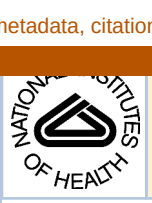

Published in final edited form as:

Exp Physiol. 2008 May ; 93(5): 658–664.

Tissue Specific Regulation of ACE/ACE2 and AT_1/AT_2 Receptor Gene Expression By Estrogen in ApoE/ERα Knock-Out Mice

K. Bridget Brosnihan[1], **Jeffrey B. Hodgin**[2], **Oliver Smithies**[2], **Nobuyo Maeda**[2], and **Patricia Gallagher**[1]

1*The Hypertension and Vascular Research Center, Wake Forest University Health Sciences, Winston-Salem, NC 27157-1932, USA*

2*Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC*

Summary

ACE and ACE2 and the AT_1 and AT_2 receptors are pivotal points of regulation in the renin-angiotensin system. ACE and ACE2 are key enzymes in the formation and degradation of Ang II and Ang-(1-7). Ang II acts at either the AT_1 or the AT_2 receptor to mediate opposing actions of vasoconstriction/vasodilation. While it is known that estrogen (E2) acts to down-regulate ACE and the AT_1 receptors, its regulation of ACE2 and the AT_2 receptor and the involvement of a specific estrogen receptor subtype are unknown. To investigate the role of estrogen receptor-α (ERα) in estrogen's regulation of ACE/ACE2 and AT_1/AT_2 mRNAs in lung and kidney, ovariectomized female mice lacking apolipoprotein E (ee) with the ERα (AAee) or without the ERα (ααee) were treated with 17-β estradiol (6 μg/day) or placebo for 3 months. ACE,ACE2 and AT1/AT2 receptor mRNAs were measured using reverse transcriptase, real-time polymerase chain reaction (RT/RT-PCR). In the kidney, 17-β estradiol showed 1.7 fold down-regulation of ACE mRNA in AAee mice, with 2.1-fold up-regulation of ACE mRNA in ααee mice. 17-β estradiol showed 1.5 and 1.8 fold down-regulation of ACE2 and AT_1 receptor mRNA in AAee mice; this regulation was lost in ααee mice. 17-β estradiol showed marked (81-fold) up-regulation of the AT_2 receptor mRNA in AAee mice. In the lung 17-β estradiol treatment had no effect on AT_1 receptor mRNA in AAee mice, but resulted in a 1.5-fold decreased regulation of AT_1 mRNA in ααee. There was no significant interaction of estrogen with ER in the lung for ACE, ACE2, and AT2 receptor genes. These studies reveal tissue specific regulation by 17-β estradiol of ACE/ACE2 and AT_1/AT_2 receptor genes with the ERα receptor primarily responsible for the regulation of kidney ACE2 , AT_1 receptor, and AT_2 receptor genes.

Keywords

renin-angiotensin system; lung and kidney; ACE; ACE2; AT_1 and AT_2 receptors; ERKO mice

Introduction

In previous studies the inhibitory effects of estrogen (E2) on atherosclerosis were well-documented in numerous animal models (Clarkson *et al.*, 1996;Clarkson *et al.*, 1994). A mouse model of atherosclerosis that results in severe atherosclerotic lesions is the apolipoprotein E-deficient (ApoE−/−) mouse. Long term estrogen treatment was highly effective in inhibiting lesion progression (Hodgin *et al.*, 2001). The long term effects of E2 are generally ascribed to transcriptional modulation of target genes through estrogen receptors (ERs). Two estrogen

Correspondence: K. Bridget Brosnihan, Ph. D., The Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1032, Tel: 336-716-2795, bbrosnih@wfubmc.edu Fax: 336-716-245.

receptors, ERα and ERβ, encoded by two separate genes were characterized, are distinct structurally and functionally, and have tissue specific expression patterns. Both receptors are expressed in a variety of cardiovascular cells types. Using the ApoE−/− that lack the ERα, Hodgin et al (Hodgin *et al.*, 2001) showed that 17-β estradiol treatment of ovariectomized ApoE−/− mice with the ERα attenuated the artherosclerosis, but that ApoE−/− mice without the ERα caused minimal reduction in artherosclerosis with E2 treatement. These studies demonstrated that ERα is a major mediator of the atheroprotective effects of E2. This mouse model was instrumental in uncovering the receptor subtype that mediates E2 effects on atherosclerosis.

Many components of the renin-angiotensin system (RAS) are regulated by E2. ACE mRNA and activity, as well as the AT_1 receptor, are down-regulated by E2 (Brosnihan *et al.*, 1999;Gallagher *et al.*, 1999;Brosnihan *et al.*, 2000;Wu *et al.*, 2003;Krishnamurthi *et al.*, 1999;Nickenig *et al.*, 1998). The role of E2 in the regulation of ACE2, a recently described homologue of ACE that shows high catalytic activity in the degradation of Ang II (Burrell *et al.*, 2004;Shaltout *et al.*, 2007), has not been determined, but we showed that ACE2 mRNA and activity are increased in the kidney and uterus of pregnant animals (Joyner *et al.*, 2007;Neves *et al.*, 2007) making estrogen a likely modulator for ACE2 regulation, although other hormones could be involved. There are few studies describing the effect of E2 on AT_2 receptor regulation. The objective of these studies was to study the role of E2 in the regulation of ACE and ACE2 as well as AT_1 and AT_2 receptor genes in ApoE/estrogen receptor α (ERα) knock-out mice.

Experimental Procedures

Animals

Frozen mouse tissues were obtained from Dr. Jeffrey B. Hodgin and the results from his study are described in a previous publication (Hodgin *et al.*, 2001). The protocols for the mouse experiments were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill, NC. The experimental animals used for investigating the role of the ERα were female ApoE−/− (designated ee) with or without ERα [designated AA or αα] on a C57Bl6/J genetic background. The four groups were 1) animals with the ERα and ee (AAee) and no 17-β estradiol treatment; 2) AAee animals plus 17-β estradiol treatment; 3) animals without the ERα (αaee) without 17-β estradiol treatment; and 4) αaee animals plus 17-β estradiol treatment. At 30 days of age, female mice were weighed, ovariectomized and randomly implanted subcutaneously with either pellets designed to release 17-β estradiol at 6 μg/day (0.36 mg/pellet with 60-day release) for 60 days or a placebo control (Innovative Research of America, Sarasota, FL). Pellets (additional pellets with 60-day release) were replaced 60 days after surgery and the mice were sacrificed 30 days later at 4 months of age. Mice were maintained in a specific pathogen free condition on standard mouse chow (Prolab Isopro RMH3000; PMI Nutrition International, Brentwood, MI).

RNA Isolation and Reverse Transcriptase/Real-time Polymerase Chain Reaction (RT-RTPCR)

The concentration and integrity of RNA isolated from mouse kidney or lung tissue using the TRIZOL reagent was assessed with an Agilent 2100 Bioanalyzer. Total RNA was reverse transcribed and the resultant cDNA was amplified with an ABI 7000 Sequence Detection System. The primer/probe sets for AT_1 receptor, AT_2 receptor, and ACE were purchased from Applied Biosystems (Foster City, CA); the ACE2 primer/probe set consisted of forward primer 5'-CCCAGAGAACAGTGGACCAAAA-3', reverse primer 5'-GCTCCACCACACCAACGAT-3', and probe 5'-FAM-CTCCCGCTTCATCTCC-3'. The mixtures were heated at 50°C for 2 min, at 95°C for 10 min followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. All reactions were performed in triplicate and 18S ribosomal RNA

served as an internal control. Undegraded total RNA samples were used to evaluate the integrity of 18S and 28S rRNA, which show distinct 18s and 28S rRNA subunit peaks with a ratio of 1.85. The results were quantified as Ct values, where Ct is defined as the threshold cycle of PCR at which amplified product is first detected, and expressed as the ratio of target/control (Relative Gene Expression).

Radioimmunoassay of 17-β Estradiol

17-β estradiol was assayed on serum using a radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX).

Statistical analysis

Comparisons between the groups were performed using a 2-way ANOVA. Fisher LSD multiple comparison test was used (Number Cruncher Statistical Systems, Kaysville, Utah). A p value less than 0.05 was considered statistically significant. All values are presented as mean±SEM.

Results

Serum 17-β estadiol levels of the placebo treated mice were < 20 pg/ml; whereas serum 17-β estadiol levels of the estrogen treated animals were 72 ± 20 pg/ml in the AAee and 104 ± 17 pg/ml in the ααee groups, as previously reported (Hodgin *et al.*, 2001).

Estrogen effects on kidney ACE/ACE2 and AT_1/AT_2 mRNAs

Figure 1 shows that chronic estrogen treatment reduced ACE mRNA by 1.7-fold in ApoE−/− mice possessing the ERα (AAee) agreeing with previously reported findings in ovariectomized Sprague Dawley rats treated with estradiol (Brosnihan *et al.*, 2000;Gallagher *et al.*, 1999). 17-β estradiol treatment resulted in a 2.1-fold up-regulation of kidney ACE mRNA in the ApoE −/−ERα KO mice (ααee). 17-β estradiol treatment down-regulated kidney ACE2 and AT_1 receptor mRNAs by 1.5-and 1.8-fold, respectively, in AAee; and this regulation by 17-β estradiol treatment was lost in ααee mice (Figure 1), suggesting that ERα is the primary regulator of the ACE2 and the AT_1 receptor mRNAs. 17-β estradiol showed marked up-regulation by 81-fold of the AT_2 receptor mRNA in AAee mice, which was markedly reduced in ααee mice, suggesting that ERα contributes to the up-regulation of the kidney AT_2 receptor. The increase with 17-β estradiol treatment in ααee did not reach statistical significance.

Estrogen effects on lung ACE/ACE2 and AT_1/AT_2 mRNAs

In the lung 17-β estradiol treatment resulted in a 1.5 fold down-regulation of AT_1 mRNA in lung in the absence of ERα, suggesting that ERβ mediates the 17-β estradiol effect on AT_1 mRNA (Figure 2). There was no significant interaction between 17-β estradiol and the ER for ACE, ACE2 and AT_2 receptor mRNAs in lung. However, an effect of estrogen was observed in ααee mice for ACE and ACE2 mRNA.

Discussion

Estrogen exerts complex regulation over the components of the RAS. The most clear demonstration of estrogen's effects on the RAS are the reports that E2 up-regulates angiotensinogen in the liver (Nasjletti *et al.*, 1969) and down-regulates ACE (Brosnihan *et al.*, 2000;Gallagher *et al.*, 1999) and AT_1 receptor in the kidney, adrenal and vasculature (Wu *et al.*, 2003;Krishnamurthi *et al.*, 1999;Nickenig *et al.*, 1998). With the development of ER knock-out mice, it is possible to uncover the role of a specific subtype of the ER that regulates the expression of the genes of the RAS. In these studies using the ApoEKO mice with and without the ERα, we demonstrated that the ERα receptor down-regulates ACE2 and the AT_1

receptor mRNA in the kidney, since these effects of 17-β estradiol were absent in the ααee mice treated with 17-β estradiol. 17-β estradiol also down-regulated ACE mRNA in kidney tissues, but in the ααee mice the down-regulation was reversed to up-regulation. By inference, it appears that ERβ may contribute to the up-regulation of kidney ACE mRNA, which becomes uncovered in the absence of ERα. The most striking effect of 17-β estradiol in this study was the demonstration that 17-β estradiol resulted in 81-fold up-regulation of the AT_2 receptor in the kidney. Because the increase in AT_2 receptor mRNA in the kidney with 17-β estradiol treatment was lost in the ApoE/ERα KO mice as compared to AAee mouse, a primary role for ERα is likely. However, because there was a trend for the AT_2 receptor mRNA to be influenced by 17-β estradiol in the ααee mice the contribution of ERβ on its regulation cannot be eliminated. In the lung only the AT_1 receptor mRNA showed a significant interaction of 17-β estradiol with ER. Because the regulation by estrogen was only uncovered in the ααee mice, it appears that ERβ may contribute to the down-regulation of the AT_1 receptor gene in the lung. Because these studies were not done in ERβKO mice, the direct participation of the ERβ awaits studies in the ERβKO mice, but the ααee model allows us to suggest a role of ERβ by inference. These studies reveal tissue specific regulation by 17-β estradiol of ACE/ACE2 and AT_1/AT_2 receptor genes with the ERα receptor primarily responsible for the regulation of kidney ACE2, AT_1 receptor, and AT_2 receptor genes. The participatory role of ERβ seems likely in regard to the regulation of kidney ACE mRNA and lung AT_1 receptor mRNA.

The down-regulation of ACE by estrogen is well established (Brosnihan *et al.*, 2000;Gallagher *et al.*, 1999;Seely *et al.*, 2004;Proudler *et al.*, 2003). 17-β estradiol treatment in ovariectomized rats decreased ACE mRNA and activity in renal cortex and medulla and aorta (Brosnihan *et al.*, 2000;Gallagher *et al.*, 1999). Post-menopausal women on hormone replacement also show a decreased serum ACE activity (Seely *et al.*, 2004;Proudler *et al.*, 2003). While no estrogen response element has been found in the ACE coding sequence, it is thought that an ACE promoter may be responsive to actions of gene transcription factors independent of the hormone response element. The ACE promoter does contain a consensus AP1 site that could mediate the effects of exogenous estrogen (Goraya *et al.*, 1994;Howard *et al.*, 1993). Our studies demonstrated that kidney ACE mRNA was reduced with 17-β estradiol treatment. In mice lacking the ERα, estrogen replacement resulted in increased kidney ACE mRNA. This finding indicates that ERα is not the only receptor subtype involved in the regulation of ACE mRNA. It is known that ααee mice do not express the wild type gene encoding ERα; however, a small splice variant of the disrupted ERα gene along with a residual level of ERα binding was described. Since the binding was detected only in the uterus and not in other tissue, such as kidney, liver, and brain (Couse *et al.*, 1995), it is unlikely that the ERα splice variant is involved in kidney ACE mRNA regulation. The most likely candidate to explain the E2 effect in the absence of ERα is the ERβ. Because ACE mRNA was up-regulated by E2 in mice lacking ERα, this finding also suggests the involvement of ERβ contributing to the up-regulation of the ACE gene.

Most of the traditionally observed effects of the RAS are mediated by the AT_1 receptor. Like ACE, the AT_1 receptor is down-regulated by 17-β estradiol replacement therapy (Wu *et al.*, 2003;Krishnamurthi *et al.*, 1999;Nickenig *et al.*, 1998). Studies in humans as well as experimental animals report this action of E2 at the level of the gene, receptor density, and protein (Wu *et al.*, 2003;Krishnamurthi *et al.*, 1999;Nickenig *et al.*, 1998). Our studies on the AT_1 receptor demonstrate down-regulation by 17-β estradiol in the kidney, but no effect of 17-β estradiol on the lung AT_1 receptor mRNA in AAee The significant down-regulation of the lung AT_1 receptor that appears in the ααee mice with 17-β estradiol replacement suggests a role for the ERβ that may be masked by the ERα.

Both the kidney and lung express ACE2 activity, mRNA and protein (Gembardt *et al.*, 2005;Riviere *et al.*, 2005). The kidney and ileum of the mouse show the highest level as

compared to all tissues, with the lung showing ACE2 expression to a lesser degree (Gembardt *et al.*, 2005). Few studies have been conducted to evaluate the regulation of the ACE2 gene. It is known that there is up-regulation of ACE2 in heart failure (Goulter *et al.*, 2004). Both Ang II through activation of the AT_1 receptor (Gallagher *et al.*, 2006) and aldosterone down-regulate ACE2 (Keidar *et al.*, 2005). The role of estrogen in its regulation is not so clear. Recently, Ojeda et al (Ojeda *et al.*, 2007) showed that female offspring of intrauterine growth restricted (IUGR) mothers displayed a 6-fold increase in ACE2 activity as compared to control females, which was reduced by ovariectomy in IUGR female offspring, but not control females. The lack of effect in ovariectomized controls was suggested to reflect a permanent alteration in the regulation of RAS components in the offsping of IUGR animals. 17-β estradiol treatment was associated with up-regulation of renal ACE2 in the renal wrap model of hypertension (Ji *et al.*, 2007). Finally, pregnant animals showed an increase in ACE2 immunocytochemical expression and activity in renal cortex and medulla (Joyner *et al.*, 2007) in late gestation, the up-regulation being consistent with the high levels of estrogen that are found in pregnancy. These findings suggest that estrogen up-regulates ACE2 gene expression, but that the regulation may differ under pathological conditions. The current studies using 17-β estradiol replacement in ApoE−/− ovariectomized mice showed that 17-β estradiol down-regulated kidney ACE2 mRNA. This effect appeared to be mediated by ERα, since it was lost in the ααee animals treated with 17-β estradiol. Because of the discrepancies of our data with previous studies showing a positive effect of estrogen treatment on ACE2 regulation, it is not clear if the findings in our study are related to the pathological background of the animals or to species differences, since most previously reported studies were conducted in rats rather than mice. Further assessment of the regulation of renal ACE2 by estrogen is warranted. There was no significant interaction of estrogen with the ER for lung ACE2 mRNA. However, in ααee mice 17-β estradiol down-regulated lung ACE2 mRNA.

The AT_2 receptor is highly expressed in the fetal rat kidney but its expression is markedly reduced 3 months after birth (Kakuchi *et al.*, 1995). In the adult rat kidney a low level of expression of the AT_2 receptor has been described (Ichiki & Inagami, 1995); however, in adult mice kidney AT_2 receptors are clearly expressed and associated with renal blood vessels, glomeruli and tubular structures (Baiardi *et al.*, 2005;Armando *et al.*, 2002). Armando et al. (Armando *et al.*, 2002) found that female mice expressed much higher levels of AT_2 receptors and the expression of renal AT_2 receptor in female mice was 17-β estradiol dependent. These investigators describe a 60-fold increase in AT_2 receptor binding in the inner medulla of ovariectomized mice treated with 17-β estradiol, with additional but smaller increases in other renal regions, including the capsule, glomeruli, and medullary rays. Our study showing a marked increase in kidney AT_2 receptor mRNA confirms the estrogen effect on AT_2 receptor in mice kidney. Because this increase in kidney AT_2 receptor mRNA was lost in the ααee mice, a primary role for the ERα receptor is likely. However, a role for ERβ cannot be eliminated, since there was a trend for estrogen to increase the AT_2 receptor mRNA in ααee mice, although to a much lesser extent. Lefebvre et al (Lefebvre *et al.*, 2006) showed that lung expresses the AT_2 receptor and that the expression of the AT_2 receptor was unchanged with congestive heart failure. To our knowledge, there have been no studies evaluating the role of estrogen on the expression of AT_2 receptors in the lung. Our study demonstrates that there is no significant interaction of estradiol and ER in the regulation of the AT_2 receptor mRNA in lung.

The studies were conducted in ApoEKO mice which is an excellent model of atherosclerosis. Thus the studies evaluating estrogen's regulation of RAS components in this model are relevant to studies showing E2 protection in pathological conditions. However, it should be pointed out that discrepancies between reports in the literature concerning E2 regulation of RAS components may reflect the different models studied and thus may be a result of different pathologies. In this study the findings may be influenced by the fact that they were conducted in ApoEKO mice with and without an ERα receptor and, thus the results may be influenced

by this background. Another consideration is that estrogen's action in ovariectomized mice may not necessarily reflect endogenous estrogen's action in the intact female. In addition, it remains possible that E2 loss via ovariectomy has no effect on RAS components when compared to intact animals. Furthermore, a direct assessment of the role of ERβ needs to be done in ERβKO mice to confirm the inferences made.

In conclusion, our study demonstrates that 17-β estradiol replacement exerts differential regulation on components of the RAS. ERα was the primary receptor subtype responsible for the regulation of kidney ACE2, AT_1 receptor and AT_2 receptor genes. The most dramatic effect of 17-β estradiol was on the up-regulation of kidney AT_2 receptor gene which may contribute to the gender specific renal protective effects of estrogen. Finally, our data suggest that ERβ may be primarily involved or contribute to the regulation of the ACE gene in the kidney and the AT_1 receptor gene in the lung.

Acknowledgements

This work was supported by grants from the National Institutes of Health, NHLBI-P01 HL51952-11 and NICHD HD42631. The authors gratefully acknowledge grant support in part provided by Unifi, Inc. Greensboro, NC and Farley-Hudson Foundation, Jacksonville, NC.

References

Armando I, Jezova M, Juorio A, Terron JA, Falcon-Neri A, Semino-Mora C, Imboden H, Saavedra JM. Estrogen upregulates renal angiotensin II AT(2) receptors. Am J Physiol Renal Physiol 2002;283:F934–F943. [PubMed: 12372768]

Baiardi G, Macova M, Armando I, Ando H, Tyurmin D, Saavedra JM. Estrogen upregulates renal angiotensin II AT1 and AT2 receptors in the rat. Regul Pept 2005;124:7–17. [PubMed: 15544836]

Brosnihan KB, Li P, Gallagher PE. Down regulation of angiotensin converting enzyme mRNA by estrogen. Menopause Digest 2000;2:22–23.

Brosnihan KB, Senanayake PS, Li P, Ferrario CM. Bi-directional actions of estrogen on the renin-angiotensin system. Brazilian Journal of Medical and Biological Research 1999;32:373–381. [PubMed: 10347798]

Burrell LM, Johnston CI, Tikellis C, Cooper ME. ACE2, a new regulator of the renin-angiotensin system. TRENDS in Endocrinology and Metabolism 2004;15:166–169. [PubMed: 15109615]

Clarkson TB, Anthony MS, Klein KP. Hormone replacement therapy and coronary artery atherosclerosis: the monkey model. British Journal of Obstetrics & Gynaecology 1996;103

Clarkson TB, Prichard RW, Morgan TM, Petrick GS, Klein KP. Remodeling of coronary arteries in human and nonhuman primates. Journal of the American Medical Association 1994;271:289–294. [PubMed: 8295288]

Couse JF, Curtis SW, Washburn TF, Lindzey J, Golding TS, Lubahn DB, Smithies O, Korach KS. Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. Mol Endocrinol 1995;9:1441–1454. [PubMed: 8584021]

Gallagher PE, Chappell MC, Ferrario CM, Tallant EA. Distinct roles for Ang II and Ang-(1-7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. AJP - Heart and Circulatory 2006;290:420–426.

Gallagher PE, Li P, Lenhart JR, Chappell MC, Brosnihan KB. Estrogen regulation of angiotensin-converting enzyme mRNA. Hypertension 1999;33:323–328. [PubMed: 9931124]

Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Schultheiss HP, Siems WE, Walther T. Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. Peptides 2005;26:1270–1277. [PubMed: 15949646]

Goraya TY, Kessler SP, Kumar RS, Douglas J, Sen GC. Identification of positive and negative transcriptional regulatory elements of the rabbit angiotensin-converting enzyme gene. Nuclei Acids Research 1994;22:1194–1201.

Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. BMC Medicine 2004;2:19–26. [PubMed: 15151696]

Hodgin JB, Krege JH, Reddick RL, Korach KS, Smithies O, Maeda N. Estrogen receptor alpha is a major mediator of 17 beta-estradiol's atheroprotective effects on lesion size in Apoe−/− mice. J Clin Invest 2001;107:333–340. [PubMed: 11160157]

Howard T, Balogh R, Overbeek P, Bernstein KE. Sperm-specific expression of angiotensin-converting enzyme (ACE) is mediated by a 91-base-pair promoter containing a CRE-like element. Molecular and Cellular Biology 1993;13:18–27. [PubMed: 8380220]

Ichiki T, Inagami T. Expression, genomic organization, and transcription of the mouse angiotensin II type 2 receptor gene. Circulation Research 1995;76:693–700. [PubMed: 7728985]

Ji H, Pesce C, Menini S, Wu X, Zheng W, Sandberg K. 17β-estradiol (E2)-mediated protection from renal injury is associated with angiotensin converting enzyme 2 (ACE2) up regulation. Hypertension 2007;48(4):e28.

Joyner J, Neves LA, Granger JP, Alexander BT, Merrill DC, Chappell MC, Ferrario CM, Davis WP, Brosnihan KB. Temporal-spatial expression of angiotensin-(1-7) and angiotensin converting enzyme 2 in the kidney of normal and hypertensive pregnant rats. Am J Physiol Regul Integr Comp Physiol 2007;239:R169–R177. [PubMed: 17428896]

Kakuchi J, Ichiki T, Kiyama S, Hogan BL, Fogo A, Inagami T, Ichikawa I. Developmental expression of renal angiotensin II receptor genes in the mouse. Kidney Int 1995;47:140–147. [PubMed: 7731139]

Keidar S, Gamliel-Lazarovich A, Kaplan M, Pavlotzky E, Hamoud S, Hayek T, Karry R, Abassi Z. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. Circ Res 2005;97:946–953. [PubMed: 16179584]

Krishnamurthi K, Verbalis JG, Zheng W, Wu Z, Clerch LB, Sandberg K. Estrogen regulates angiotensin AT1 receptor expression via cytosolic proteins that bind to the 5′ leader sequence of the receptor mRNA. Endocrinology 1999;140:5435–5438. [PubMed: 10537176]

Lefebvre F, Prefontaine A, Calderone A, Caron A, Jasmin JF, Villeneuve L, Dupuis J. Modification of the pulmonary renin-angiotensin system and lung structural remodelling in congestive heart failure. Clin Sci (Lond) 2006;111:217–224. [PubMed: 16640505]

Nasjletti A, Matsunaga M, Masson GMC. Effects of estrogens on plasma angiotensinogen and renin activity in nephrectomized rats. Endocrinology 1969;85:967–970. [PubMed: 4185920]

Neves LAA, Stovall K, Joyner J, Valdes G, Gallagher PE, Ferrario CM, Merrill DC, Brosnihan KB. ACE2 and Ang-(1-7) in the uterus during early and late gestation. Am J Physiol Regul Integr Comp Physiol. 2007 October 31;

Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosenkranz S, Stablein A, Beckers F, Smits JF, Daemen MJ, Vetter H, Bohm M. Estrogen modulates AT1 receptor gene expression in vitro and in vivo. Circulation 1998;97:2197–2201. [PubMed: 9631868]

Ojeda NB, Grigore D, Robertson EB, Alexander BT. Estrogen protects against increased blood pressure in postpubertal female growth restricted offspring. Hypertension 2007;50:679–685. [PubMed: 17724277]

Proudler AJ, Cooper A, Whitehead M, Stevenson JC. Effects of oestrogen-only and oestrogen-progestogen replacement therapy upon circulating angiotensin I-converting enzyme activity in postmenopausal women. Clin Endocrinol (Oxf) 2003;58:30–35. [PubMed: 12519409]

Riviere G, Michaud A, Breton C, Vancamp G, Laborie C, Enache M, Lesage J, DeLoof S, Corvol P, Vieau D. Angiotensin-converting enzyme 2 (ACE2) and ACE activities display tissue-specific sensitivity to undernutrition-programmed hypertension in the adult rat. Hypertension 2005;46:1169–1174. [PubMed: 16203874]

Seely EW, Brosnihan KB, Jeunemaitre X, Okamura K, Williams GH, Hollenberg NK, Herrington DM. Effects of conjugated oestrogen and droloxifene on the renin-angiotensin system, blood pressure and renal blood flow in postmenopausal women. Clin Endocrinol 2004;60:315–321.

Shaltout H, Westwood B, Averill DB, Ferrario CM, Figueroa J, Diz DI, Chappell MC. Angiotensin metabolism in renal proximal tubules, urine, and serum of sheep: evidence for ACE2-dependent processing of angiotensin II. Am J Physiol Regul Integr Comp Physiol 2007;292:F82–F91.

Wu Z, Zheng W, Sandberg K. Estrogen regulates adrenal angiotensin type 1 receptors by modulating adrenal angiotensin levels. Endocrinology 2003;144:1350–1356. [PubMed: 12639918]

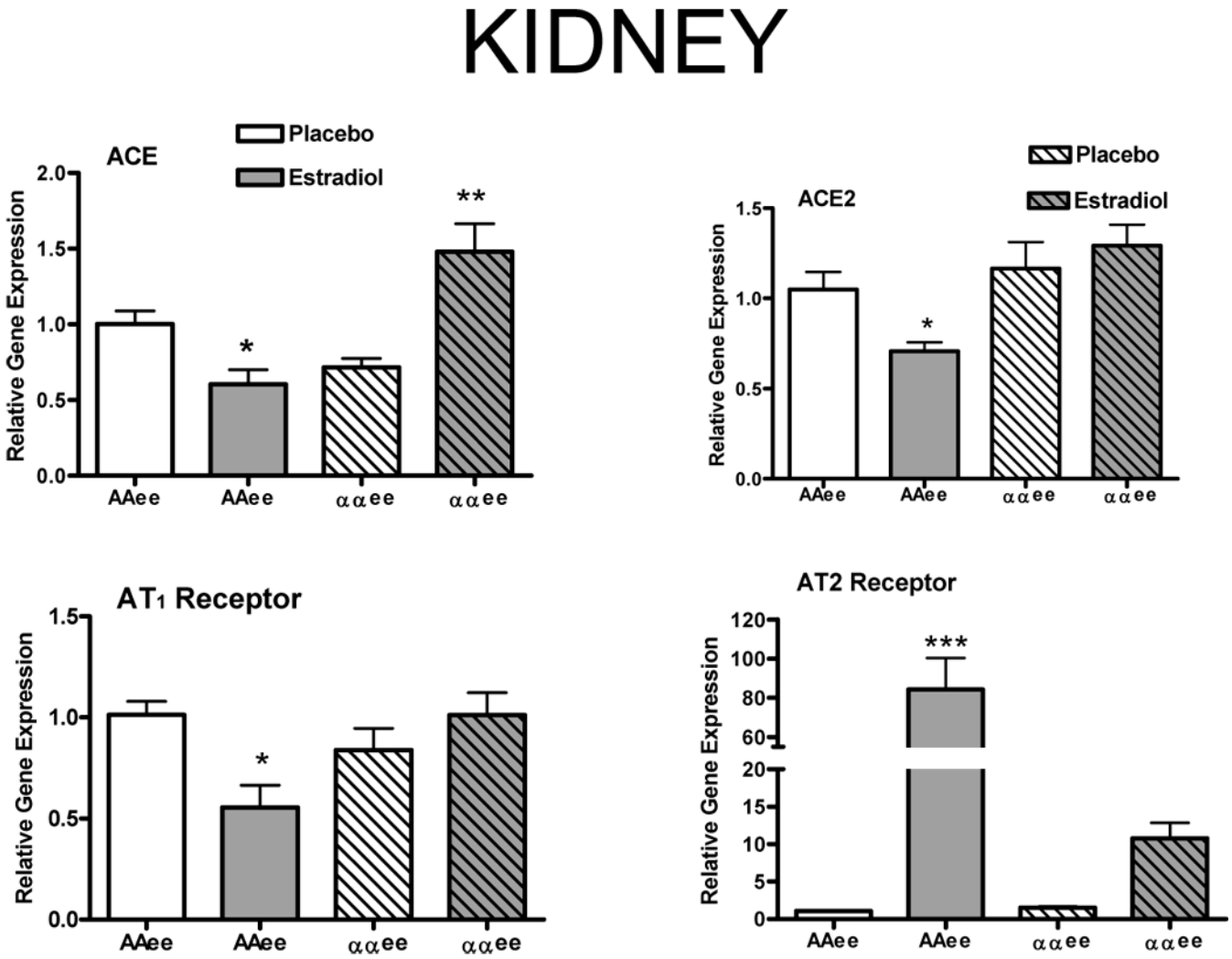

Figure 1.

ACE, ACE2, AT_1 receptor and AT_2 receptor mRNAs (n=7-8/group) in the kidney of ovariectomized ApoE knock-mice (ee) with (AA) (solid bar) and without (αα) (crosshatch bar) the ERα receptor and either treated with placebo (white bar) or 17-β estradiol (grey bar). Values expressed as mean ± SEM. Statistical analysis using the 2-way ANOVA revealed a significant interaction of estrogen ($p < 0.05$) with ER for ACE, ACE2, AT_1 receptor and AT_2 receptor mRNA. Fisher LSD multiple comparisons are indicated by * $p < 0.05$ vs placebo; ** $p < 0.01$ vs placebo; ***$p < 0.001$ vs placebo.

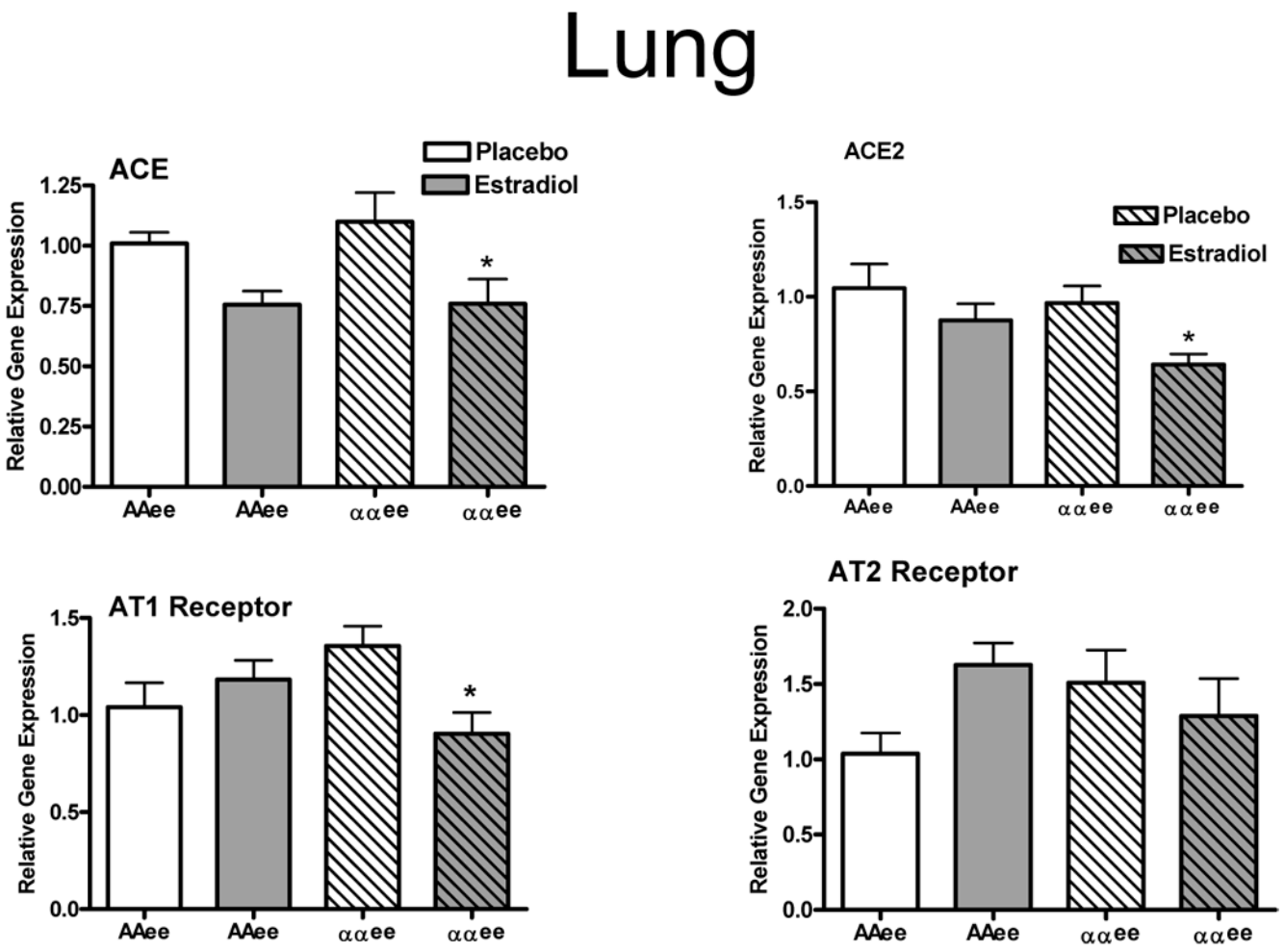

Figure 2.

ACE, ACE2, AT_1 receptor and AT_2 receptor mRNAs (n=7-8/group) in the lung of ovariectomized apoE knock-out mice (ee) with (AA) and without (αα) the ERα receptor and either treated with placebo (white bar) or 17-β estradiol (grey bar). Values are expressed as mean ± SEM. Statistical analysis using the 2-way ANOVA revealed a significant interaction of estrogen ($p < 0.05$) with ER for AT_1 receptor mRNA, whereas there was only an effect of 17-β estradiol that was localized to ααee for ACE and ACE2 mRNA. * $p<0.05$ vs placebo.