due to exogenous norepinephrine was assessed by comparing the average of the two contractions before the addition of estradiol to the average of the two contractions in the presence of estradiol.

In experiments where the effects of the two vehicles, dimethyl sulfoxide (DMSO) and ethanol, were compared, tissues were allowed to reach a steady level of contraction in the presence of a single concentration of norepinephrine (3×10^{-7} M). This contraction to norepinephrine was then repeated, and tissues were subsequently perfused with vehicle, either DMSO or ethanol. Two contractions to norepinephrine were again repeated in the presence of vehicle, and tissues were subsequently perfused with 17ß-estradiol (10^{-5} M), dissolved in the appropriate vehicle, for 30 min. Two additional contractions to the same concentration of norepinephrine were then performed in the presence of 17ß-estradiol. The acute effect of estradiol dissolved in different vehicles was assessed by comparing the average of the two contractions in the presence of vehicle before the addition of estradiol to the average of the two contractions in the presence of estradiol.

A 10^{-2} M stock solution of 17ß-estradiol in DMSO was prepared, and dilutions were made in DMSO such that a 200 µl volume was added to 200 ml of Krebs' buffer for each concentration of estradiol used. In comparison experiments, a 10^{-1} M stock solution of 17ß-estradiol in ethanol was prepared by sonication, and a 20 µl volume was added to 200 ml of Krebs' buffer. Norepinephrine was diluted in 0.001 M HCl.

2.3. Drugs and chemicals

(−)-Norepinephrine bitartrate and 17ß-estradiol were purchased from Sigma Chemical (St. Louis, MO, USA).

3. Results

3.1. Effects of estradiol

17ß-Estradiol relaxed female arteries that were precontracted with 3×10^{-7} M norepinephrine. The contractile response to this concentration of norepinephrine was not significantly different between males and females. A steady level of relaxation was achieved after approximately 15 min exposure to 17ß-estradiol (Fig. 1A). A similar exposure to 17ß-estradiol had no effect, however, on male arteries precontracted with norepinephrine. Time-matched control arteries exposed to vehicle alone maintained a steady level of contraction to norepinephrine over the course of the experiment. The endothelium appeared to be functional in that acetylcholine (10^{-6} M) relaxed contractile responses to norepinephrine by more than 30% in all arteries studied.

Fig. 1B is a typical tracing showing the magnitude of the perfused rat tail artery responses to norepinephrine and 17ß-estradiol in female arteries. Perfusion of 17ß-estradiol (10^{-7} and 10^{-5} M) did not change basal perfusion pressure in either male or female tail arteries. In female artery segments, acute exposure to 17ß-estradiol, at concentrations of 10^{-7} and 10^{-5} M, significantly attenuated the contractile response to norepinephrine (Fig. 2A). In male artery segments, the same concentrations of 17ß-estradiol had no significant effect (Fig. 2B).

3.2. Effect of vehicle

The relaxing effect of 10^{-5} M 17ß-estradiol in female arteries was the same regardless of vehicle, ethanol or DMSO. Compared to arteries contracted with norepinephrine plus vehicle, relaxations of $33 \pm 7\%$ and $29 \pm 8\%$

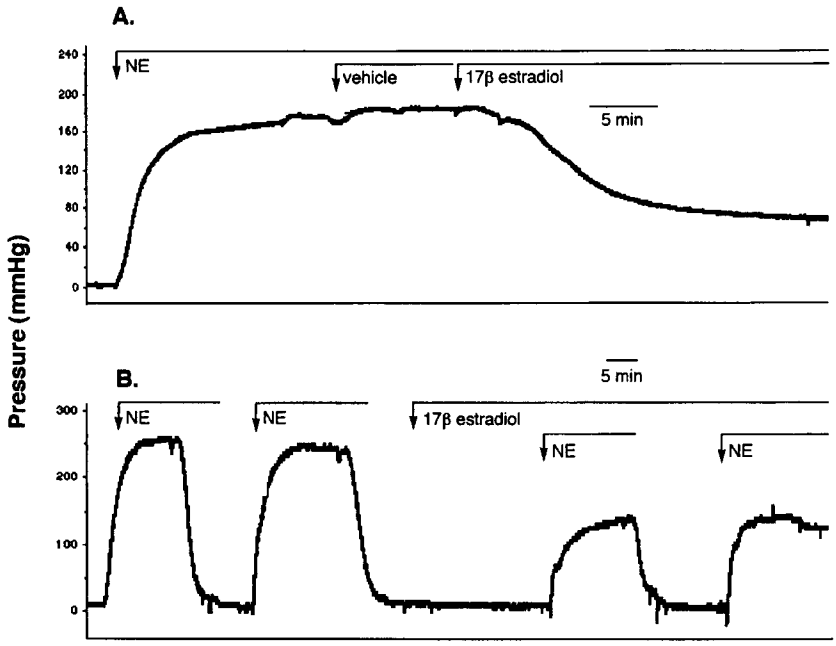

Fig. 1. (A) Time course of effect of 17ß-estradiol (10^{-5} M) on female rat tail artery precontracted with norepinephrine (NE, 3×10^{-7} M). The effect of the vehicle (ethanol) is also shown. (B) Typical tracing showing the contractile response to norepinephrine (NE, 3×10^{-7} M) and norepinephrine in the presence of 17ß-estradiol (10^{-5} M).

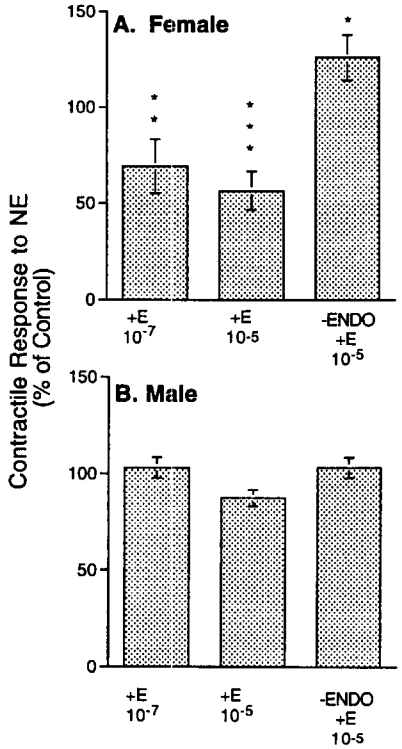

Fig. 2. Effect of 17ß-estradiol (+E 10^{-7}, 10^{-5} M) on the contractile response to norepinephrine (3×10^{-7} M) in intact and endothelium-denuded (−ENDO), female (A, $n = 8$, 4 and 7, respectively) and male (B, $n = 5$, 6 and 4, respectively) arteries. Data are expressed as percentage of the response to norepinephrine alone for tissues with and without endothelium as appropriate and are shown as mean ± S.E.M. * $P < 0.05$ ** $P < 0.01$ compared to control responses with norepinephrine alone.

were found when arteries were perfused with 17ß-estradiol dissolved in either ethanol or DMSO, respectively ($P > 0.4$, $n = 5$). In male arteries, no relaxation to 10^{-5} M 17ß-estradiol was found with either vehicle. Both ethanol and DMSO vehicles caused variable increases in the contractile response to norepinephrine; however, these effects were not significantly different between male and female artery segments.

3.3. Endothelium removal

In arteries where the endothelium was rubbed away, acetylcholine (10^{-6} M) produced little or no relaxation (< 20%) of the contraction induced by norepinephrine (3×10^{-7} M). The contractile response to norepinephrine was significantly greater in endothelium-denuded arteries compared to endothelium-intact arteries in both males and females. In males, the contractile response was 66 ± 11 mm Hg in endothelium-intact arteries and 165 ± 19 mm Hg in denuded arteries, while in females the contractile response was 50 ± 6 mm Hg in endothelium-intact arteries and 83 ± 12 mm Hg in denuded arteries. Perfusion of 17ß-estradiol did not change basal perfusion pressure in either male or female denuded arteries. In female denuded arteries, 17ß-estradiol no longer attenuated responses to norepinephrine; instead, contractile responses were increased by 26 ± 12% ($P < 0.05$, $n = 7$, Fig. 2A). In male denuded tail artery segments, acute exposure to 17ß-

estradiol (10^{-5} M) had no effect on the contractile response to norepinephrine (Fig. 2B).

4. Discussion

Our results show that acute exposure to 17ß-estradiol attenuates the contractile response to norepinephrine in female, but not male, rat tail arteries in a concentration-dependent manner. This suggests that 17ß-estradiol may have a modulatory role on the vascular adrenergic response to sympathetic stimulation in females. The effect of 17ß-estradiol was dependent on an intact endothelium and occurred on a time-scale too rapid to be consistent with a genomic mechanism of action. Previous work in our laboratory has shown that contractions due to adrenergic nerve stimulation in the rat tail artery are also attenuated by brief exposures to 17ß-estradiol. A similar sex difference was found for this effect, i.e., the attenuation was greater in female than male arteries (García-Villalón et al., submitted). We have also found that 17ß-estradiol acutely attenuates the response of arteries precontracted with potassium chloride; however, in contrast, this effect of 17ß-estradiol was not different between males and females (García-Villalón et al., submitted). Together, these data suggest that the effect of 17ß-estradiol on the adrenergic response is greater in females than males, and is mediated by a mechanism different from the effect of estradiol on arteries precontracted with potassium chloride.

The effect of 17ß-estradiol on the adrenergic response is likely to be mediated post-junctionally, as contractile responses due to exogenous addition of norepinephrine or nerve stimulation were both attenuated by 17ß-estradiol to a similar extent. Since acute addition of 17ß-estradiol has no significant effect on electrically stimulated norepinephrine release from vascular nerve terminals (García-Villalón et al., submitted), it seems likely that the effect of 17ß-estradiol on the contractile response to nerve stimulation would be mediated by the same mechanism as that of the effect of 17ß-estradiol on the contractile response to exogenously added norepinephrine.

In vivo, long-term treatment with 17ß-estradiol has been shown to have effects on vascular responses to adrenergic stimulation (Colucci et al., 1982; Miller and Vanhoutte, 1990). In vitro, short-term effects of 17ß-estradiol on the vasculature have also been reported, but these reports vary greatly. Acute exposure to 17ß-estradiol increases the contractile responses to norepinephrine, K^+, or the thromboxane A_2 mimic U-46619, but not electrical field stimulation, in the male rat mesentery (Vargas et al., 1995). Acute exposure to 17ß-estradiol attenuates contractile responses to prostaglandin $F_{2\alpha}$, K^+, or Bay K 8644 in both male and female rabbit coronary arteries in an endothelium-independent manner (Jiang et al., 1991). Estrogen relaxes porcine coronary arteries in an endothelium-independent manner by opening BK_{Ca} channels through a cGMP-dependent mechanism (White et al., 1995). 17ß-Estradiol also relaxes the sustained phase of contraction in male Sprague-Dawley rat tail artery helical strips precontracted with [Arg^8]vasopressin, K^+, or norepinephrine (Shan et al., 1994). Most of these studies looked at male arteries only, and the present investigation is the first to report an endothelium-dependent relaxation to 17ß-estradiol which is greater in females than males. This suggests that previous reports may reflect other mechanisms than the one underlying the effects of 17ß-estradiol shown in the present study.

In the present study, the ability of 17ß-estradiol to relax female arteries contracted with norepinephrine was shown to be dependent on an intact endothelium. Acute exposure to 17ß-estradiol has been shown recently to enhance carbachol-induced, endothelium-dependent relaxation of the female rat aorta precontracted with phenylephrine (Paredes-Carbajal et al., 1995). This suggests that 17ß-estradiol may act to increase receptor-mediated release of nitric oxide from the endothelium of the rat aorta in the presence of an adrenergic agonist. 17ß-Estradiol has been shown to greatly increase intracellular calcium concentration in chicken and pig ovarian granulosa cells in less than 5 s (Morley et al., 1992). If a similar mechanism is present in endothelial cells, it would result in an increase in nitric oxide release mediated by 17ß-estradiol. In vivo, the acute effects of 17ß-estradiol were assessed in coronary arteries of postmenopausal atherosclerotic female monkeys (Williams et al., 1992). These arteries constricted in response to acetylcholine before 17ß-estradiol infusion but dilated in response to acetylcholine 20 min after 17ß-estradiol infusion. Because there is a basal sympathetic tone associated with the in vivo responses, and because acetylcholine mediates its vasodilatory effects through the endothelium, these results are also consistent with the idea that 17ß-estradiol may acutely influence adrenergic responses via an endothelium-dependent mechanism.

It should be noted that the concentrations of 17ß-estradiol used in this experimental situation are pharmacological, and much higher than endogenous circulating levels. Nevertheless, this study demonstrates that 17ß-estradiol is able to relax blood vessels, an action which is consistent with epidemiological data. Indeed, sublingual administration of 17ß-estradiol has acute effects in the peripheral vasculature of postmenopausal women, causing a reduction of forearm resistance and increasing mean blood flow (Volterrani et al., 1995). Furthermore, the pharmacological concentrations used in this study caused a substantial relaxation of arteries. The physiological action of 17ß-estradiol as a vasodilatory modulator may be more subtle in nature and therefore require lower concentrations.

In summary, these results suggest that 17ß-estradiol may have an acute modulatory role on vascular responses to sympathetic stimulation in females. This effect is likely to be mediated by a post-junctional response that is dependent on an intact endothelium and occurs on a relatively rapid time-scale.

Acknowledgements

This work was supported in part by a grant from the National Institutes of Health, HL 50775.

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