Published in final edited form as:

Vascul Pharmacol. 2011 ; 55(1-3): 42–49. doi:10.1016/j.vph.2011.05.002.

Estrogens and Selective Estrogen Receptor Modulators Regulate Gene and Protein Expression in the Mesenteric Arteries

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Abstract

Estrogen has both beneficial and detrimental effects on the cardiovascular system. Selective estrogen receptor modulators (SERMs) exhibit partial estrogen agonist/antagonist activity in estrogen target tissues. Gene targets of estrogen and SERMs in the vasculature are not well-known. Thus, the present study tested the hypothesis that estrogens (ethinyl estradiol, estradiol benzoate, and equilin) and SERMs (tamoxifen and raloxifene) cause differential gene and protein expression in the vasculature. DNA microarray and real-time RT-PCR were used to investigate gene expression in the mesenteric arteries of estrogen and SERM treated ovariectomized rats. The genes shown to be differentially expressed included stearoyl-CoA desaturase (SCD), soluble epoxide hydrolase (sEH), secreted frizzled related protein-4 (SFRP-4), insulin-like growth factor-1 (IGF-1), phospholipase A2 group 1B (PLA2-G1B), and fatty acid synthase (FAS). Western blot further confirmed the differential expression of sEH, SFRP-4, FAS, and SCD protein. These results reveal that estrogens and SERMs cause differential gene and protein expression in the mesenteric artery. Consequently, the use of these agents may be associated with a unique profile of functional and structural changes in the mesenteric arterial circulation.

Keywords

ethinyl estradiol; equilin; tamoxifen; raloxifene; microarray

1. Introduction

The vasculature is clearly a target for estrogen (Ling et al., 2006; Miller and Duckles, 2008; Xing et al., 2009), and estrogens can directly affect vascular function (Li and Stallone, 2005; Li M., et al., 2008; Mark et al., 2007). Nevertheless, the precise effects of estrogen in the vasculature remain contradictory and controversial. One potential explanation for these disparate findings is the concept that estrogen is protective to a healthy vasculature (Miller and Duckles, 2008) but may exacerbate disease when vascular disease such as

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Disclosures

The authors of this manuscript have no conflicts of interest to report.

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atherosclerosis is present (Clarkson, 2007; Dubey et al., 2005). Alternatively, because of potential unique interactions with estrogen receptors and recruitment of different coactivators and corepressors, each type of estrogen may have characteristic effects on gene and protein expression and consequently function in the vasculature. However, information regarding the global effects of different formulations of estrogens on arterial gene expression remains sparse. Work from our laboratory (Eyster et al., 2007; Rodrigo et al., 2003a; Rodrigo et al., 2003b) and others (Gao et al., 2006; Nakamura et al., 2004; Watanabe et al., 2004) have identified moderate effects of estrogen on gene expression in arterial tissue and cells.

Selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene, lack the steroid structure of estrogens but possess a tertiary structure that allows them to bind to estrogen receptors and modulate estrogen receptor-mediated gene transcription (Nilsson and Koehler, 2005). This characteristic of SERMs gives them distinct estrogen-agonist/antagonist profiles. Tamoxifen is used for estrogen-responsive breast cancer (Decensi et al., 2005). Raloxifene is approved for the treatment of osteoporosis in post-menopausal women (Lewiecki, 2009). Information on the effects of these SERMs in the vasculature remains limited and contradictory. Both exhibited positive effects on local blood flow in some (Leung et al., 2007) but not other (Mark et al., 2007) studies, and both tamoxifen and raloxifene increased the risk of venous thromboembolic events (Cuzick et al., 2003, Decensi et al., 2005).

Three formulations of estrogen and two selective estrogen receptor modulators (SERMs) were chosen for this study. The estrogens used were ethinyl estradiol, equilin, and estradiol benzoate. Ethinyl estradiol is used clinically as a component of oral contraceptives. Equilin is one component of conjugated equine estrogens used clinically for management of menopausal symptoms in women. Estradiol benzoate is an estrogen commonly used in laboratory studies. The SERMs selected for this study were tamoxifen and raloxifene. These formulations of estrogens and SERMs are commonly used and little information is currently available regarding the genomic action of these agents on the regional vascular beds. The current study tested the hypothesis that various formulations of estrogen and SERMs differentially regulate gene and protein expression in the mesenteric arteries. The mesenteric arterial tree was chosen for this study because it is an example of resistance blood vessels that contributes substantially to the regulation of total peripheral resistance and blood pressure (Christensen and Mulvany, 1993).

2. Methods

2.1. Animal treatments

All experiments and protocols were performed in accordance with regulations established by the National Institutes of Health (NIH) Council on Animal Care and were approved by the Institutional Animal Care and Use Committee of the University of South Dakota. For gene expression studies, prepubertal female Sprague Dawley rats (Harlan, Indianapolis, IN) were ovariectomized at 4 weeks of age, prior to the initiation of regular ovarian cycles. The animals were allowed to recover for 2 weeks before the experimental protocol was initiated. The rats were randomly divided into 6 groups (n=8/group) and treated by daily gavage for 3 weeks with the vehicle (20% 2-hydroxypropyl- β -cyclodextrin, Sigma, St Louis, MO), ethinyl estradiol (EE, 0.15 mg/kg; Sigma), estradiol benzoate (EB, 0.15 mg/kg; Sigma), equilin (EQ, 0.15 mg/kg; Sigma), tamoxifen (TAM, 3 mg/kg; Sigma), or raloxifene (RAL, 3 mg/kg; Eli Lilly & Co., Indianapolis, IN). Doses were chosen from published work of other laboratories (Sato et al., 1996) as well as our own (Eyster et al., 2007; Mark et al., 2007; Rodrigo et al., 2002) that were demonstrated to have physiological effects.

For the protein expression experiments, a separate cohort of animals (n=6/group) was used. This group was also ovariectomized at four weeks of age. After a two week recovery period, the rats were treated by daily gavage for four days with 2-hydroxypropyl- β -cyclodextrin (vehicle, Sigma), EE (0.15 mg/kg; Sigma), EQ (0.15 mg/kg; Sigma), or TAM (3 mg/kg; Sigma). The purpose of shortening the treatment protocol from 3 weeks to 4 days was to determine whether the changes in expression observed at 3 weeks could be observed after only 4 days of treatment, or whether these expression changes required the full 3 weeks of treatment. Additionally, this comparison also controlled for the possibility that some of the changes observed after three weeks of treatment were compensatory in nature and not primary actions of the drug treatments.

2.2. Tissue collection and RNA extraction

After the final gavage treatment on day 21 or day 4, the rats were deeply anesthetized with isoflurane and euthanized by decapitation. For both cohorts of rats, the mesenteric arterial tree was isolated and harvested as described by our laboratory (Rodrigo et al., 2002) and stored at -80°C .

Mesenteric artery RNA was extracted and purified using an RNeasy Mini Kit (Qiagen, Germantown, MD). Any potentially contaminating DNA was removed by treatment with RNase-free DNase (Qiagen) on the RNeasy column. Total RNA was quantified and RNA integrity was assessed using the Agilent Bioanalyzer (Santa Clara, CA). Following analysis, the RNA was stored at -80°C until it was used for gene expression studies.

2.3. DNA microarray

CodeLink Whole Rat Genome Bioarrays (Applied Microarrays, Tempe, AZ) were used. Five μg total RNA was used from each mesenteric arterial sample for the microarray analysis. The microarray protocol was carried out as described (Eyster et al., 2007). A set of bacterial RNAs was added to each total RNA sample and carried through the synthesis and labeling reactions as controls and for normalization of the data. The microarrays were scanned with the GenePix 4000B (Axon Instruments, Union City, CA) with GenePix Pro 5.0 software (Axon Instruments). Analysis of the microarrays was performed with the CodeLink Expression Analysis software (Applied Microarrays) and Acuity 3.1 software (Axon), and statistical analysis was performed with GeneSpring 7.1 software (Agilent, Santa Clara, CA). A sample of n=3 was completed with the microarrays for each treatment.

2.4. Real time RT-PCR

The TaqMan Gold RT-PCR kit (Applied Biosystems, Foster City, CA) was used to conduct real time RT-PCR (Applied Biosystems). The primers and minor groove binding probes for real-time RT-PCR were purchased as pre-designed primer and probe sets from Applied Biosystems (TaqMan® Gene Expression Assays). For a relative quantitation assay, an endogenous control (housekeeping gene, i.e., cyclophilin B (Weisinger et al., 1999)) was selected. Changes in expression of the target gene were calculated relative to the endogenous control. The software program qBase was used for quantitation of gene expression (Hellemans et al., 2002). The real time RT-PCR studies were performed with an n of 6–8.

2.5. Immunoblot

Immunoblots were used to examine the content of specific proteins in the mesenteric arteries as described (Rodrigo et al., 2002). Proteins (50 μg) were separated by electrophoresis on 10% SDS polyacrylamide gels. The proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Biorad, Hercules, CA). The membranes were blocked overnight in

Aquablock (EastCoast Bio, North Berwick, ME) then incubated with primary antibody. The following primary antibody dilutions were used: stearyl-CoA desaturase goat polyclonal 1:100 (Santa Cruz Biotechnology, Santa Cruz, CA), soluble epoxide hydrolase goat polyclonal, 1:200 (Santa Cruz Biotechnology), secreted frizzled related protein-4, 1:100 (Santa Cruz Biotechnology), fatty acid synthase rabbit polyclonal, 1:100 (Abcam, Cambridge, MA), β -actin mouse polyclonal, 1:8000 (Calbiochem, Darmstadt, Germany). β -actin was used as a loading control for each sample. After incubation with primary antibody, the membranes were washed and proteins were detected by incubating the membrane with a 1:1500 dilution of IRDye800-conjugated, affinity purified fluorescent secondary antibody (Rockland Immunochemicals, Gilbertsville, PA). The protein bands were imaged with the Odyssey Infrared Imager (Li-Cor Biosciences, Lincoln, NE) and quantified using densitometric analysis software accompanying the imager. Data are expressed as relative densitometric units after subtraction of the background.

2.6. Statistical analysis

Microarray analysis was performed for 3 individual samples for each treatment; no mesenteric artery samples were pooled. Statistical analysis of DNA microarray data was performed using GeneSpring 7.1 software (Agilent Technologies, Santa Clara, CA); statistical significance was accepted at p value < 0.05 . Genes that showed a significant p value were further analyzed by fold-expression compared to the control group. For real time RT-PCR and immunoblot, results are expressed as mean $\pm$ SEM. Relative expression values from real time RT-PCR and protein expression values from western blot were examined by analysis of variance followed by Dunnett's Multiple Comparison test (GraphPad Prism 4 Software, San Diego CA).

3. Results

CodeLink Whole Rat Genome Bioarrays contain 33,849 probes. Statistical analysis (ANOVA, GeneSpring 7.1 software) indicated that 6,870 genes showed differential expression in the treatment groups compared to vehicle control with p values < 0.05 . Of those, 100 were named genes that exhibited more than 1.5-fold difference (increase or decrease) in expression and for which relative expression values were greater than 0.2. Unnamed genes outside these parameters were not further analyzed. The named genes were sorted into the following gene ontologies: signal transduction, receptors, transcription/translation, channels/transporters, energy synthesis/storage, enzymes, immune response, membrane associated, cytochrome P450 family, and miscellaneous genes. The list of differentially expressed genes sorted by function in vehicle control (CTL), EE, EB, EQ, TAM, and RAL treated groups is shown in Supplementary Table 1.

The complete data set for these DNA microarrays has been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) as recommended by Minimum Information About a Microarray Experiment (MIAME) standards (Brazma et al., 2001) and can be accessed through GEO Series accession number GSE23037.

Genes were chosen for confirmation studies based on their relationships to each other, relationship to previously identified genes of interest in our laboratory (Eyster et al., 2007), or known effects on vessel function. Six genes were chosen from the DNA microarray data for confirmation of differential expression by real time RT-PCR using these criteria.

Fatty acid synthase (FAS) expression was significantly increased in EE and EQ treatment groups compared to CTL by DNA microarray (Figure 1A). There was no effect of the other treatment groups on FAS expression. Real-time RT-PCR confirmed the increase in the

expression of FAS in the EE and EQ treatment groups in comparison to CTL, (Figure 1B) as well as the lack of response to the other treatments. Immunoblot confirmed an increase in protein expression of FAS in the EE and EQ treatment groups in comparison to CTL (Figure 1C).

Stearoyl-CoA desaturase (SCD) expression was significantly increased in response to EE and EQ treatment compared to CTL as measured by DNA microarray (Figure 2A). Real-time RT-PCR confirmed the significant increases in SCD mRNA expression in response to EE and EQ (Figure 2B) as well as the lack of effect of the other treatments on SCD expression. SCD protein expression was significantly increased in the EQ treatment group compared to CTL (Figure 2C), but the increase in SCD protein in response to EE was not statistically significant.

Soluble epoxide hydrolase (sEH) was upregulated in response to EE, TAM, and RAL compared to CTL (Figure 3A) as shown with DNA microarray but not with the other estrogens. The effect of EE, but not that of TAM or RAL, was confirmed by real-time RT-PCR (Figure 3B). Immunoblot analysis showed that protein expression of sEH was upregulated in the EE treatment group (Figure 3C).

DNA microarray demonstrated a significant decrease in secreted frizzled related protein-4 (SFRP-4) expression in EE, EQ, TAM, and RAL treated animals compared to CTL (Figure 4A). Real time RT-PCR analysis detected a statistically significant down-regulation of SFRP-4 for EE and TAM treatments, but not for EQ or RAL (Figure 4B). The variation in gene expression patterns between DNA microarray and real-time RT-PCR may be explained by different sensitivity of the probes used for the two different techniques. Immunoblot confirmed the inhibition of SFRP-4 protein expression in the mesenteric arteries in response to EE and TAM, as well as the lack of effect by EQ compared to CTL (Figure 4C).

DNA microarray analysis showed that insulin-like growth factor-1 (IGF-1) expression was significantly decreased in the EE, EQ, TAM, and RAL treatment groups compared to CTL (Figure 5A). When analyzed by real time RT-PCR, the decrease in IGF-1 mRNA in response to EE as well as an increase in response to EB compared to CTL reached statistical significance (Figure 5B). The effects of EQ, TAM, and RAL observed in the microarray data were not confirmed by real time RT-PCR. As above, it is likely that the disparities between DNA microarray and real time RT-PCR data are due to differences in probe sensitivity.

Phospholipase A₂, group 1B (PLA2-G1B) was upregulated in the TAM treatment group compared to CTL by DNA microarray (Figure 6A). There was no effect of the other treatments on PLA2-G1B expression. The differential expression patterns were similar in the real-time RT-PCR data (Figure 6B).

4. Discussion

The most important finding of this study is that EE, EQ, and EB, the three formulations of estrogen that were tested in this study, exhibit differential effects on gene and protein expression in the mesenteric arteries. This study reinforces previous work in our laboratory that demonstrated significantly different effects of these formulations of estrogen on vascular reactivity in the mesenteric vascular bed (Mark et al., 2007). Similarly, others have shown differing vascular responses to different formulations of estrogens (Okano et al., 2006). Potential mechanisms by which the differing responses to the various formulations of estrogens may be mediated include differences in the ability of each to interact with and activate the genomic estrogen receptors (ER) ER α and ER β , differential interaction with nongenomic estrogen receptors/signaling pathways, or differing effects of metabolites of

each of the estrogen preparations (Dubey and Jackson, 2001). The overall effects of estrogen on gene expression were moderate, in keeping with our previous report (Eyster et al., 2007).

A second important finding of this study is that the SERMs, TAM and RAL, showed similar effects to estrogens in regulation of some genes but not others. These data support the concept that TAM and RAL are partial agonists of estrogen in the vasculature.

In the current experiments, estrogens, as well as SERMs, were administered by gavage for 3 weeks or 4 days rather than by intraperitoneal injection for 4 days as in the previous study (Eyster et al., 2007). Oral administration is a common means of administration of pharmaceutical estrogens and is therefore relevant to human usage. Of the 6 genes shown to be differentially expressed in our previous study, up-regulation of fatty acid synthase and 3 α -hydroxysteroid dehydrogenase were confirmed in the current study. The differences in mode of administration may explain the differences in gene expression in response to estrogen observed in the two studies. It seems unlikely that the length of treatment with estrogens was responsible for the differences in expression observed in the two studies since two treatment periods were used in the current study. The data demonstrate that acute expression changes in response to estrogens and SERMs at 4 days were also observed when the estrogenic treatments were given for 3 weeks. Thus, the observed differential effects of these estrogenic compounds on gene expression are likely primary effects and not compensatory responses.

FAS mRNA and protein were upregulated in the mesenteric artery of EE and EQ treated rats. This finding is consistent with our previous work which showed that FAS was differentially expressed in rat mesenteric arteries in response to injected estradiol benzoate (Eyster et al., 2007). Administration of EB by gavage in the current study did not up-regulate FAS as previously shown, possibly because of the different route of administration or because of an outlier data point from one animal. FAS is responsible for the synthesis of saturated fatty acids, myristate, palmitate, and stearate (Smith et al., 2003) and is highly expressed in tissues such as liver, adipose, and lactating mammary glands (Smith et al., 2003). Although FAS is not typically considered to be a mainstream regulator of vascular control, it is interesting to note that it has been shown to be important for endothelial cell proliferation and angiogenesis (Browne et al., 2006). Moreover, other work showed that free fatty acids can potentiate adrenergic constriction (Stepniakowski et al., 1996) and inhibit endothelial dilator mechanisms (Shankar and Steinberg, 2005). Thus, FAS may represent a novel pathway by which estrogens influence mesenteric arterial structure and function.

SCD gene expression was upregulated in the mesenteric artery of EE and EQ treated rats. SCD is located downstream from FAS in the pathway of fatty acid synthesis. Because of the multiple physiological roles of monounsaturated fatty acids, changes in SCD expression could affect many key pathophysiological processes, including metabolism, adiposity and atherosclerosis (Paton and Ntambi, 2009). Recent evidence also suggests a role for SCD in endothelial cell functions. Qin and coworkers have shown that laminar shear stress can up-regulate the expression of SCD in vascular endothelial cells in culture (Qin et al., 2007). In addition, SCD increased conversion of stearic acid to oleic acid, which resulted in suppression of endothelial cell activation, and inhibition of pro-inflammatory cytokines and adhesion molecules (Carluccio et al., 1999). These studies indicate a potential beneficial role of SCD in vascular endothelial cells. We hypothesize that upregulation of SCD expression with EE and EQ treatment may contribute to a possible protective role of these estrogens in the mesenteric arteries. The expression patterns of SCD and FAS are consistent with the possibility that EE and EQ modify fatty acid synthesis in the mesenteric arterial tree.

Soluble EH (sEH) plays a role in the conversion of epoxyeicosatrienoic acids (EETs) into their corresponding dihydroxyeicosatrienoic acids (DHETs). Hydrolysis of the EETs to DHETs is generally regarded as a mechanism whereby the biological effects of the EETs are attenuated or eliminated (Zeldin et al., 1993). EETs play important roles in the regulation of vascular function and are potent vasodilators (Campbell et al., 1996; Hu and Kim, 1993), thereby suggesting a role for sEH in blood pressure regulation. Additionally, 11(12)-EET displays anti-inflammatory properties in vascular endothelial cells (Node et al., 1999). This activity was diminished in the corresponding DHET, suggesting a pro-inflammatory role for sEH. Indeed, inhibitors of sEH prevent the development of some models of hypertension (Li J, et al., 2008) and hypertension-related end organ damage (Imig, 2010). In a study by Olearczyk and coworkers, sEH was identified in the mesenteric vasculature of adult male rats and treatment with a sEH inhibitor resulted in concentration-dependent relaxation of the precontracted mesenteric vessel (Olearczyk et al., 2006), confirming the vasodilator actions of EETs in this vascular bed. In our study, EE treatment up-regulated sEH expression, which would be expected to reduce vasodilator function. This may account for our earlier observation that EE treatment increased mesenteric vascular bed sensitivity to the constrictor effects of norepinephrine (Mark et al., 2007). In contrast, other studies have shown up-regulation of EET in response to estrogen (Sun et al., 2010).

Secreted frizzled related protein-4 (SFRP-4) is an endogenous inhibitor of the Wnt pathway, a signaling pathway involved in diverse functions such as embryonic development, cancer, and metabolism (Seth and Vidal-Puig, 2010). SFRP-4 expression has been identified in the ovary, brain, kidney, lung, and endometrium (Jones and Jomary, 2002). In the vasculature, the Wnt pathway has been implicated in vascular remodeling and angiogenesis (Goodwin and D'Amore, 2002; Zerlin et al., 2008). Our study is the first to show the presence of this component of the Wnt pathway, SFRP-4, in the mesenteric arteries. The significant decrease in SFRP-4 expression in the mesenteric artery of EE and TAM treated rats indicates an ability of these treatments to affect Wnt signaling activity, and possibly vascular remodeling.

IGF-1 has been shown to have both positive and negative effects in the cardiovascular system (Conti et al., 2004). It is an important mediator of cell growth, differentiation, and transformation in blood vessels (Delafontaine et al., 2004). In cell culture, IGF-1 stimulated migration of vascular smooth muscle cells and had potent mitogenic and antiapoptotic effects on the cells (Arnqvist et al., 1995). The vasoactive effects of IGF-1 indicate that it can affect blood pressure and regional blood flow via nitric oxide. Short-term injection of IGF-1 decreased mean arterial pressure and this effect was inhibited by preinfusion of a nitric oxide synthase inhibitor, indicating that the decrease in blood pressure in response to IGF-1 was mediated by nitric oxide (Pete et al., 1996). In a separate study, treatment with IGF-1 resulted in vasorelaxation in normotensive rats but not in hypertensive rats (McCallum et al., 2005). In contrast, smooth muscle-targeted overexpression of IGF-1 resulted in enhanced vascular contractility, possibly via regulation of contractile protein expression (Zhao et al., 2001). Moreover, IGF-1 has been associated with the development of atherosclerosis (Bayes-Genis et al., 2000). Estrogen decreased IGF-1 expression in rat aortic smooth muscle cells (Scheidegger et al., 2000). In contrast, estrogen increased IGF-1 expression in uterine smooth muscle (Swartz et al., 2005). Our data indicate an increase in the expression of IGF-1 in the mesenteric artery of EB treated rats but a decrease in response to EE. This divergence in the response to two different formulations of estrogen support the pleiotropic nature of the effects of estrogens and of IGF-1 on the vasculature. Further research to clarify the effects of estrogens on IGF-1 in the vasculature is warranted.

Phospholipase A₂ group 1B (PLA₂-G1B) is a member of the large PLA₂ superfamily which has an important role in the activation of the inflammatory process (Verheij et al., 1981).

Our study is the first to demonstrate the presence of PLA2-G1B in blood vessels. PLA2-G1B expression was significantly increased in the mesenteric artery with TAM treatment, which indicates a potential ability of TAM to modulate signal transduction pathways that utilize lipid mediators.

5. Conclusions

These data demonstrate that various formulations of estrogens and SERMs, despite similarities in structure and action, have disparate effects on the mesenteric arteries. Apparently small differences in the structures of estrogens and SERMs allow them to have selective effects on gene and protein expression in the mesenteric arteries. Further examination is needed to determine the physiological and/or pathophysiological roles of these genes in the function of the mesenteric arteries.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank the personnel of the USD Laboratory Animal Services for their assistance with the animal treatments. This work was supported in part by NIH HL 69886 (C.M.-K. and K.M.E.) and by NIH HL 63053 (D.M.).

List of Abbreviations

TAM	Tamoxifen
RAL	Raloxifene
SERMs	Selective estrogen receptor modulators
EE	Ethinyl estradiol
EQ	Equilin
EB	Estradiol benzoate
FAS	Fatty acid synthase
SCD	Stearoyl-CoA desaturase
sEH	Soluble epoxide hydrolase
SFRP-4	Secreted frizzled-related protein 4
IGF-1	Insulin-like growth factor 1
PLA2-G1B	Phospholipase A2 group 1B

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Figure 1.

Differential expression of fatty acid synthase (FAS) in the mesenteric arteries of control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), tamoxifen (TAM), and raloxifene (RAL) treated ovariectomized rats. (A) DNA microarray data (relative expression, $n=3/\text{group}$); (B) real-time reverse transcription-PCR (RT-PCR) relative expression data ($n=8/\text{group}$); (C) Images of immunoblot data for FAS and actin protein for control (lanes 1–3), TAM (lanes 4–6), EE (lanes 7–9), and EQ (lanes 10–12) treated animals. (D) FAS protein expression divided by the actin loading control (relative density, $n=6/\text{group}$). Differential expression for DNA microarray, real time RT-PCR, and immunoblot data in (A), (B) and (D) was determined by ANOVA followed by Dunnett's Multiple Comparison Test ($p<0.05$).

Figure 2.

Differential expression of stearoyl-CoA desaturase (SCD) in the mesenteric arteries of control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), tamoxifen (TAM), and raloxifene (RAL) treated ovariectomized rats. (A) DNA microarray data (relative expression, $n=3/\text{group}$); (B) real-time reverse transcription-PCR (RT-PCR) relative expression data ($n=8/\text{group}$); (C) Images of immunoblot data for SCD and actin protein for control (lanes 1–2), TAM (lanes 3–4), EQ (lanes 5–6), and EE (lanes 7–8) treated animals. (D) SCD protein expression divided by the actin loading control (relative density, $n=6/\text{group}$). Differential expression for DNA microarray, real time RT-PCR, and western blot data in (A), (B) and (D) was determined by ANOVA followed by Dunnett's Multiple Comparison Test ($p<0.05$).

Figure 3.

Differential expression of soluble epoxide hydrolase (sEH) in the mesenteric arteries of control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), tamoxifen (TAM), and raloxifene (RAL) treated ovariectomized rats. (A) DNA microarray data (relative expression, n=3/group); (B) real-time reverse transcription-PCR (RT-PCR) relative expression data (n=8/group); (C) Images of immunoblot data for sEH and actin for control (lanes 1–2), TAM (lanes 3–4), EQ (lanes 4–5), and EE (lanes 7–8) treated rats. (D) sEH protein expression divided by the actin loading control (relative density, n=6/group). Differential expression for DNA microarray, real time RT-PCR, and western blot data in (A), (B) and (D) was determined by ANOVA followed by Dunnett's Multiple Comparison Test ($p < 0.05$).

Figure 4.

Differential expression of secreted frizzled related protein-4 (SFRP-4) in the mesenteric arteries of control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), tamoxifen (TAM), and raloxifene (RAL) treated ovariectomized rats. (A) DNA microarray data (relative expression, $n=3/\text{group}$); (B) real-time reverse transcription-PCR (RT-PCR) relative expression data ($n=8/\text{group}$); (C) Immunoblot images for control (lanes 1–3), TAM (lanes 4–6), EE (lanes 7–9), and EQ (lanes 10–12) treated animals. (D) SFRP4 protein expression divided by the actin loading control (relative density, $n=6/\text{group}$). Differential expression for DNA microarray, real time RT-PCR, and western blot data in (A), (B) and (C) was determined by ANOVA followed by Dunnett's Multiple Comparison Test ($p<0.05$).

Figure 5.

Differential expression of insulin-like growth factor-1 (IGF-1) in the mesenteric arteries of control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), tamoxifen (TAM), and raloxifene (RAL) treated ovariectomized rats. (A) DNA microarray data (relative expression, n=3/group); (B) real-time reverse transcription-PCR (RT-PCR) relative expression data (n=8/group); Differential expression for DNA microarray and real time RT-PCR data in (A) and (B) was determined by ANOVA followed by Dunnett's Multiple Comparison Test ($p < 0.05$).

Figure 6.

Differential expression of phospholipase A2 group 1B (PLA2-G1B) in the mesenteric arteries of control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), tamoxifen (TAM), and raloxifene (RAL) treated ovariectomized rats. (A) DNA microarray data (relative expression, n=3/group); (B) real-time reverse transcription-PCR (RT-PCR) relative expression data (n=8/group); Differential expression for DNA microarray and real time RT-PCR data in (A) and (B) was determined by ANOVA followed by Dunnett's Multiple Comparison Test ($p < 0.05$).