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Article

Vasorelaxant Mechanism of Estriol: Endothelium-Dependent and -Independent Pathways in an Ex Vivo Rat Aortic Model

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RESUMO

Este estudo investigou o efeito vasorrelaxante do estriol (E3) em comparação com o do estradiol (E2) em anéis de aorta torácica de ratos, com ênfase nos mecanismos dependentes e independentes do endotélio. Anéis aórticos isolados de ratos Wistar, com ou sem endotélio, foram pré-contraídos com fenilefrina e submetidos a curvas de concentração-resposta cumulativas para E3 e E2. A participação do óxido nítrico sintase endotelial (eNOS), de canais de potássio, de prostanoides e do influxo de cálcio foi avaliada por meio de inibidores farmacológicos $N^{\omega}N\omega$-nitro-L-arginina – L-NAME; tetraetilamônio; indometacina e de soluções despolarizantes livres de Ca^{2+}. O E3 promoveu vasorrelaxamento de forma dependente da concentração, porém com menor potência e eficácia do que o E2. Em preparações com endotélio íntegro, o relaxamento induzido pelo E3 foi significativamente reduzido por L-NAME e TEA, sugerindo a participação do óxido nítrico e dos canais de potássio no mecanismo de ação. A indometacina não modificou o relaxamento induzido por E3, indicando ausência de envolvimento relevante dos prostanoides. Em condições despolarizadas e isentas de cálcio, tanto E3 quanto E2 atenuaram as contrações induzidas por $CaCl_2$, revelando uma modulação do influxo de cálcio, independente da endotelial. Esses resultados demonstram que o estriol induz vasorrelaxamento por meio de múltiplos mecanismos, incluindo a ativação da eNOS, o envolvimento de canais de K^+ e a inibição da entrada de cálcio. Em conjunto, os resultados ampliam a compreensão dos mecanismos vasculares mediados pelo estriol.

Palavras-chave: óxido nítrico; canais de cálcio; canais de potássio; receptores estrogênicos; músculo liso vascular.

ABSTRACT

This study investigated the vasorelaxant effects of estriol (E3) compared to estradiol (E2) in rat thoracic aortic rings, focusing on endothelium-dependent and independent mechanisms. Isolated aortic rings from Wistar rats, with or without endothelium, were pre-

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contracted with phenylephrine and subjected to cumulative concentration–response curves for E3 and E2. The involvement of endothelial nitric oxide synthase (eNOS), potassium channels, prostanoids, and calcium influx was assessed using pharmacological inhibitors $N^{\omega}N\omega$-nitro-L-arginina – L-NAME, tetraethylammonium (TEA), indomethacin, and depolarizing Ca^{2+}-free solutions. E3 promoted concentration-dependent vasorelaxation, but with lower potency and efficacy than E2. In endothelium-intact preparations, the E3-induced relaxation was significantly reduced by L-NAME and TEA, suggesting the involvement of nitric oxide and potassium channels in its mechanism. Indomethacin did not alter E3-induced relaxation, indicating no significant involvement of prostanoids. In depolarized, calcium-free conditions, both E3 and E2 attenuated $CaCl_2$-induced contractions, indicating endothelium-independent modulation of calcium influx. These results demonstrate that estriol induces vasorelaxation through multiple mechanisms, including the activation of eNOS, involvement of K^+ channels, and inhibition of calcium entry. Taken together, the results broaden the understanding of estriol-mediated vascular mechanisms.
Keywords: nitric oxide signaling; calcium channel modulation; potassium channels; estrogen receptors; and vascular smooth muscle.

Introduction

Hormone replacement therapy (HRT) in postmenopausal women has long been considered a therapeutic option for alleviating menopausal symptoms and offering cardiovascular protection. Evidence suggests that HRT may reduce the risk of coronary heart disease and overall mortality (Langer 2017; Low et al. 2002). Estrogens are a core component of HRT regimens, with conjugated equine estrogens (CEEs) among the most prescribed formulations (Bhavnani and Stanczyk 2014). Despite their widespread use, the cardiovascular benefits of CEEs have been the subject of ongoing debate. Notable studies, such as the Heart and Estrogen/progestin Replacement Study (HERS), raised concerns regarding the safety and efficacy of CEEs, particularly in older women or those with pre-existing cardiovascular conditions (Hulley et al. 1998; Rossouw et al. 2002).

One key issue with CEEs is their non-bioidentical nature. Derived from the urine of pregnant mares, CEEs contain a mixture of estrogens not naturally found in human physiology, leading to potentially distinct effects compared to endogenous estrogens (Novensa et al. 2010). This has sparked growing interest in bioidentical hormone replacement therapy (BHRT), which utilizes hormones identical in structure to those naturally produced by the human body. Estriol (E3), a bioidentical estrogen, is frequently used in BHRT either alone or in combination with estradiol (E2) and estrone (E1), offering an alternative to traditional HRT regimens (Holtorf 2009). Estriol's appeal lies in its weaker estrogenic activity, which is believed to confer benefits with a potentially lower risk of adverse effects.

Despite the increasing use of BHRT in clinical practice, particularly for the management of menopausal symptoms, the cardiovascular effects of E3 remain inadequately explored. Current evidence on E3 vascular impact is inconsistent, and comprehensive studies are limited. Research into the pharmacodynamics of E3, particularly its interactions with vascular tissues, is necessary to elucidate its potential role in cardiovascular protection. Estriol has been shown to exhibit vasorelaxant properties similar to those of E2, with both endothelium-dependent and independent mechanisms implicated (Oliveira et al. 2018). These vasorelaxant effects involve nitric oxide (NO) production and the activation of signaling pathways, such as PI3K/Akt, which are critical for maintaining vascular homeostasis (Hisamoto et al. 2001; Haynes et al. 2000).

Given the increasing prevalence of cardiovascular disease (CVD) among postmenopausal women and the need for safer therapeutic alternatives, further investigation of E3 is warranted. Studies have shown that estradiol can induce rapid NO synthesis and activate endothelial nitric oxide synthase (eNOS), leading to vasodilation (Haynes et al. 2000). However, the extent to which E3 exerts similar effects, particularly compared with E2, remains unclear. Additionally, it is crucial to determine whether estriol's weaker estrogenic activity translates into differential modulation of the cardiovascular system, potentially offering a therapeutic advantage by reducing the risk of adverse events, such as hypertension.

In this sense, this study aims to investigate the vascular effects of E3 in the thoracic aorta of rats, focusing on its endothelium-dependent and independent actions. Findings from this study could have significant implications for the development of BHRT-based strategies aimed at reducing cardiovascular risk in postmenopausal women, addressing an important gap in the current understanding of estrogen therapy and vascular health.

Materials and Methods

Animals

Male Wistar rats (200-300g) were used to eliminate any potential hormonal alterations that could interfere with the experimental protocol. The animals were housed in controlled conditions (22 ± 2°C, 45 ± 15% humidity, 12-h light–dark cycle), with ad libitum access to water and commercial feed. All procedures adhered to the National Council for the Control of Animal Experimentation (CONCEA), followed guidelines, and were approved by the Ethics Committee for Animal Use of the Federal University of the Jequitinhonha and Mucuri Valleys, protocol number CEUA/UFVJM Nº 09/2022. On the day of the experiments, rats were killed by decapitation, under anesthesia with 50 $mg.kg^{-1}$ of ketamine and 10 $mg.kg^{-1}$ of xylazine administered intraperitoneally (i.p.), with a small animal guillotine, and aortas were removed. Drugs and Reagents.

The following drugs and reagents were used in the experimental protocols: sodium chloride (Synth - Brazil), potassium chloride (Synth - Brazil), calcium chloride dihydrate (Vetec - Brazil), magnesium sulfate (Synth - Brazil), potassium phosphate monobasic (Impex - Brazil), sodium bicarbonate (Vetec - Brazil), glucose (Synth - Brazil), disodium EDTA (Synth - Brazil), ethanol (Vetec - Brazil), dimethyl sulfoxide (DMSO) (Synth - Brazil), estriol (Sigma - USA), 17β-estradiol (Sigma - USA), phenylephrine (Phe) (Sigma - USA), acetylcholine (ACh) (Sigma - USA), Nω-nitro- L-arginine methylester (L-NAME) (Sigma - USA), indomethacin (Sigma - USA), tetraethylammonium (TEA) (Sigma - USA), sodium nitroprusside (NPS) (Sigma - USA). Indomethacin, estriol, and 17β-estradiol were dissolved in ethanol. Krebs-Henseleit modified solution and other substances were prepared in distilled water.

Experimental Protocols

Vascular function studies

After euthanasia, thoracic aortas were carefully removed and immediately placed in ice-cold (4 °C) Krebs-Henseleit-modified solution [composition (in mM): 130 NaCl, 14.9 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4 \cdot 7H_2O$, 5.5 glucose, 1.56 $CaCl_2 \cdot 2H_2O$, and 0.026 EDTA], gassed with 5% CO_2/95% O_2 to maintain a pH of 7.4. The connective and fat tissue were removed, and vessels were sectioned into transverse rings of 4 mm in length. For experiments using aortas without vascular endothelium, the intimal layer was removed by gently rubbing the lumen with a cotton-covered metal cannula.

Aortic vascular rings were prepared and suspended using two stainless steel wires. One end was attached to a force transducer (TIM-200, MOB IOB, Brazil), which was connected to a computer system (AECAD 04 amplifier, AVS Projects, Brazil) to record contraction and relaxation responses. The opposite end of each ring was placed in a glass chamber (either 6 mL or 20 mL) filled with modified Krebs-Henseleit solution, maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2), ensuring that the vessel remained submerged.

A resting tension of 1.5 g was applied to each aortic ring, followed by a 60-minute stabilization period. During this period, the nutrient solution was refreshed, and tension was adjusted every 15 minutes to maintain optimal conditions for the tissue.

After stabilization, a vascular contractile response test was performed using phenylephrine (Phe - 1 μM). Acetylcholine (ACh - 10 μM) was subsequently administered to assess endothelial integrity. Endothelial integrity was classified based on the ACh-induced relaxation response: preparations were deemed endothelial-intact if relaxation was ≥ 90%, and endothelial-denuded if relaxation was ≤ 10%. Following the endothelial integrity assessment, the nutrient solution was replaced every 15 minutes for 45 minutes, or until the preparation returned to baseline.

Evaluation of Endothelium-Dependent Vasorelaxation Induced by Estriol

To evaluate the role of the endothelium in the vasorelaxant effect of estriol or 17β-estradiol (as a control), both hormones were tested in aortic rings with and without intact endothelium. The presence or absence of endothelium in each preparation was confirmed as described above.

The preparations were precontracted with Phe (1 μM). During the tonic phase of the contraction, cumulative concentration-effect curves (CCEs) were constructed for estriol or 17β-estradiol at 0.1, 0.3, 1, 3, 10 and 100 μM. After the CCE, the preparations were washed and stabilized for 60 minutes, with solution changes every 15 minutes. A new contraction was induced with Phe, followed by the addition of ACh (10 μM) to endothelial-intact preparations to ensure tissue viability. For endothelial-denuded preparations, sodium nitroprusside (NPS - 1μM) was added to induce endothelium-independent relaxation to confirm the preparation's viability. The vehicle (ethanol) was also tested, and its final concentration in the baths did not exceed 1%.

Analysis of eNOS Pathway Involvement in Estriol-Induced Vasorelaxation

Endothelial-intact preparations were pre-incubated for 30 minutes with L-NAME (100μM; a non-selective eNOS inhibitor) and contraction with Phe (0.1μM). After 10 minutes of stabilization, CCEs for estriol or 17β-estradiol (0,01 - 1 mM) were constructed.

Investigation of Hyperpolarizing Factor Involvement in Estriol-Induced Vasorelaxation

Endothelial-intact preparations were pre-incubated for 30 minutes with tetraethylammonium (TEA, 1mM; a non-selective potassium channel inhibitor). After pre-contraction with Phe (0.1μM) and stabilization for 10 minutes, CCEs for estriol or 17β-estradiol (0,01 - 1 mM) were constructed.

Analysis of Prostanoid Involvement in Estriol-Induced Vasorelaxation

Endothelial-intact preparations were pre-incubated for 30 minutes with indomethacin (10μM; a non-selective COX inhibitor), followed by contraction with Phe (0.1μM). After 10 minutes of stabilization, CCEs for estriol or 17β-estradiol (0,01 - 1 mM) were constructed.

Evaluation of Smooth Muscle and Ca^{2+} Channel Involvement in Estriol-Induced Vasorelaxation

To assess whether estriol- or 17β-estradiol-induced relaxation involved Ca^{2+} influx, endothelial-denuded rings were pre-contracted with a depolarizing solution (120 mM KCl). After a 30-minute stabilization period and confirmation of endothelial absence, the preparations were incubated with vehicle or 0.01, 0.2, or 1 mM of estriol or 17β-estradiol in Ca^{2+}-free Krebs-Henseleit solution. After 20 minutes, the tissues were placed in a depolarizing solution (60 mM) for 10 minutes, followed by CCEs for $CaCl_2$ (10 - 0.1 mM). Results were expressed as percentages of the maximum contractile response to KCl (120 mM), and the curves were statistically compared.

Statistical Analysis

The data obtained were analyzed and expressed as mean ± standard error of the mean (SEM) percentage of vascular relaxation relative to the reduction in maximal Phe-induced contraction (Emax) from 6 to 8 preparations per group. Results were subjected to the unpaired two-tailed Student's t-test for comparative analyses between two groups. For comparisons involving more than two groups, results were analyzed using analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Differences were considered significant when $P < 0.05$. All data were analyzed using GraphPad Prism® version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Estriol (E3) induced concentration-dependent relaxation in endothelium-intact (E+) aortic rings, achieving a maximum relaxation (Emax) of 59.90 ± 3.41% (Figure 1A). This effect was significantly different from the relaxation induced by the vehicle (Emax = 2.19 ± 0.73%) (Figure 1A) and estradiol (E2), which displayed a higher maximum relaxation (Emax = 85.09 ± 1.25%) (Figure 1B). The EC50 values for E3 and E2 were determined to be 404.70 ± 5.71 μM and 157.41 ± 6.57 μM, respectively. Under the experimental conditions of this study, E2 demonstrated 2.5 times greater efficacy than E3.

In endothelium-denuded (E−) preparations, E3 also induced concentration-dependent relaxation; however, this effect was significantly reduced compared to that observed in E+ preparations (Figure 1A). The maximum relaxation in E-aortic rings was 29.75 ± 1.46% (Figure 1A). This response was significantly different from the relaxation promoted by the vehicle (Emax = 2.19 ± 0.73%) (Figure 1A) and from that of E2, which exhibited an Emax of 49.22 ± 0.80% (Figure 1B).

These findings suggest that E3 and E2 promote vascular relaxation, with a more pronounced effect in endothelium-intact aortic rings. However, E2 shows superior potency and efficacy compared to E3 in both endothelium-intact and endothelium-denuded preparations.

The analysis of the involvement of endothelial nitric oxide synthase (eNOS) in the vasorelaxant effect of estriol showed that incubation with L-NAME (100 μM) for 30 minutes inhibited the relaxation induced by E3 (Emax = 30.07 ± 1.67%) (Figure 2A) and E2 (Emax = 59.42 ± 2.31%) (Figure 2B). These values were significantly different from those observed for E3 (Emax = 59.90 ± 3.41%) (Figure 2A) and E2 (Emax = 85.09 ± 1.25%) (Figure 2B), in the absence of inhibitors.

The influence of endothelium-derived hyperpolarizing factor (EDHF) on the vasorelaxant effect of estriol in rat thoracic aorta was investigated using intact endothelium aortic rings precontracted with phenylephrine (Phe, 1.0 μM). Incubation with TEA, a blocker of Ca^{2+}-activated K^+ channels (BKCa), at a concentration of 1 mM reduced the E3-induced relaxation (Emax = 46.52 ± 0.60%, Figure 3A) but did not significantly alter the E2-induced vasorelaxation (Emax = 80.24 ± 0.73%, Figure 3B). This suggests that, compared to E2, E3 may involve distinct mechanisms, specifically K^+ channel involvement.

The investigation of vasodilatory prostanoids in the effect of estriol on intact endothelium aortic rings revealed that pre-incubation with indomethacin (10 μM) did not inhibit the E3-induced vasorelaxation (Emax = 63.22 ± 3.91%, Figure 4A). Similarly, under the experimental conditions tested, E2 showed no inhibition with this antagonist (Emax = 88.94 ± 0.90%, Figure 4B).

The study of endothelial-independent mechanisms focused on calcium channels. Aortic rings devoid of functional endothelium were placed in a hyperpolarizing solution free of Ca^{2+} (KCl 60 mM), and the effects of E3 at concentrations of 0.01, 0.2 and 1.0 mM were investigated. At concentrations of 0.2 and 1.0 mM, E3 significantly blocked the contractions induced by cumulative concentrations of $CaCl_2$ (Figure 5A). Similarly,

E2 also effectively inhibited the contractions induced by cumulative concentrations of $CaCl_2$ at the same concentrations (0.2 and 1.0 mM) (Figure 5A).

To further enhance the analysis, Table I presents the maximum effect (Emax) obtained from the cumulative $CaCl_2$ curves in the presence of E2 or E3 at concentrations of 0.01, 0.2 and 1.0 mM, compared to the solvent control. This data underscores the capacity of both E2 and E3 to modulate calcium-induced contractions, indicating a potential role in calcium channel inhibition and a pathway independent of endothelial influence.

Discussion

The present study demonstrates that estriol (E3) induces concentration-dependent vasorelaxation in rat thoracic aorta through both endothelium-dependent and endothelium-independent mechanisms. Our findings extend previous observations on estrogen-mediated vascular regulation by providing a systematic mechanistic comparison between E3 and estradiol (E2) in an ex vivo model. Significantly, the interpretation of these results is restricted to experimental and mechanistic implications, without extrapolation to clinical efficacy or therapeutic benefit.

Both E3 and E2 promoted relaxation in endothelium-intact and endothelium-denuded preparations, indicating that these hormones target both endothelial and vascular smooth muscle compartments. However, E3 consistently exhibited lower efficacy and lower apparent potency than E2, as reflected by reduced maximal relaxation and higher EC50 values. This pharmacodynamic profile highlights intrinsic differences among these estrogens in receptor interactions, intracellular signaling efficiency, and downstream effector activation. Such differences should be interpreted within the controlled conditions of an ex vivo system and do not imply clinical inferiority or superiority.

The marked attenuation of E3-induced relaxation by L-NAME strongly supports the involvement of nitric oxide (NO) signaling in its endothelium-dependent component. Estrogen-induced activation of endothelial nitric oxide synthase (eNOS) represents a central mechanism underlying rapid vasodilatory responses. Previous studies demonstrated that estradiol stimulates eNOS phosphorylation and NO production through PI3K/Akt and MAPK-dependent pathways (Haynes et al. 2000). Our data indicate that estriol shares this fundamental signaling route, although with reduced efficacy. The partial persistence of relaxation in endothelium-denuded rings further demonstrates that NO-dependent signaling does not fully account for the vasorelaxant effect of E3, indicating that an endothelium-independent mechanism is operative.

Endothelium-independent estrogenic vasorelaxation has been attributed primarily to direct actions on vascular smooth muscle cells, including modulation of intracellular calcium handling and membrane ion channel activity (Freay 1997; Gonzales and Kanagy 1999). In this context, the inhibitory effect of E3 on $CaCl_2$-induced contractions in depolarized, calcium-free preparations provides strong evidence for direct interference with calcium influx pathways. Voltage-dependent L-type calcium channels are a significant determinant of vascular tone, and their inhibition reduces cytosolic calcium availability and induces relaxation. Several studies demonstrated that estradiol modulates L-type calcium channel activity via both genomic and non-genomic mechanisms (Brewer et al. 2009; Vega-Vela et al. 2017). Our findings indicate that estriol retains this capacity, reinforcing the concept that calcium channel modulation constitutes a conserved estrogenic mechanism in vascular smooth muscle.

The contribution of potassium channels to estrogen-induced vasorelaxation has been widely investigated, yet results remain heterogeneous across vascular beds, species, and experimental conditions. In the present study, blockade of potassium channels by tetraethylammonium significantly reduced E3-induced relaxation but exerted a limited effect on E2 responses. This differential sensitivity suggests that E3 may rely more strongly

on hyperpolarizing mechanisms than E2. Activation of Ca^{2+}-activated K^+ channels promotes membrane hyperpolarization, closure of voltage-dependent calcium channels, and subsequent relaxation (Rosenfeld et al. 2000). Previous studies reported variable involvement of K^+ channels in estradiol-induced vasodilation, with some authors demonstrating significant inhibition by TEA and others observing minimal contribution (Gonzales and Kanagy 1999; Oliveira et al. 2018). Our data indicate that estriol preferentially engages this pathway, possibly reflecting distinct receptor coupling or differential activation of downstream signaling cascades.

In contrast, inhibition of cyclooxygenase with indomethacin did not modify the vasorelaxant responses to either E3 or E2, indicating that prostanoids do not contribute significantly under the present experimental conditions. This observation is consistent with previous reports showing that estrogen-induced vasorelaxation is mainly independent of prostaglandin synthesis (Filgueira et al. 2012; Oliveira et al. 2018). Although prostacyclin (PGI_2) is a major endothelial vasodilator, its role appears secondary to NO-dependent and ion channel–mediated mechanisms in estrogen-mediated responses (Rosenfeld et al. 1996).

The coexistence of endothelial and smooth muscle mechanisms underscores the pleiotropic nature of estrogen signaling in the vasculature. Estrogens activate multiple receptor populations, including nuclear estrogen receptors (ERα and ERβ) and membrane-associated receptors such as G protein–coupled estrogen receptor (GPER), which trigger rapid non-genomic signaling events (Levin 2009; Prossnitz and Barton 2011). Activation of these receptors initiates complex intracellular networks involving PI3K/Akt, MAPK, and cyclic nucleotide pathways that converge on eNOS activation, potassium channel opening, and calcium channel inhibition. The lower efficacy of E3 compared with E2 may reflect weaker receptor affinity, reduced membrane receptor activation, or diminished coupling efficiency to these signaling pathways.

Notably, the present experimental design imposes relevant limitations that restrict the scope of interpretation. The exclusive use of male rats precludes the influence of the female hormonal milieu. It does not recapitulate the endocrine environment of postmenopausal women, which represents the main clinical context of estriol administration. Sex-specific differences in endothelial function, receptor expression, and estrogen responsiveness are well documented (Reckelhoff and Fortepiani 2004; Stanhewicz et al. 2018). Consequently, the vascular effects observed in this model may not accurately reflect responses in female or ovariectomized animals.

Another major limitation concerns the concentration required to elicit vasorelaxation. The effective range of estriol and estradiol extended into the micromolar to millimolar domain, far exceeding circulating plasma levels achieved during hormone replacement therapy. Physiological concentrations of estrogens are typically in the nanomolar range (Langer 2017; Khalil 2005). At suprapharmacological concentrations, estrogens may engage non-physiological targets, alter membrane fluidity, or exert nonspecific effects on ion channels. Therefore, the present findings should be interpreted strictly as mechanistic observations obtained under experimental conditions rather than predictors of in vivo vascular efficacy.

Despite these limitations, the present study provides important mechanistic insights. The demonstration that estriol activates NO signaling, engages potassium channels, and inhibits calcium influx indicates that this weak estrogen retains the core molecular machinery associated with estrogenic vasorelaxation. These findings are consistent with emerging evidence that estriol, despite its lower estrogenic potency, may exert biologically relevant vascular actions (Novensa et al. 2010). Nevertheless, the reduced efficacy and the requirement for high concentrations indicate that its vascular actions should be regarded primarily as experimental phenomena.

Future investigations should address these limitations by employing physiologically relevant concentrations, female or ovariectomized models, and in vivo approaches. Direct assessment of receptor-subtype involvement, selective potassium-channel blockers, and intracellular signaling intermediates will be essential to establish a

more precise molecular framework. Such studies will clarify whether estriol exerts meaningful vascular effects under clinically relevant conditions and whether it may contribute to cardiovascular modulation during hormone replacement therapy.

Taken together, the present data demonstrates that estriol shares major signaling pathways with estradiol, including nitric oxide generation, potassium channel activation, and calcium channel modulation. However, its lower efficacy, reduced apparent potency, and dependence on suprapharmacological concentrations indicate that its vasorelaxant properties should be interpreted as mechanistic rather than translational. These findings refine the understanding of estrogen signaling diversity in the vasculature and provide a rigorous experimental foundation for future translational investigations.

Conclusion

Estriol induces vasorelaxation in isolated rat aortic rings via combined endothelium-dependent and endothelium-independent mechanisms involving nitric oxide signaling, potassium channel activation, and calcium influx inhibition. Compared with estradiol, estriol displays lower efficacy and reduced apparent potency under the present experimental conditions.

These findings provide mechanistic insight into estrogen-induced vascular relaxation but do not support direct clinical extrapolation. The use of male animals, the ex vivo experimental design, and the pharmacological concentrations employed represent essential limitations. Future studies using physiologically relevant concentrations, female or ovariectomized models, and in vivo approaches will be necessary to clarify the translational relevance of estriol in the context of vascular physiology and hormone therapy.

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References

Bhavnani BR, Stanczyk FZ 2014. Pharmacology of conjugated equine estrogens: efficacy, safety, and mechanism of action. *J Steroid Biochem Mol Biol* 142:16-29.

Brewer LD, Dowling AL, Curran-Rauhut MA, Landfield PW, Porter NM, Blalock EM 2009. Estradiol reverses a calcium-related biomarker of brain aging in female rats. *J Neurosci* 29(19):6058-6067.

de Oliveira TS, de Oliveira LM, de Oliveira LP, Costa RMD, Tostes RC, Georg RC, Costa EA, Lobato NS, Filgueira FP, Ghedini PC 2018. Activation of Pl3K/Akt pathway mediated by estrogen receptors accounts for estrone induced vascular activation of cGMP signaling. *Vascul Pharmacol* 110:42-48.

Filgueira FP, Lobato NS, dos Santos RA, Oliveira MA, Akamine EH, Tostes RC, Fortes ZB, Carvalho MH 2012. Endogenous testosterone increases leukocyte-endothelial cell interaction in spontaneously hypertensive rats. *Life Sci* 15;90(17-18):689-694.

Freay AD, Curtis SW, Korach KS, Rubanyi GM 1997. Mechanism of Vascular Smooth Muscle Relaxation by Estrogen in Depolarized Rat and Mouse Aorta : Role of Nuclear Estrogen Receptor and Ca2+ Uptake. *Circulation Research* 81(2): 242-248.

Gonzales RJ, Kanagy NL 1999. Endothelium-Independent Relaxation of Vascular Smooth Muscle by 17beta-Estradiol. *J Cardiovasc Pharmacol Ther* 4(4):227–234.

Haynes MP, Sinha D, Russell KS, Collinge M, Fulton D, Morales-Ruiz M, Sessa, WC, Jeffrey R 2000. Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells. *Circ Res* 87(8):677-682.

Hisamoto K, Ohmichi M, Kurachi H, Hayakawa J, Kanda Y, Nishio Y, Adachi K, Tasaka K, Miyoshi E, Fujiwara N, Taniguchi N, Murata Y 2001. Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. *J Biol Chem* 276(5):3459-3467.

Holtorf K 2009. The Bioidentical Hormone Debate: Are Bioidentical Hormones (Estradiol, Estriol, and Progesterone) Safer or More Efficacious than Commonly Used Synthetic Versions in Hormone Replacement Therapy? *Postgraduate Medicine* 121(1):73–85.

Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E 1998. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA* 280(7):605-613.

Khalil RA 2005. Sex Hormones as Potential Modulators of Vascular Function in Hypertension. *Hypertension* 46(2):249-254

Langer RD 2017. The evidence base for HRT: what can we believe? *Climacteric* 20(2):91–96.

Levin ER 2009. Plasma membrane estrogen receptors. *Trends Endocrinol Metab* 20(10):477-482.

Low, AK.; Russel, Lori DR; Holman, HE; Shepherd, JM.; Hicks, GS; Brown, CA. Hormone replacement therapy and coronary heart disease in women: a review of the evidence. American Journal of the Medical Sciences, v. 324, n. 4, p. 180–184, 2002.

Novensa L, Selent J, Pastor M, Sandberg K, Heras M, Dantas AP 2010. Equine Estrogens Impair Nitric Oxide Production and Endothelial Nitric Oxide Synthase Transcription in Human Endothelial Cells Compared With the Natural 17β-Estradiol. *Hypertension* 56(3):405-411.

Prossnitz ER, Barton M 2011. The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol* 7(12):715–726.

Reckelhoff JF, Fortepiani LA 2004. Novel mechanisms responsible for postmenopausal hypertension. *Hypertension* 43(5):918-923.

Rosenfeld CR, Cox BE, Roy T, Magness RR 1996. Nitric oxide contributes to estrogen-induced vasodilation of the ovine uterine circulation. *J Clin Invest* 98(9):2158–2166.

Rosenfeld CR, White RE, Roy T, Cox BE 2000. Calcium-activated potassium channels and nitric oxide coregulate estrogen-induced vasodilation. *Am J Physiol Heart Circ Physiol* 279(1):H319-H328.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA* 288(3):321-333.

Stanhewicz AE, Wenner MM, Stachenfeld NS. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am J Physiol Heart Circ Physiol*. 2018; 315 (6): H1569–H1588.

Vega-Vela NE, Osorio D, Avila-Rodriguez M, Gonzalez J, García-Segura LM, Echeverria V, Barreto GE 2017. L-Type Calcium Channels Modulation by Estradiol. *Mol Neurobiol* 54:4996–5007.