Relaxation to oestrogen receptor subtype selective agonists in male and female mouse aorta.

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Abstract

It has been reported that the oestrogen receptor (ER) alpha agonist 4,4,4-(4-propyl-[^{1}H]-pyrazole-1,3,5-triyl) tris-phenol (PPT) is more potent than the ER-beta receptor agonist 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN) at producing relations in rat mesenteric artery. We have investigated the relaxant actions of the ER-alpha agonist PPT and the ER-beta agonist DPN in aorta from male and female mouse. Rings of blood vessel were set up in small vessel myographs for recording of isomeric contractions. Tissues were contracted with KCl (40 mM) and tested for relaxations to acetylcholine, then again contracted with KCl and tested for relaxations to increasing concentrations of ER agonists. In male and female mouse aorta, the ER-beta agonist DPN produced significantly greater relaxations than the ER-alpha agonist PPT.

Key words:Oestrogen receptor, ER-beta receptor.

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1. Introduction

Oestrogens have affinity for specific intracellular binding proteins known as oestrogen receptors (ERs). There are two oestrogen receptors, both of which are members of the superfamily of steroid hormone receptors (1). In addition to the classic oestrogen receptor, discovered by Elwood Jensen in 1960 (now known as ER-α) (2), a homolog and novel oestrogen receptor subsequently called ER-β was cloned (3). These receptors represent two separate gene products, which are nuclear receptors, the distribution ratio

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of these two receptors varies between tissues and species and they show different biological roles (4). Both act as transcription factors to alter gene expression when they are activated by oestrogen (1). Once bound to oestrogen, the oestrogen receptor undergoes a conformational change allowing the receptor-oestrogen complex to activate oestrogen-responsive genes to achieve the biological activity required (5); this genomic signaling pathway can take hours or more to occur (6). Further studies into oestrogen signaling mechanisms led to the discovery of cell surface oestrogen receptors, these receptors are coupled to cytosolic signal transduction proteins and act by mechanisms independent of the classical genomic pathway to induce rapid cellular events (6). Both ER-α and ER-β, appear to be expressed in vascular tissues (4).

Mechanical, chemical, or immune injury to blood vessels stimulates the proliferation of the smooth muscle cells leading to a lesion in the blood vessels; this process can be inhibited by the antiproliferative effects of oestrogen (7). Oestradiol induced inhibition of the vascular injury response occurs in mice lacking the oestrogen receptor α (ERα-KO) (8) and in mice lacking the oestrogen receptor β (ER β-KO) (9), although other studies implicate the oestrogen receptor α in this response (10). 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/βoestrogen receptor -KO mice (11). Taken together, these finding suggest that oestrogen receptor β may compensate for oestrogen receptor α in oestrogen receptor α KO mice, and possibly vice versa. In the present study we have investigated the

relaxant actions of the ER-alpha agonist PPT and the ER-beta agonist DPN in aorta from male and female mice.

2. Materials and Methods

2.1. Drugs

potassium chloride (KCl) (Merck Darmstadt, Germany); Acetylcholine chloride (Sigma, Poole, U.K); PPT (4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl) tris -phenol; Tocris); DPN (2, 3-bis (4-hydroxyphenyl)-propionitril.

2.2. Animals

Male and female adult wild-type black mice (18 - 28g), animals were housed in controlled environment with 12-h light and 12-h dark cycle. They were fed a standard mouse diet. Animal were killed by CO_2 overdose.

2.3. Mounting the vessels in the small vessel myograph

Rings of mouse aorta 1-1.5 mm 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) linked to a Macintosh computer. The myograph chamber was filled with Krebs-Henseleit solution (KHS) 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, ascorbic acid 0.28, and gassed with 95% O_2 and 5% CO_2 at 37ºC with pH 7.4. propranolol (3µM) was added to block β-adrenoceptors. The vessels were set to a tension equivalent to that generated at 0.9 times diameter of the vessel at 100 mm Hg transmural pressure (12). To allow tissues to equilibrate under resting force, the fluid (KHS) in the chamber was changed at regular intervals (15 min) for 30 minutes.

The gap between the myograph jaws is 2 mm, but the length of vessels was 1-1.5 mm (usually near to 1 mm). The

length of vessels was measured in some experiments using an eyepiece with graticule, but in most experiments it was found that estimates of vessel length based on comparison of vessel length with size of jaws gave sufficient accuracy.

The diameter of aortic rings was 753±17 µm (n=21 animals) in vessels from wild-type mice.

2.4. Statistics

Values are mean ± S.E.M. from number of experiments (n). Contractions to KCl (mM) or relaxations (%) to agonists (negative relaxation indicates contraction) were compared between the variance by Student's *t* -test. Differences between groups were considered significant when $P< 0.05$. Statistical and graphical analysis was carried out using Instat for Macintosh and GraphPad Prism.

3. Results

In aorta from wild-type mice, KCl (40 mM) produced contractions of 4.25±0.98 mM (n=12) and 4.36±0.44 mM (n=14), in male and female. The contraction to KCl (40 mM) was 78.0±6 % (n=5) and 64.8±8 % (n=5) of maximum in aorta from male and female mice, respectively (Figure 1).

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 i.e. a contraction of 9.1±4.1% of KCl contraction, n=5; for DPN and PPT, respectively) (Figures 2, 3). In fact, PPT failed to produce significant relaxations in mice aorta as compared to the effects of vehicle. Vehicle had little effect except the highest concentration (100% ethanol; final bath concentration 1%), which contracted aorta. There were

no differences between male and female mice in aortic responses (Figure 4).

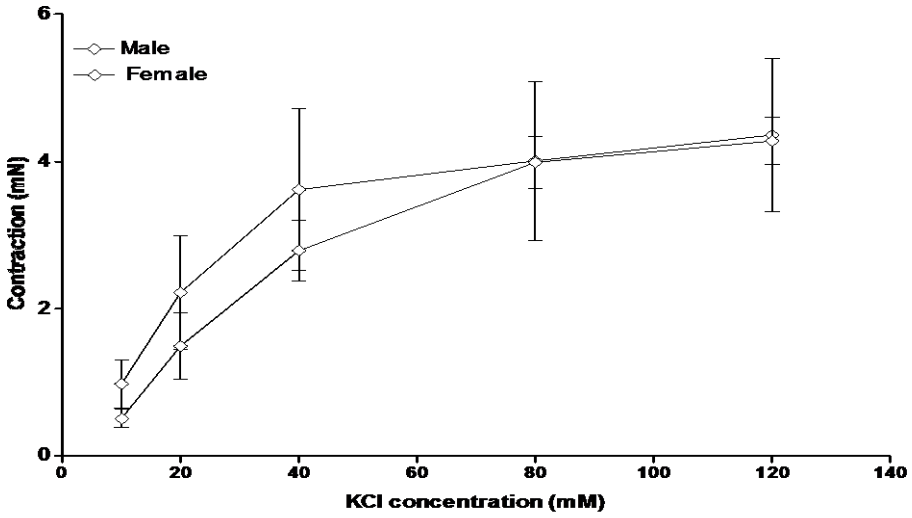

Figure 1. A concentration-contractile response curves to KCl in male and female aorta from wild-type mice. Vertical bars represent S.E of mean from 5 (male, female) experiments.

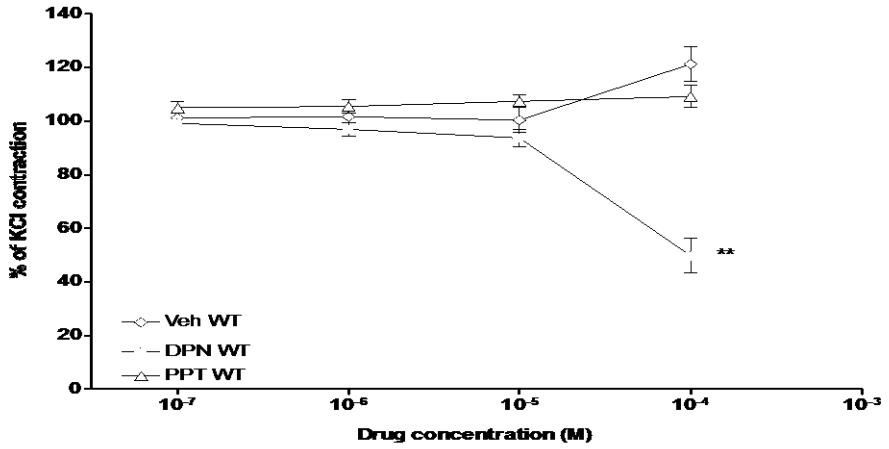

Figure 2.The effects of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN or vehicle (veh) in female aorta from wild-type (WT) precontracted with KCl 40 mM. Vertical bars represent S.E of mean from 5 (PPT, veh) or 9 (DPN) experiments. (★★ Indicates $p < 0.01$ compared to vehicle).

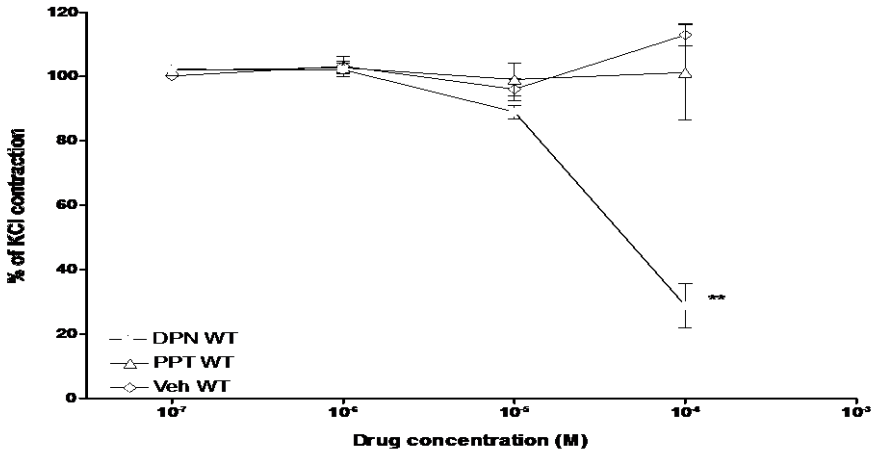

Figure 3.The effects of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN or vehicle (veh) in male aorta from wild-type (WT) precontracted with KCl 40 mM. Vertical bars represent S.E of mean from 6 (PPT, DPN) or 5 (veh) experiments. (★★ Indicates $p< 0.01$ compared to vehicle).

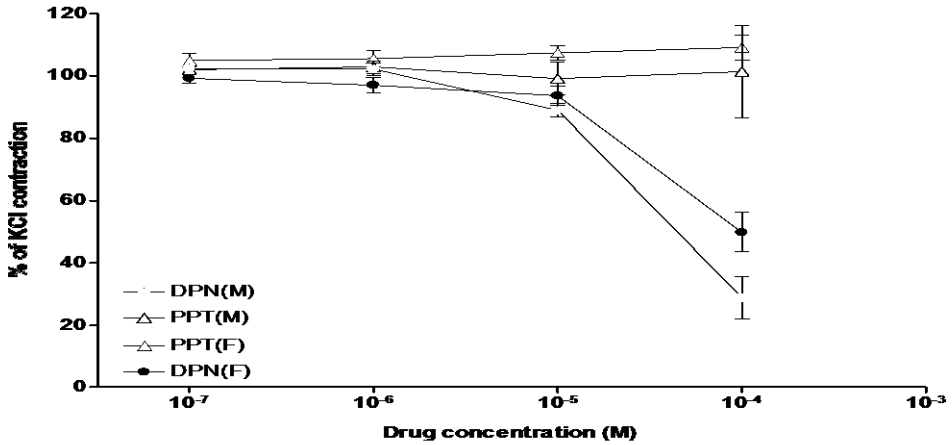

Figure 4. The effects of the oestrogen receptor α agonist PPT and the oestrogen receptor β agonist DPN in male (M) and female (F) aorta from wild-type (WT) mice, precontracted with KCl 40 mM. Vertical bars represent S.E of mean from 6 (PPT, DPN) experiments in male and 5 (PPT) or 9 (DPN) experiments in female. Data taken from Figures 2, 3. For vehicle experiments, see Figures 2, 3.

4. Discussion

In aorta from both male and female WT mice, our findings demonstrated that the oestrogen receptor β agonist DPN induced relaxation was significantly greater than that of the oestrogen receptor α agonist PPT. In fact, in both male and female mice, PPT failed to produce a significant relaxation in concentrations of up to 100 μM. Two studies have been reported that theoestrogen receptor (ER) alpha agonist PPT is more potent than the ER-beta receptor agonist DPN at producing relations in rat mesenteric artery which is at variance with our results (13, 14). Both ER-α and ER-β, appear to be expressed in vascular tissues, in tissue and species dependent manner.

استرخاء الشريان الأورطي في ذكور وإناث الفئران بواسطة النواهض المنتقية للأنواع الفرعية من مستقبلات هرمون الاستروجين

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الملخص:

أفادت بعض الدراسات أن مستقبلات هرمون الاستروجين ناهض ألفا-PPT هو أكثر فعالية من مستقبلات هرمون الاستروجين ناهض بيتا-DPN في تكوين صلات فى الشريان الأورطي للفئران. هنا ناقشنا مدى تاثير الارتخاء عند استخدام ناهض ألفا-PPT و ناهض بيتا-DPN على الشريان الأورطي للفئران وذلك بوضع حلقات من الاوعية الدموية في myographsvessel صغيرة لتسجيل التقلصات الايزوميرية عليها. كذلك غمرت انسجة فى كلوريد البوتاسيوم (40 mM) ثم اختبر الارتخاء بالنسبة للاستيل كولين ثم اعيدت التجربة و اختبر الارتخاء عند الزيادة فى تركيز ناهضات هرمون الاستروجين. النتائج اثبتت ان التأثير على الشريان الأورطي للفئران (ذكور وإناث) كان اكثر فى حالة ناهضER-بيتاDPN منه فى حالة ناهض ألفا-PPT.

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5. Reference

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