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Nongenomic responses to 17β-estradiol in male rat mesenteric arteries abolish intrinsic gender differences in vascular responses

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1 The aim of the present study was to investigate the gender differences in the acute effects of 17β-estradiol on the rat superior mesenteric artery.

2 Isometric tension was measured in rings of mesenteric arteries from both male and female Sprague–Dawley rats.

3 Relaxation to acetylcholine was not significantly different between arteries (with endothelium) from male and female rats in the absence or presence of 17β-estradiol. After blockade of endothelium-dependent hyperpolarizations with apamin (0.3 μM) plus charybdotoxin (0.1 μM), acute exposure to 17β-estradiol (1 nM) for 30 min resulted in enhancement of relaxation to acetylcholine in arteries from male but not female rats.

4 After acute exposure to 17β-estradiol, mesenteric arteries from male rats were more sensitive to sodium nitroprusside than arteries from female rats.

5 Contractions of mesenteric arteries to phenylephrine and 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U46619) were greater in arteries from male rats than female rats. This difference was not detected after acute exposure to 17β-estradiol.

6 In preparations without endothelium, the enhancement of relaxation and reduction in contraction in arteries from male rats were preserved.

7 These results suggest that there exists a gender difference in the response to the acute nongenomic modulatory effect of 17β-estradiol in rat mesenteric arteries. Arteries from male rats seem to be more sensitive to the modulatory effects of 17β-estradiol than arteries from female rats. The effect appears to be mainly at the level of the vascular smooth muscles.
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Abbreviations: ATP, adenosine triphosphate; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; KCl, potassium chloride; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; U46619, 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$

Introduction

While men are generally at a higher risk for cardiovascular and renal diseases than age-matched premenopausal women, their arterial blood pressures are also higher than that of women of similar ages (Khoury *et al*., 1992; Wiinber *et al*., 1995). One factor that may contribute to the gender difference in arterial blood pressure is a difference in vascular reactivity. A significant part of this difference is due to the endothelium. Nitric oxide (NO) production (Forte *et al*., 1998) and endothelial NO release (Rosano *et al*., 1993) are greater in premenopausal women than in men. Estrogen may also augment the production of vasodilator derivatives of cyclooxygenase. For example, the level of prostacyclin is increased in female subjects (Geary *et al*., 2000). In contrast, the production of cyclooxygenase-derived vasoconstrictors, such as thromboxane A_2, is increased in the male (Kähönen *et al*., 1998). The production of other endothelium-derived vasoconstrictors, such as endothelin, is also greater in men than in women (Akishita *et al*., 1998).

Besides the endothelium, vascular smooth muscles contribute significantly to gender differences in vascular reactivity. Responses to α-adrenoceptor agonists are smaller in mesenteric arteries of female than male rats (Stallone, 1993). This may, at least in part, be due to a decrease in the expression of α_1-adrenoceptors in the arteries (Zhang & Davidge, 1999). The intracellular calcium level in female arteries is lower than in male (Murphy & Khalil, 2000). The expression of protein kinase C is also higher in the vascular smooth muscles in male than in female rats (Kanashiro & Khalil, 2001).

While a great part of the observed gender differences in vascular reactivity has been attributed to genomic modulation by sex hormones, nongenomic effects of those hormones also exist (Kelly & Levin, 2001). Not only is the endothelial nitric oxide synthase (eNOS) upregulated by estrogen genomically, acute administration of estrogen may also activate eNOS (Stefano *et al*., 2000). In vascular smooth muscles, estrogen has also been shown to modulate ATP-sensitive K^+

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(Martinez *et al*., 2001) and Ca^{2+}-activated K^+ (White *et al*., 1995) channels nongenomically.

Arteries from both male and female rats respond to the acute administration of estrogen, with arteries from male having a higher sensitivity than arteries from female (Crews & Khalil, 1999). However, the origin of this differential effect is unclear. The sexual dimorphism of the effect of estrogen has only been demonstrated at high, pharmacological concentrations. Whether this sexual dimorphism can also be demonstrated at low, more physiological concentrations of estrogen is unknown. The present study was designed to investigate whether or not differential effects of vasoactive substances are observed in male and female mesenteric arteries after acute exposure to physiological concentrations of 17β-estradiol and whether such effects are endothelium dependent.

Methods

Animals

The use of animals was approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. Sprague–Dawley rats of either sex at 10 weeks were employed in the study.

Tissue preparation

Rats were killed by an overdose of sodium pentobarbital (70 mg kg^{-1} i.p.). The main branch of the superior mesenteric artery was dissected free. After removal of the surrounding connective tissues, arteries were cut into 3 mm wide rings and suspended between stainless steel hooks in 4 ml jacketed organ baths filled with oxygenated Krebs–Henseleit solution (composition in mM: NaCl 120, KCl 4.76, $NaHCO_3$ 25, NaH_2PO_4 1.18, $CaCl_2$ 1.25, $MgSO_4$ 1.18, glucose 5.5; control solution) maintained at 37°C. In experiments where arterial rings without endothelium were used, arteries were perfused with 0.5 ml of 0.1% Triton X-100 in control solution before 3 mm rings were cut. The rings from both male and female rats were then placed under 1 g of resting tension, which was determined to be the approximate optimal tension by length-tension experiments in preliminary studies. Rings were equilibrated for 90 min during which the bathing solution was changed every 15 min with readjustment of baseline tension when necessary. Isometric tension was measured by force transducers (FT03, Grass Instrument Co., Quincy, U.S.A.) coupled to an amplifier and a personal computer for data collection (PICO Data Logger, Pico Technology Ltd, Cambridge, U.K.).

Experimental protocols

After the equilibration period, the rings were challenged twice with KCl (50 mM). The viability of each rat mesenteric arterial ring was determined by contracting with phenylephrine (1 μM) before relaxing with acetylcholine (1 μM). Rings that failed to produce a contraction $\geqslant$0.5 g when challenged with phenylephrine and $\geqslant$90% relaxation to acetylcholine were excluded from the study. In preparations without endothelium, rings with $\geqslant$5% relaxation were discarded. Drugs were then removed by repeated changes of bath solution. After baseline tension was re-established, the rings were incubated again with various drugs. In preliminary studies, indomethacin (10 μM) was found to have no apparent effect on the relaxation to both the baseline tension as well as the relaxation to acetylcholine. In subsequent experiments, indomethacin (10 μM) was included in order to rule out the possible involvement of cyclooxygenase products. 17β-Estradiol and indomethacin (10 μM) were added 30 min prior to testing. Indomethacin (10 μM) was present throughout the experiment. Where necessary, antagonists were introduced into the baths 20 min before the addition of vehicle solvent or the studied agonist. Except where noted, all drugs remained present throughout the experiment. Relaxations and contractions were produced by a stepwise addition of drugs at half-log intervals. Each tissue was exposed to only one contracting or one relaxing agent.

Data analysis

Data are reported as means$\pm$standard error of the mean (s.e.m.) with n indicating the number of rats from which arterial rings were obtained. Data are expressed as tension in grams of contraction in case of contraction studies. In preliminary studies, mesenteric arterial rings from both male and female rats were found to be not significantly different in protein content (data not shown) and therefore no correction for the difference of ring size was attempted. Maximal contractions and pD_2 values were determined with the aid of a curve-fitting program (SigmaPlot, SPSS Inc., Chicago, IL, U.S.A.). pD_2 values$\pm$s.e.m were converted to EC_{50} with 95% confidence interval (95% CI). Statistical tests were performed using a computer statistical package (SPSS, SPSS Inc., Chicago, IL, U.S.A.). Independent sample t-test was applied to determine individual differences between groups of data. P-values of <0.05 were considered to indicate statistically significant differences.

Drugs and chemicals

9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619) was obtained from Biomol, PA, U.S.A. Phenylephrine hydrochloride, acetylcholine hydrochloride, 17β-estradiol and the remaining chemicals were obtained from Sigma, St Louis, MO, U.S.A. Stocks of 17β-estradiol and U46619 were made up in ethanol. The final concentration of ethanol in each bath did not exceed 0.2% of total bath volume. Indomethacin (1 mM) stock solution was dissolved in a 1 mM sodium carbonate solution before diluting to a working concentration. All other drugs were dissolved in deionized water and all working solutions were obtained by dilution in control solution.

Results

Effect of gender on relaxation to 17β*-estradiol*

Mesenteric arteries from both male and female rats were contracted with phenylephrine (1 μM). 17β-Estradiol elicited a concentration-dependent relaxation. At high concentrations (10, 30 and 100 μM), significant relaxations were observed (data not shown). No significant relaxation was observed in the physiological range of 17β-estradiol concentrations (1–10 nM concentrations).

Effects of 17β-estradiol on endothelium-dependent relaxations

Acetylcholine elicited a concentration-dependent relaxation in mesenteric arteries from both male and female rats (Figure 1). There was no apparent difference in both the maximal relaxation (Figure 1a) and the EC_{50} (15.8 nM, 95% CI 12.6–19.9 nM in male and 7.9 nM, 95% CI 5.2–12.0 nM in female rats). Exposure of the arteries to 17β-estradiol (1 nM) for 30 min did not significantly affect either the maximal relaxation or the EC_{50} to acetylcholine. No significant difference was found between all four groups when relaxations were expressed as area under concentration–response curves (Figure 1b).

Blockade of NO production by N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM) for 30 min reduced the maximal relaxation to acetylcholine in both male (61.3 $\pm$ 7.4%) and female (73.4 $\pm$ 11.0%) rats (Figure 2a). Exposure of the arteries to 17β-estradiol (1 nM) for 30 min did not cause any difference in maximal relaxation or EC_{50}. No significant difference was found between the four groups when relaxations were expressed as area under concentration–response curves (Figure 2b).

Blockade of endothelium-derived hyperpolarizing factor (EDHF)-mediated responses by a combination of apamin (0.3 μM) and charybdotoxin (0.1 μM) reduced the maximal relaxation of the arteries to acetylcholine in both male (74.2 $\pm$ 5.6%) and female (87.5 $\pm$ 2.3%) rats (Figure 3a). Exposure to 17β-estradiol (1 nM) for 30 min enhanced the relaxation to acetylcholine in arteries from male rats with an EC_{50} significantly reduced from 66.1 nM (95% CI 52.3–83.27 nM) to 24.5 nM (95% CI 16.6–23.4 nM). Relaxation to acetylcholine in arteries from female rats after exposure to 17β-estradiol was not significantly different from control (Figure 3a). The area under the concentration–response curve for the male control group was significantly ($P<0.05$) smaller than for arteries from male rats after acute exposure to 17β-estradiol and arteries from female rats with or without acute 17β-estradiol exposure (Figure 3b).

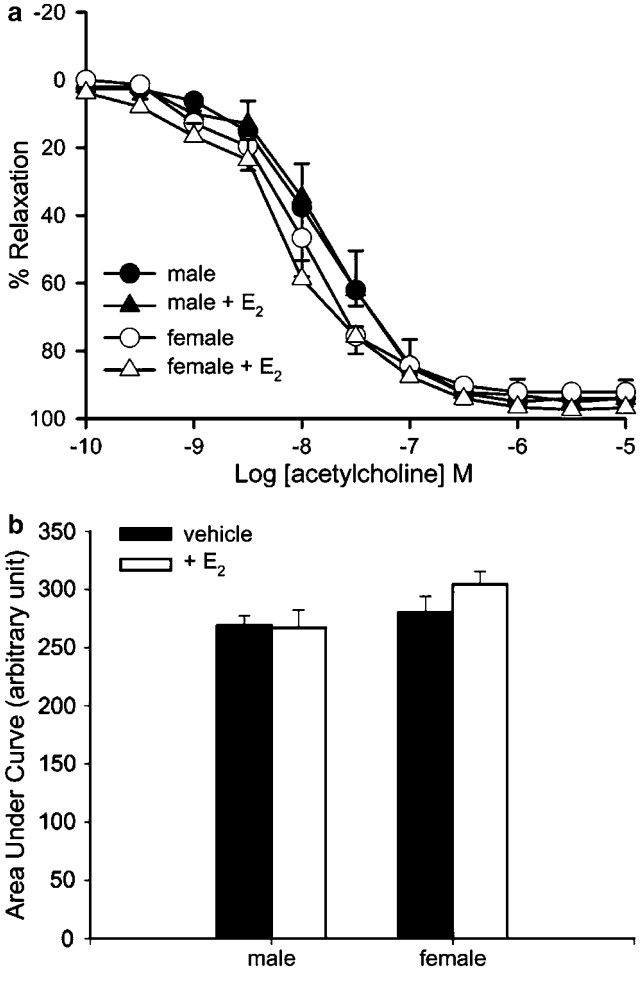

Figure 1 Effects of 17β-estradiol on acetylcholine-induced relaxation. Rings from superior mesenteric arteries of male and female rats were incubated with either vehicle control or 17β-estradiol (1 nM) for 30 min. Rings were then contracted with phenylephrine (1 μM) before acetylcholine was added cumulatively. Data are expressed as mean $\pm$ s.e.m. with $n = 5$–7. (a) Concentration–response curves to acetylcholine after exposure to 17β-estradiol. (b) Relaxation expressed as area under concentration–response curves.

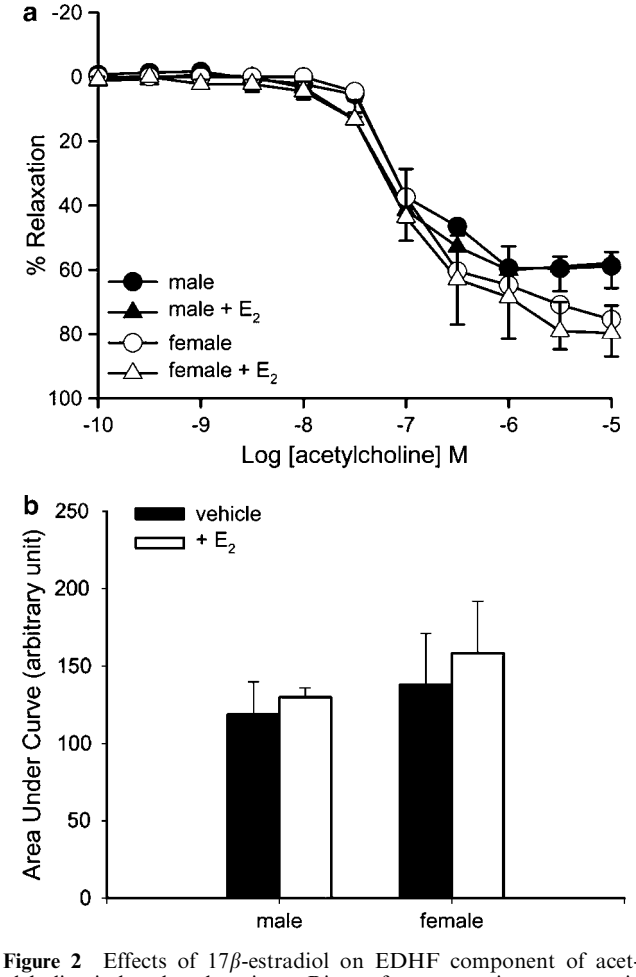

Figure 2 Effects of 17β-estradiol on EDHF component of acetylcholine-induced relaxation. Rings from superior mesenteric arteries of male and female rats were incubated with L-NAME (300 μM) together with either vehicle control or 17β-estradiol (1 nM) for 30 min. Rings were then contracted with phenylephrine (1 μM) before acetylcholine was added cumulatively. Data are expressed as mean $\pm$ s.e.m. with $n = 6$–7. (a) Concentration–response curves to acetylcholine after exposure to 17β-estradiol. (b) Relaxation expressed as area under concentration–response curves.

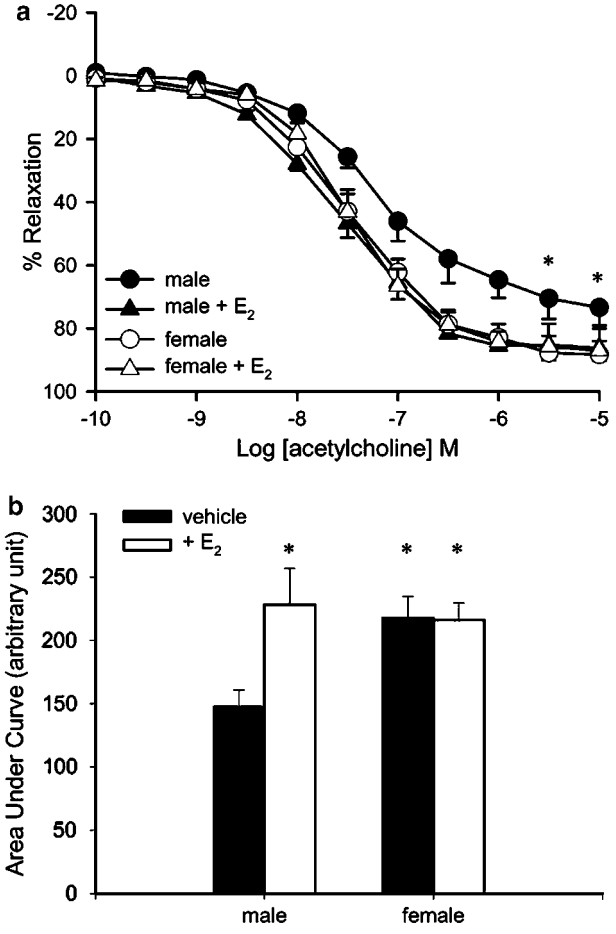

Figure 3 Effects of 17β-estradiol on NO component of acetylcholine-induced relaxation. Rings from superior mesenteric arteries of male and female rats were incubated with apamin (300 nM) and charybdotoxin (100 nM) together with either vehicle control or 17β-estradiol (1 nM) for 30 min. Rings were then contracted with phenylephrine (1 μM) before acetylcholine was added cumulatively. Data are expressed as mean$\pm$s.e.m. with $n=6$–7. (a) Concentration–response curves to acetylcholine after exposure to 17β-estradiol. *$P<0.05$ vs all groups. (b) Relaxation expressed as area under concentration–response curves. *$P<0.05$ vs male control group (ANOVA followed by *post hoc* Dunnett's test).

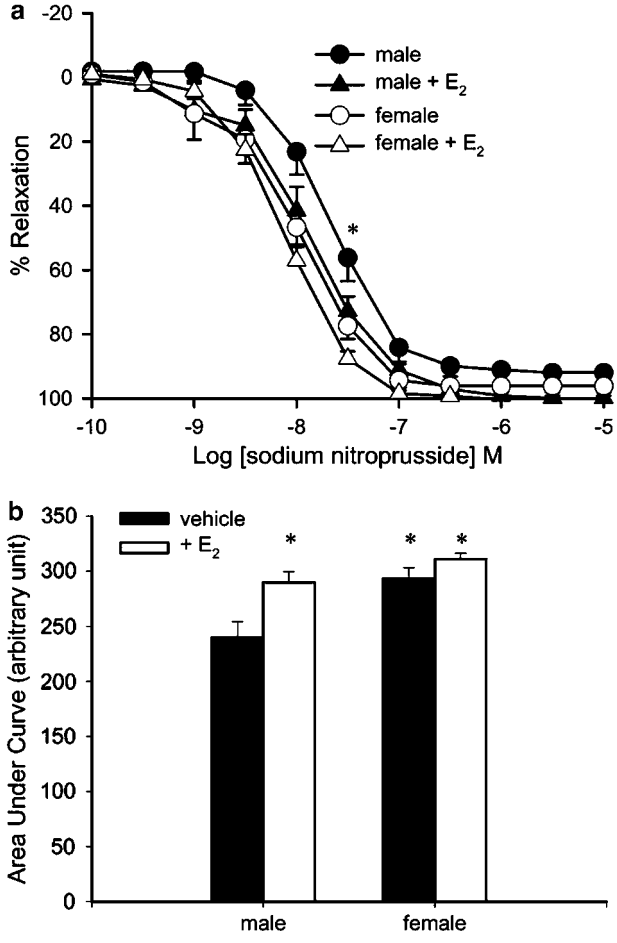

Figure 4 Effects of 17β-estradiol on sodium nitroprusside-induced relaxation. Rings from superior mesenteric arteries of male and female rats were incubated with either vehicle control or 17β-estradiol (1 nM) for 30 min. Rings were then contracted with phenylephrine (1 μM) before sodium nitroprusside was added cumulatively. Data are expressed as mean$\pm$s.e.m. with $n=6$–7. (a) Concentration–response curves to sodium nitroprusside after exposure to 17β-estradiol. *$P<0.05$ vs all groups. (b) Relaxation expressed as area under concentration–response curves. *$P<0.05$ vs male control group (ANOVA followed by *post hoc* Dunnett's test).

*Effect of 17*β*-estradiol on endothelium-independent relaxations*

Sodium nitroprusside elicited a concentration-dependent relaxation in arteries from both male and female rats (Figure 4a). Mesenteric arteries from female rats were slightly but significantly more sensitive to sodium nitroprusside than arteries from male rats. The EC_{50} in control male arteries was 20.9 nM (95% CI 17.0–25.7 nM), and that in female arteries was 9.5 nM (95% CI 7.9–11.5 nM). Relaxation to sodium nitroprusside was enhanced in arteries from male rats after exposure to 17β-estradiol (1 nM) for 30 min to a level that is comparable to arteries from female rats. The concentration–response curve to sodium nitroprusside in arteries from male rats after exposure to 17β-estradiol was not significantly different from that obtained in arteries from female rats (Figure 4a; EC_{50} 13.2 nM (95% CI 10.2–17.0 nM) in male and 6.2 nM (95% CI 5.1–7.4 nM) in female rats, respectively). The area under the concentration–response curve in the vehicle-treated male group was significantly ($P<0.01$) smaller than in arteries from male rats after acute exposure to 17β-estradiol, as well as arteries from female rats with ($P<0.005$) or without ($P<0.005$) acute 17β-estradiol exposure (Figure 4b).

*Effect of 17*β*-estradiol on contractions*

Phenylephrine elicited a concentration-dependent contraction in mesenteric arteries from both male and female rats (Figure 5a). Exposure to 17β-estradiol (1 nM) decreased the maximal contraction in arteries from male but not from female rats. The reduction in contraction after exposure to 17β-estradiol in arteries from male rats resulted in maximal responses similar in magnitude to that of arteries from female rats (Figure 5a). The area under concentration–response curve of arteries from vehicle-treated male rats was significantly ($P<0.05$) larger than the other three groups (Figure 5b).

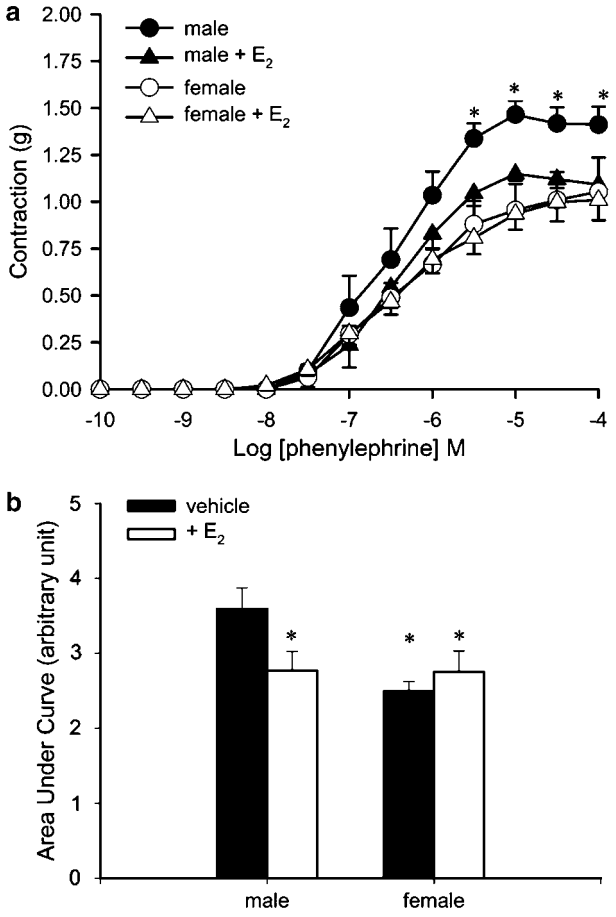

Figure 5 Effects of 17β-estradiol on phenylephrine-induced contraction. Rings from superior mesenteric arteries of male and female rats were incubated with either vehicle control or 17β-estradiol (1 nM) for 30 min. Phenylephrine was then added cumulatively. Responses were expressed as tension in grams. Data are expressed as mean$\pm$s.e.m. with $n=6$–7. (a) Concentration–response curves to phenylephrine after exposure to 17β-estradiol. *$P<0.05$ vs all groups. (b) Contraction expressed as area under concentration–response curves. *$P<0.05$ vs male control group (ANOVA followed by *post hoc* Dunnett's test).

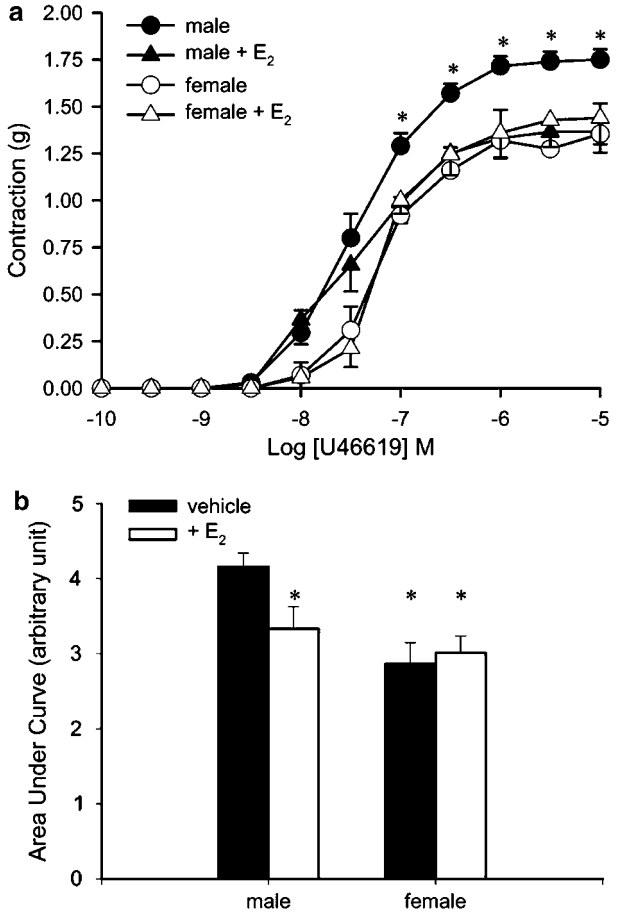

Figure 6 Effects of 17β-estradiol on U46619-induced contraction. Rings from superior mesenteric arteries of male and female rats were incubated with either vehicle control or 17β-estradiol (1 nM) for 30 min. U46619 was then added cumulatively. Responses were expressed as tension in grams. Data are expressed as mean$\pm$s.e.m. with $n=6$–7. (a) Concentration–response curves to U46619 after exposure to 17β-estradiol. *$P<0.05$ vs all groups. (b) Contraction expressed as area under concentration–response curves. *$P<0.05$ vs male control group (ANOVA followed by *post hoc* Dunnett's test).

Responses to U46619 showed a similar pattern with that of phenylephrine in arteries from the two genders (Figure 6a). U46619 elicited a greater maximal contraction in arteries from male than female rats. Exposure to 17β-estradiol (1 nM) for 30 min resulted in a decrease in maximal contraction in arteries from male rats (Figure 6a). However, this effect was not observed in arteries from female rats. The area under concentration–response curve of arteries from vehicle-treated male rats was significantly larger than that from the other groups ($P<0.05$ vs male estradiol group; $P<0.005$ vs female control and female estradiol group) (Figure 6b).

Effect of endothelium

Relaxations to sodium nitroprusside in arteries without endothelium were significantly enhanced in arteries from male rats when incubated with 17β-estradiol for 30 min (Figure 7a).

Removal of endothelium did not affect the effect of 17β-estradiol on contractions in mesenteric arteries from male rats. Thus, in arteries without endothelium, the maximal contraction to phenylephrine was reduced after incubation with 17β-estradiol (Figure 7b).

Discussion

This study confirmed that arteries from male rats elicited poorer relaxation and stronger contraction when compared to arteries from female rats. However, this intrinsic difference in responses to vasoactive substances could be abolished by acute exposure of arteries from male rats to 17β-estradiol. The results also demonstrated that this could be achieved using a physiologically relevant concentration of 17β-estradiol (nanomolar concentration). This finding may help to provide an insight to the gender differences in cardiovascular events such as hypertension and alterations in vascular reactivity.

The present study shows that mesenteric arteries from both male and female rats elicited a concentration-dependent relaxation to 17β-estradiol. In arteries from both male and

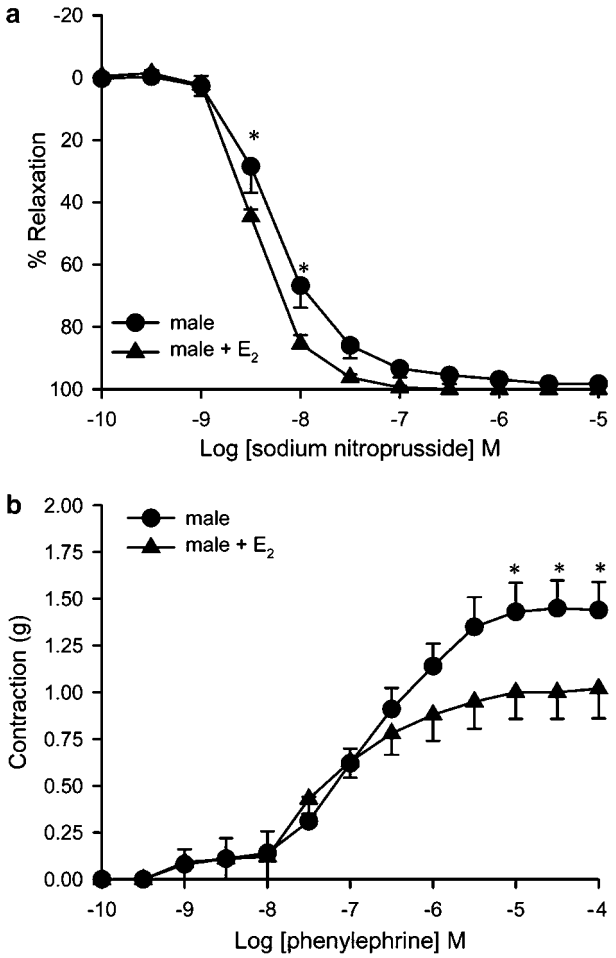

Figure 7 Effects of 17β-estradiol on phenylephrine-induced contraction and sodium nitroprusside-induced relaxation. Endothelium disrupted rings from superior mesenteric arteries of male rats were incubated with either vehicle or 17β-estradiol for 30 min. (a) Rings were contracted with phenylephrine (1 μM) before sodium nitroprusside was added cumulatively. (b) Phenylephrine was added cumulatively. Responses were expressed as tension in grams. Data are expressed as mean$\pm$s.e.m. with $n=6$–7. *$P<0.05$ vs male control group (ANOVA followed by *post hoc* Dunnett's test).

female rats, 17β-estradiol at 1 nM, the concentration chosen for the subsequent experiments, did not elicit a direct relaxation. The results obtained from the present study demonstrate a modulating effect of 17β-estradiol on vascular responses, rather than a direct relaxing response.

Relaxations to acetylcholine were not different between arteries from male and female rats, in accord with previous studies (Sakuma *et al*., 2002). Relaxation to acetylcholine was not affect by the acute administration of 17β-estradiol for 30 min in both male and female rats. This is in contrast to the results from a previous study (Paredes-Carbajal *et al*., 1995), where there was an increased relaxation to carbachol after exposure of the aortae from ovariectomized rats to 17β-estradiol at 1 nM for 40–60 min. However, this discrepancy could be due to the use of different arteries from rats of different hormonal status, which may affect the contribution of different relaxation pathways. The endothelium-dependent relaxation of the rat mesenteric artery is mediated predominantly by NO and EDHF (McCulloch & Randall, 1998). However, the two pathways have been shown to inhibit each other (Olmos *et al*., 1995; Bauersachs *et al*., 1996; McCulloch *et al*., 1997). An attempt was made to investigate the two pathways individually, using selective blockers. The present results show that arteries from male rats had a smaller maximal relaxation to acetylcholine than in female rats when the EDHF pathway was blocked by the combination of apamin plus charybdotoxin (Zygmunt & Högestätt, 1996). Significant enhancement of relaxation to acetylcholine by 17β-estradiol was observed in arteries from male rats treated with the combination of apamin plus charybdotoxin. Furthermore, relaxation was enhanced to a level that is close to that of arteries from female rats. This is in line with the findings in another study where the relaxation to 17β-estradiol was found to be attenuated following administration of L-NAME to aortae from male rats but not female rats (Tep-areenan *et al*., 2003). Acute treatment of the arteries with 17β-estradiol did not affect the relaxation in arteries treated with L-NAME. The fact that the augmentation was seen only in arteries treated with the combination of apamin plus charybdotoxin suggests that 17β-estradiol selectively affects the NO pathway. Thus, the EDHF pathway is not involved in the acute response of 17β-estradiol.

Several studies suggest that 17β-estradiol can acutely activate endothelial NO synthase, leading to an increased NO production (Stefano *et al*., 2000). 17β-Estradiol may act *via* a putative cell membrane estrogen receptor (Figtree *et al*., 2003) linked to the PI_3-kinase-Akt pathway (Haynes *et al*., 2000). However, the enhanced NO-mediated relaxation can also be due to effects on the vascular smooth muscles. In confirmation with earlier observations (Kähönen *et al*., 1998; Mayhan *et al*., 2002), the present results show that the relaxation induced by an exogenous NO donor is significantly higher in female than in male arteries. Again, when arteries from male rats were treated with 17β-estradiol acutely, the relaxation was enhanced to the same level as in female rats. The effect was observed irrespective of the presence or absence of endothelial cells, suggesting that the effect is localized in the vascular smooth muscles and does not involve an increase in NO availability. Measurement of cGMP in arteries with endothelium from both male and female rats before and after 17-estradiol administration for 30 min revealed that there was no difference in cGMP production either with or without sodium nitroprusside stimulation (data not shown). It is therefore unlikely that the effect of 17β-estradiol affect relaxation of the arteries by an increased production of either NO in the endothelium or an increased production of cGMP. The idea that the nongenomic effect of 17β-estradiol in vascular cells resides in the vascular smooth muscles is far from novel. It has been reported that 17β-estradiol could acutely relax rat small arteries even in the presence of the NOS inhibitor L-NNA, indomethacin or high K^+ ions (Shaw *et al*., 2000). In both male and female rat mesenteric arteries precontracted with methoxamine, high concentrations of 17β-estradiol has been shown to cause relaxation in the presence of either L-NAME or indomethacin, suggesting that the relaxation observed is largely endothelium independent. In our laboratory, we demonstrated that exposure of porcine coronary arteries to 17β-estradiol at 1 nM acutely enhances relaxation to sodium nitroprusside in preparations with and without endothelium, suggesting that the effect of

17β-estradiol is contributed by the vascular smooth muscles rather than the endothelium (Teoh *et al*., 1999).

While chronic estrogen treatment in ovariectomized rats reduces agonist-induced contractions in rat blood vessels (Paredes-Carbajal *et al*., 1995), the present study shows that acute treatment of mesenteric arteries of male rats with the female hormone also reduces agonist-induced contractions. Indeed, phenylephrine-induced contraction was reduced in mesenteric arteries from female when compared to male rats and this gender difference was abolished by acute treatment of the arteries from male rats with 17β-estradiol. Phenylephrine, being an α-adrenoceptor agonist, may produce a larger contraction in arteries from male due to a higher density of α-adrenoceptors in the blood vessels (Zhang & Davidge, 1999). However, contraction to U46619, a thromboxane A_2 analog, was also significantly higher in arteries from male than female rats. Acute treatment of the arteries from male rats with 17β-estradiol also abolished the observed difference in response to U46619 between arteries from the two genders. Thus, the effect of acute 17β-estradiol treatment did not appear to be agonist specific, although the contraction elicited by KCl *via* nonreceptor-mediated mechanism was similar between male and female rats (data not shown).

The gender difference seen in phenylephrine-induced contraction has been shown to be endothelium dependent and appears to involve cyclooxygenase products (McKee *et al*., 2003). The present results, however, showed that the gender difference in the contraction to both phenylephrine and U46619 was not abolished in arteries without endothelium. Recent findings suggest that chronic estrogen treatment can suppress the effect of Rho kinase in the vascular smooth muscles, which phosphorylate the regulatory subunit of myosin light chain phosphatase which leads to a decrease in dephosphorylation of myosin light chain kinase leading to contraction (Somlyo & Somlyo, 2000). However, 17β-estradiol does not seem to affect Rho kinase activity when administered acutely, as the effect of 17β-estradiol on contraction to phenylephrine in preparations without endothelium was not abolished by the rho kinase inhibitor Y 27632 (data not shown). Previously in our laboratory, we demonstrated that the effect of 17β-estradiol (1 nM) on contraction in porcine coronary arteries can be abolished by adenylyl cyclase inhibitors, suggesting a role for the cAMP cascade (Keung *et al*., 2005). Whether the same mechanism can account for the effect seen in the present study requires further confirmation. Nonetheless, it appears that other than the endothelium, vascular smooth muscles also contribute to the gender difference in agonist-induced contraction. More importantly, this gender difference in agonist-induced contraction can be abolished by acute treatment of the arteries with 17β-estradiol. This effect appears to be independent of the mechanism of action of 17β-estradiol in the endothelium as the estrogen receptor antagonists ICI 182 780 and tamoxifen failed to inhibit its effect in the vascular smooth muscle (data not shown), but has been shown to inhibit the effect of 17β-estradiol in the endothelium (Chen *et al*., 1999).

The nature of this nongenomic effect of 17β-estradiol is unknown. However, the rapidity and the specificity of the effect suggest that it is receptor mediated. A membrane-bound estrogen receptor exists in endothelial cells, which acutely activates eNOS (Chen *et al*., 1999). This receptor, in human endothelial cells, may be a truncated protein, 46 kDa in size, of the full-length 66 kDa genomic ERα receptor (Figtree *et al*., 2003). Whether the effects observed in the present study can be attributed to this putative membrane estrogen receptor is uncertain. One possible explanation for the observed difference in response to acute exposure to 17β-estradiol between the two genders may be the difference in sex hormone levels. Indeed, hormone levels affect the expression of different estrogen receptor subtypes in a number of cell types. While ERα is upregulated with increased concentration of estradiol, ERβ expression may be downregulated by it (Maggi *et al*., 2003). On the other hand, testosterone upregulates estrogen receptor expression in the prostate gland of the rat (Asano *et al*., 2003) and efferent ducts of the goat (Goyal *et al*., 1998). Whether the present result observed in arteries from male rats is a result of the presence of testosterone or the lack of estrogen remains to be determined.

In summary, the results obtained from the present study are unique in that the acute nongenomic effect of 17β-estradiol on the vascular smooth muscles can be demonstrated in male but not in female rats. This acute nongenomic effect of 17β-estradiol in the male rats may help to abolish gender differences in vascular responses. Thus, 17β-estradiol may act on the vascular system to provide protection not only in female but also in male rats. Whereas 17β-estradiol usually acutely affects the endothelium, the present results suggest that vascular smooth muscle is also involved.

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