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Decidualized Pseudopregnant Rat Uterus Shows Marked Reduction in Ang II and Ang-(1-7) Levels

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Abstract

Previous studies showed that angiotensin (Ang) II and Ang-(1-7) concentrations were reduced in the implantation site at day 7 of pregnancy in Sprague-Dawley rats as compared to the site immediately adjacent to it, which does not have the embryo attached, clearly showing the importance of the blastocyst in the regulation of renin-angiotensin system (RAS).

Objective—The objective of this study was to evaluate the regulation of the RAS in the decidualized uterus in the pseudopregnant rat, a model without the presence of a conceptus.

Methods—Ovariectomized, adult female rats were sensitized for the decidual cell reaction with steroid treatments; decidualization was induced by oil-injection of the right horn; the left horn served as a control. The uterine content of Ang I, Ang II, and Ang-(1-7) was examined in the decidualized and non-decidualized uteri.

Results—Both Ang-(1-7) and Ang II and ACE and ACE2 mRNA were significantly reduced in the decidualized horn as compared to the non-decidualized horn. Immunocytochemical characterization of Ang II, Ang-(1-7), ACE and ACE2 demonstrated that Ang-(1-7), Ang II, and ACE2 polarize to the anti-mesometrial pole with decidualization.

Conclusion—The decidualization process elicits marked reduction in uterine Ang II and Ang-(1-7) content as compared to the non-decidualized horn. The differential immunocytochemical expression of Ang II and Ang-(1-7) with ACE2, but not ACE in the anti-mesometrial pole of the decidualized horn may favor the formation and action of Ang-(1-7) in the anti-mesometrial pole, an area which plays a role in triggering the decidualization process.

Keywords

angiotensin peptides; uterus; decidualization; ACE; ACE2; pseudopregnancy

INTRODUCTION

The outcome of pregnancy depends greatly on the success of the implantation process. Blastocyst implantation is a complex process that requires synchronized interaction of the competent blastocyst and the receptive uterus. One of the first signs of implantation is an increase in endometrium vascular permeability, which is localized to the sites where the

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blastocysts attach. Once the blastocyst is attached, the decidual cell reaction occurs. The onset of decidualization can be induced by embryo attachment or an artificial means, i.e. air bubble or bolus injection of oil after exposure of the uterus to an appropriate hormonal environment of progesterone and 17-β estradiol. [1] During this period, the uterus undergoes increases in endometrial permeability and angiogenesis; the decidual cells undergo proliferation, differentiation and apoptosis.[2;3] Cycloxygenase derived prostanoids for example participate in the angiogenesis during implantation and decidualization by regulating VEGF signaling. [4] Caspases and apoptotic protease activating factor 1(APAF) are some of the important proteases responsible for apoptosis.

Recently we described the renin-angiotensin system (RAS) in early pregnancy at day 5 and 7 of gestation and showed 1) that both the implantation site and the inter-implantation sites showed decreased content of Ang II as compared to the virgin uterus at both days 5 and 7 of gestation; 2) that the implantation site showed independent regulation from the inter-implantation site with an increase in Ang II at day 5 of gestation and a decrease in both Ang II and Ang-(1-7) at day 7 of gestation in the implantation site; and 3) that there was overall up-regulation of ACE and ACE2 mRNA in both the inter-implantation and implantation sites as compared to the virgin uterus but down-regulation of both mRNAs occurring in the implantation site as compared to the inter-implantation site. [5] The previous studies clearly showed the importance of the blastocyst in the regulation of the implantation site RAS. [5]

From these studies it appears that the regulation of the RAS in early pregnancy can be time, hormone, and blastocyst dependent. The pseudopregnant rat is a tool that can be used to evaluate the regulation of the RAS and its relationship to mechanisms that are involved in the decidualization process in the absence of a conceptus. We anticipate that the inter-implantation site would be regulated most comparably to the decidualized uterus. The objective of this study is to evaluate the regulation of the RAS in the decidualized uterus in the pseudopregnant rat.

METHODS

Animals

Adult 9-week-old Virgin female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley Inc (Indianapolis, IN, USA). The animals were housed individually under a 12-h light/dark cycle in an AAALAC-approved facility. All protocols were approved by the Animal Care and Use Committee of Wake Forest School of Medicine and are in compliance with NIH guidelines.

Surgical procedures

Ten week old female rats were anesthetized with 2% isoflurane and underwent bilateral ovariectomy through flank incisions. After a 5 day recovery period, ovariectomized rats were treated with subcutaneous injections of hormones [progesterone (P) 1–4 mg or 17β-estradiol (E2) 0.1–0.3 μg] which sensitized the uterus for optimal decidualization, as previously described.[2] On day 8 of hormone treatment, animals were anesthetized with 2% isoflurane and the right flank incision was reopened. After exposing the uterus, a single bolus of 0.2 mL sesame oil was injected into the right uterine lumen and a small ligature was placed around the needle insertion site to prevent leakage. The left horn was not infused and served as a control. Five days later, the animals were sacrificed by decapitation, the entire uterine horns were quickly removed and weighed, and either snap-frozen on dry ice for peptide and mRNA analysis or fixed in 4% paraformaldehyde for immunohistochemical analysis.

Uterine permeability

In an additional group (n=6) of similarly treated animals uterine permeability was assessed. Animals were anesthetized with isoflurane and 2 μCi of [^{125}I]-BSA/100g body weight in 0.2 mL saline was injected into the heart. [6;7] Sixty minutes later, the animals were decapitated and trunk blood was collected. The uterus was removed, cleaned and radioactivity in the infused and non-infused uteri was counted. Radioactivity in samples of serum and skeletal muscle were also counted. A permeability index of labeled albumin was determined by dividing the tissue radioactivity (cpm/mg) of uterus by the muscle radioactivity (cpm/mg).

Immunohistochemistry

After 24 h in fixative the uterus was sliced, and the tissue was left in 4% paraformaldehyde for an additional 12 h and then transferred to 70% ethanol. Immunocytochemical distribution of Ang II, Ang-(1-7), ACE, and ACE2 was obtained using the avidin-biotin method, as previously published [8;9]. The primary antibodies used were an affinity-purified rabbit polyclonal antibody to Ang-(1-7) (1:25 dilution), an affinity purified polyclonal rabbit antibody to Ang II (Phoenix Pharmaceutical, Inc, Phoenix, AZ) (1:750 dilution), a monoclonal antibody to ACE2 produced by the Hypertension and Vascular Research Center (1:150 dilution), and a purified mouse monoclonal antibody to ACE (Millipore Company, Billerica, MA) (1:25 dilution). The secondary antibody was biotinylated goat anti-rabbit and goat anti-mouse (Vector Laboratories, Burlingame, CA), (1:400 dilution) for Ang-(1-7), Ang II, and ACE2 and for ACE, respectively. Sections were stained with 3,3′-diaminobenzidine (DAB, Sigma-Aldrich Chemical Co. St. Louis, MO) in Tris-buffered saline (0.05mol/L, pH-7.6–7.7), and counterstained with hematoxylin before being dehydrated and mounted. To validate the staining procedure, uterus sections were incubated with the secondary antibody alone without the primary antibody and by pre-absorption of the antibody with 10 μM Ang-(1-7) and Ang II (Bachem, San Carlos, CA), ACE2 peptide (GenScript Corporation, Piscataway, NJ), and ACE (Millipore Company, Billerica, MA) as previously described. [10–12] Quantification of the staining was done on a Leica light microscope (DM4000B, Leica Microsystems, Wetzlar, Germany). Illumination settings were held constant for image capture session (Retiga 1300R camera, QImaging, Surrey, BC, Canada; SimplePCI v6.0, Compix Inc., Cranberry Twp., PA), and image channel input levels were windowed (50–255) uniformly in Adobe Photoshop (CS4 v11.0, Adobe Systems Inc., San Jose, California). With the lasso tool, the ABC stained regions were carefully selected at the mesometrial and anti-meosmetrial regions and the number of pixels noted and expressed as a mesometrial to anti-mesometrial ratio.

Tissue and plasma measurement of angiotensin peptides

Frozen uterus was rapidly weighed and homogenized, as described by Allred et al. [13] Tissue homogenates were extracted using Sep-Pak columns. [13–15] The eluate was divided for three radioimmunoassays (RIAs) [Ang I, Ang II and Ang-(1-7)] and the solvent evaporated. Ang I was measured using Peninsula RIA kit (San Carlos, CA). Ang II was measured using the Alpco Diagnostic kit, (Windham, NH). Ang-(1-7) was measured using the antibody produced by our laboratory.[14;15] Blood was collected in a cocktail of inhibitors at the time of sacrifice and samples frozen for RIAs of angiotensin peptides.

Reproductive hormone assays

17-β estradiol and progestereone were measured by radioimmunoassay (Polymedco, Courtland Manor, NY) after ethanol extraction.

RNA isolation and reverse transcriptase/real-time polymerase chain reaction

RNA was isolated from tissue, using the TRIZOL reagent (GIBCO Invitrogen, Carlsbad, CA), as directed by the manufacturer. The RNA concentration and integrity were assessed using an Agilent 2100 Bioanalyzer with an RNA 6000 Nano LabChip (Agilent Technologies, Palo Alto, CA). The primer/probe sets for angiotensin converting enzyme (ACE), renin, neprilysin (NEP), angiotensinogen, cyclooxygenase 1 and 2 (COX-2, COX-1), vascular endothelial growth factor A (VEGF-A), apoptotic protease activating factor 1 (APAF), caspase 3 and caspase 9 were purchased from Applied Biosystems, while ACE2 was designed by the laboratory (forward primer 5′-CCCAGAGAACAGTGGACCAAAA-3′; reverse primer 5′-GCTCCACCACACCAACGAT-3′; and probe 5′-FAM-CTCCCGCTTCATCTCC-3′). All reactions were performed in triplicate and 18S ribosomal RNA served as an internal control. The results were quantified as Ct values, where Ct was defined as the threshold cycle of PCR at which amplified product was first detected, and defined as relative gene expression (the ratio of target/control). Neprilysin is a metalloprotease that converts Ang I to Ang-(1-7), thus its measurement with ACE and ACE2 contributes to understanding the balance between Ang II and Ang-(1-7).

Statistical analysis

Comparisons between the groups were performed using one way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test or unpaired Student's t-test (GraphPad Software, San Diego, CA). A p value less than 0.05 was considered statistically significant. All values are presented as mean±SEM.

RESULTS

The baseline circulating levels of angiotensin peptides (Ang I:144.1 ±15.4, Ang II: 21.9 ± 16, Ang-(1-7): 89.1 ± 11.5 pmol/L, n =16–18), 17-β estradiol (48.7 ± 25 pg/ml, n=8) and progesterone (157 ± 26 ng/mL, n=11) in pseudopregnant rats characterizes the systemic environment that is common for the two uterine hornes. Figure 1A shows an H&E staining of the non-infused and decidualized horn. Five days after oil injection uterine horn weight increased 9-fold as compared to the non-infused horn (1543 ± 133 mg vs 171 ± 7 mg, $p<0.00001$, n=21) and permeability index increased 2-fold (7.1 ± 0.52 vs 3.1 ± 0.21, $p<0.001$, n=12). Other markers of decidualization, including VEGF-A (n = 9–10) and Cox-2 mRNAs (n = 9–10) (Figure 1D–E) were increased in the infused horn, whereas markers of apotosis, APAF (1.0 ± 0.05 vs 1.0 ± 0.4, n = 8), caspase 3 (1.04 ± 0.06 vs 1.18 ± 0.08, n = 8) and caspase 9 (1.0 ± 0.07 vs 1.1 ± 0.05, n = 8) mRNAs were unchanged.

RAS components in the decidualized and non-decidualized uterine horns

Figure 2A shows the angiotensin peptide profile in decidualized uterine horn as compared to the non-decidualized (non-infused) horn. Ang-(1-7) is the predominant angiotensin peptide in the non-infused uterine horn (Ang-(1-7) > Ang II > Ang I), but in the oil-infused horn (Ang-(1-7) = Ang II > Ang I). The concentration of Ang I, Ang II and Ang-(1-7) (n = 12) are markedly decreased in the oil-infused uterine horn as compared to non-infused. The changes in peptides are associated with marked down-regulation of ACE and ACE2 mRNA in the decidualized horn (n = 8) (Figure 2B and 2C), whereas neprilysin mRNA (n = 8) is unchanged in the decidualized horn (Figure 2D). Renin and angiotensinogen mRNAs were too low to detect in the non-infused and oil-infused horns.

Immunohistochemical expression of Ang-(1-7), Ang II, ACE2, and ACE in the pseudopregnant decidualized rat uterus as compared to the non-infused uterus

Figure 3 shows the immunocytochemical distribution of Ang-(1-7), Ang II, ACE and ACE2 in cross sections of the non-decidualized vs the decidualized horn. In the non-infused horn, Ang-(1-7) and ACE2 are localized to the mesometrial pole, whereas Ang II and ACE are distributed throughout the horn. The mesometrial to anti-mesometrial ratio of staining quantified in Table 1 reflects the uniformity of staining for Ang II and ACE throughout the non-decidualized horn (ratios of 1.1 and 1.2, respectively) and the regionalization of the staining to the mesometrial pole for Ang-(1-7) and ACE2 (1.9 and 2.6, respectively). In the decidualized horn, the staining for Ang II, Ang-(1-7) and ACE2 shows more pronounced staining in the anti-mesometrial pole as compared to the mesometrial pole (M/AM ratios <1). ACE staining shows a greater prominence at the mesometrial pole in the decidualized horn.

At higher magnification, Ang-(1-7), Ang II, ACE and ACE2 staining is present in the luminal epithelial and glandular epithelial cells of the non-infused rat uterus (Figure 4 and 5). In the decidualized horn, the staining for Ang-(1-7) and Ang II is patchy in the luminal epithelium. In the glandular epithelium, the staining for Ang-(1-7) and ACE2 persists, while that for Ang II and ACE is less intense.

DISCUSSION

It is clear from the results of this investigation that the rat uterus expresses all three angiotensin peptides and three of the main generating enzymes for these peptides, ACE, ACE2 and NEP. In addition, the genes for ACE and ACE2 and the peptide content are down-regulated during the decidualization process. The observation of this phenomenon in the pseudopregnant rat reveals that the regulation occurs in the absence of trophoblastic contribution, since the blastocyst was not present. Because the systemic/hormonal environment is the same for the two uterine horns, it appears that the paracrine stimulation of the oil infused horn comprises a potent trigger of the decidualization process, which initiates the release of mediators that induce stromal cell proliferation and differentiation and increased permeability, processes that have been previously described as characteristic of decidualization.[1;3;16] Our data indicate that these mediators also inhibit the expression of RAS. This reduction of the RAS may be necessary for the early events of gestation.

The finding of reduced Ang peptides in the decidualized uterus is somewhat similar to what was found in the pregnant uterus at the time of implantation;[5] however, comparison of the two studies reveal important differences. The previous study in pregnant animals revealed 1) that both the implantation and the inter-implantation sites showed decreased content of Ang II as compared to the virgin uterus at both days 5 and 7 of gestation; 2) that the implantation site showed independent regulation from the inter-implantation site with an increase in Ang II at day 5 of gestation and a decrease in both Ang II and Ang-(1-7) at day 7 of gestation in the implantation site; and 3) that there was overall up-regulation of ACE and ACE2 mRNA in both the inter-implantation and implantation sites as compared to the virgin uterus but down-regulation of both mRNAs occurring in the implantation site as compared to the inter-implantation site. What the new study using the pseudopregnant rat uncovered is 1) that the decidualization process reduced the content of all three peptides not just Ang II and this was associated with marked down-regulation of ACE and ACE2 mRNA expression. Although we had anticipated that the inter-implantation site without the blastocyst attachment would be most comparable to the decidualized uterus of the pseudopregnant rat, our study revealed differences between the inter-implantation site and the decidualized uterus. Taken together the studies show that both the blastocyst and the decidualized environment exert independent control over the regulation of RAS components. In the normal pregnant animal,

this may be attributed to the paracrine influence of the attached blastocyst and/or the presence of trophoblast cells in the decidualized environment in the inter-implantation site. In the pseudopregnant animals, where there is no blastocyst or invading trophoblasts and the systemic hormonal environment is the same for both uteri, the paracrine environment of the various cell types in the decidualized uterus must release inhibitory factors that contribute to the down-regulation of the RAS. Consideration must be given to leukaemia inhibitory factor, IL-11, and prostaglandins which have been shown to be locally produced in the decidualized uterus. [17]

The two regions of the decidualized or pregnant uterine horn are programmed for different roles with the anti-mesometrial pole participating in implantation (embryo, oil), while the mesometrial pole is fundamental for placental blood flow. [18] Our studies revealed that there were regional differences in the immunocytochemical distribution of Ang-(1-7), Ang II, ACE and ACE2 in the non-infused and decidualized uterus. In the non-infused uterus, Ang-(1-7) and ACE2 showed more enhanced staining in the mesometrial pole; whereas staining for Ang II and ACE was distributed throughout the horn. In the decidualized uterus, Ang-(1-7), Ang II, and ACE2 were predominately localized to the anti-mesometrial pole, a finding consistent with their having a role in the regulation of implantation. Srivastava et al [19] reported that the anti-mesometrial decidua is formed by giant-sized polyploidy and closely packed cells, and this region shows the characteristics of an endocrine organ in that they secrete several hormones and growth factors. The more localized staining for Ang II, Ang-(1-7) and ACE2 in the anti-mesometrial pole is consistent with a pathway that may favor the formation of Ang-(1-7), suggesting that it may play a prominent role in triggering decidualization and the implantation process. On the other hand, the mesometrial pole showed faint staining for Ang II, Ang-(1-7) and ACE2. This is the region where blood vessels gain access to the placenta and these cells secrete α-macroglubulin, which binds growth factors. Our data would suggest that the RAS plays a lesser role in the mesometrial pole. Future studies should dissect the uterus into mesometrial and anti-mesometrial subregions before measuring the peptide or gene content, to reveal whether there are localized concentrated areas of RAS peptides within the decidualized uterus.

In addition, radioimmunoassay of the peptides and immunostaining revealed that both active peptides Ang II and Ang-(1-7) were in the uterus and co-localized. Because the peptides have antagonist actions against each other with Ang II, showing proliferative, angiogenic, and vasoconstrictive properties [20;21] and Ang-(1-7) showing antiproliferative, anti-angiogenic and vasodilator properties [22;23] the predominate peptide may exert the predominate effect. Thus in the non-decidualized uterus Ang-(1-7) is the predominate peptide expressed, but in the decidualized uterus Ang II and Ang-(1-7) are present in equal concentration, thus suggesting an equal balance their actions.

One would anticipate that a reduction in expression would indicate a lesser role for the RAS in decidualization. Yet studies by Squires and Kennedy suggest a requirement for ACE and Ang II production for the expression of decidual cell reaction in rats.[24] Treatment of the uterine horn with the ACE inhibitor enalaprilat resulted in reduced permeability and uterine weight, suggesting the dependency of the decidualization on the production of Ang II. Addition of Ang II with the enalaprilat treatment, however, did not reverse the effects. The authors state that the failure of Ang II to reverse the effects of enalaprilat is most likely due to an insufficient dose or permeability factors that may have limited Ang II actions. In spite of this finding, their overall conclusion was that local production of Ang II is required for decidualization. This conclusion was supported by *in vitro* studies in which endometrial stromal cells in culture treated with enalaprilat inhibited alkaline phosphatase activity, a biochemical measure of stromal cell growth. The inhibition was reversed with Ang II treatment. Previous studies demonstrated that Ang II increases uterine decidualization,

endometrial vascular permeability, and stimulates angiogenesis. [20;21;24] The role of Ang-(1-7) in the decidualization process has not been addressed, but its previously described antiangiogenic [22;25] and antiproliferative [26] actions make it a candidate to counterbalance the actions of Ang II in this site. Our studies showing lower levels of Ang II and Ang-(1-7) in the decidualized uterus do not eliminate the possibility of local regional enrichment of peptide content, where the peptides could actively participate in decidualization.

The reduction in Ang II and Ang-(1-7) during decidualization differs from the enhanced expression of kallikrein,[27] PGE and PGF2α [24] and PLA2, [28] all of which have been implicated as key mediators of endometrial vascular permeability and decidualization. Our finding of increased COX-2 mRNA is consistent with the increase in PGE and PGF2α in the decidualized horn.[6] In addition, VEGF-A mRNA was increased in the decidualized horn as compared to the non-decidualized horn. VEGF-A was first characterized as a vascular permeability factor and its increase is consistent with the increase in permeability of the decidualized horn. Its actions in regard to angiogensis may also come into play in the decidualized horn. These studies illustrate that there may be multiple factors that contribute to the decidualization process or a complex network of regulatory and growth factors that participate in decidualization. From our studies, it appears that the mRNA for markers of apoptosis, including caspase 3 and 9, and APAF, were unchanged with decidualization. This may indicate that they are not involved in the decidualization process, that the process of cell death may be localized to either the mesometrial or anti-mesometrial poles and the effects would be better demonstrated by dissection of the separate poles, or that their involvement may be elicited at different times in the decidualization process.

In summary, decidualizaton is a process that is linked with maternal-fetal relationships, whereby it can limit trophoblast invasion, provide nutrition for the embryo and provide endocrine/autocrine secretions that promote growth and development. In these studies we have demonstrated that the uterine RAS is inhibited during the decidualization process. However we also showed that there is polarization of the expression of the peptides to the anti-mesometrial pole, a finding that suggests that within the decidualized uterus, there may be regions exerting a functional role consistent with implantation rather than blood flow regulation. Future studies are warranted to dissect the role of the RAS in these micro-uterine environments.

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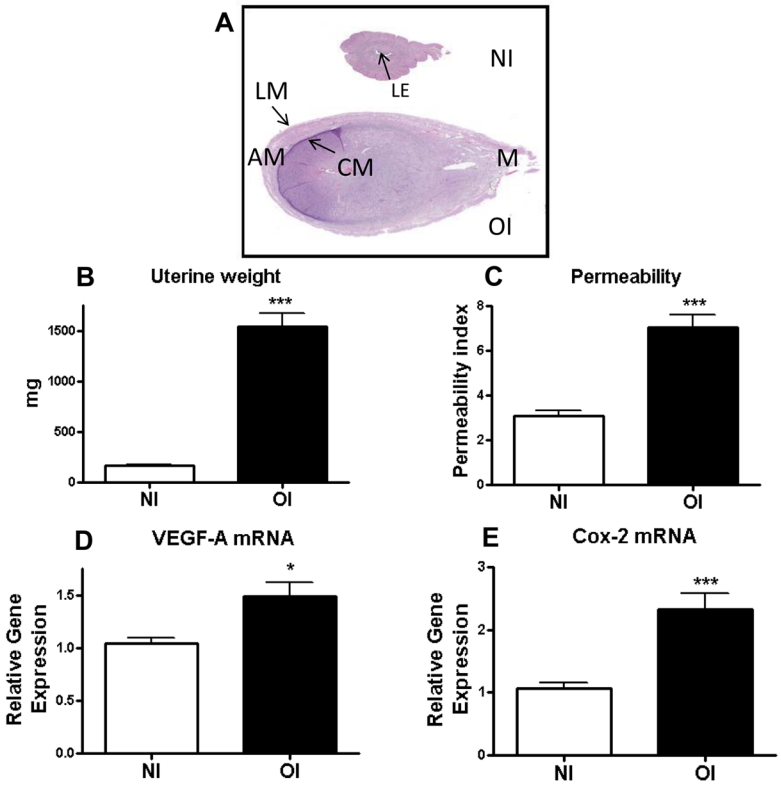

Figure 1.

A. H & E staining of the non-infused (NI) and oil-infused (OI) uterine horns of the pseudopregnant rats. M = Mesometrial pole, AM = Anti-mesometrial pole, LE = Luminal epithelial, CM = circular smooth muscle, LM = Longitudinal smooth muscle. Comparison of uterine weight (B), permeability (C), VEGF-A mRNA (D), and COX-2 mRNA (E) in the NI and OI uterine horns. * $p<0.05$ and *** $p<0.001$ vs NI.

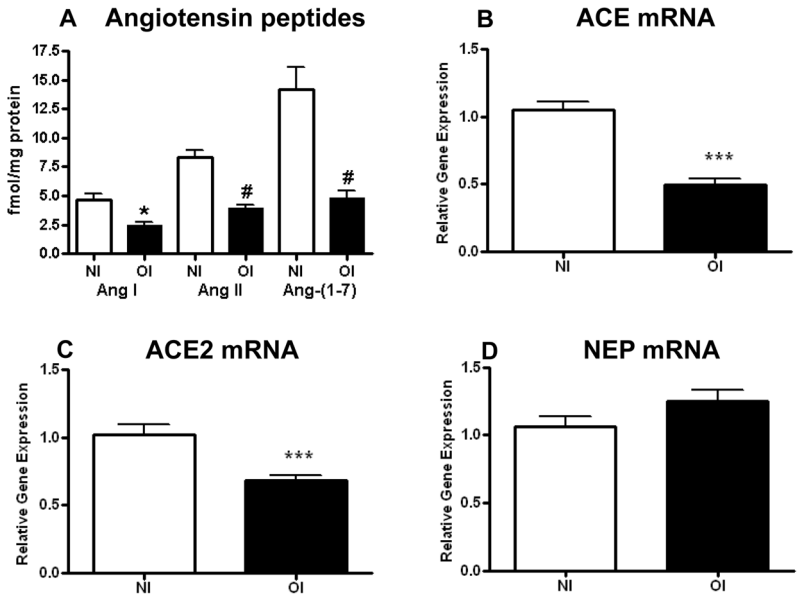

Figure 2.

A. Concentration of angiotensin peptides (Ang I, Ang II, and Ang-(1-7) and relative gene expression of (B) ACE mRNA,(C) ACE2 mRNA, and (D) neprilysin (NEP) mRNA in oil-infused (OI) or non-infused (NI) uterine horns of pseudopregnant rats. Decidualization is associated with marked reductions in Ang I, Ang II, and Ang-(1-7) and ACE and ACE2 mRNAs without a change in NEP mRNA. * $p<0.05$, # $p<0.01$, *** $p<0.001$ vs NI horn.

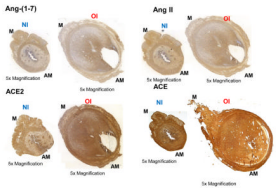

Figure 3.

Immunohistochemical expression of Ang-(1-7), Ang II, ACE2, and ACE in the pseudopregnant decidualized (OI) rat uterus as compared to the non-infused (NI) uterus. The NI horn is presented at x 5 magnification, whereas the OI horn is presented at x1 magnification. In the NI horn, Ang-(1-7) and ACE2 show more enhanced staining in the mesometrial (M) pole; whereas staining for Ang II and ACE is distributed throughout the horn. Decidualization is associated with more concentrated staining of Ang-(1-7), Ang II, and ACE2 at the anti-mesometrial pole (AM). There is more intense staining of ACE in the mesomemetrial pole (M).

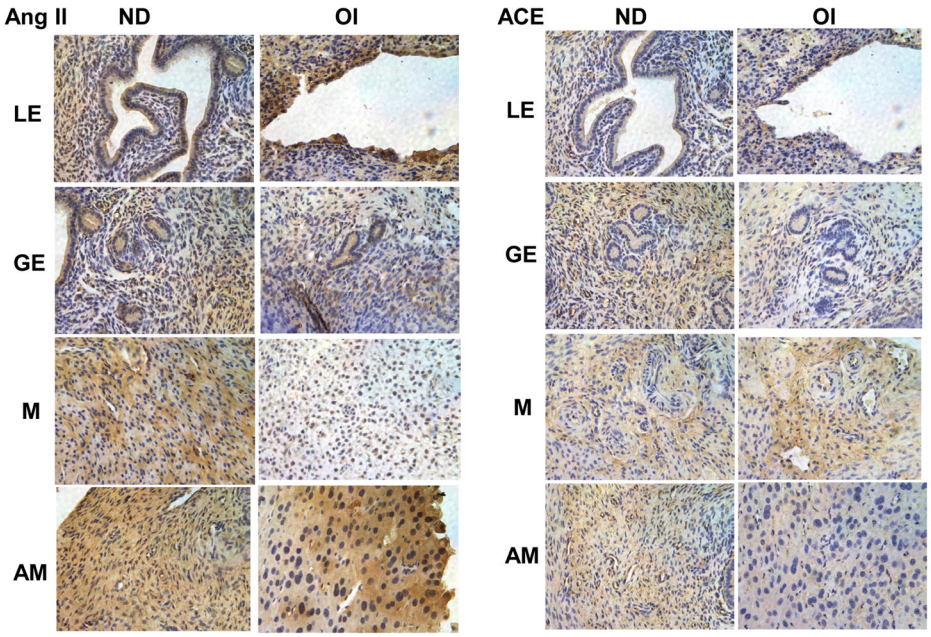

Figure 4.

Immunohistochemical expression of Ang II and ACE at greater magnification (x40) illustrating their presence in the luminal epithelium (LE), glandular epithelium (GE), mesometrial pole (M) and anti-mesometrial pole (AM) of the pseudopregnant decidualized (OI) rat uterus as compared to the non-infused (NI) uterus. Ang II and ACE staining is present in the LE and GE of the non-infused rat uterus. Also, Ang II and ACE are distributed throughout the horn. In the OI horn, while Ang II is localized in the AM pole ACE is localized more at the M pole. Notice the patchy and attenuated LE in OI indicating collapse of the LE.

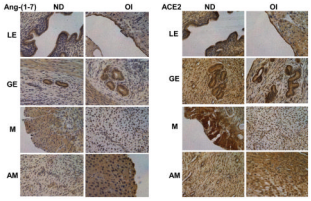

Figure 5.

Immunohistochemical expression of Ang-(1-7) and ACE2 at greater magnification (40X) illustrating their presence in the luminal epithelium (LE), glandular epithelium (GE), mesometrial pole (M), and anti-mesometrial pole (AM) of the pseudopregnant decidualized (OI) rat uterus as compared to the non-infused (NI) uterus. Ang-(1-7) and ACE2 staining is present in the LE and M of the non-infused rat uterus. In the OI uterus more intense Ang-(1-7) and ACE-2 staining is seen in the AM pole. Notice the patchy and attenuated LE in OI indicating collapse of the LE.

Table 1

Semi-quantification of the immunohistochemical staining distribution at the mesometrial and the anti-mesometrial poles

peptide/enzyme Uterine horn	Ang II NI	Ang II OI	Ang-(1-7) NI	Ang-(1-7) OI	ACE2 NI	ACE2 OI	ACE NI	ACE OI
M/AM	1.1 ± 0.1	0.3 ± 0.1**	1.9 ± 0.3	0.3 ± 0.05**	2.6 ± 0.4	0.3 ± 0.1**	1.2 ± 0.1	6.3 ± 2.2*

NI = Non-induced; OI = Oil-induced; M= Mesometrial pole; AM= Anti-mesometrial pole.

M/AM = Ratio of staining intensity in M to AM. n = 3.

Values are mean ± SEM.

** p<0.001 vs NI;

* p<0.05 vs NI