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Effects of 17β-Oestradiol on Rat Isolated Coronary and Mesenteric Artery Tone: Involvement of Nitric Oxide

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Abstract

Pre- and post-menopausal women receiving oestrogen replacement therapy have a significantly reduced risk of cardiovascular disorders. It has been suggested that this protection might be partly a result of a direct relaxant effect of oestrogens on coronary arteries. This study examines and directly compares the effects of 17β-oestradiol on rat isolated coronary and mesenteric vessels. The influence of nitric oxide on these responses was also investigated.

17β-Oestradiol caused similar concentration-dependent relaxation of isolated coronary and mesenteric resistance arteries pre-contracted with either KCl (60 mM) or 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin (U46619; 1 μM). The relaxation responses to 17β-oestradiol were significantly reduced, but not totally inhibited, in the presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase; they were not altered by indomethacin, an inhibitor of prostaglandin synthesis. The responses to 17β-oestradiol in the presence of L-NAME were not dependent on the vessel studied or the pre-contracting agent used.

These results suggest that nitric oxide might contribute to the vasodilatory effects of 17β-oestradiol in rat isolated coronary and mesenteric resistance arteries.

It is generally accepted that during pre-menopausal years women have a lower risk of developing coronary heart disease than men. After the menopause, the incidence rapidly increases to a level which is similar to that for men of comparable age (Barrett-Connor & Bush 1991). In post-menopausal women oestrogen replacement therapy has been shown to reduce the incidence of coronary events by approximately 50%, with the greatest benefit being observed for women with coronary artery disease (Stampfer et al 1991).

Several potential mechanisms have been proposed for this protective effect of oestrogens. Oestrogen replacement has been shown to modify the lipid profile beneficially by reducing levels of low-density lipoprotein (LDL) and increasing those of high-density lipoprotein (HDL) and cholesterol (Bush et al 1987). In addition oestrogens have been shown to inhibit cholesterol deposition and atherosclerotic plaque formation in the arterial wall (Adams et al 1990); it appears, however, that these effects account for only 25–50% of the observed risk reduction, suggesting that additional factors are involved (Bush et al 1987). It has been suggested that one such factor might be a direct vasodilatory effect on coronary artery tone.

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Infusion of 17β-oestradiol has been shown to cause coronary artery vasodilation in rabbit isolated perfused hearts (Raddino et al 1986) and to increase myocardial perfusion in non-pregnant ovariectomized ewes (Magness & Rosenfeld 1989). Clearly these effects might contribute to the cardioprotection offered by the hormone. Infusion of acetylcholine into atherosclerotic coronary arteries of ovariectomized monkeys has been shown to cause contraction whereas relaxation was observed after treatment with 17β-oestradiol (Williams et al 1990). Similar results have also been obtained with post-menopausal women, leading to the conclusion that 17β-oestradiol might alter vascular tone in-vivo, at least partly, by a nitric oxide-dependent mechanism (Collins et al 1995). In isolated coronary arteries from rabbit (Jiang et al 1991) or man (Mugge et al 1993), however, the relaxation induced by 17β-oestradiol was not altered either by removal of the endothelium or by addition of inhibitors of nitric oxide synthesis, suggesting a direct effect on the vascular smooth muscle. Although it is unclear why results from these in-vivo and in-vitro studies are different, it should be noted that both the in-vitro studies described above used fairly large epicardial

coronary arteries which might react differently from the smaller coronary resistance vessels.

It is clear that oestrogens might also vasodilate systemic arterial beds by a mechanism which again appears to be, in-vivo at least, partly endothelium-dependent (Rosenfeld et al 1996; Sudhir et al 1996). A role of nitric oxide and endothelial factors in the relaxant effects of oestradiol on isolated femoral and aortic vessels has been supported by some workers (Gisclard et al 1988; Williams et al 1988) but not all (Salas et al 1994). The responses of isolated systemic resistance vessels to oestradiol have not previously been investigated.

Thus it appears that oestradiol might dilate both coronary and systemic vascular beds. Although it seems that vasodilation of the coronary vessels, and thus increased myocardial perfusion, might contribute to the cardioprotective effect of the hormone, it is unclear whether coronary vessels are abnormally highly sensitive to oestrogens or whether the protection is because of general vasodilation. Differences between the reactivity of coronary and systemic vessels to oestrogens have been suggested by the study of Gilligan et al (1994), who reported that oestradiol increased the vasodilator response to sodium nitroprusside, an endothelium-independent vasodilator, in the forearm but not the coronary circulation of post-menopausal women. Comparisons of endothelium-dependent response with oestrogens have not previously been made.

The aim of this study was therefore to compare the effect of 17β-oestradiol on the tone of isolated rat coronary and mesenteric resistance vessels and to determine the involvement of endothelial factors, if any, in the responses.

Materials and Methods

Drugs and chemicals

All drugs and chemicals were obtained from Sigma (St Louis, MO). A stock solution of 17β-oestradiol was prepared by dissolving in 100% ethanol and then diluting 1 : 2000 with Krebs solution to give a concentration of 100 μM. Stock solutions (10 μM) of indomethacin and U46619 were also prepared by dissolving in 100% ethanol or a 1 : 2 mixture of 100% ethanol and 1 mg mL^{-1} sodium carbonate. All other drugs were dissolved directly in Krebs solution.

Animals and tissues

Male Wistar rats, 250–300 g, were killed by stunning and exsanguination. The mesentery and the heart were removed and third-order mesenteric vessels or the septal coronary artery, or both, were dissected out and mounted on a Mulvany-Halpern wire myograph for measurement of isometric tension. Where possible both coronary and mesenteric vessels were taken from each animal to enable direct comparison of the vessels. The vessels were bathed in Krebs solution of composition (mM): NaCl 119, KCl 4·7, $MgSO_4.7H_2O$ 1·2, $NaHCO_3$ 25, KH_2PO_4 1·17, K_2EDTA 0·03, glucose 5·5, calcium 2·5 at pH 7·4. The myograph chamber was covered and the solutions were heated to 37 °C and oxygenated with 95% O_2–5% CO_2.

After mounting and equilibration, the ratio of the resting tension to the internal circumference of the arteries was determined and the vessels set to a normalized internal circumference of l_0 where $l_0 = 0{\cdot}9l_{100}$ and l_{100} is the internal circumference of the vessel under an effective transmural pressure of 100 mmHg. It has previously been shown that maximum active tension is developed under these conditions (Mulvany & Halpern 1977). After normalization, vessels were subjected to a routine run-up procedure (3 × 2-min exposures to 60 mM KCl) before experiments were started.

In all tissues the presence or absence of a functionally intact endothelium was tested by measuring the relaxation response to acetylcholine or carbachol. If relaxation was less than 15% it was assumed that the endothelium was damaged but not totally removed; such tissues were excluded from the study.

To study the effects of oestrogens on vascular tone, tissues were contracted with either 60 mM KCl (KCl being isosmotically substituted for NaCl) or 1 μM 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin (U46619), or a dose adjusted to give a similar contraction to that induced by 60 mM KCl. When a stable contraction was obtained, concentration–response curves to 17β-oestradiol (10 nM–30 μM) were constructed. Although it is recognized that maximum relaxation in response to 17β-oestradiol might not always have been obtained, the concentration could not be increased further because of the poor solubility of the oestrogen.

Tissues were then washed and the tone left to return to baseline. Tissues were then incubated with 50 μM N^{ω}-nitro-L-arginine methyl ester (L-NAME) for approximately 30–40 min and the experiments were repeated. We have previously shown (unpublished observations) that inhibitors of nitric oxide synthase completely block the responses to acetylcholine and carbachol of coronary vessels of the size used in this experiment. In mesenteric vessels of this size the responses were not completely blocked although the sensitivity of the vessels to acetylcholine, as determined from EC50 values (the concentration inducing half the maximum response) obtained from full concentration–

response curves, was reduced almost threefold. In other experiments tissues were incubated with the prostaglandin synthesis inhibitor indomethacin (10 μM), alone or after the addition of L-NAME, before construction of the second concentration–response curve to 17β-oestradiol. In some vessels the endothelium was removed by passing a thin hair through the lumen of the vessel. In these experiments concentration–response curves to 17β-oestradiol were again constructed after pre-contraction with KCl. To avoid the possibility of vascular smooth-muscle damage during endothelium removal, vessels were excluded from the study if contraction to KCl was < 50% of endothelium-intact vessels.

Analysis of data

All results are expressed as means ± s.e.m. with n representing the number of experiments. Changes in tension were expressed as changes in active wall tension ($mN\,mm^{-1}$). All responses were normalized as a percentage of the contraction to 60 mM KCl or 1 μM U46619, as appropriate. Differences between groups were compared by analysis of variance and Student's *t*-test (paired or unpaired), Welch's non-parametric test or Student-Neumann Keuls test for multiple comparisons. Preliminary studies revealed no significant difference, i.e. time effect, between first and second curves constructed to 17β-oestradiol. Solvent-only controls also had no effect on vascular tone. All curves constructed in the presence of inhibitors of nitric oxide synthase and prostaglandin synthesis were therefore compared with the first curves constructed for the same tissue. It should be noted that because full concentration–response curves could not always be obtained, EC50 values could not be accurately determined and statistical significance was therefore determined at each individual dose.

Results

Resting parameters

The mean diameter of the rat coronary and mesenteric arteries used in this study were not significantly different (311·87 ± 12·91 μm (n = 24) and 279·91 ± 12·37 μm (n = 28), respectively). Near-maximum, maintained contractions were obtained for all tissues in response to addition of 60 mM KCl or 1 μM U46619. The magnitudes of the contractions induced by both agents were not significantly different (1·67 ± 0·14 (n = 19) and 1·60 ± 0·21 (n = 5) $mN\,mm^{-1}$, respectively, for coronary vessels and 2·29 ± 0·22 (n = 22) and 2·23 ± 0·37 (n = 6) $mN\,mm^{-1}$, respectively, for mesenteric vessels in response to KCl and U46619).

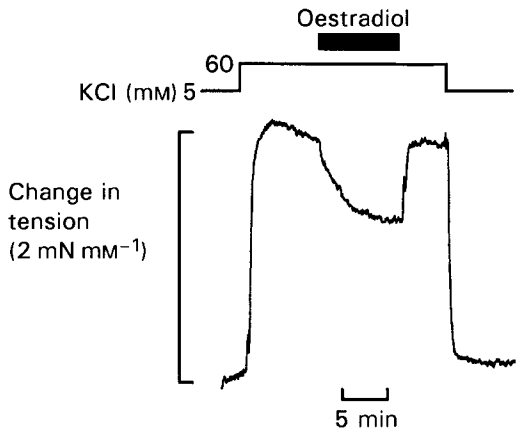

Figure 1. The response of rat isolated coronary artery, pre-contracted with KCl (60 mM), to a single concentration (3 μM) of 17β-oestradiol. The response is rapid and is reversed by removal of the oestrogen by washing.

Effects of 17β-oestradiol

Vessels pre-contracted with 60 mM KCl. Addition of 17β-oestradiol induced relaxation of both coronary and mesenteric vessels pre-contracted with 60 mM KCl. Figure 1 shows an example of one experiment in which a single dose of the oestrogen was added. Note that the tissue began to relax almost immediately upon addition of 17β-oestradiol and that the response reached a steady state within 10 min. After removal of the oestrogen by washing with fresh KCl solution the tissues returned to their original pre-contracted level, i.e. the response to 17β-oestradiol was reversible.

For all tissues the relaxation observed in response to addition of 17β-oestradiol was found to be concentration-dependent over the range 0·3–30 μM; no response was observed for oestradiol concentrations below 0·3 μM. For the majority of tissues studied near-maximum relaxation was induced by 30 μM oestradiol. There was no significant differences between the responses of rat coronary and mesenteric vessels to the hormone at any concentration (Figure 2).

Influence of L-NAME, indomethacin and endothelium removal on responses to 17β-oestradiol

Addition of L-NAME to resting tissues induced contraction in 56% of coronary tissues and 14% of mesenteric tissues. This response began within 2–3 min of addition of the L-NAME and reached a maximum after approximately 30 min. The mean contractions of the coronary arteries were 91·93 ± 6·44% of the KCl or U46619 contraction; those of the mesenteries were significantly smaller, 42·39 ± 8·17% ($P < 0·005$). When contraction was observed addition of KCl or U46619 increased it only as far as was observed in the absence of the

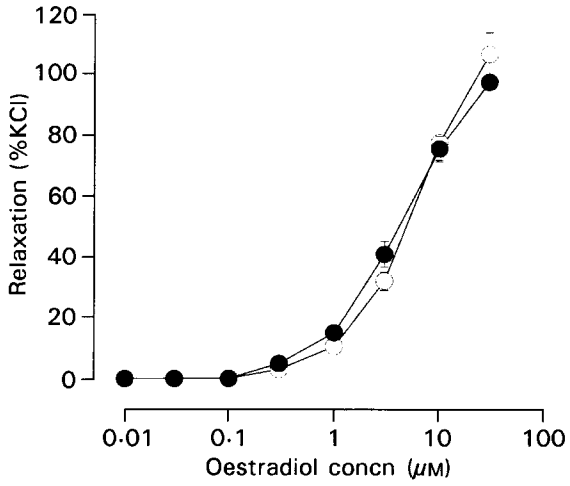

Figure 2. Concentration–response curves to 17β-oestradiol (10 nM–30 μM) of rat isolated coronary (○) and mesenteric (●) resistance vessels pre-contracted with 60 mM KCl. The relaxation responses are expressed as a percentage of the contraction of each tissue obtained in response to KCl.

nitric oxide inhibitor in the same tissue. There were no significant differences between the magnitude of contractions to KCl in the presence of L-NAME and those in the absence of the inhibitor.

In the presence of L-NAME the relaxation induced by 17β-oestradiol in both types of tissue was reduced relative to those observed in the absence of the inhibitor of nitric oxide synthase; this was observed at most concentrations (Figure 3A; 17β-oestradiol doses of 1, 3 and 10 nM, for which no responses were observed, have been omitted for clarity). It should be noted, however, that although the responses to 17β-oestradiol were reduced by L-NAME the majority of the relaxation was not affected by the inhibitor. The responses of coronary vessels observed in the presence of L-NAME were not significantly different from those of mesenteric vessels (Figure 3A).

Addition of the prostaglandin-synthesis inhibitor indomethacin had no effect on the tone of resting tissues. In the presence of indomethacin relaxation induced by 17β-oestradiol in both types of vessel was no different from those observed in the absence of the inhibitor (Figure 3B).

Although indomethacin alone did not affect resting tissues, when added to the resting tissues after L-NAME it caused a reduction in the L-NAME-induced contraction. In coronary vessels indomethacin reduced the contraction by 16·62 ± 2·78% (n = 11) whereas in mesenteric vessels the contraction was reduced by 53·76 ± 12·24% (n = 5). The actual reductions in wall tension observed for the two types of vessel were 0·228 ± 0·043 and 0·608 ± 0·247 mN mm^{-1} for coronary and mesenteric vessels, respectively (not significant). Subsequent addition of KCl again induced a contraction similar in magnitude to that observed in control tissues or tissues incubated with L-NAME alone. In the presence of both indomethacin and L-NAME the responses to oestradiol were not significantly different from those observed in the presence of L-NAME alone (Figure 3C).

Contractions induced by KCl in endothelium-denuded vessels used in this study were similar to those induced in endothelium-intact vessels (1·67 ± 0·31 mN mm^{-1} (n = 5) for coronary arteries and 2·82 ± 0·60 mM mm^{-1} (n = 6) for mesenteric. Removal of the endothelium from both coronary and mesenteric vessels resulted in significantly reduced responses to 17β-oestradiol compared with endothelium-intact vessels (Figure 3D). The responses of endothelium denuded arteries to 17β-oestradiol were similar to those obtained from arteries incubated with L-NAME and indomethacin.

Vessels pre-contracted with U46619

In vessels pre-contracted with U46619 17β-oestradiol again induced concentration-dependent relaxation similar in magnitude to that observed for tissues pre-contracted to the same level with KCl. For both types of vessel the magnitude of the relaxation was significantly reduced in the presence of L-NAME and indomethacin (Table 1). These responses were similar to those observed for KCl-contracted tissues.

Discussion

The results of this study show that 17β-oestradiol has a direct relaxant effect on rat coronary and mesenteric arteries pre-contracted with either 60 mM KCl or 1 μM U46619. Both types of vessel respond to the oestrogen in a similar manner. In all circumstances the relaxant effects of oestradiol were significantly reduced (but not fully inhibited) by L-NAME, an inhibitor of nitric oxide synthase. Addition of indomethacin, either alone or in the presence of L-NAME, had no effect on responses to 17β-oestradiol. In endothelium-denuded vessels the relaxation induced by the oestrogen was less than that observed for endothelium-intact vessels but similar to that observed in the presence of L-NAME and indomethacin.

Direct relaxant effects of 17β-oestradiol have previously been demonstrated on isolated coronary arteries from man (Mugge et al 1993; Chester et al 1995) and rabbit (Jiang et al 1991). These studies concluded that the relaxation induced by the oestrogens was a consequence of the direct effect of the hormone on smooth muscle because it was little affected by inhibitors of nitric oxide synthase and still persisted in endothelium-denuded vessels. This

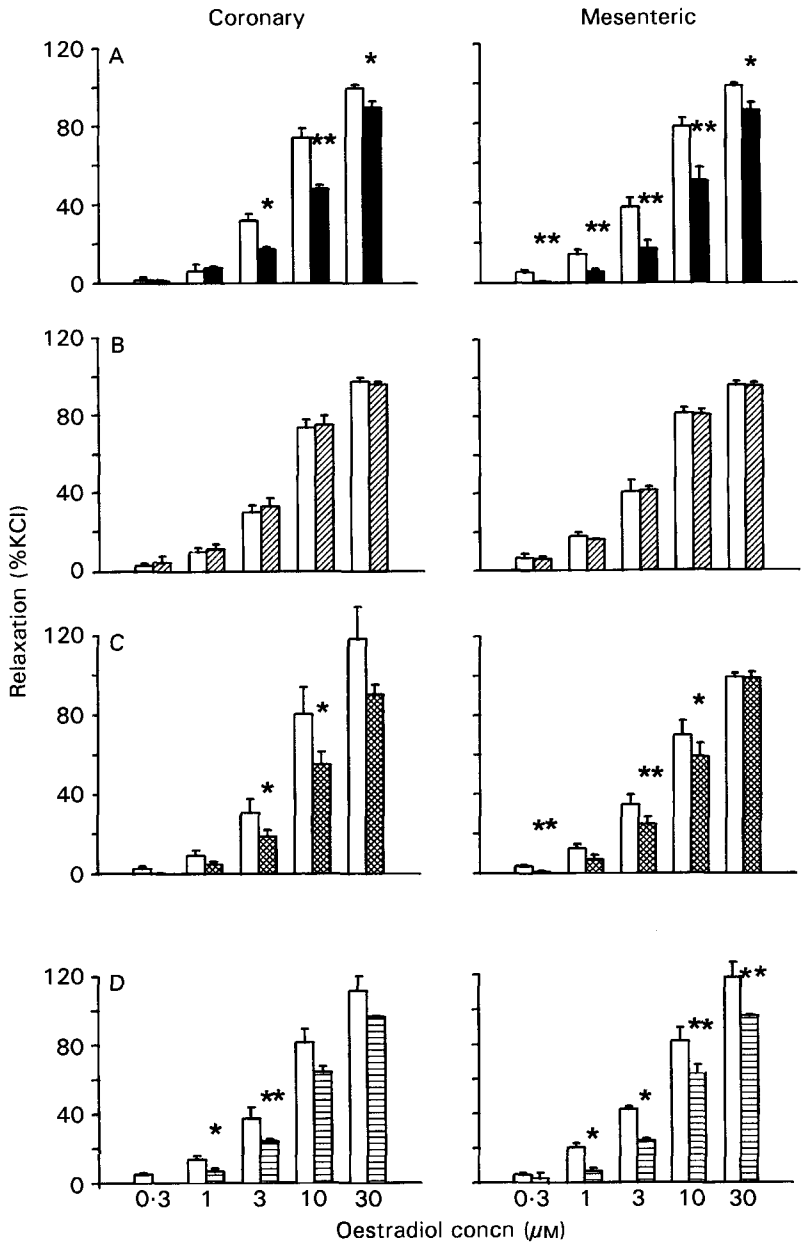

Figure 3. The effect of A. L-NAME (50 μM); B. indomethacin (10 μM); C. L-NAME (50 μM) + indomethacin (10 μM); D. endothelium removal, on the relaxation responses to 17β-oestradiol of rat isolated coronary and mesenteric arteries pre-contracted with 60 mM KCl. Open bars represent control data. All data are expressed as percentage relaxation of the contraction induced by KCl. * $P < 0.05$, ** $P < 0.01$ significantly different from controls.

is in contrast with our investigations which have shown that the relaxation induced by 17β-oestradiol in both coronary and mesenteric vessels was partially dependent on nitric oxide. The reasons for these differences are unknown but clearly factors such as species differences and the size of the vessel under investigation might contribute. It is interesting to note that this is the only study to investigate the direct effects of oestrogens on resistance vessels (other studies used larger conduit vessels).

The involvement of nitric oxide in the vasodilatory effects of oestrogens has, however, been supported by several in-vivo studies. In the ovine uterine circulation the increase in blood flow after infusion of 17β-oestradiol has been shown to be partially inhibited by L-NAME, indicating involvement of nitric oxide (Rosenfeld et al 1996). Abnormal constrictory responses to acetylcholine observed both in post-menopausal women (Reis et al 1994) and in hypercholesterolaemic ovariectomized cynomolgus monkeys (Williams et al 1990) have been shown to be attenuated or reversed after infusion of oestradiol. Acute intra-arterial infusion of the hormone has also been shown to potentiate forearm vasodilation induced by acetyl-

choline but this effect was not seen after three-week chronic oestrogen administration. The authors concluded that these differences might be a result of the lower plasma levels achieved with chronic oestrogen administration (Gilligan et al 1995). It is now recognized, however, that vasodilatory responses to acetylcholine are mediated not just by release of nitric oxide but also, in some tissues at least, by the release of an as yet unidentified endothelium-derived hyperpolarizing factor (EDHF) (Bolton et al 1984). In the studies described above it should be noted that the specificity of the acetylcholine response for nitric oxide was not tested.

As mentioned above, endothelium-dependent relaxation might be a consequence of a combination of release of both endothelium-derived nitric oxide and EDHF. In addition, the endothelium might release a number of prostaglandins, either vasodilatory or constrictory, which might contribute to responses including those to 17β-oestradiol. In this study, however, it was found that the relaxation responses were not affected by indomethacin, an inhibitor of prostaglandin synthesis. It has previously been shown that there might be an interaction between the synthesis of nitric oxide and prostaglandins such that inhibition of the synthesis of one might upregulate the synthesis of the other (Barker et al 1996). Such a phenomenon might explain the small reduction in tone we observed upon addition of indomethacin to tissues which had contracted upon addition of L-NAME and inhibition of basal nitric oxide synthesis. In contrast, addition of indomethacin when nitric oxide synthesis was not inhibited produced no change in tone. The responses to 17β-oestradiol observed in the presence of both L-NAME and indomethacin were, however, similar to those in the presence of L-NAME alone, suggesting that prostaglandins are not involved in the relaxant effects of the hormone. This observation has been supported by Rosenfeld et al (1996) who found that the partially L-NAME-sensitive relaxation of ovine uterine arteries to oestradiol was not affected further by addition of indomethacin. Although long-term (12–24 h) incubation with 17β-oestradiol has been shown to stimulate the production of the vasodilatory prostaglandin prostacyclin (PGI_2) in cultured endothelial cells from man (Mikkola et al 1995) shorter incubation times had no effect (Corvazier et al 1984). The synthesis of the vasoconstrictory compound endothelin was not altered by oestradiol under any conditions (Mikkola et al 1995). These studies further support our observation of a lack of involvement of prostaglandins in the rapid effects of oestrogens on vascular tone.

We have previously shown (see methods) that the response to acetylcholine of coronary arteries (of similar size to those used in this study) pre-contracted with U46619 could be completely blocked by the nitric oxide synthesis inhibitor L-nitroarginine whereas that of mesenteric vessels was only partially blocked, suggesting the involvement of EDHF. Although we cannot completely discount the possibility that EDHF might contribute to the endothelium-dependent component of the relaxation of mesenteric vessels to 17β-oestradiol in this study, we believe it is unlikely. This is because the responses were similar in tissues pre-contracted with high K^+ (conditions under which hyperpolarization would not occur) and U46619, and the responses to 17β-oestradiol after inhibition of nitric oxide and prostaglandin synthesis were similar to those in endothelium-denuded vessels, suggesting that other endothelial factors are not contributing to the response.

Thus the results of our study show that relaxation of small rat isolated coronary and mesenteric vessels to 17β-oestradiol was partly a result of stimulation of nitric oxide synthesis or release, or both, but did not seem to involve other endothelial factors. It should be noted, however, that the majority of the response persisted in the presence of

Table 1. Effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME) and indomethacin on the responses of rat isolated coronary and mesenteric arteries to 17β-oestradiol.

	17β-Oestradiol concn							
	10 nM	30 nM	100 nM	300 nM	1 μM	3 μM	10 μM	30 μM
Coronary artery control (n = 5)	–	–	–	4·57 ± 1·84	13·17 ± 2·77	29·66 ± 2·26	60·57 ± 3·69	83·97 ± 2·34
L-NAME + indomethacin	–	–	–	0·79 ± 0·54	4·98 ± 1·51*	18·63 ± 4·10*	49·64 ± 8·92	81·38 ± 9·69
Mesentery artery control (n = 6)	–	0·17 ± 0·17	2·01 ± 2·01	5·20 ± 2·28	19·39 ± 4·65	34·09 ± 5·51	57·19 ± 4·19	79·79 ± 3·63
L-NAME + indomethacin	–	–	–	–	4·29 ± 1·36*	16·00 ± 2·94*	42·02 ± 6·45	75·20 ± 5·00

* $P < 0.05$ significantly different from respective control. Responses are expressed as a percentage of the KCl pre-contraction.

L-NAME, indicating that the major means by which oestradiol relaxes these tissues is by a direct effect on the smooth muscle. Han et al (1995) have recently reported that the relaxation of porcine coronary arteries by 17β-oestradiol could at least partly be explained by a reduction in the concentration of intracellular free Ca^{2+} as a result of inhibition of Ca^{2+} influx across the plasma membrane via voltage-gated Ca^{2+} channels. Similar effects of oestrogens on Ca^{2+} influx have also been reported in isolated smooth muscle cells from a variety of vascular beds (Kitazawa et al 1997). It is thought that there is no contribution to the relaxant responses of sequestration of Ca^{2+} into intracellular stores (Kitazawa et al 1997). A reduction in myosin light-chain phosphorylation in the presence of 17β-oestradiol has also been observed (Kitazawa et al 1997), although others have been unable to demonstrate any effect on myofilament sensitivity (Han et al 1995). Thus although it is clear that oestrogens can have a direct effect on vascular smooth muscle the mechanisms remain to be fully elucidated.

It is believed that the relaxant effects of oestrogens are mediated by a mechanism independent of the classical genomic pathway of steroid activation. There is some evidence for this: the response speed is much quicker than the effects of oestrogens on gene expression (Orimo et al 1993); inhibitors of RNA and protein synthesis and cytosolic/nuclear oestrogen receptor antagonists have been shown to have no effect on the vascular relaxant effects of oestrogens (Kitazawa et al 1997); and 17α-oestradiol, a steroid that has no genomic effects (Merrlam et al 1980), has been shown to have vasodilatory effects similar to those of 17β-oestradiol (Salas et al 1994). It is thought that these non-genomic effects are mediated by binding to cell-surface receptors, because oestrogen receptors have been identified in smooth muscle cells of coronary arteries from both rat (Lavigne et al 1995) and man (Losordo et al 1994) and in aortae from a number of species (Horowitz & Horowitz 1982; Lin et al 1986). There is also evidence that the vascular endothelium contains oestrogen receptors (Coburn & Buonassisi 1978; Bayard et al 1995) clearly supporting our observations that the vascular effects of oestrogens are mediated in part by nitric oxide.

Clearly the vasodilatory effects of oestrogens could contribute to the cardioprotective effects of the hormone. The results of this study show, however, that the responsiveness of coronary vessels to oestradiol is no greater than that of systemic vessels. The protective effect therefore appears to be a consequence of general vasodilation rather than a specific relaxation of coronary vessels. Differences between the effects of oestrogens on different vascular beds have previously been reported by Gilligan et al (1994), who found that infusion of oestradiol increased the vasodilator response to sodium nitroprusside in the forearm but not the coronary circulation suggesting, as sodium nitroprusside is an endothelium-independent vasodilator, different sensitivities of vascular smooth muscle to the hormone. This might suggest that the similar effects we observed for coronary and systemic vessels were because of differences in the effects of oestrogens on endothelial factors; we could, however, find no differences between the effects of L-NAME or indomethacin on the two types of vessel, suggesting that this is not so.

It should be noted that the doses of oestrogens used in the current experiments are much higher than circulating plasma levels of 17β-oestradiol in pre-menopausal women, which are generally within the range 50–250 $pg\,mL^{-1}$ (Abraham et al 1972). The plasma concentration of oestradiol, however, might not reflect the concentration at the smooth muscle because it might be reduced by binding to carrier molecules and lipophilic oestrogen molecules might accumulate in smooth-muscle cells. Thus high local concentrations of 17β-oestradiol might be influencing contraction of vascular tissues.

In summary, we have shown that 17β-oestradiol has a direct relaxant effect on rat isolated coronary and mesenteric resistance arteries. In contrast with other in-vitro studies on larger vessels we have shown that this response is partly mediated by nitric oxide. Thus the reduced risk of cardiac disorders observed in pre- or post-menopausal women receiving oestrogen replacement therapy might be partly because of vasodilation of the coronary arteries and thus increased myocardial blood flow. However, we could find no evidence of the coronary artery being especially sensitive to the vasodilatory effects of the hormone—mesenteric vessels responded in a similar manner suggesting that this protection is a consequence of a general relaxant effect on the vasculature rather than a specific effect on the coronary circulation.

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