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A NOVEL ROLE FOR AN ENDOTHELIAL ADRENERGIC RECEPTOR SYSTEM IN MEDIATING CATECHOLESTRADIOL-INDUCED PROLIFERATION OF UTERINE ARTERY ENDOTHELIAL CELLS

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Abstract

Sequential conversion of estradiol-17β to its biologically active catecholestradiols 2-hydroxyestradiol (2-OHE_2) and 4-hydroxyestradiol (4-OHE_2) contributes importantly to its angiogenic effects on uterine artery endothelial cells derived from pregnant (P-UAECs), but not nonpregnant (NP-UAECs) ewes via estrogen receptor-independent mechanism. Because catecholestradiols and catecholamines exhibit structural similarities and have high affinity for α- and β-adrenergic receptors (ARs), we investigated if the endothelial α- or β-ARs mediate catecholestradiols-induced proliferation of P-UAECs and whether catecholamines alter these responses. Western analyses revealed expression of specific AR subtypes in NP-UAECs and P-UAECs including α_2-, β_2- and β_3-ARs; not α_1- and β_1-ARs. Levels of β_2-ARs and β_3-ARs were unaltered by pregnancy; whereas α_2-ARs were decreased. Norepinephrine and epinephrine increased P-UAEC, but not NP-UAEC proliferation and these effects were suppressed by propranolol (β-AR blocker) but not phentolamine (α-AR blocker). Catecholamines combinations with 2-OHE_2 or 4-OHE_2 enhanced P-UAEC mitogenesis. Catecholestradiol-induced P-UAECs proliferation was also inhibited by propranolol but not phentolamine. β_2-AR and β_3-AR antagonists (ICI 118,551and SR 59230A respectively) abrogated the mitogenic effects of both 2-OHE_2 and 4-OHE_2. Stimulation of β_2-ARs and β_3-ARs using Formoterol and BRL37344 dose-dependently stimulated P-UAEC proliferation which was abrogated by ICI 118,551and SR 59230A, respectively. Proliferation effects of both catecholamines and catecholestradiols were only observed in P-UAEC (not NP-UAEC) and were mediated via β_2-ARs and β_3-ARs. We demonstrate for the first time convergence of the endothelial AR and estrogenic systems in the regulating endothelial proliferation, thus providing a distinct evolutionary advantage for modulating uterine perfusion during stressful pregnancies.

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Keywords

catecholamines; catecholestradiols; adrenergic receptors; endothelial proliferation

Introduction

Estradiol-17β ($E_2β$) has physiologic/pathophysiologic effects on the cardiovascular system via diverse mechanisms including local conversion to catecholestradiols 2-hydroxyestradiol (2-OHE_2) and 4-hydroxyestradiol (4-OHE_2 by cytochrome P450s (CYP450s).[1,2] Catecholestradiols improve endothelial function and alleviate hypertension in ZSF1 rats; animal model for hypertension and metabolic syndrome.[2,3] We reported that $E_2β$, 2-OHE_2 and 4-OHE_2 increase proliferation in ovine uterine artery endothelial cells derived from pregnant (P-UAECs) but not the nonpregnant ewes (NP-UAECs).[4] Unlike $E_2β$, 2-OHE_2 and 4-OHE_2 induced P-UAEC proliferation were not blocked by ICI-182,780 demonstrating that catecholestradiols induce P-UAEC proliferation was estrogen receptor (ER)-independent.[4]

Catecholestradiols exhibit close structural similarities (Figure 1) and functional interactions with the norepinephrine and epinephrine.[5] Owing to the shared phenolic A ring "catechol" moiety, catecholestradiols interact directly with catecholamine responses, a property not shared by $E_2β$.[5] Catecholestradiols compete with high affinity for binding to neuroendocrine enzymes and α-adrenergic receptors (ARs) and β-ARs in hypothalamus, anterior pituitary, corpus striatum and liver [5,6,7,8,9,10]. 3D structural and functional analyses demonstrate that catecholamines "catechol" moiety is functionally important in AR activation.[11,12] Thus, it is conceivable that the ER-independent mitogenic effects of 2-OHE_2 and 4-OHE_2 on the uterine endothelium may be mediated by α-ARs or β-ARs and they may directly interact with the catecholamines which endogenously activate ARs. There are no reports on the potential role of an endothelial AR system in catecholestradiol-induced proliferation.

We hypothesized that catecholestradiols stimulate P-UAECs proliferation via α-ARs and/or β-ARs and that catecholamines which directly activate these ARs will alter these responses. We investigated: 1)NP-UAEC versus P-UAEC subtype specific expression of $α_1$-, $α_2$-, $β_1$-, $β_2$- and $β_3$-ARs; 2) whether norepinephrine and epinephrine stimulate NP-UAEC versus P-UAEC proliferation and the interactive effects of catecholamines and catecholestradiol in mitogenesis; 3) whether catecholestradiols and catecholamines stimulate P-UAEC proliferation via α-ARs or β-ARs; 5) if ARs exhibit subtype specificity in catecholestradiol-induced P-UAEC proliferation; and 6) subtype specific pharmacological activation of ARs on P-UAEC proliferation. We report for the first time specific $β_2$-AR and $β_3$-AR subtype-mediated mechanisms for the novel convergence of the endothelial AR system with estrogen metabolites in regulating P-UAEC not NP-UAEC proliferation. We discovered in P-UAECs specific a co-dependence via conserved catechol phenolic moieties (derivatives of both $E_2β$ and tyrosine) of a unique β-AR coupled system. This may provide for a distinct gestational evolutionary advantage by modulating uterine angiogenesis to help/maintain fetal developmental well-being during periods of repeated bouts of stress releases of catecholamines as seen during "fight or flight" responses.

Methods

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Cell Preparation and Culture

Protocols were approved by the University of Wisconsin-Madison Animal Care Committee.[4,13] NP-UAECs and P-UAECs were isolated and cultured from nonpregnant

(n=6) and late gestation (n=6) ewes.[4] At passage 4 (~70%) confluence, UAECs were lysed for Western Analyses or transferred to 96-well plates for experimental treatments.

Western Analyses

Western Analyses were performed [4] using rabbit anti-α_1-AR, anti-α_2-AR, anti-β_1-AR, anti-β_2-AR, or anti-β_3-AR antibodies (1:500) and secondary antibodies (1:2000). β-actin/GAPDH used as loading controls.

5-Bromodeoxyuridine Cell Proliferation Assays

5-Bromodeoxyuridine assay was performed and validated as previously described. [4]

Experimental Treatments

Proliferation experiments were performed in quadruplicates and replicated in ≥ four preparations. NP-UAECs and P-UAECs in 96-well plates were serum starved (24 hours) and washed in endothelial basal media (EBM) and medium was replaced with EBM containing 0, 0.1, 1, 10 or 100 nmol/L norepinephrine or epinephrine (24 hours). Additional P-UAEC studies investigating interactions were performed by combination treatments (0.1 nmol/L) of 2-OHE_2 or 4-OHE_2 with norepinephrine or epinephrine. α-ARs and/or β-ARs were blocked nonselectively by pretreating P-UAECs (10 μmol/L; 1 hour) with phentolamine (α-AR blocker) or propranolol (β-AR blocker) propranolol followed by norepinephrine, epinephrine or 2-OHE_2 or 4-OHE_2 (0.1 nmol/L; 24 hours). Based on Western analyses of specific AR subtypes, we conducted AR subtype specific blockade using (10 μmol/L; 1 hour) yohimbine (α_2-AR), ICI 118,551(β_2-AR) and SR 59230A (β_3-AR) followed by catecholestradiol treatments (0.1 nmol/L; 24 hours). We validated AR-subtype specific inhibition for P-UAEC mitogenic responses (0, 0.1, 1, 10, 100 nmol/L) using specific β_2-AR agonist Formoterol and β_3-AR agonist BRL 37344. ICI 118,551 and SR 59230A (1 μmol/L) effects respectively on Formoterol and BRL 37344 (100 nmol/L) for specificity validation of β_2-AR and β_3-AR agonists in P-UAECs. For antagonists/agonist specificities, please see http://hyper.ahajournals.org.

Statistical Analysis

Data as Means ± SEM are presented as a fold of untreated control. Overall group differences were determined by one-way or two-way ANOVA (SigmaPlot 11 Statistical Software) followed by post hoc multiple pairwise comparison Student-Newman-Keuls/Bonferroni tests. Level of significance was established a *priori* at $P<0.05$.

Results

P-UAECs Express Distinct Adrenergic Receptor Subtypes

Western immunoblotting showed α_2-AR, β_2-AR and β_3-AR not α_1-AR or β_1-AR subtypes in P-UAECs (Figure 2A). Positive controls show protein expressions of α_1-ARs, α_2-ARs, β_1-ARs, β_2-ARs and β_3-ARs in vascular smooth muscle cells (VSM), left ventricular cardiomyocytes (CMC) and adipose tissue (AT), thus validating the absence of α_1-ARs or β_1-ARs. Western analyses and densitometric analyses (data not shown) showed the equal expression of β_2-ARs and β_3-ARs between NP-UAECs and P-UAECs; however, α_2-ARs were higher in NP-UAECs versus P-UAECs.

Catecholamines Increase P-UAEC but not NP-UAEC Proliferation and Increase Catecholestradiol-Induced of P-UAECs Proliferation

Neither norepinephrine nor epinephrine stimulated in NP-UAECs proliferation (Figure 3A). Concentration-dependent norepinephrine responses were observed in P-UAEC (Figure 3B)

with maximum proliferation (2.08 ± 0.03 fold) observed at 100 nmol/L. At a low physiologic concentrations (0.1 nmol/L) norepinephrine significantly elevated proliferation (1.78 ± 0.028 fold). The highest P-UAEC proliferative responses to epinephrine were 1.89 ± 0.035 fold observed at 1 nmol/L; however, at 10 and 100 nmol/L, there was no further increase in proliferation to epinephrine and this was slightly less than that was observed with norepinephrine.

We then determined their interactive effects using combination treatments (0.1 nmol/L) of norepinephrine or epinephrine with 2-OHE_2 or 4-OHE_2 (Figure 3C). At this dose, the magnitude of P-UAEC proliferative responses to catecholamines were similar to catechoestradiols (1.86± 0.02-, vs. 1.81± 0.02-fold, respectively). Combination treatments with norepinephrine or epinephrine with either 2-OHE_2 or 4-OHE_2 further increased P-UAEC proliferation versus either catecholamine or catecholestradiol treatments alone ($P < 0.05$). Proliferative responses for combination treatments of norepinephrine or epinephrine with either 2-OHE_2 (2.22 ± 0.07- and 2.24±0.07-fold, respectively) or 4-OHE_2 (2.15±0.07- and 2.23±0.07-fold, respectively).

β-ARs but not α-ARs Mediate Catecholamine and Catecholestradiol-Induced P-UARC Proliferation

Phentolamine and propranolol antagonism (10μmol/L) of P-UAEC proliferation using both 0.1 (Figure 4A) and 100 nmol/L (not shown) doses of catecholamines yielded identical results. Neither antagonist alone altered basal P-UAEC proliferation. Increases in proliferation seen with norepinephrine or epinephrine were unaltered ($P >0.05$) by nonselective inhibition of α-ARs using phentolamine. In contrast, nonselective blockade of β-ARs using propranolol completely abrogated catecholamine mediated P-UAEC proliferation ($P < 0.05$).

Confirming our previous report,[4] the magnitude of proliferation of P-UAECs in responses to 2-OHE_2 (1.89±0.02-fold), and 4-OHE_2 (1.88±0.02-fold) were similar (Figure 4B). Increases in P-UAEC proliferation seen with 0.1 nmol/L 2-OHE_2 and 4-OHE_2 were unaltered ($P >0.05$) by nonselective inhibition of α-ARs using phentolamine. In contrast, nonselective blockade of β-ARs using propranolol completely abrogated catecholestradiol-mediated P-UAEC proliferation ($P < 0.05$). To determine the putative role of adrenergic G-protein coupled receptors in E_2β-induced P-UAEC proliferation, we evaluated these α-AR and β-AR antagonists on E_2β-induced proliferation of P-UAECs. The E_2β-induced rise (0.1 nmol/L) in P-UAEC proliferations was not altered ($P >0.05$) by either phentolamine or propranolol.

$β_2$-ARs and to a Lesser Extent $β_3$-ARs Mediate Catecholestradiol-Induced P-UAEC Proliferation

We then evaluated subtype specific α-AR and β-AR inhibition (10 μmol/L) on the P-UAEC proliferative responses to catecholestradiols. None of the inhibitors alone altered basal control P-UAEC proliferation (Figure 5A). Inhibition of $α_2$-AR subtype with Yohimbine did not inhibit 2-OHE_2 and 4-OHE_2-induced P-UAEC proliferation. In contrast, the selective antagonists of $β_2$-AR (ICI 118,551) or $β_3$-AR (SR 59230A) respectively either blocked ($P < 0.01$) or only partially attenuated ($P<0.05$) the proliferation induced by 0.1 nmol/L 2-OHE_2 and 4-OHE_2. .

We further evaluated additive effects of AR subtypes and putative AR heterodimerization in regulating catecholestradiol-mediated P-UAEC proliferation (Figure 5B). Combination of ICI 118,551 with either yohimbine or SR 59230A inhibited catecholestradiol-induced proliferation of P-UAECs demonstrating primary involvement of $β_2$-ARs. In contrast

combination of SR59230A and yohimbine only partially decreased catecholestradiol-induced P-UAEC proliferation demonstrating only partial β_3-AR subtype involvement. These combination inhibitor studies neither support dimerization nor significant cross talk between these AR subtypes.

Stimulation of β_2- and β_3-ARs Promote P-UAEC Proliferation

To further evaluate β_2-ARs vs. β_3-ARs, we tested the actions of specific β-AR agonists. Both of the subtype specific β_2-AR (Formoterol) and β_3-AR agonist (BRL 37344) produced concentration-dependent and similar P-UAEC proliferative responses (Figure 6A). Formoterol and BRL 37344 (100 nmol/L) produced maximal P-UAEC proliferations of 1.89 ± 0.07- and 1.90 ± 0.07-fold respectively. We then validated specificities of these agonists on their respective ARs (Figure 6B) P-UAEC proliferation by Formoterol was completely attenuated by β_2-AR (ICI 118,551) but not β_3-AR (SR 59230A) antagonist. β_3-AR antagonist completely abrogated responses by BRL 37344, not by Formoterol.

Discussion

Recently we reported that unlike their parent substrate hormone $E_2\beta$, 2-OHE_2 and 4-OHE_2 do not stimulate P-UAEC proliferation via classical ERs.[4] Herein we hypothesized that catecholestradiols mediate P-UAEC proliferation via either α-ARs or β-ARs and that the catecholamines will modify/interact with these proliferative effects. We describe the first report of a complete and coupled AR system in P-UAECs (not NP-UAECs) that are responsible for catecholestradiol- and catecholamine-mediated proliferation, a critical process for angiogenic-mediated uterine perfusion during gestation. These data provide a novel previously not described model by which estrogen metabolites function as potential circulating β-AR mimetic agonists. Therefore, modifying phenolic A ring of estrogens to a “catechol” moieties generate an endogenous β-mimetic agent with angiogenic and possibly other cardiovascular capabilities. Our key findings are that: 1) in NP-UAECs and P-UAECs there are distinct AR subtypes expressed including α_2-ARs, β_2-ARs and β_3-ARs but only in P-UAECs do norepinephrine and epinephrine increase proliferation; 2) catecholamines play a complementary and a conserved role to 2-OHE_2 and 4-OHE_2 by acting as positive modulators of P-UAEC proliferation; 3) neither catecholestradiols nor catecholamines induce P-UAEC proliferation α-ARs, but rather solely via β-ARs; 4) 2-OHE_2 and 4-OHE_2 modulate P-UAEC proliferation primarily via β_2-ARs and to a lesser-extent via β_3-ARs; and 5) pharmacologic agonists for either β_2-ARs or β_3-ARs specifically stimulate P-UAEC proliferation suggesting similar coupling mechanisms and/or signaling convergence.

We report, for the first time, *in vitro* expressions of several specific AR subtypes α_2-ARs, β_2-ARs, and β_3-ARs in NP-UAECs and P-UAECs, findings consistent with reports demonstrating distinct individual AR subtypes in endothelia of aorta, choroid, placenta, femoral artery and retina.[14,15,16,17,18,19] When compared to NP-UAECs, β_2-AR and β_3-AR expressions were unaltered by pregnancy status, whereas α_2-ARs were reduced. It is unclear whether co-expression of different specific ARs within the same endothelial cells represents unappreciated signaling complexity or just simply a functional redundancy. Using high throughput proteomic analyses of P-UAECs, we observed that β_2-ARs are abundantly localized in the P-UAEC caveolae domain, a “hub” for compartmentalizing signal transduction for regulation of multiple functions (Ramadoss and Magness, unpublished data, [2011]). Therefore, demonstration of specific AR expression relative to the subcellular localization of α_2, β_2 and β_3-ARs in NP-UAECs versus P-UAECs needs to be determined. This may fulfill distinct physiologic and pathophysiologic functional significance for expression relative to localization of multiple AR subtypes in endothelium. .

Since ARs are present on the endothelium, they are undoubtedly exposed to circulating endogenous norepinephrine and epinephrine released from the adrenal medulla. Normal physiologic circulating catecholamine concentrations are 1-2 nmol/L [20,21,22] and increase dramatically in pathologic cardiovascular conditions and during "fight or flight" stress responses. Hence, we demonstrated that even at a low physiologic concentration (0.1 nmol/L) of both norepinephrine and epinephrine significantly increases P-UAEC, not NP-UAEC, proliferation suggesting that catecholamines indeed may play roles in regulating physiologic angiogenesis during gestation. Consistent with these finding, catecholamines augment *in vivo* angiogenesis in dopamine β-hydroxylase knockout mice deficient in plasma catecholamines.[23] Confirming our recent report, a low physiologic concentration (0.1 nmol/L) of 2-OHE_2 and 4-OHE_2 stimulate P-UAEC proliferation.[4] We report herein for the first time that catecholamine and catecholestradiol combinations induced significantly higher P-UAEC proliferation. We further demonstrate for the first time that both catecholamines and catechoestradiols individually elevate P-UAEC proliferation only via β-ARs suggesting that functional β-ARs are likely important for regulating physiologic and/or pathologic angiogenesis during gestation. These data therefore demonstrate that catecholamines play a complementary and even an additive role to 2-OHE_2 and 4-OHE_2 as positive β-AR-mediated modulators of physiologic angiogenesis. These data also implied that catecholamines and catechoestradiols should exhibit similar AR-subtype-specific signaling pathways to induce P-UAEC proliferation. Catecholestradiols have been previously shown to competitively bind to AR subtypes in rat cerebral cortex, striatum, and anterior pituitary as well as to guinea-pig hypothalamic membranes.[9,10] Therefore, our data show that "catechol" moieties of catecholestradiols and catecholamines are very important for the binding and activation of β-ARs signaling.

The lack of alteration of P-UAEC proliferation when the nonspecific α-AR antagonist phentolamine and α_2-AR specific blocker yohimbine was used show that α_2-ARs that were reduced by pregnancy do not play a role in catecholestradiol-induced angiogenesis in P-UAECs. There are no reports showing a role of α_2-ARs regulating endothelial cell proliferation. However, α_2-ARs have been closely associated with nitric oxide signaling in endothelial cells [24], suggesting functional relevance of α_2-AR expression in endothelial-mediated vasodilatation.

Consistent with our novel findings that propranolol abrogated 2-OHE_2 and 4-OHE_2–induce P-UAEC proliferation, are reports showing that stimulation of β-ARs by various pharmacokinetic compounds stimulate proliferation of endothelial cells.[15,19,25] Classically, β-ARs are prototypical G-protein coupled receptors triggering diverse signaling cascades through α, β and γ G-protein subunits, adenylate cyclase, intracellular cAMP and protein kinase A and C.[26] However, new layers of complexity in signaling suggest that β-AR activation can induce a myriad of cellular responses via p38 and p42/44 mitogen-activated protein kinases independent of adenylate cyclase, cAMP and protein kinase A and C. [27,28,29,30] Therefore signal transduction studies are needed to further elucidate the potential differences in β-AR-mediated molecular mechanism of action of the catecholestradiol versus catecholamines in endothelial cells.

The current observation that subtype specific β_2-AR antagonist ICI 118,551 abolished P-UAEC proliferation stimulated by 2-OHE_2 and 4-OHE_2 suggests β_2-AR coupling whereas the partial inhibition by β_3-AR blocker SR 59230A also implies potential involvement of β_3-ARs. In contrast, both the specific β_2-AR (Formoterol) and β_3-AR (BRL 37344) agonists equally induced P-UAEC proliferation which were specifically blocked by their specific antagonists (Figure 6), suggesting that both β-ARs may regulate these proliferative effects. Thus, the partial inhibitory effects of SR 59230A on catecholestradiol responses (Figure 5A) do not point to a lack of potency or effective concentration since a similar concentration of

SR 59230A induced significant abrogation of β_3-ARs in response to BRL 37344. Activation of either β_2-AR and/or β_3-AR have been shown to play a role in endothelial cell proliferation from human umbilical vein, retina and bovine aorta.[14,15,16,18]. However, ours is the first report to demonstrate that β_2-AR and β_3-AR mediate the catecholestradiol-induced proliferation of endothelial cells. P-UAECs express similar levels of β_2-ARs and β_3-ARs compared to NP-UAECs, demonstrating that the AR-mediated effects are not dependent on expression levels, but rather on other gestational-programming factors at the level of signaling. These data therefore provide a broader understanding of the mechanism of action of catecholestradiols in endothelial cell proliferation. Importantly, these results also point to the potential relevance of previously unappreciated complexities of estrogen signaling in the cardiovascular system via interactions of steroid metabolites and endothelial AR system.

Overall, the present study indicates that actions of catecholestradiols and catecholamines via endothelial ARs represent an evolutionary conserved and highly versatile signaling mechanism for regulating endothelial proliferation. During gestation angiogenic processes are to a great extent responsible for the dramatic 30-50 fold-rises in uterine blood flow, such that by term the uterine vascular bed receives nearly 20% of the also greatly expanded cardiac output and blood volume. [31,32] Furthermore, maternal uterine perfusion is maintained 1-2 fold in excess of the needs of the parallel, but separate, fetoplacental circulation.[33] We previously suggested that during an acute gestational “flight or fight” response when catecholamines are greatly elevated -far in excess of the efficacious levels utilized herein- cardiac output redistributes away from the uterine vascular bed (α-AR-mediated) to the muscles and other tissues (β-AR-mediate) for survival of the mother and her fetus, thus providing a distinct short term survival advantage for placental mammals.[34,35]

Perspectives

The current study sheds new light on the existence of a previously unrecognized two ligand system for a single AR family representing a mechanism by which the same physiological regulators of the “flight or fight” responses that protect the mother during a state of acute but repeated physiologic stress will indeed act as an angiogenic switch to subsequently induce maintenance in uterine relative to fetoplacental blood flows. This provides for a marked evolutionary advantage of maintaining delivery of oxygen and nutrients through the uteroplacental circulation thus protecting the growing fetus from subsequent stress-induced profound reductions in uterine blood flow. These data also uncover novel complexities of estrogen signaling in the cardiovascular system via ARs and necessitates the further investigation of estrogen metabolites such as catecholestradiols in the vascular system which do not signal via the classical estrogen receptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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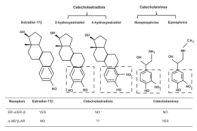

Figure 1.

Adding one OH group modifies the estrogenic phenolic A ring forming “catechol” moieties potentially making it an “AR-mimetic” agent for inducing ER-independent angiogenesis. The boxes outline shared phenolic A ring “catechol” moiety between 2-hydroxyestradiol, 4-hydroxyestradiol, norepinephrine and epinephrine. ?? indicates hypotheses tested.

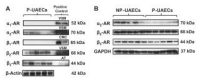

Figure 2. AR subtypes in UAECs

(A) Western blots demonstrating expression of α_2-ARs, β_2-ARs and β_3-ARs; but not α_1-ARs or β_1-ARs subtypes in P-UAECs. Positive control lanes are vascular smooth muscle cells (VSM), left ventricle cardiomyocytes (CMC) and adipose tissue (AT). **(B)** Expression α_2-ARs, β_2-ARs and β_3-ARs in **NP-UAECs versus P-UAECs**. Blots are representative of two independent experiments from individual UAEC cell lines.

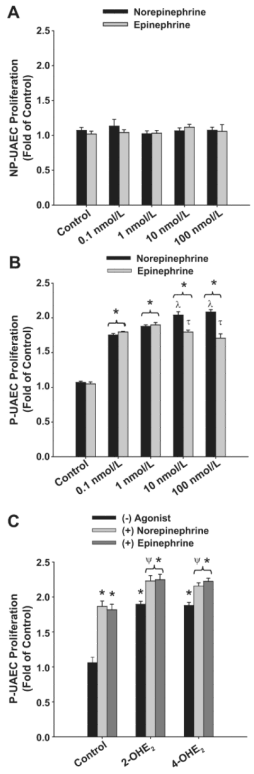

Figure 3. Catecholamines stimulate of P-UAECs but not NP-UAECs Proliferation and Augement Catecholestradiol-induced P-UAEC Proliferation

(A) Concentration response of NP-UAECs to 0, 0.1, 1, 10 and 100 nmol/L norepinephrine and epinephrine (two-way ANOVA; concentration x group; $F_{8,45} = 0.306$, $P = 0.960$; $n = 6$). **(B)** Concentration response of P-UAECs to 0, 0.1, 1, 10 and 100 nmol/L norepinephrine and epinephrine (two-way ANOVA; concentration x group; $F_{8,45} = 10.52$, $P < 0.001$; $n = 6$). **(C)** Combinations of 0.1 nmol/L norepinephrine or epinephrine with either 2-OHE_2 or 4-OHE_2 augmented P-UAEC proliferation responses (two-way ANOVA; group x agonist; $F_{4,27} = 3.73$, $P = 0.015$; $n = 4$) *Increase vs. control. λ Increase vs. 0.1 and 1 nmol/L. τ norepinephrine > epinephrine. ψ Increase vs. catecholestradiols or catecholamines alone

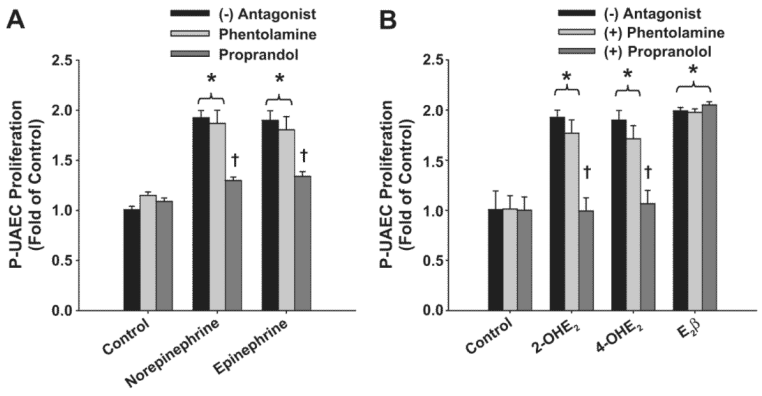

Figure 4. β-ARs, but not α-ARs, mediate Catecholamine and Catecholestradiol-induced P-UAEC Proliferation

(A) Effects of phentolamine or propranolol on P-UAECs proliferation to 0.1 nmol/L norepinephrine or epinephrine. Phentolamine had no effect; whereas propranolol completely abrogated catecholamine-induced P-UAEC proliferation (two-way ANOVA; antagonist x group; $F_{8,45} = 9.12$, $P < 0.001$; $n = 4$). **(B)** Effects of phentolamine or propranolol on P-UAECs proliferative responses to 2-OHE_2, 4-OHE_2 or $E_2\beta$ (0.1 nmol/L). Phentolamine had no effect; whereas propranolol completely abrogated 2-OHE_2- and 4-OHE_2-, but not $E_2\beta$-induced P-UAECs proliferative responses (two-way ANOVA; antagonist x group; $F_{6,36} = 7.88$, $P < 0.001$; $n = 4$). *Increase vs. untreated. † Complete inhibition.

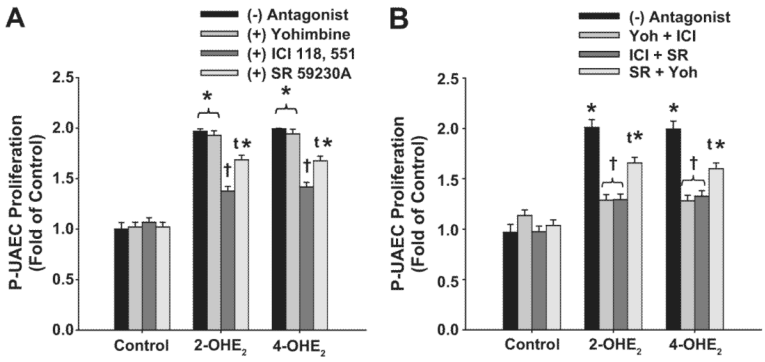

Figure 5. β_2-ARs and to a Lesser Extent β_3-ARs Mediate Catecholestradiol-induced P-UAEC Proliferation

(A) Effects of yohimbine, ICI 118,551 or SR59230A on P-UAEC proliferation to 2-OHE_2 or 4-OHE_2 (0.1 nmol/L). Yohimbine had no effect; whereas ICI 118,551 attenuated and SR59230A partially inhibited catecholestradiol-mediated P-UAEC proliferation (two-way ANOVA; antagonist x group; $F_{8,33}$= 7.871, $P < 0.001$; n = 4). **(B)** Effects of yohimbine, ICI 118,551, and SR59230A combinations on P-UAEC proliferative responses to catecholestradiols. ICI 118,551 in all combinations completely blocked P-UAEC proliferation responses to catecholestradiols (two-way ANOVA; antagonist combination x group; $F_{8,33} = 9.551$, $P < 0.001$; n = 4). *Increase vs. untreated. † Complete inhibition. τ Partial inhibition.

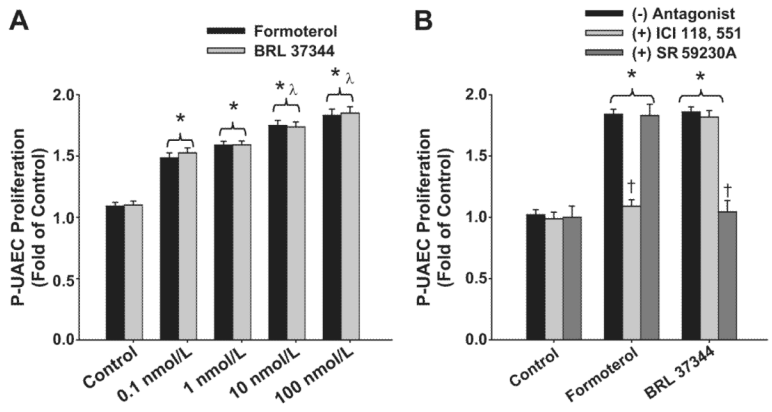

Figure 6. Stimulation of β_2- and β_3-ARs promotes P-UAEC proliferation

(**A**) Concentration response of P-UAECs to 0, 0.1, 1, 10 and 100 nmol/L of β_2- and β_3-AR agonists Formoterol and BRL 37344 (two-way ANOVA; concentration x group; $F_{4,30}$ = 3.01, $P < 0.001$; n = 6). (**B**) Effects of ICI 118, 551 or SR59230A on P-UAEC proliferative responses to Formoterol and BRL 37344 (two-way ANOVA; antagonist x group; $F_{8,45}$ = 20.53, $P < 0.001$; n = 6). *Increase vs. untreated. λ Increase vs. 0.1 and 1 nmol/L. † Complete inhibition.