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Relaxations to oestrogen receptor subtype selective agonists in rat and mouse arteries

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Abstract

It has been recently reported that the oestrogen receptor α agonist PPT (4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl) tris-phenol) is more potent than the oestrogen receptor β agonist DPN (2,3-bis(4-hydroxyphenyl)-propionitrile) at producing relaxations in rat mesenteric artery. We have investigated the relaxant actions of PPT and DPN in rat and mouse aorta and mesenteric artery. In rat aortic rings contracted with KCl (40 mM), the oestrogen receptor β agonist DPN produced significantly greater relaxations than the oestrogen receptor α agonist PPT. In wild-type (WT) mouse aorta, the same result was found, but in WT mouse mesenteric artery, as in rat mesenteric artery, DPN was significantly less potent than PPT in females but had similar potency to PPT in males. Relaxations to DPN also occurred in aorta from nitric oxide synthase-3-knockout (NOS-3-KO) mice, and in denuded aorta from both mouse and rat. Hence, in the mouse mesenteric artery, as in the rat mesenteric artery, PPT is at least as potent as DPN at producing relaxations; however, DPN was much more potent than PPT in the rat and mouse aorta. Effects of oestrogen receptor subtype selective agonists are tissue dependent. In addition, actions are largely endothelium-independent.

Keywords: Oestrogen receptor α; Oestrogen receptor β; Oestrogen receptor; PPT (4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl) tris-phenol); DPN (2,3-bis (4-hydroxyphenyl)-propionitrile); Mouse aorta; Mouse mesenteric artery

1. Introduction

Although pre-menopausal women have lower incidences of cardiovascular disease (Farhat et al., 1996), due presumably to the effects of oestrogen, trials of hormone replacement therapy have failed to find beneficial effects to outweigh risks (Beral et al., 2003). Studies of agonists for subtypes of oestrogen receptor may lead to development of alternative therapies, by employing subtype selective agonists.

Oestrogen receptors have been subdivided into oestrogen receptor α (Jensen and Jacobson, 1960) and oestrogen receptor β (Kuiper et al., 1997), with selective agonists PPT (4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl) tris-phenol) and DPN (2,3-bis(4-hydroxyphenyl)-propionitrile), respectively. The oestradiol induced inhibition of the vascular injury response occurs in mice lacking the oestrogen receptor α receptor (ERα-KO) (Iafrati et al., 1997) and in mice lacking the oestrogen receptor β receptor (oestrogen receptor β-KO) (Karas et al., 1999), although other studies implicate the oestrogen receptor α receptor in this response (Brouchet et al., 2001). Pare et al. (2002) found that oestrogen inhibited the vascular injury response in wild type, oestrogen receptor α-KO and in oestrogen receptor β-KO mice, but not in the double gene knockout oestrogen receptor α/oestrogen receptor β-KO mice. Knockout studies also implicate the oestrogen receptor α in stimulation of NOS synthase expression (Geary et al., 2001) and NO production (Darblade et al., 2001). Lindner et al. (1998) demonstrated that removal of the endothelial cell layer is accompanied by a large increase in oestrogen receptor β expression in smooth muscle cells and endothelial cells. Taken together, these finding suggest that that oestrogen

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receptor β may compensate for oestrogen receptor α in oestrogen receptor α KO mice, and possibly vice versa.

The vasodilator effects of oestrogen have been ascribed to endothelium-dependent (Gisclard et al., 1988; Herrington et al., 1994) and endothelium-independent components (Gerhard and Ganz, 1995; Crews and Khalil, 1999; Harder and Coulson, 1979). Release of NO from the endothelium is elevated in arteries of females compared with males (Wellman et al., 1996; Kauser and Rubanyi, 1994), and oestrogen increases NOS expression or activity (Gisclard et al., 1988; Knot et al., 1999; Geary et al., 2000). The advent of knockout technology allows us to investigate the role of nitric oxide (NO) in responses to oestrogen receptor agonists by employing nitric oxide synthase-3-knockout (NOS-3-KO) mice.

The present study began as an investigation of the actions of PPT and DPN in rat and in wild-type and nitric oxide synthase-3 knockout (NOS-3-KO) mouse aorta, but prior to completion of the manuscript, results using oestrogen receptor α and oestrogen receptor β agonists were reported in rat mesenteric artery (Montgomery et al., 2003) which were at variance with our data obtained in the aorta. This led us to repeat our study in rat and mouse mesenteric artery with the hypothesis that effects of oestrogen receptor subtype agonists may be tissue dependent, and independent of NOS-3.

Some of these results have been published in abstract form (Razak and Docherty, 2002; Al Zubair et al., 2004).

2. Methods

2.1. General

Male and female adult Wistar rats (250–400 g) were obtained from Trinity College Dublin. Male and female adult wild-type and NOS-3-KO C57 Black mice (18–28 g) were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). Animals were housed in a controlled environment with a 12-h light and 12-h dark cycle. They were fed a standard rat/mouse diet. Animals were killed by CO_2 overdose. The studies were carried out in accordance with the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee.

2.2. Rat aorta

The thoracic aorta was removed, cleared from surrounding connective tissue and cut transversely into 3 mm rings. In some experiments, the endothelium was removed by gently rubbing of the intimal surface with a rod. The aortic rings were mounted in 20 ml organ baths, attached between a fixed rod and an isometric tension transducer under 10 mN tension. Baths contained Krebs–Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, D-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, EDTA 0.03 and ascorbic acid 0.28. Additionally, propranolol (3 μM) was present to block β-adrenoceptors; indomethacin (10 μM) to inhibit prostaglandin synthesis and corticosterone (40 μM) to block uptake of noradrenaline into smooth muscle. Aortic rings were maintained at 37 °C and continuously gassed with 95% O_2/5% CO_2. Bathing fluid was changed every 15 min except during responses to vasoactive agents.

To establish the viability of the endothelium, after a 60 min equilibration period, the rings were contracted with KCl (40 mM) followed by exposure to acetylcholine (10 μM) to induce relaxation. KCl was directly added to the bath without altering other salts, so that the bathing solution became hyperosmotic. Tissues which failed to produce a 2.5 mN increase in tension and/or failed to relax after addition of acetylcholine were rejected. In experiments using endothelium-denuded aortae, successful removal of endothelial cells was confirmed by the inability of acetylcholine (10 μM) to induce relaxation in tissues precontracted with KCl (40 mM). In some experiments, concentration–response curves were obtained to KCl (10–120 mM) to assess potency of KCl.

After a further hour, aortic rings (intact or denuded endothelium) were precontracted with KCl (40 mM). At the plateau of contraction, 17β-oestradiol, oestrogen receptor α agonist PPT or the oestrogen receptor β agonist DPN (0.01–30 μM) or vehicle was added cumulatively to the bath.

2.3. Small vessel myograph studies

Rings of second order branches of male rat mesenteric artery, or rings of mouse aorta or mesenteric artery, 1.5 mm in length, were mounted in a small vessel myograph with 40 μm tungsten wires. Data were recorded on a dual channel electronic display recorder (Myo-Interface Model 400A) and analog acquisition system (MacPacq. MP100, Biopac Systems). Vessels were allowed to equilibrate at 37 °C in Krebs–Henseleit solution (95% O_2/5% CO_2) of the same composition as for rat aorta. Propranolol (3 μM) was also present to block β-adrenoceptors. The vessel was set to a tension generated at 0.9 times the diameter of the vessel at 100 mm Hg transmural pressure (Mulvany and Warshaw, 1977). Arteries were allowed to equilibrate for 30 min under this passive tension.

Tissues were contracted with KCl (40 mM) or phenylephrine (0.1 μM) and acetylcholine (10 μM) was added at the plateau of contraction. After 30 min (mouse vessels) or 60 min (rat mesenteric artery) washing out, KCl (40 mM) was again added at the plateau of contraction, and a concentration–response curve to an oestrogen receptor agonist (0.1 to 100 μM) was then performed in a cumulative manner. In some experiments, concentration–response curves were obtained to KCl (10–120 mM) to assess potency of KCl.

In some experiments, tissues were exposed to the NOS-inhibitor L-NAME (100 μM) or the oestrogen receptor antagonist ICI 182,780 (1 μM) for 30 min prior to contraction with KCl (40 mM) and addition of oestrogen receptor agonist.

2.4. Drugs

Acetylcholine chloride (Sigma, Poole, UK); DPN (2,3-bis(4-hydroxyphenyl)-propionitrile; Tocris); 17-β-estradiol (Sigma); ICI 182,780 (7α[9-[(4,4,5,5,5,-pentafluoropentyl)-sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17β-diol; Tocris); L-NAME hydrochloride (N^G-nitro-L-arginine methyl ester hydrochloride; Tocris); Phenylephrine hydrochloride (Sigma); PPT (4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl) tris-phenol; Tocris); propranolol hydrochloride (Sigma). Drugs were dissolved in distilled water, except for DPN, 17-β-estradiol, ICI 182,780 and PPT which were dissolved in ethanol (100%) and diluted with distilled water.

2.5. Statistics

Values are mean ± S.E.M. from *n* experiments. Contractions to KCl (mN) or relaxations (%) to agonists (negative relaxation indicates contraction) were compared between groups by one-way analysis of variance (ANOVA) plus Dunnett's test, or by Student's *t*-test for unpaired data when only 2 groups were involved. Statistical and graphical analysis was carried out using Instat for Macintosh and GraphPad Prism for IBM-compatible computer.

3. Results

3.1. Rat aortic rings

KCl (40 mM) caused a contraction that reached a plateau at approximately 15–20 min. The KCl-induced contraction was significantly greater in aortic rings (12.5 ± 2.1 mN, $n=11$) from male as compared to female rats (7.6 ± 0.8 mN, $n=15$, $P<0.05$). The contraction to KCl (40 mM) was 87.2 ± 3.9% ($n=7$) and 81.8 ± 3.7% ($n=4$) of maximum KCl contraction in aorta from male and female rats, respectively.

In both male and female rat aortic rings with intact endothelium, the oestrogen receptor β agonist DPN (30 μM) produced significantly greater relaxation of KCl (40 mM) evoked contractions than the oestrogen receptor α agonist PPT (30 μM) (female: 66.4 ± 8.5% vs. 27.8 ± 5.4%, respectively, $n=6$; male: 63.1 ± 6.10% vs. 35.1 ± 5.65%, respectively) (Fig. 1). The maximum relaxation to the oestrogen receptor β agonist DPN was similar to that to 17β-oestradiol (17β-oestradiol, female: 81.3 ± 7.0%,; male: 69.1 ± 2.0%, $n=6$, respectively; no significant difference between female and male; no significant difference between effects of DPN and 17β-oestradiol in the same gender).

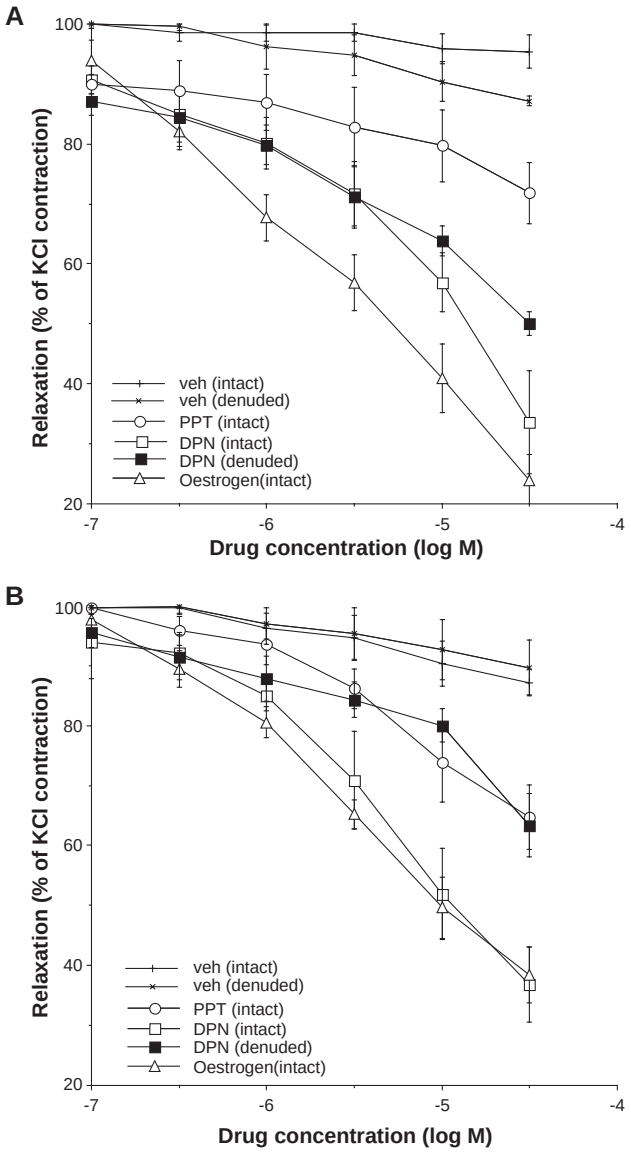

Fig. 1. The effects of the oestrogen receptor α agonist PPT, the oestrogen receptor β agonist DPN, 17β-oestradiol (Oe) or vehicle (veh) in (A) female and (B) male rat aorta, with and without endothelium, precontracted with KCl 40 mM. Vertical bars represent S.E. of mean from at least 5 experiments.

Relaxations to DPN also occurred in endothelium-denuded aorta from female rat (30 μM: 50.2 ± 2.0%, $n=5$, not significantly different from effects in presence of endothelium) (Fig. 1A). In male rat aorta, denudation significantly reduced the relaxation by the oestrogen receptor β agonist to that of the oestrogen receptor α agonist (Fig. 1B). Hence, removal of the endothelium had a greater effect on relaxations to DPN in male than female aorta. The small relaxations to PPT were unaffected by removal of endothelium in both male and female (data not shown).

3.2. Rat mesenteric artery

In male rat mesenteric arteries with intact endothelium, KCl (40 mM) caused a contraction of 4.22 ± 1.11 mN ($n=16$)

that reached a plateau at approximately 15–20 min. The contraction to KCl (40 mM) was 58.0 ± 3.6% (n = 4) of the maximum KCl contraction in mesenteric artery from male rats. The oestrogen receptor α agonist PPT (10 μM) produced significantly greater relaxation of KCl (40 mM) evoked contractions than the oestrogen receptor β agonist DPN (10 μM) (57.4 ± 16.4% vs. 17.2 ± 6.06%, respectively, n = 5–6), although responses were similar at 100 μM (Fig. 2).

3.3. Mouse aorta

In aorta from wild-type mice, KCl (40 mM) produced contractions of 4.25 ± 0.98 mN (n = 12) and 4.36 ± 0.44 mN (n = 14), in vessels from male and female, respectively. The contraction to KCl (40 mM) was 78.0 ± 5.7% (n = 5) and 64.8 ± 7.5% (n = 5) of maximum in aorta from male and female mice, respectively. The oestrogen receptor β agonist DPN (100 μM) produced significantly greater relaxations of KCl evoked contractions than the oestrogen receptor α agonist PPT (100 μM), in vessels from both male and female (e.g. in female: 50.2 ± 6.4%, n = 9 versus −9.1 ± 4.1% relaxation/9.1 ± 4.1% contraction, n = 5; for DPN and PPT, respectively) (Fig. 3). In fact, PPT failed to produce significant relaxations in WT mouse aorta as compared to the effects of vehicle (Fig. 3). Vehicle had little effect except the highest concentration (100% ethanol; final bath concentration 1%), which contracted both aorta and mesenteric artery. There were no differences between male and female mice in aortic responses.

In aorta from NOS-3-KO mice, KCl (40 mM) produced contractions of 3.58 ± 081 mN (n = 11) and 3.12 ± 0.46 mN (n = 15) in vessels from male and female, respectively. Acetylcholine (10 μM) failed to produce a significant relaxation in either group. The contraction to KCl (40 mM) was 75.4 ± 5.0% (n = 5) and 77.2 ± 4.2% (n = 5) of maximum KCl contraction in aorta from male and female mice, respectively. Relaxations to DPN occurred in aorta from NOS-3-KO mice and, when corrected for changes occurring in vehicle experiments, were significantly greater than relaxations in wild-type in females (DPN 100 μM: WT, 54.3 ± 6.4%, n = 9; NOS-3-KO, 77.3 ± 7.9%, n = 5; P < 0.05)., but not in males (DPN 100 μM: WT, 74.5 ± 6.9%, n = 6; NOS-3-KO, 67.3.0 ± 4.0%, n = 5) (Fig. 3). Although PPT (100 μM) failed to produce a significant relaxation in WT mouse aorta, it produced a significant relaxation as compared to the effects of vehicle in aorta from

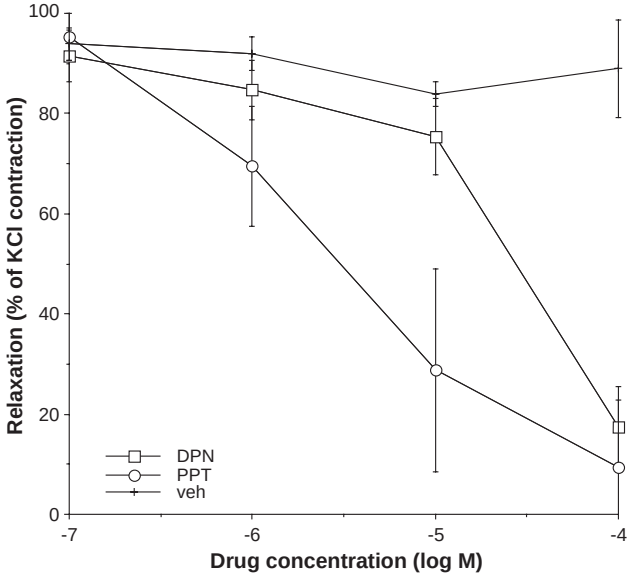

Fig. 2. The effects of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN or vehicle (veh) in mesenteric arteries from male rats, precontracted with KCl 40 mM. Vertical bars represent S.E. of mean from at least 5 experiments.

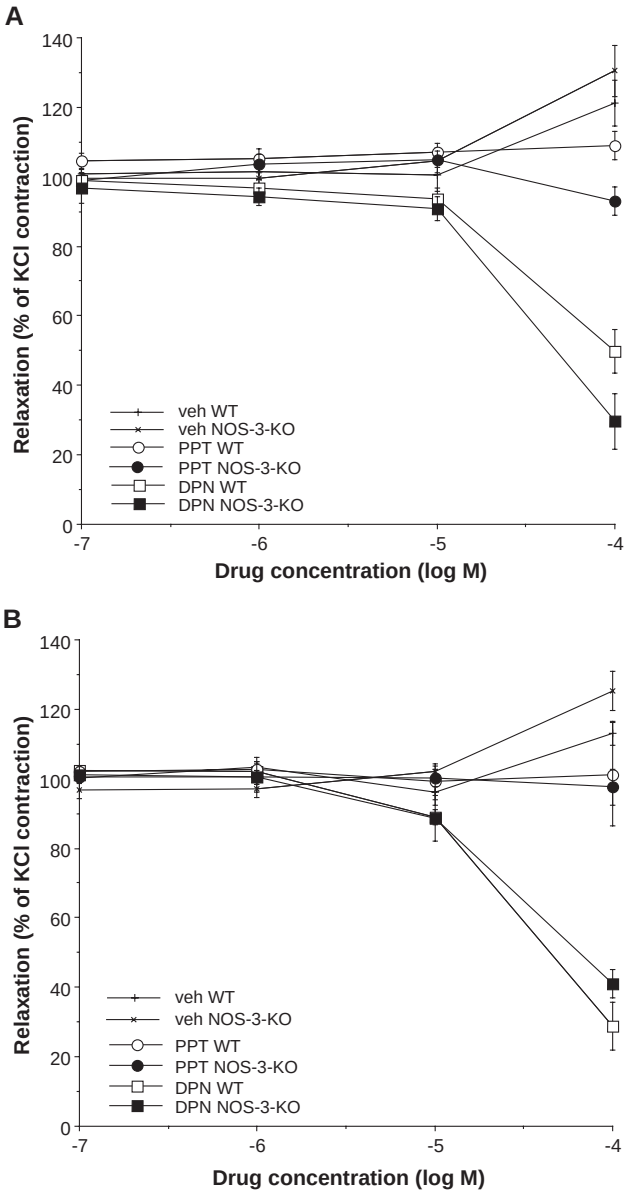

Fig. 3. The effects of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN or vehicle (veh) in (A) female and (B) male aorta from wild-type (WT) and NOS-3-KO female mice, precontracted with KCl 40 mM. Vertical bars represent S.E. of mean from at least 5 experiments.

both male and female NOS-3-KO mice. The oestrogen receptor antagonist ICI 182,780 (1 μM) did not significantly affect relaxations to DPN (DPN 100 μM: 64.7 ± 8.1%, $n = 3$) in aorta from female NOS-3-KO mice.

In aorta from female WT mice, KCl (40 mM) produced contractions of 6.95 ± 0.90 mN ($n = 4$) and 6.34 ± 2.13 mN ($n = 4$) in vessels exposed to L-NAME or denuded, respectively. The contraction to KCl (40 mM) was 91.0 ± 4.3% ($n = 4$) and 99.6 ± 0.2% ($n = 5$) of maximum in vessels exposed to L-NAME or denuded, respectively. Relaxations to DPN in aorta from WT female mice were unaffected by removal of endothelium or addition of L-NAME (100 μM). DPN (100 μM) produced relaxations of 50.2 ± 6.4% of control ($n = 9$) in WT vessels with intact endothelium; 61.5 ± 13.3% ($n = 4$) in WT endothelium denuded vessels; 70.2 ± 3.4% ($n = 4$) in WT vessels with intact endothelium in presence of L-NAME; 70.4 ± 7.9% ($n = 5$) in NOS-3-KO vessels with intact endothelium, respectively.

3.4. Mouse mesenteric artery

In mesenteric artery from wild-type mice, KCl (40 mM) produced contractions of 3.13 ± 0.49 mN ($n = 15$) and 5.50 ± 0.89 mN ($n = 16$) in vessels from male and female mice, respectively (response significantly greater in vessels from female mice, $P < 0.05$). The contraction to KCl (40 mM) was 49.8 ± 8.5% ($n = 5$) and 62.2 ± 8.0% ($n = 5$) of maximum in vessels from male and female mice, respectively. In wild-type female mouse mesenteric artery, PPT was significantly more potent than DPN at producing relaxations of KCl evoked contractions (10 μM: 31.8 ± 9.1%, $n = 5$ versus 11.1 ± 4.9% relaxation, $n = 6$, for PPT and DPN, respectively) (Fig. 4). The relaxations to both agonists at the concentration of 100 μM were similar (Fig. 4). In female mouse mesenteric artery, 17β-oestradiol showed low potency, similar to that of DPN, with maximum relaxation of 35.0 ± 8.0% ($n = 6$) at a concentration of 100 μM (Fig. 4). In wild-type male mouse mesenteric artery, PPT and DPN were about equipotent (Fig. 4), mainly due to an increased potency of DPN as compared to females.

In mesenteric artery from NOS-3-KO mice, KCl (40 mM) produced contractions of 2.19 ± 0.49 mN ($n = 14$) and 3.14 ± 0.41 mN ($n = 16$), in vessels from male and female mice, respectively (no significant difference). Acetylcholine (10 μM) failed to produce a significant relaxation in either group. The contraction to KCl (40 mM) was 71.4 ± 3.2% ($n = 7$) and 82.2 ± 6.1% ($n = 5$) of maximum KCl contraction in mesenteric artery from male and female mice, respectively. DPN produced relaxations of KCl induced contractions which were not significantly different from responses in wild-type animals (e.g. in female, 100 μM: 56.6 ± 8.8%, $n = 6$ in wild-type; 65.0 ± 10.0%, $n = 6$ in NOS-3-KO) (Fig. 4). The oestrogen receptor antagonist ICI 182,780 (1 μM) did not significantly affect relaxations to DPN (DPN 100 μM: 55.7 ± 4.0% relaxation, $n = 3$) in mesenteric artery from female NOS-3-KO mice.

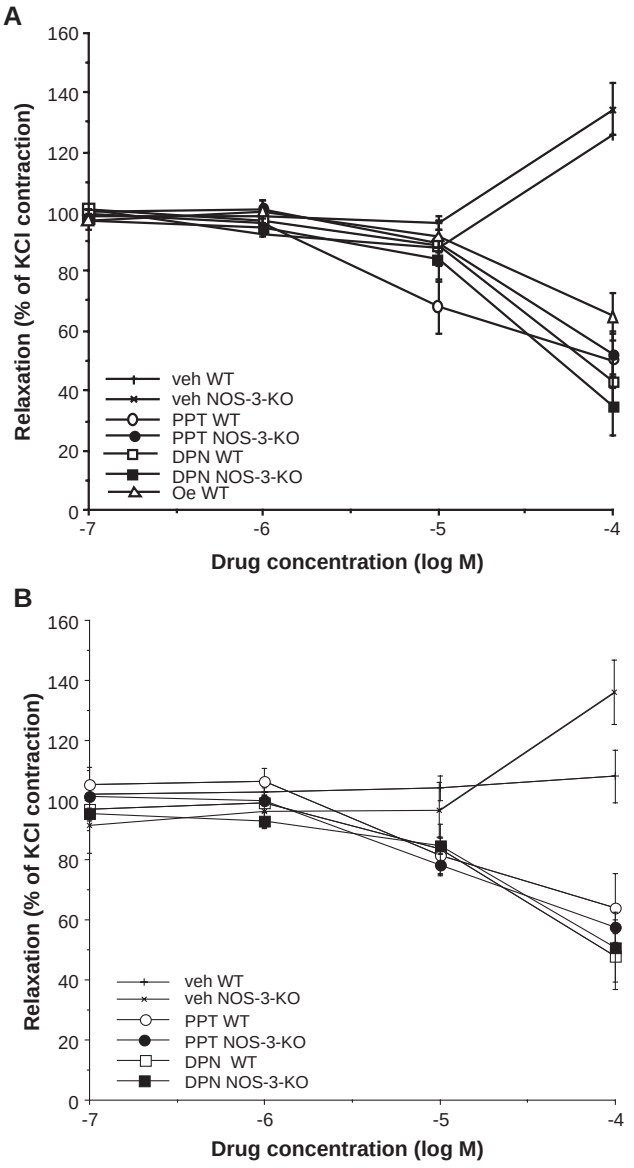

Fig. 4. The effects of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN, 17β-oestradiol (Oe) or vehicle (veh) in (A) female and (B) male mesenteric arteries from wild-type (WT) and NOS-3-KO female mice, precontracted with KCl 40 mM. Vertical bars represent S.E. of mean from at least 5 experiments.

4. Discussion

In this study, we have examined the actions of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN in aorta and mesenteric artery from rat and wild-type and NOS-3-KO mice. Studies in each blood vessel will be described separately.

In the first part of this study, ER receptor subtype selective agonists were examined in rat aorta. The oestrogen

receptor β agonist DPN has a 70-fold selectivity in ligand binding affinity for oestrogen receptor β over oestrogen receptor α (Meyers et al., 2001), and the oestrogen receptor α agonist PPT has a 410-fold affinity for the oestrogen receptor α over oestrogen receptor β (Stauffer et al., 2000). Our findings demonstrated that the oestrogen receptor β agonist-induced relaxation is significantly greater than that to the oestrogen receptor α agonist, in both male and female rat aorta with intact endothelium. Removal of endothelium in male, but not female, aorta reduced the relaxation produced by oestrogen receptor β agonist to that of oestrogen receptor α agonist. Hence, the relaxation to the oestrogen receptor β agonist is partly endothelium-dependent and partly endothelium-independent in male, but largely endothelium-independent in female, rat aorta. Of course, the small relaxation seen to the oestrogen receptor α agonist does not necessarily involve the oestrogen receptor α receptor. In rat aorta, Andersen et al. (1999) found that acute administration of oestrogen had actions mainly on the smooth muscle, whereas chronic oestrogen treatment had actions which were endothelium-dependent. Zhu et al. (2002) found that chronic oestrogen exposure decreases contractions to phenylephrine by induction of iNOS in WT mice aortic rings, but in oestrogen receptor α-KO mice chronic oestrogen exposure increased the contractions.

Since Montgomery et al. (2003) have reported that the oestrogen receptor α agonist PPT was significantly more potent than the oestrogen receptor β agonist DPN at producing relaxations in male rat mesenteric artery, a result which is at variance with our rat (and mouse) aorta findings, we also examined male rat mesenteric artery. Our results in rat mesenteric artery were in agreement with those of Montgomery et al. (2003). In our studies, PPT caused a significant relaxation at 1 μM and DPN caused a significant relaxation at 100 μM, and equivalent effects were obtained at 0.3 μM and 30 μM, respectively, in the studies of Montgomery et al. (2003).

Further studies employed mouse blood vessels, in which the agonists were always less potent than in the equivalent rat vessels. In both male and female mouse, results obtained in the aorta were largely similar to those obtained in rat aorta: the oestrogen receptor β agonist-induced relaxation was significantly greater than that to the oestrogen receptor α agonist. The relaxation was also present in NOS-3-KO mice, and in female WT mice following the NOS inhibitor L-NAME or following removal of the endothelium. However, in the mouse mesenteric artery different results were obtained: the oestrogen receptor α agonist-induced relaxation was significantly greater than that to the oestrogen receptor β agonist at low agonist concentrations in females but not males; i.e. PPT was more potent in females (equipotent in males). In females, 17β-oestradiol also showed lower potency than PPT, but similar to DPN. Montgomery et al. (2003), employing male rat small mesenteric artery, found that PPT was more potent than 17β-oestradiol, which was more potent than DPN. Hence, there are differences between aorta and mesenteric arteries in the types of receptor mediating relaxations to ER receptor agonists. The potency of DPN in the rat aorta (present study) was similar to the potency of PPT in rat mesenteric artery (Montgomery et al., 2003) (relaxations occurring starting at 0.3–1 μM), but both agonists were less potent in the mouse aorta and mesenteric artery (present results).

In the present study, the contraction to KCl was greater in the aorta of male than of female rats. This agrees with previous studies (Stallone et al., 1991; Crews and Khalil, 1999; Tostes et al., 2000; Murphy and Khalil, 2000); however, differences in muscle mass of aorta may also explain differences between male and female. Since we did not carry out morphometric analysis, we cannot comment further on this. There were no significant male/female differences in KCl contractions in mouse aorta, but in mesenteric artery from WT mice, contractions to KCl (40 mM) were significantly greater in vessels from females. However we have previously found that the maximum contraction to KCl is extremely variable in mouse mesenteric artery, but not in aorta (Bexis et al., 2004). Some other differences were seen between males and females in this study. In rat aorta, part of the relaxation to the oestrogen β receptor agonist DPN was endothelium-dependent in males but not in females. In mouse aorta, relaxations to DPN were increased in female but not male NOS-3-KO mice as compared to wild type: this might suggest that, as in rat aorta, a component of the relaxation in vessels from male animals involves endothelial NOS-3. In mouse mesenteric artery, DPN potency was increased in males so that it was equipotent with PPT.

Experiments were carried out using KCl (40 mM) as contractile agent. KCl (40 mM) produced 81–87% of the maximum contraction to KCl in rat aorta, but 58% in rat mesenteric artery. KCl (40 mM) produced 64–78% of maximum in WT mouse aorta, and 75–77% of maximum in NOS-KO mouse aorta (no significant difference). KCl (40 mM) evoked contractions were 91% of maximum in female WT mouse aorta following L-NAME and 99% of maximum in WT without endothelium. KCl (40 mM) produced 49–62% of maximum in WT mouse mesenteric artery, but 71–82% of maximum in NOS-3-KO mouse mesenteric artery. These results are broadly consistent with our previous findings that potency of KCl is increased in NOS-3-KO aorta and mesenteric artery, as compared to the equivalent WT (Bexis et al., 2004). Differences in the degree of contraction with KCl may affect the level of relaxation obtained to oestrogen receptor agonists, but this does not seem to be a major factor in this study since, although deletion of NOS-3 increased the level of KCl contraction, it did not reduce the relaxations obtained.

A number of studies point to actions of 17β-oestradiol to restrict calcium entry. In experiments carried out in calcium-free solution with calcium stores depleted, 17β-oestradiol (10–30 μM) significantly reduced the contraction to calcium

restoration in rat tail artery (Shan et al., 1994) and aorta (Browne et al., 1999). A calcium antagonistic action of 17β-oestradiol, not linked to specific oestrogen receptors, has been reported in human isolated myocardial tissue (Sitzler et al., 1996). In patch-clamp studies of porcine coronary myocytes, 17β-oestradiol was found to activate K^+ channels by a cGMP-dependent mechanism (White et al., 1995). Such an action would result in decreased calcium availability. Studies in cultured vascular smooth muscle cells (Zhang et al., 1994) and in myocytes from rat ileum (Kitazawa et al., 1997) demonstrate that oestrogen inhibits voltage dependent calcium influx. Wynne et al. (2004) demonstrated that ageing in female spontaneously hypertensive rats decreased the inhibitory effects of oestrogen on calcium entry in rat denuded aorta. In rabbit basilar artery, oestrogen produces an endothelium-independent relaxation by inhibition of calcium influx (Salom et al., 2001). Hence, endothelium-independent actions of 17β-oestradiol may involve inhibition of calcium entry.

In the present study, the oestrogen receptor antagonist ICI 182,780 (Wakeling et al., 1991) had no significant effect on relaxations to DPN (100 μM) in mouse aorta or mesenteric artery from female NOS-3-KO mice. ICI 182,780 is reported to block endothelium-dependent relaxations to oestrogen receptor agonists in rat aorta (Garban et al., 2004; Bucci et al., 2002), but endothelium-independent relaxations in rat aorta (Garban et al., 2004), rat mesenteric arteries (Shaw et al., 2000), rabbit basilar artery (Salom et al., 2001) and dog coronary artery (Sudhir et al., 1995) are not blocked by ICI 182,780. Hence, endothelium-independent relaxations to oestrogen and oestrogen receptor agonists have been shown to be mediated by mechanisms not affected by the classical oestrogen receptor antagonists. The present study agrees with these findings.

In summary, in the mouse mesenteric artery, as in the rat mesenteric artery, DPN is less potent than PPT at producing relaxations, at least in females However, in the rat and mouse aorta only DPN produced marked relaxations. Effects of oestrogen receptor subtype selective agonists are tissue dependent. This may have implications for the overall cardiovascular effects of oestrogen receptor subtype selective agonists, and may represent a novel therapeutic strategy, by employing subtype selective agonists.

References

Al Zubair, K., Razak, A., Bexis, S., Docherty, J.R., 2004. Relaxations to oestrogen receptor subtype selective agonists in rat and mouse arteries. www.pa2online/Vol2Issue2abst053P.

Andersen, H.L., Weis, J.U., Fjalland, B., Korsgaard, N., 1999. Effect of acute and long-term treatment with 17β-estradiol on the vasomotor response in the rat aorta. Br. J. Pharmacol. 126, 159–168.

Beral, V., Million Women Study Collaborators, 2003. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 362, 419–427.

Bexis, S., Vandeputte, C., McCormick, P.A., Docherty, J.R., 2004. Deletion of inducible nitric oxides synthase decreases mesenteric vascular responsiveness in portal hypertensive mice. Eur. J. Pharmacol. 499, 325–333.

Brouchet, L., Krust, A., Dupont, S., Chambon, P., Bayard, F., Arnal, J.F., 2001. Estradiol accelerates reendothelialization in mouse carotid artery through estrogen receptor-α but not estrogen receptor-β. Circulation 103, 423–428.

Browne, M., Connolly, C., Docherty, J.R., 1999. Vascular actions of 17beta-oestradiol in rat aorta and mesenteric artery. J. Auton. Pharmacol. 19, 291–299.

Bucci, M., Roviezzo, F., Cicala, C., Pinto, A., Cirino, G., 2002. 17-Beta-oestradiol-induced vasorelaxation in vitro is mediated by eNOS through hsp90 and akt/pkb dependent mechanism. Br. J. Pharmacol. 135, 1695–1700.

Crews, J.K., Khalil, R.A., 1999. Antagonistic effects of 17β-estradiol, progesterone and testosterone on Ca2+ entry mechanisms of coronary vasoconstriction. Arterioscler. Thromb. Vasc. Biol. 19, 1034–1040.

Darblade, B., Pendaries, C., Krust, A., Dupont, S., Fouque, M.J., Rami, J., Chambon, P., Bayard, F., Arnal, J.F., 2001. Estradiol alters nitric oxide production in the mouse aorta through the α-, but not β-oestrogen receptor. Circ. Res. 90, 413–419.

Farhat, M.Y., Lavigne, M.C., Ramwell, P.W., 1996. The vascular protective effects of estrogen. FASEB J. 10, 615–624.

Garban, H.J., Buga, G.M., Ignarro, L.J., 2004. Estrogen receptor-mediated vascular responsiveness to nebivolol: a novel endothelium-related mechanism of therapeutic vasorelaxation. J. Cardiovasc. Pharmacol. 43, 638–644.

Geary, G.G., Krause, D.N., Duckles, S.P., 2000. Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. Am. J. Physiol.: Heart Circ. Physiol. 279, H511–H519.

Geary, G.G., Mc Neill, A.M., Ospina, J.A., Krause, D.N., Korach, K.S., Duckles, S.P., 2001. Selected contribution: cerebrovascular NOS and cyclooxygenase are unaffected by estrogen in mice lacking estrogen receptor-alpha. J. Appl. Physiol. 91, 2391–2399.

Gerhard, M., Ganz, P., 1995. How do we explain the clinical benefits of oestrogen? Circulation 92, 5–8.

Gisclard, V., Miller, V.M., Vanhoutte, P.M., 1988. Effect of 17β-oestradiol on endothelium-dependent responses in rabbit. J. Pharmacol. Exp. Ther. 244, 19–22.

Harder, D.R., Coulson, P.B., 1979. Estrogen receptors and effects of estrogen on membrane electrical properties of coronary vascular smooth muscle. J. Cell. Physiol. 100, 375–382.

Herrington, D.M., Braden, G.A., Williams, J.K., Morgan, T.M., 1994. Endothelial-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. Am. J. Cardiol. 73, 951–952.

Iafrati, M.D., Karas, RH., Aronovitz, M., Kim, S., Sullivan Jr., T.R., Lubahn, DB., O'Donnell Jr., T.F., Korach, K.S., Mendelsohn, M.E., 1997. Estrogen inhibits the vascular injury response in estrogen receptor α-deficient mice. Nat. Med. 3, 545–548.

Jensen, E.V., Jacobson, H.I., 1960. Basic guide to the mechanism of oestrogen action. Recent Prog. Horm. Res. 18, 387–414.

Karas, R.H., Hodgin, J.B., Kwoun, M., Krege, J.H., Aronovitz, M., Mackey, W., Gustafsson, J.A., Korach, K.S., Smithies, O., Mendelsohn, M.E., 1999. Estrogen inhibits the vascular injury response in estrogen receptor β-deficient female mice. Proc. Natl. Acad. Sci. U. S. A. 96, 15133–15136.

Kauser, K., Rubanyi, G.M., 1994. Gender differences in bioassayable endothelium-derived nitric oxide from isolated rat aorta. Am. J. Physiol. 267, H2317–H3211.

Kitazawa, T., Hamada, E., Kitazawa, K., Gaznabi, A.K.M., 1997. Non-genomic mechanism of 17β-Oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. J. Physiol. 499, 497–511.

Knot, H.J., Lounsbury, K.M., Brayden, J.E., Nelson, M.T., 1999. Gender differences in coronary artery diameter reflect changes in both endothelial Ca^{2+} and ecNOS activity. Am. J. Physiol. 276, H961–H969.

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.-Å., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 3, 863–870.

Lindner, V., Kim, S.K., Karas, R.H., Kuiper, G.G.J.M., Gustafsson, J.-Å., Mendelsohn, M.E., 1998. Increased expression of estrogen receptor-β mRNA in male blood vessels after vascular injury. Circ. Res. 83, 224–229.

Meyers, M.J., Sun, J., Carlson, K.E., Marriner, G.A., Katzenellenbogen, B.S., Katzenellenbogen, J.A., 2001. Estrogen receptor-beta potency-selective ligands: structure–activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. J. Med. Chem. 44, 4230–4251.

Montgomery, S., Shaw, L., Pantelides, N., Taggart, M., Austin, C., 2003. Acute effects of oestrogen receptor subtype-specific agonists on vascular contractility. Br. J. Pharmacol. 139, 1249–1253.

Mulvany, M.J., Warshaw, D.M., 1977. The active tension-length of vascular smooth muscle related to its cellular components. J. Gen. Physiol. 74, 85–104.

Murphy, J.G., Khalil, R.A., 2000. Gender-specific reduction in contractility and $[Ca^{2+}]_i$ in vascular smooth muscle cells of female rat. Am. J. Physiol. 278, C834–C844.

Pare, G., Krust, A., Karas, R.H., Dupont, S., Aronovitz, M., Chambon, P., Mendelsohn, M.E., 2002. Estrogen receptor-α mediates the protective effects of estrogen against vascular injury. Circ. Res. 90, 1087–1092.

Razak, A., Docherty, J.R., 2002. Gender differences in relaxations of rat aorta. Pflugers Arch.: Eur. J. Physiol. 443, S332.

Salom, J.B., Burguete, M.C., Perez-Asensio, F.J., Torregrosa, G., Alborch, E., 2001. Relaxant effects of 17-beta-estradiol in cerebral arteries through Ca(2+) entry inhibition. J. Cereb. Blood Flow Metab. 21, 422–429.

Shan, J., Resnick, L.M., Liu, Q.Y., Wu, X.C., 1994. Vascular effects of 17 beta-estradiol in male Sprague–Dawley rats. Am. J. Physiol. 266, H967–H973.

Shaw, L., Taggart, M.J., Austin, C., 2000. Mechanisms of 17 beta-oestradiol induced vasodilatation in isolated pressurized rat small arteries. Br. J. Pharmacol. 129, 555–565.

Sitzler, G., Lenz, O., Kilter, H., La Rosee, K., Bohm, M., 1996. Investigation of the negative inotropic effects of 17β-oestradiol in human isolated myocardiac tissues. Br. J. Pharmacol. 119, 43–48.

Stallone, J.N., Crofton, J.T., Share, L., 1991. Sexual dimorphism in vasopressin-induced contraction of rat aorta. Am. J. Physiol. 260, H453–H458.

Stauffer, S.R., Coletta, C.J., Tedesco, R., Nishiguchi, G., Carlson, K., Sun, J., Katzenellenbogen, B.S., Katzenellenbogen, J.A., 2000. Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonist. J. Med. Chem. 43, 4934–4947.

Sudhir, K., Chou, T.M., Mullen, W.L., Hausmann, D., Collins, P., Yock, P.G., Chatterjee, K., 1995. Mechanisms of estrogen-induced vasodilation: in vivo studies in canine coronary conductance and resistance arteries. J. Am. Coll. Cardiol. 26, 807–814.

Tostes, R.C., David, F.L., Carvalho, M.H., Nigro, D., Scivoletto, R., Fortes, Z.B., 2000. Gender differences in vascular reactivity to endothelin-1 in deoxycorticosterone-salt hypertensive rats. Hypertension 34, 1232–1236.

Wakeling, A.E., Dukes, M., Bowler, J., 1991. A potent specific pure antiestrogen with clinical potential. Cancer Res. 51, 3867–3873.

Wellman, G.C., Bonev, A.D., Nelson, M.T., Brayden, J.E., 1996. Gender differences in coronary artery diameter involve estrogen, nitric oxide and Ca^{2+}-dependent K^+ channels. Circ. Res. 79, 1024–1030.

White, R.E., Darkow, D.J., Lang, J.L., 1995. Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism. Circ. Res. 77, 936–942.

Wynne, F.L., Payne, J.A., Cain, A.E., Reckelhoff, J.F., Khalil, R.A., 2004. Age-related reduction in estrogen receptor-mediated mechanisms of vascular relaxation in female spontaneously hypertensive rats. Hypertension 43, 405–412.

Zhang, F., Ram, J.L., Standley, P.R., Sowers, J.R., 1994. 17β-Estradiol attenuates voltage-dependent Ca2+ currents in A7r5 vascular smooth muscle cell line. Am. J. Physiol. 266, C975–C980.

Zhu, Y., Bian, Z., Lu, P., Karas, R.H., Bao, L., Cox, D., Hodgin, J., Shaul, P.W., Thorn, P., Smithies, O., Gustafsson, J.K., Mendelsohn, M.E., 2002. Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta. Science 295, 505–508.