Extent of Vascular Remodeling Is Dependent on the Balance Between Estrogen Receptor α and G-Protein–Coupled Estrogen Receptor

Robert Gros, Yasin Hussain, Jozef Chorazyczewski, J. Geoffrey Pickering, Qingming Ding, Ross D. Feldman

Abstract—Estrogens are important regulators of cardiovascular function. Some of estrogen’s cardiovascular effects are mediated by a G-protein–coupled receptor mechanism, namely, G-protein–coupled estrogen receptor (GPER). Estradiol-mediated regulation of vascular cell programmed cell death reflects the balance of the opposing actions of GPER versus estrogen receptor α (ERα). However, the significance of these opposing actions on the regulation of vascular smooth muscle cell proliferation or migration in vitro is unclear, and the significance in vivo is unknown. To determine the effects of GPER activation in vitro, we studied rat aortic vascular smooth muscle cells maintained in primary culture. GPER was reintroduced using adenoviral gene transfer. Both estradiol and G1, a GPER agonist, inhibited both proliferation and cell migration effects that were blocked by the GPER antagonist, G15. To determine the importance of the GPER-ERα balance in regulating vascular remodeling in a rat model of carotid ligation, we studied the effects of upregulation of GPER expression versus downregulation of ERα. Reintroduction of GPER significantly attenuated the extent of medial hypertrophy and attenuated the extent of CD45 labeling. Downregulation of ERα expression comparably attenuated the extent of medial hypertrophy and inflammation after carotid ligation. These studies demonstrate that the balance between GPER and ERα regulates vascular remodeling. Receptor-specific modulation of estrogen’s effects may be an important new approach in modifying vascular remodeling in both acute settings like vascular injury and perhaps in longer term regulation like in hypertension. (Hypertension. 2016;68:1225-1235. DOI: 10.1161/HYPERTENSIONAHA.116.07859.)

Key Words: apoptosis ■ estradiol ■ estrogen ■ inflammation

Estrogens are important physiological and pathophysiological regulators of cardiovascular function.1 Beneficial cardiovascular effects have long been ascribed to estrogens—in part based on the lower risk of cardiovascular events seen in premenopausal women versus age-matched men.2 Thus, estrogen replacement therapy for postmenopausal women was expected to have beneficial cardiovascular effects. Counterintuitively, in randomized clinical trials, postmenopausal estrogen treatment increased cardiovascular events,3,4 suggesting unanticipated detrimental effects of estrogens. Furthermore, in the setting of vascular injury, potential detrimental effects of estrogens have been inferred. This inference has been primarily based on women’s increased risk for vascular restenosis after stent placement5-8—especially younger women9 and especially with the earlier bare metal stents.7 Thus, estrogens might mediate both beneficial and detrimental vascular effects.

Work from our laboratory and those of others have suggested that the balance between the beneficial and detrimental vascular effects of estrogens may be based on the activation of distinct receptors with opposing mechanisms of action. Estrogens like estradiol (E2) were first thought to act only via the estrogen receptors (ER) functioning exclusively as transcriptional regulators. However, it is now known that some estrogen actions occur more rapidly and via a more recently identified G-protein–coupled receptor,8-10 G-protein–coupled estrogen receptor (GPER, aka GPR30).11 GPER has been suggested to be important in rapid, nonclassic, estrogen-induced growth regulation in some tissues.15-19 GPER’s growth-regulating effects have been extensively studied,13,18,20-26 primarily in reproductive tissues and in cancer cells. Notably, GPER activation has also been shown to attenuate adverse cardiac ventricular remodeling in mRen2 Lewis rats.27

The role of GPER in mediating the growth-regulating effects of estrogen in the vasculature remains unclear. In other tissues, both pro- and antiapoptotic effects of estrogens have been reported28 as have pro- and antiproliferative effects.29 Studies from our laboratory have highlighted the opposing effects on apoptosis of GPER versus ERα in vascular cells.
Our initial focus on ERα (versus ERβ, which is also expressed in rat aortic vascular smooth muscle cells [VSMCs]) was based on our demonstration that in isolated rat VSMCs, estradiol’s (E2) antiapoptotic effects were mediated via ERα.30 In contrast, E2 acting via GPER was proapoptotic. However, the in vivo implications of these receptor-specific opposing effects of estradiol on growth regulation have yet to be established.

In these studies, we examined the process of vascular remodeling to understand how E2 regulates vascular growth via GPER versus ERα. Remodeling occurs subsequent to several vascular stressors including hypertension,11 atherosclerosis,32 and vascular injury33 and can be associated with both adaptive responses (as in vascular injury) and maladaptive responses (as in hypertension and in vascular restenosis). The VSMC hypertrophy and hyperplasia that occurs in remodeling processes has been linked to a shift in the balance between proliferative and apoptotic mechanisms and a dedifferentiation of VSMCs from a contractile phenotype, as seen under normal conditions in situ, to a so-called synthetic phenotype,34 as seen after vascular injury. This latter phenotype is characterized as having higher proliferative rates and greater migratory behavior. This synthetic phenotype is more similar to the phenotype of VSMCs maintained in primary culture,35 the model in which we have initially characterized the role of GPER versus ERα in regulating growth.30

Previous studies have suggested an important role of estrogen in the response to vascular injury36 including inhibition of neointimal proliferation22,38 and inhibition of the adventitial inflammatory response.29,40 It has been suggested that the primary estrogen-mediated response occurred via ERα activation.38 However, this conclusion was mostly based on a lack of appreciation of the existence of GPER or inferred from the effects of drugs like the estrogen antagonistICI 182, 170, which was subsequently shown to also act as GPER ligand.41

The importance in the balance between GPER and ERα expression in altering the remodeling response in vivo is unknown. On the basis of these uncertainties, we sought to determine the functional impact of GPER expression on vascular smooth muscle growth regulation in vivo. Data to be presented demonstrate that the extent of vascular remodeling with carotid ligation is dependent on the balance between GPER versus ERα expression.

Methods

Vascular Smooth Muscle Cell Culture

Rat aortic VSMCs were isolated from male rats as described previously and cultured in DMEM with 10% fetal bovine serum (Sigma-Aldrich, Oakville, Ontario, Canada) supplemented with gentamicin and amphotericin B (Invitrogen, Carlsbad, CA).37 Rats used in our studies as a source of aortic smooth muscle cells were maintained at Western University, London, Ontario, Canada, and experiments were performed following the guidelines and protocols approved by the University Council on Animal Care for Animal Research. Notably, these cells have abundant expression of GPER when freshly isolated, which is quickly downregulated when maintained in culture and with the shift of these cells from a contractile to a synthetic phenotype.42 Thus, we previously have used these cultured rat aortic VSMCs as a null background in which we could modulate relative GPER expression with adenoviral mediated gene transfer.30

Construction of Adenoviral Plasmid Constructs

Adenoviral constructs for GPER, green fluorescent protein (GFP), short hairpin (sh) ERα, shGPER, and shGFP were generated with AdMaxTM adenovirus vector creation kit (Microbix Biosystems Inc, Toronto, Ontario, Canada) as we have previously described.30,43,44 Rat VSMCs were infected with adenoviral constructs for 24 hours at 37 °C after which infection media were replaced with fresh phenol-free media (Sigma-Aldrich), as phenol red has been reported to have estrogenic activity.43 Cells were used for experimentation 48 hours post infection with serum deprivation for the last 24 hours.

Assessment of Regulation of DNA Synthesis as an Index of Proliferation by 5-Ethynyl-2′-Deoxyuridine Labeling

After 24 hours of serum starvation in phenol-free DMEM, cells were treated with the GPER agonist, G149 (Calbiochem-Novabiochem, San Diego, CA), estradiol (Sigma-Aldrich), or vehicle (control) for 2 hours, after which cells were restimulated with 10% fetal bovine serum for 18 hours and then incubated with 5-ethyl-2′-deoxyuridine (EdU, 10 μmol/L) for 2 hours. EdU incorporation was assessed by the Click-iT EdU flow cytometry assay kit (Lifetech, Carlsbad, CA). To assess the impact of the GPER antagonist, G1547 (Calbiochem-Novabiochem), on E2 or G1 effects on proliferation, cells were treated with G15 (1 μmol/L) for 15 minutes before addition of these agonists.

Assessment of Regulation of Migration

After serum starvation for 24 hours in phenol-free DMEM, cells were then treated with either vehicle or increasing concentrations of E2 (0.1–100 nmol/L) or G1 (0.01–10 μmol/L) or, in wild-type cells, angiotensin II (100 μmol/L) as a positive control. To assess the effect of G15 on migration, cells were treated for 24 hours with E2 (10 nmol/L) or G1 (1 μmol/L) in the absence or presence of G15 (1 μmol/L).

Cells were collected 24 hours after treatment. Cell migration was assessed using a Boyden Chamber assay as previously described.46 Briefly, the upper and lower wells of the Boyden Chambers (Neuro Probe, Gaithersburg, MD) were separated by a porous (10 mm pore size) polycarbonate membrane (Neuro Probe, Gaithersburg, MD) pre-coated with 100 μg/mL human fibronectin (Sigma-Aldrich). Twenty-five thousand cells in 50 μL of M199 medium containing 1% bovine serum albumin (Sigma-Aldrich) were added to the upper well of the chambers, whereas the lower well was filled with M199 medium containing 1% bovine serum albumin. Cells were incubated for 4 hours at 37 °C in 5% CO2. After those cells remaining on the upper surface of the membrane were mechanically removed, the membranes were then fixed in methanol for 5 minutes, followed by staining for 5 minutes with Harris hematoxylin (Leica Microsystems Inc, Concord, Ontario, Canada). The number of cells that had migrated to the lower surface was then counted microscopically at high power (20x objective). All experimental conditions were studied in triplicate.

Rat Carotid Artery Ligation

Carotid artery ligations were performed as we have previously described for mice—with minor modifications.99 The major differences between our model and the balloon injury or wire-induced injury techniques more typically used in rat models50 relate to the absence of inducing any other trauma to the vessel beyond ligation and the instillation of distilled water at the time of ligation (to mediate endothelial cell disruption). This approach still results in robust neointimal proliferation and adventitial inflammatory responses and allows penetration of our adenoviral constructs past the endothelial barrier. However, this less traumatic approach results in a lesser extent of intimal hemorrhage—at least compared with our findings from initial studies using a wire-induced injury model.

Wistar rats (male and female, 3 months of age; Charles River Laboratories) were used in our studies. They were cared for in accordance with the Canadian Council on Animal Care guidelines. The protocol for their use was approved by the Animal Use
Subcommittee, Western University. The rats were anaesthetized with ketamine (10 mg/kg) and xylazine (10 mg/kg), and the common carotid artery was isolated and looped proximally with a 6-0 suture for temporary vascular control and ligated distally with a 6-0 suture at the carotid artery bifurcation. A 22-gauge needle was introduced into the common carotid artery just proximal to the bifurcation, and distilled water was infused to disrupt the endothelium and enhance adeno viral mediated gene transfer. Ten minutes after, the needle was used to flush the carotid artery twice with PBS and subsequently to deliver a mixture of adenovirus in pluronic gel (Life Technologies Inc, cat#6866; Burlington, Ontario, Canada) into the lumen of the carotid. The mixture was allowed to incubate for 20 minutes in the absence of blood flow. The needle was then withdrawn, the common carotid was ligated at the bifurcation, the proximal suture was removed to restore blood flow, and the wound was closed in layers. Rats were euthanized 4 days after surgeries for assessment of GPER and ERα receptor expression and 7 days after the surgeries for morphological and immunohistochemical assessments.

**Western Blot Analysis of GPER Expression**

Injured and contralateral uninjured (as the control) carotid arteries were homogenized in lysis buffer containing 20 mmol/L Tris, pH 8.0, 1% Nonidet P-40, 0.1% SDS, 140 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, and proteinase inhibitor cocktail (Roche Diagnosis, cat#12604600; Indianapolis, IN) and clarified with centrifugation. Protein concentration was determined by Pierce BCA protein assay kit (ThermoFisher Scientific, Burlington, Ontario, Canada). Forty to sixty micrograms of lysates were then resolved on 10% SDS-PAGE. The assessment of the extent of GPER expression in injured versus uninjured vessels at 7 days after the surgery and the validation of expression of flag-tagged adeno viral constructions at 4 days after the surgery were based on immunoblotting using an anti-GPER antibody (MBL, cat#LS-A4272, lot#8970; Woburn, MA) and an antiflag M2 antibody (Sigma-Aldrich, cat#F7425) respectively, as we have previously described. Secondary antibodies were obtained from Sigma-Aldrich (cat#A0545).

**Assessment of GPER and ERα mRNA Expression**

Carotid arteries were isolated as described above. Isolation of total RNA was performed using the one-step RNA isolation reagent TRIzol (Invitrogen, Burlington, ON, Canada). One microgram of RNA was used for analysis of GPER, ERα, or glyceraldehyde 3-phosphate dehydrogenase (as a control) mRNA expression using SuperScript one-step reverse transcription–PCR kits (Invitrogen) with primers specific to GPER, ERα, or glyceraldehyde 3-phosphate dehydrogenase (for GPER: forward: 5′-GCAGGCGTCTTCTCTCTCACC-3′; reverse: 5′-ACAGCCTGAGCTTGTCCTCCGTG-3′; for ERα: forward: 5′-TCTCAATTGGCCTTGGACAGG-3′; reverse: 5′-ATCTTTGCCAGACTCCGGT-3′; for glyceraldehyde 3-phosphate dehydrogenase: forward: 5′-TGAACGGGAGCATCACGTG-3′, reverse: 5′-TCCACCCAGGTTGCTGTA-3′).

**Morphometric Analysis**

Morphometry measurements were performed as previously described. Briefly, at 7 days after injury, piperazine-2,4-bis [2-ethanesulfonic acid] (PIPES)–buffered (0.1 mol/L PIPES [pH 6.9], 2 mmol/L MgCl2) 4% paraformaldehyde was used to fix the carotid artery. Injured and control (the contralateral uninjured) carotid arteries were harvested and imbedded in paraffin.
Sections were cut from the proximal, middle, and distal thirds of the injured region of the common carotid artery. Adjacent sections were subjected to standard hematoxylin and eosin staining (Leica AutoStainer XL; Leica Microsystems Inc). Mean medial thickness was assessed from 5 measurements (at 0, 60, 120, 180, and 240 degrees) taken from each of 6 sections cut from each vessel by an observer blinded to treatment condition. Image analysis measurements were calibrated with a stage micrometer to obtain absolute dimensions. For each animal, medial thickness in the ligated vessel is expressed as a percentage of the medial thickness of the contralateral uninjured vessel.

Assessment of Cell Proliferation by Extent of Ki67 Labeling

To assess cell growth in carotid tissues, we assessed the extent of Ki67 staining. After the deparaffinization and antigen retrieval, the sections of tissues were blocked in 5% goat serum in PBS for 30 minutes at room temperature and then incubated with the Ki67 antibody (Abcam, cat#AB66155; Toronto, Ontario, Canada) in PBS at 1:100 for overnight at 4 °C. After 3 washes with PBS, the tissues were incubated with secondary biotinylated goat antirabbit IgG (Vector Laboratories, cat#BA-1000; Burlington, Ontario, Canada) at a dilution of 1:200 in PBS for 30 minutes at room temperature and visualized with ABC kits and DAB (Vector Laboratories, Inc), followed by hematoxylin counterstaining. Density of labeling was expressed as a percentage of the density of labeling in sections from the paired uninjured vessel.

Assessment of the Inflammatory Response by Extent of Leukocyte Infiltration as Assessed by CD45 Staining

The inflammatory reaction in the vessel in response to carotid ligation was determined by assessing the extent of CD45 staining (Abcam, cat#AB10558) using the same procedures as those described for the assessment of cell proliferation by the extent of Ki67 labeling.

Statistical Analyses

The significance of difference between paired groups was determined by Student test for paired data. P<0.05 on a 2-sided test was taken as a minimum level of significance. All statistical analyses were performed using Prism 3 software (GraphPad Software, San Diego, CA).

Other Materials

The antibody against Tubulin was obtained from Abcam, Inc (cat#AB4074). All other chemical reagents were obtained from Sigma-Aldrich Canada, Ltd. All steroid ligands were dissolved in dimethyl sulfoxide with a working dilution in assays of 1:1000 (dimethyl sulfoxide: H2O). Dimethyl sulfoxide at a 1:1000 dilution was also included in all control conditions tested.

Results

GPER-Mediated Inhibition of Vascular Smooth Muscle Cell Proliferation

In initial studies, we assessed the effects of GPER agonists G1 and estradiol (E2) on proliferation of VSMCs
Figure 3. Impact of carotid injury on medial thickness, Ki67 labeling, extent of CD45 staining and G-protein–coupled estrogen receptor (GPER) expression. A, The effect of carotid ligation on the development of medial thickening (the area between the arrows), depicted in the right, vs a section from an uninjured contralateral carotid artery (left), 7 d post injury. B, Carotid ligation increased the proliferation in the medial layer as assessed by Ki67 staining (representative positively stained cell depicted by arrow), shown in the right, vs a section (Continued)
maintained in primary culture. Under baseline conditions, neither of these GPER agonists had significant effects on EdU incorporation (Figure 1A). However, with recovery of VSMC GPER expression, G1 treatment inhibited EdU incorporation with a maximal effect of 34±7% of control at 10 µmol/L (n=3; Figure 1B). Similarly, E2 inhibited proliferation with a maximum effect at 100 nmol/L (67±11% of control [n=4]; Figure 1B). Pretreatment with G15 abolished the inhibitory effects of G1 and estrogen (n=3; Figure 1C).

**GPER-Mediated Inhibition of Vascular Smooth Muscle Migration**

In wild-type cells, neither estradiol (1–10 nmol/L) nor G1 (1 µmol/L) mediated any significant alterations in the extent of vascular smooth muscle cell migration (n=3; Figure 2A), although angiotensin II (100 µmol/L) mediated a 49±5% increase in cell migration. In contrast, with VSMC GPER recovery, both estradiol and G1 inhibited migration (Figure 2B). These effects were attenuated by coinubcation with G15 (1 µmol/L; Figure 2C).

**Carotid Injury Leads to an Increase in Medial Thickness**

One week after carotid ligation, carotid medial thickness was increased by 51±8% compared with the medial thickness in the uninjured contralateral artery in female rats (n=3). This was paralleled by a 75±4% increase in vascular smooth muscle cell density (Figure 3A) and by neointimal proliferation (Figure 3B). There was also evidence of an inflammatory response both morphologically and based on the infiltration of CD45-positive cells into neointimal and adventitial layers (Figure 3C).

**Carotid Injury Leads to an Attenuation of GPER Expression**

We have previously demonstrated that GPER expression is downregulated in the setting where, after primary culture, VSMCs undergo a phenotypic switch from a contractile to a synthetic cell type. This transition is analogous to what occurs after vascular injury.51–53 Thus, we considered whether GPER expression would be similarly downregulated in vivo after vascular injury and the consequent shift of VSMCs to the synthetic phenotype. On SDSPAGE, in carotid artery lysates, GPER was resolved in 2 bands, namely, a major band with a corresponding molecular weight of ≈50 kDa—as previously described in other tissues—54 and a minor band of GPER (≈43 kDa) as reported by other investigators.55,56 As we have seen in vitro, with VSMC primary culture, after carotid ligation or vascular injury, there was a detectable downregulation of GPER content both with regard to protein content (to 15±6% of control; n=3; Figure 3D) and mRNA content (to 28±3% of control; n=3; Figure 3E).

**Increasing GPER Expression Attenuates Medial Hypertrophy After Carotid Ligation**

To assess the role of GPER in injury-mediated VSMC hypertrophy, GPER was reintroduced via adenGPER gene transfer (adenGFP was used as the control condition). Abluminal adenoviral exposure (using our adenGFP construct) had no significant effect on the extent of medial hypertrophy after carotid ligation (AdenoGFP: to 153±4%, n=11 females; vehicle treated: to 151±8%, n=3 females).

Infection with the adenGPER construct was associated with upregulation of GPER mRNA content (Figure 4A) and of GPER protein content (Figure 4B). Recovery of GPER expression in ligated vessels was associated with a significant

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**Figure 3 Continued.** From the uninjured contralateral carotid artery (left). C, Carotid artery ligation enhanced the extent of CD45 labeling (representative positively stained cells depicted by arrows) seen both in the intima and in the adventitia of injured carotid arteries (right) vs a section from the uninjured contralateral carotid artery (left). D, Carotid injury led to downregulation of endogenous GPER expression (to 15±6% of control). α-Tubulin expression was used as a loading control. Representative immunoblot from 3 independent experiments performed under identical conditions.

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**Figure 4.** Increasing G-protein-coupled estrogen receptor (GPER) expression attenuates medial hypertrophy after carotid ligation in sex-independent manner. Introduction of adenoshGPER led to an increase in the expression of GPER as demonstrated by (A) reverse transcription–PCR and (B) immunoblotting. C, The upregulation of GPER expression mediated a significant attenuation in medial hypertrophy. Data represent the means±SE of 10 experiments performed for each condition (5 males and 5 females; *P<0.05). D, GPER-mediated attenuation of medial thickness is sex independent. GPER recovery reduced the medial thickness by 38% in males—similar to the extent seen in females (42%). Data represent the means±SE of 5 experiments performed for each condition. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; and GFP, green fluorescent protein.
ERα downregulation paralleled a significant reduction in the extent of the inflammatory response after carotid ligation as assessed by the proportion of CD45-positive cells (adenoshGFP: 26±3%; adenoshGPER: 13±3%; n=12; 6 males and 6 females for each condition; P<0.05). The extent of reduction in the inflammatory response with ERα downregulation was not significantly different in males (reduced to 60% of control) versus females (40%).

Notably, further downregulation of GPER expression using the adenoshGPER construct did not alter the extent of injury-mediated medial hypertrophy (shGFP 47±8% above uninjured control, n=5 females; shGPER: 53±8% above uninjured control, n=5 females; Figure 6B) nor the extent of CD45 staining (shGFP 26±4%, shGPER 27±3%, n=5 females; Figures 6C).

**Discussion**

In the current studies, we demonstrate that the balance in the expression of GPER versus ERα is a significant determinant of the vascular response to injury. The in vitro findings demonstrate that expression of GPER inhibits vascular smooth muscle cell growth and cell migration. In vivo, we demonstrate that either upregulation of GPER expression or downregulation of ERα expression attenuates the vascular response to injury.

In vitro, GPER activation inhibited vascular smooth muscle growth and cell migration—important determinants of the extent of vascular remodeling. In contrast, ER activation had no effect on proliferation or on migration—as judged by the lack of effect of E2 in primary cultured VSMCs where GPER is downregulated, whereas ERα expression is maintained.30 Our finding of the inhibitory effects of GPER activation on proliferation in rat aortic VSMCs is consistent with a previous report in coronary artery VSMCs.57

Our finding that GPER activation inhibits migration has not previously been reported in VSMCs (nor has this been reported in any other noncancer cells). However, it is notable that in cancer cells, GPER activation has been shown to have cell-specific effects on migration—stimulating renal cell cancer cells migration48 but inhibiting lung cancer cell migration.59

In aggregate, the effects of GPER to promote apoptosis in VSMCs (as we have previously shown30), inhibit proliferation, and inhibit migration might all contribute to the GPER-mediated regulation of the remodeling response to vascular injury.

It has been reported that at higher concentrations G1 might inhibit vascular smooth muscle cell growth via a GPER-independent mechanism.59 However, in the current studies, the conclusion that the effects of G1 are mediated via GPER activation are supported by 2 lines of evidence, namely, (1) the G1-mediated effect on vascular smooth muscle cell growth is dependent on the expression of GPER and (2) the effect of G1 is inhibited by the selective GPER antagonist, G15.

With vascular injury, GPER expression is downregulated as demonstrated by the reduction in RNA and protein content. From our in vitro studies,30 we have demonstrated that GPER expression is downregulated in the setting where VSMCs in primary culture undergo the switch from a contractile to a synthetic cell type.42 Notably, this phenotypic switch also
Figure 6. Estrogen receptor α (ERα) downregulation attenuated medial hypertrophy and the inflammatory response after carotid ligation. A, Reverse transcription–PCR. Introduction of adenoshERα downregulated expression of ERα (middle). AdenoshGPER introduction led to further downregulation of G-protein–coupled estrogen receptor (GPER) expression (top). B, Downregulation of ERα (left, n=12, 6 males) (Continued)
Parallels increased rates of cellular proliferation and migration, A similar switch to a synthetic phenotype occurs with vascular injury. In the current studies, we demonstrate that GPER expression is also downregulated with vascular injury—shifting the balance between GPER and ERα.

These studies further demonstrate that resetting the balance between GPER and ERα after carotid ligation attenuates the vascular injury response—either by recovering GPER expression or by downregulating ERα. The recovery of GPER expression leading to attenuation of the vascular remodeling response has not been previously described. However, the impact of ERα downregulation to attenuate the vascular remodeling response might be inferred by revisiting the findings of Pare et al 10 in their studies of the response to vascular injury in mouse ER knockout models, reported almost 15 years ago. Their conclusion was that ERα was responsible for the protective effects of exogenously administered estrogen. However, it is notable that the extent of remodeling seen in ER knockout animals without exogenous estrogen administration was attenuated versus the response seen in wild-type animals—consistent with our findings in rats with ERα knockdown without exogenous estrogen administration.

Notably, further GPER downregulation using our adenovirusGPER construct did not further alter the vascular injury response. The explanation for this finding is unclear but may represent a threshold effect for the extent of GPER expression required to inhibit proliferation (at least at physiological circulating levels of estrogen), that is, GPER expression after vascular injury has been decreased to a point where further reduction in GPER content has no further impact.

These findings of the regulation of carotid artery remodeling after vascular injury contrast with the findings from a recent study examining the receptor basis of estrogen’s effects to abrogate femoral vascular remodeling after wire injury in mice. Our studies and those of Chamblis et al 4 have emphasized the importance of rapid signaling pathways in mediating the effects of estrogen to attenuate the remodeling response after carotid vascular injury. In contrast, Smirnova et al 6 concluded that classic nuclear ERα-mediated mechanisms were paramount in their mouse femoral artery model. Notably, in neither report was the potential role of GPER activation in modulating the effects of estrogen on vascular remodeling considered. Furthermore, these 2 latter studies examined the effects of treatment with exogenous estrogen on vascular injury responses in females only, whereas we examined the impact of endogenous circulating hormonal levels on vascular injury responses in males and females. Thus, whether these differences in findings are species specific and vascular bed specific and/or reflect differences in the physiological versus pharmacological effects of estrogens are yet to be determined.

Perspectives
We demonstrate that as predicted by our in vitro studies, resetting the balance between GPER and ERα expression in vivo has significant effects on the vascular remodeling response. Either upregulating GPER expression or downregulating ERα expression inhibited the response to vascular injury. In aggregate, these findings suggest that selective modulation of one arm or the other of the balance of ERα versus GPER expression alters the process of vascular remodeling.

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Disclosures
None.

References


What Is New?

- G-protein–coupled estrogen receptor activation inhibits proliferation and migration of rat vascular aortic smooth muscle cells.
- After carotid ligation and consequent hypertrophy, G-protein–coupled estrogen receptor expression is downregulated.

What Is Relevant?

- Either upregulation of G-protein–coupled estrogen receptor expression or downregulation of estrogen receptor α attenuates the remodeling response and the associated inflammatory response after vascular injury.

Summary

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On p 1227, Methods, in the last sentence in the left column, “GPR30” is changed to “GPER” to read, “Thus, we previously have used these cultured rat aortic VSMCs as a null background in which we could modulate relative GPER expression with adenoviral mediated gene transfer.30”

On p 1228, Figure 2 is a duplicate of Figure 1. The legend as stated is correct, but the image is incorrect.

These corrections have been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/68/5/1225.