Norepinephrine exposure restrains endometrial decidualization during early pregnancy

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Abstract

Previous studies have focused on the role of norepinephrine on arrhythmias, generalized anxiety disorder, and cancer. This study aimed to investigate the effect of norepinephrine on endometrial decidualization. Artificial decidualization and norepinephrine-treated mice were established in vivo. In vitro, human endometrial stromal cells were treated with MPA and cAMP to induce decidualization. Decidual markers and important signaling molecules during decidualization were detected using quantitative real-time PCR and Western blot. RNA sequencing was performed to determine related signaling pathways. Exposure to excess norepinephrine significantly restricted the induced expression of decidualized markers Dtprp, BMP2, WNT4, and Hand2 in mice. In vitro, 10 µM norepinephrine markedly downregulated the expressions of prolactin, IGFBP1, and PLZF, which are the specific markers of decidual stromal cells during decidualization. The gene set enrichment analysis showed a significant enrichment in neuroactive ligand–receptor interactions of norepinephrine treatment group. The α1b-adrenergic receptor expression was upregulated by norepinephrine. Interestingly, norepinephrine did not inhibit the expression of IGFBP1 in endometrial stromal cells after silencing α1b-adrenergic receptor, while significantly suppressed the induced decidualization with overexpression of α1b-adrenergic receptor. When α1b-adrenergic receptor was activated, endometrial p-PKC was significantly increased under post-treatment with norepinephrine in vivo and in vitro. In addition, norepinephrine treatment inhibited embryo and fetal development using a normal pregnancy model. Therefore, norepinephrine exposure inhibited endometrial decidualization through the activation of the PKC signaling pathway by upregulating α1b-adrenergic receptor. Our study could explain some female reproductive problems due to stress and provide some novel strategies for this disorder.

Key Words
- norepinephrine
- endometrial decidualization
- stress
- α1b-adrenergic receptors

Introduction

In modern society, young women face extensive social and life pressures. Long-term sustained or acute stress can lead to a pathological state that impacts the reproductive system (Frazier et al. 2018, Wang et al. 2019). Physiological or psychological stressors that induce the sympathetic nervous system’s ‘fight-or-flight’ response can rapidly increase the norepinephrine (NE) level (Tank & Lee Wong 2015). However, the effect of NE on reproductive toxicity in early pregnancy had not been reported.
Endometrial decidualization is an important step in embryonic development and fetal growth. In particular, this process is critical in establishing maternal–fetal communication and provide a good nutritional environment for embryonic development (Ng et al. 2020). Deficient decidualization can cause restricted embryonic growth (Schatz et al. 2016). Analyzing the regulation of NE on decidualization will help us understand the influence of stress on endometrial decidualization and fetal growth.

In early pregnancy, endometrial stromal cells (ESCs) undergo extensive proliferation and differentiation and transform into secretory decidual stromal cells, leading to decidualized endometrium. The decidual/trophoblastic PRL-related proteins (Dtrprp) (Kimura et al. 2001), Bmp2 (Li et al. 2007), Wnt4 (Li et al. 2013), and Hand2 (Murata et al. 2020) are well-known markers for uterine stromal differentiation during decidualization in mice. In vitro, in response to P4-mediated activation of cyclic adenosine monophosphate (cAMP) signaling, human ESCs undergo vast expansion and differentiation. Prolactin (Eyal et al. 2007), IGFBP1 (Tabanelli et al. 1992), and promyelocytic leukemia zinc finger encoded by Zbtb16 (PLZF) (Brosens & Gellersen 2006) have been found as well-established stromal differentiation markers during ESC decidualization. Besides, forkhead box O1 (FOXO1) (Buzzio et al. 2006, Park et al. 2016) and CCAAT enhancer-binding protein β (C/EBPβ) (Mantena et al. 2006, Wang et al. 2010, Tamura et al. 2018) are core decidual transcription factors that control cell cycle exit of ESCs in response to differentiation signals, activating expression of decidual marker genes, such as PRL and IGFBP1.

In this study, we hypothesized that NE inhibited endometrial decidualization. To test this hypothesis, a series of in vivo and in vitro experiments were conducted with NE to mimic stress. Sesame oil was injected into the uterine horn of mice to induce endometrial decidualization (Andrade et al. 1996); therefore, its influence on the embryo may be ruled out. Further, it was intraperitoneally injected with NE to observe its effect on decidualization. In vitro, primary stromal cells were isolated from human endometrium, treated them with cAMP and medroxyprogesterone acetate (MPA) for 48 h to induce decidualization (Salsano et al. 2017), and simultaneously added with NE to observe its effect on decidualization of human ESCs. Our results demonstrated that NE treatment inhibited endometrial decidualization in early pregnant mice through the activation of the PKC signaling pathway by upregulating α1b-adrenergic receptor (α1b-AR). This study would provide treatment ideas for endometrial decidualization disorders caused by stress.

Materials and methods

Animals and tissue collection

CS7 female and male mice, aged 8–10 weeks, were obtained from the Shanghai Laboratory Animal Center (Shanghai, China). All mice were caged at a controlled temperature of 22–25°C under a 14 h light:10 h darkness photoperiod. The Human Research Ethics Committee of Obstetrics and Gynecology Hospital of Fudan University approved the study protocol. To observe the effects of NE on normal female pregnancy, adult female mice were allowed to mate with fertile males to achieve pregnancy. The day on which sperms were detected in the vaginal smear was defined as gestational day 0.5 (GD 0.5) of pregnancy. Pregnant female mice were divided into two groups according to the dosage in previous articles (Wang & Yang 2007): the NE group was injected with 5 mg/kg NE intraperitoneally daily from GD 5.5 for three consecutive days, whereas the control group was injected with the same volume of PBS. Uterine tissue was collected from each pregnant mouse on GD 8.5 and GD 13.5. The number and size of the embryos were recorded.

Artificial decidualization induction

To examine the effects of NE on endometrial decidualization, adult female mice were allowed to mate with vasectomized adult males to achieve pseudopregnancy. Artificial decidualization was induced by infusing 25 μL of sesame oil intraluminally into one uterine horn on GD 3.5 of pseudopregnancy; the contralateral uninjected horn was considered as the control (Andrade et al. 1996). Pseudopregnant female mice were divided into two groups (NE and control) treated identically with that of the pregnant mice. NE (5 mg/kg) was injected daily for 3 days, beginning at 24 h after the oil stimulation or saline injection that served as the control. These mice were sacrificed at GD 7.5 of pseudopregnancy. Decidualization was confirmed by weighing the uterine horns and histological examination of the uterine sections.

Culture of human ESCs

Human endometrial tissue was obtained from 12 healthy volunteers of reproductive age. The Ethics Committee of the Obstetrics and Gynecology Hospital of Fudan University approved the study protocols. All participants provided written informed consents. Human ESCs were
isolated as previously described (Li et al. 2011). Briefly, the endometrial tissue was minced and digested with collagenase type IV (0.1%; Sigma-Aldrich) and 15 units/mL of deoxyribonuclease (Sigma-Aldrich) for 30 min at 37°C. The dispersed cells were passed through a 40-µm filter (BD Biosciences). After centrifugation at 800 g for 5 min, ESCs were resuspended and maintained in DMEM/F-12 containing 10% fetal bovine serum (FBS; Gemini, Calabasas, USA), 100 IU/mL penicillin (Sigma), 100 µg/mL streptomycin (Sigma), and 1 µg/mL amphotericin B (Sangon, Shanghai, China) at 37°C in 5% CO₂. The cultured stromal cells were 95% pure, as determined using vimentin staining.

**Induction of in vitro decidualization and cell viability assay**

To induce decidualization, approximately 3 × 10⁶ cells were plated per well in 6-well culture plates. After reaching 70–80% confluency, cells were treated with 1 µM MPA (Sigma) and 0.5 mM N6, 2′-O-dibutyryladenosine cAMP sodium salt (Sigma) for 48 h (Salsano et al. 2017). Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (Dojingo-Japan, CK04).

**Cell transfection**

Transfection of α1b-AR small-interfering (si)RNA. Human ESCs were transfected with α1b-AR siRNA or nontargeting siRNA (N.C. siRNA) (GenePharma, Shanghai, China) diluted in transfection medium. The sequence of α1b-AR siRNA was as follow: sense 5′-GGAGUCAUGAAGGAGAUG UTT-3′, antisense 5′-ACAUCUCCUAUGACUUCCCTT-3′. Transfection was performed using the Lipofectamine TM 3000 reagent (Invitrogen). The knockdown efficiency of α1b-AR in human ESCs was assessed using Western blotting.

Transfection of α1b-AR overexpression plasmid. All plasmids were purchased from PPL (Genebio Technology, Inc, Nanjing, China): α1b-AR overexpression plasmid pLenti-CMV-α1b-AR-GFP-Puro (Flag-α1b-AR) with a corresponding negative control (pLenti-CMV-GFP-Puro, vector). The aforementioned plasmids were transfected into human ESCs using the Lipofectamine TM 3000 reagent (Invitrogen) according to the manufacturer’s protocol. At 48 h post-transfection, the medium was gently exchanged to the new neuronal culturing medium supplemented with cAMP and MPA. After 48 h, cells were harvested for Western blot analysis.

**mRNA sequencing data analysis**

To explore the NE mechanism affecting decidualization, mRNA sequencing on induced ESCs treated with or without NE was performed to determine related signaling pathways. Sequenced reads were aligned to the human reference genome using the STAR software package. Exons from all gene isoforms were merged to create one metagene. The number of reads falling in the exons of this metagene was counted using HTSeq-count, and differential expression analysis was conducted using DE-Seq. P<0.05 were considered as the significance threshold. Volcano Plot and Heatmap analysis of differential genes was performed using the online gene set enrichment analysis (GSEA) (Subramanian et al. 2005, Anders & Huber 2010, Liu et al. 2020).

**Immunohistochemistry staining**

Decidual tissue sections were immunostained with primary antibodies against α1b-AR (1:200, Abcam) overnight at 4°C. On the next day, sections were incubated with a goat anti-rabbit (rabbit ABC detect kit, ZSbio, Beijing, China) secondary antibody at 37°C for 30 min. Next, they were stained with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. Control sections were run concurrently with experimental sections using nonspecific rabbit IgG and were similarly pretreated. Nonspecific staining was not detected in the controls.

**Western blotting analyses**

Whole-cell protein extracts were prepared by lysing cells in radioimmunoprecipitation assay buffer supplemented with proteinase inhibitors (Beyotime Institute of Biotechnology, Haimen, China). The protein yield was quantified using the bicinchoninic acid protein assay. After denaturation, equal amounts of protein were separated by SDS–PAGE before the wet-transfer onto polyvinylidene difluoride membranes. Nonspecific binding sites were blocked by incubating membranes with 5% BSA in tris-buffered saline with 0.1% Tween 20 (TBS-T) for 2 h. Then, membranes were rinsed and incubated overnight at 4°C with the following primary antibodies diluted in blocking buffer (5% BSA and 1× TBS-T): insulin-like growth factor binding protein 1 (IGFBP1, Abcam, ab181141), FOXO1 (Cell Signaling Technology, #2880), CCAAT enhancer-binding protein beta (CEBPB, Cell Signaling Technology, #90081), α1b-AR (Abcam, ab169523), α2b-AR (Abcam, ab151727), β2-AR (Abcam, ab182136), GADPH
(Abcam, ab8245), β-actin (Beyotime; AA128), protein kinase C (PKC)α (Abcam, ab32376), phospho (p)-PKCα/β II (Thr638/641) (Cell Signaling Technology, #9375), AKT (Cell Signaling Technology, #4691), and p-AKT (Ser473) (Cell Signaling Technology, #4060). Primary antibodies were removed by washing the membranes three times in TBS-T. Then, these membranes were incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (Beyotime; A0208 and A0216). After three washes with TBS-T, immunopositive bands on blots were visualized using an enhanced chemiluminescence detection system (Merck Millipore).

### Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA from uterine tissues or ESCs was extracted with TRizol reagent (Invitrogen) and then reverse-transcribed into the first strand complementary (c) DNA (Qiagen) according to the manufacturer’s instructions. The synthesized cDNA was amplified with specific primers and detected with SYBR Green (Qiagen) using a PRISM 7900 Sequence Detection System (Applied Biosystems). Triplicate samples were examined in each condition. A comparative threshold cycle (CT) value was normalized for each sample using the $2^{-\Delta\Delta CT}$ method. Primer sequences used for qRT-PCR are shown in Table 1.

### Statistical analyses

Prism 8 software (GraphPad) was used for all data analyses. All values are presented as means ± S.E.M. determined from at least three independent experiments. Analysis of measurement data was performed with Bonferroni’s post hoc test or repeated measures ANOVA with Bonferroni’s post hoc test. Analysis of count data was performed with Chi-square test. $P < 0.05$ was considered statistically significant.

### Results

#### NE exposure during early pregnancy restrains artificially induced decidualization

To assess the effects of NE on decidualization, an artificial decidualization model that excluded interference from embryo was used (Fig. 1A). At 96 h of oil stimulation, the deciduoma in the NE-treated group were much smaller than those in the control group (Fig. 1B). Decreased decidual tissue weight was observed following the NE exposure (Fig. 1B and C). As shown in Fig. 1D, HE staining of deciduoma confirmed the negative effect of NE on decidualization. We also detected the Dtppr, Bmp2, Wnt4, and Hand2 expressions, which are regarded as endometrial decidualization markers. Results in Fig. 1E, F, G and H showed that NE treatment inhibited the expression of all four molecules on GD 7.5 as compared with that of the control group (Fig. 1E, F, G and H). These results demonstrated that NE exposure restrained endometrium decidualization of mice.

#### NE inhibits decidualization of human ESCs induced by cAMP and MPA in vitro

Human ESCs were used to observe the effects of NE on decidualization process in vitro. First, the CCK-8 assay was used to determine whether treatment with 0.1-, 1-, or 10-µM NE affected cell viability. Compared to the control group, none of these NE concentrations affected cell viability (Fig. 2A).

To elucidate the effects of NE on stromal cell differentiation, cAMP and MPA were used to induce decidualization with or without 0.1-, 1-, or 10-µM NE. The expression of IGFBP1, a well-known decidualization marker (Tabanelli et al. 1992), was significantly increased after cotreatment of cAMP and MPA for 48 h. However, in the presence of 10µM NE, the upregulated expression of IGFBP1 decreased significantly (Fig. 2B). qRT-PCR results showed that 10-µM NE markedly downregulated
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NE inhibits decidualization by upregulating α1b-AR

To explore the mechanism of NE affecting decidualization, RNA sequencing was performed to determine the related signaling pathways. Human ESCs were induced decidualization with or without NE treatment in vitro. Differentially expressed genes were demonstrated between two groups according to a heatmap (Fig. 3A). The GSEA showed a significant enrichment in neuroactive ligand–receptor interactions of NE treatment group, compared to the control group (Fig. 3B). Previous reports indicated that α1b-, α2b-, and β2-AR were expressed in the mouse endometrium during decidualization (Wu et al. 2019). We found that α1b-AR, α2b-AR, and β2-AR were also expressed in human ESCs; however, only α1b-AR was upregulated after the NE treatment (Fig. 3C). Immunohistochemistry and Western blot were performed on artificially induced decidual tissue of mice, α1b-AR expression in the NE treatment group was found to be significantly upregulated as compared with that in the control group (Fig. 3D and E). Pretreating ESCs with 10-µM prazosin (an α1b-AR inhibitor) (Murata et al. 1999) for 2 h reversed the NE effect on IGFBP1 expression (Fig. 3F). Then, we knocked down α1b-AR by transfecting ESCs with α1b-AR siRNA. After verifying the silence efficiency, we found that NE did not inhibit the IGFBP1 expression after knocking down α1b-AR (Fig. 3G). Further, we over-expressed α1b-AR in ESCs to observe the induced decidualization. As shown in Fig. 3H, IGFBP1 expression was downregulated under the
decidualization induction after α1b-AR overexpression as compared with the vector group. Altogether, these results indicated that NE inhibited decidualization via upregulating α1b-AR.

**NE inhibits decidualization by activating PKC signaling pathway**

After activating α1b-AR, NE can further activate the PKC signaling pathway (Wang & Ashraf 1998). Previous studies showed that PKCa expression was downregulated during decidualization (Shyu et al. 1997a,b). Consistent with this, p-PKC level in ESCs was found to be reduced after cAMP and MPA treatment, which were used to induce decidualization. Treatment with NE recovered the p-PKC level downregulated by cAMP and MPA for 48 h, 10µM NE couldn’t reverse the downregulated expression of p-AKT (Fig. 4A). Phorbol myristate acetate (a PKC pathway agonist, 200 nM) (Abdullah et al. 2003) significantly inhibited the IGFBP1 expression, whereas in the pretreatment with Ro 31-8220 mesylate (a PKC pathway Inhibitor, 0.25 µM) (Besson et al. 2019), NE could not inhibit IGFBP1 expression (Fig. 4B). Western blot analysis also revealed a significantly increased p-PKC level in the decidua tissue of the NE-treated mice, as compared with the control group (Fig. 4C). These results indicated that the ability of NE to inhibit decidualization was achieved by activating the PKCα signaling pathway after upregulating α1b-AR.

**NE inhibits fetal growth in the normal pregnancy mice**

To assess the impact of NE on pregnancy outcomes, the fetal growth and post-implantation mice injected with NE were examined (Fig. 5A). At GD 8.5, the average weight of the embryo implantation site (IS) was decreased in mice injected with NE compared with control mice (Fig. 5B).
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Figure 3

NE inhibits decidualization by upregulating α1b-AR. (A) Heatmap result of an unsupervised hierarchical clustering of genes that is significantly different (P < 0.01) in induced ESCs treated with or without NE. Each column represents a group, and each row represents a gene. The heatmap indicates the level of row normalized gene expression. Red, high expression; Blue, low expression; a, ESCs treated with cAMP and MPA for 48 h; b, ESCs treated with cAMP, MPA, and 10μM NE for 48 h. (B) The gene set enrichment analysis of ESCs treated with cAMP and MPA with or without 10 μM NE. The expression of α1b-AR, α2b-AR and β2-AR in ESCs was analyzed by Western blot (C). Analysis was performed with Student's unpaired two-tailed t-test. ***P < 0.001. (D) The protein expressions of α1b-AR on artificially induced decidual tissue of mice between the NE treatment group and the control group were detected by Western blot. (E) Immunohistochemical analysis of α1b-AR protein expression on artificially induced decidual tissue in mice. (F) The protein levels of IGFBP1 in ESCs after pretreatment with or without prazosin for 30 min. Analysis was performed with one-way ANOVA and Bonferroni's post hoc test. *P < 0.05, ***P < 0.001. (G) Representative images of the protein levels of α1b-AR after transfection with N.C siRNA or α1b-AR siRNA. Western blot analysis of the protein levels of IGFBP1 as described. Analysis was performed with one-way ANOVA and Bonferroni's post hoc test. *P < 0.05, ***P < 0.001. (H) Overexpression of Flag-α1b-AR was sufficient to decrease IGFBP1 at the protein level in human ESCs. Analysis was performed with one-way ANOVA and Bonferroni's post hoc test. *P < 0.05, ***P < 0.001. A full color version of this figure is available at https://doi.org/10.1530/JOE-20-0479.

and C). However, NE administered during the early pregnancy had no effect on the number of embryos (Fig. 5D). Further, embryonic development was observed on GD 13.5. These results showed that the average weights of embryos and placenta were significantly lower in mice injected with NE than that in the control mice (Fig. 5E, F and G), whereas no significant difference was observed in the number of absorbed embryos between the two groups (Fig. 5H). These results demonstrated that NE exposure inhibited embryonic growth.

Discussion

Women with excessive stress have reduced fertility and abnormal embryonic development; however, the underlying mechanism remains unknown. Under stress, after activating the sympathetic nervous system, the adrenal medulla synthesizes and secretes NE, which plays a biological role by binding to adrenergic receptors (ARs) on target cells and mainly results in vasoconstriction, increased blood pressure, accelerated heart rate, and excitable heart (Tank & Lee Wong 2015, Pelliccia et al. 2017, Levy et al. 2018). NE is a catecholamine that functions both as a neurotransmitter and a hormone (Chrousos 2009). Previous studies on excess NE mainly focused on arrhythmias, generalized anxiety disorder, and cancer (Gellersen & Brosens 2014, Gardner et al. 2016, Ricon et al. 2019). Endometrial ARs were observed to be expressed on stromal cells in decidualization. It implied that endometrium could be regulated by NE. The NE effect on endometrial decidualization has not yet been reported. In this study, NE was found to restrain uterine decidualization in early pregnancy. This study focused on the effect of NE on endometrial decidualization and showed that NE inhibited uterine decidualization by activating the PKC signaling pathway via α1b-AR. This study will provide
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Decidualization is a crucial event in early pregnancy. After implantation, ESCs begin to undergo decidualization for subsequent development, placentation, and growth of the embryo. Improper decidualization can trigger pathological changes and lead to adverse pregnancy outcomes (Patterson et al. 2017, Chen et al. 2019, Zheng et al. 2020). The mouse model of artificial decidualization demonstrated that NE could downregulate related markers and affect uterine decidualization. In vitro, NE was also found to inhibit decidual markers of human ESCs induced by cAMP and MPA.

To explore the mechanism of NE in inhibiting human ESCs decidualization, omics screening and GSEA analyses were performed. These results showed a significant enrichment in neuroactive ligand–receptor interactions of NE treatment group. ARs include several subtypes, and different intracellular signaling pathways are activated when NE binds with these different subtypes (Bylund et al. 1994). Previous studies have shown that the endometrium expresses α1b-AR, α2b-AR, and β2-AR (Wu et al. 2019). Western blots suggested that the α1b-AR expression level increased after the NE treatment during decidualization of ESCs in vitro. Similarly, α1b-AR was activated by NE during endometrial decidualization in vivo. To confirm the role of α1b-AR, α1b-AR inhibitor, α1b-AR siRNA, and α1b-AR overexpression plasmid transfection technology with ESCs were used. Results of both approaches suggested that activation of α1b-AR may play an important role on NE effects. However, whether NE can directly bind to α1b-AR of ESCs still needs further exploration. In summary, NE could activate α1b-AR and inhibited endometrial decidualization both in vivo and in vitro.

In the PKC system, various hormones, neurotransmitters, etc. bind to receptors of cell membrane to activate phospholipase Cβ (PLC), which decompose phosphatidylinositol biphosphate (PIP2) into diacylglycerol (DAG) and 1,4,5-inositol triphosphate (IP3). They act as second messengers and then trigger a series of intracellular reactions (Newton 2018). Previous studies revealed that PKCa was present in the decidua, and the PKCa signaling pathway was inhibited during the endometrial decidualization in rats (Shyu et al. 1997a,b, Liu et al. 1998). Similarly, we found p-PKCα expression was downregulated after decidualization was induced in

![Figure 4](https://joe.bioscientifica.com)

Figure 4
NE inhibits decidualization by activating the PKC signaling pathway. (A) PKC, p-PKC, AKT and p-AKT levels in ESCs treated with or without cAMP, MPA and NE were detected by Western blot. Analysis was performed with one-way ANOVA and Bonferroni’s post hoc test. *P < 0.05, **P < 0.01. (B) Western blot analysis of the protein expressions of IGFBP1 in of ESCs pretreated with or without PKC pathway agonist (PMA, 200 nM) or inhibitor (Ro 31-8220, 0.25 μM). Analysis was performed with one-way ANOVA and Bonferroni’s post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001. (C) The protein expressions of PKC and p-PKC of mice treated with or without NE daily for 3 days were detected by Western blot. Data are the means ± s.e.m., n = 3, *P < 0.05, **P < 0.01, ***P < 0.001. A full color version of this figure is available at https://doi.org/10.1530/JOE-20-0479.
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human ESCs, and activating the PKC signaling pathway with PMA inhibited this decidualization. Because the PKC signaling pathway is activated when NE upregulates to α1b-AR (Wang & Ashraf 1998), we explored the effects of NE on the PKC signaling pathway in decidualized ESCs. Results showed that the p-PKC not total PKC expression level was significantly upregulated after the NE treatment both in vitro and in vivo, suggesting NE activated PKC signaling pathway. Furthermore, Pretreatment with Ro31-8220, a PKC pathway inhibitor, reverted the inhibitory effect of NE on decidualization. These data indicated NE inhibited endometrial decidualization by activating the PKC signaling pathway.

Unlike the PKC system, extracellular signals of PKA system are combined with corresponding receptors to induce intracellular responses by regulating the level of the second messenger cAMP. Previous studies showed that p-AKT was inhibited in endometrial decidualization (Wei et al. 2018), but the effect of NE on the PKA signaling pathway was still unclear. Neha J Patel found that NE could promote cell survival signaling in hippocampal neurons, significantly upregulated, whereas PI3K/Akt inhibitor LY294002 significantly downregulated p-Akt immunoreactivity with respect to vehicle-treated controls (Patel et al. 2010). However, Mar Coll demonstrated Droxidopa, an oral norepinephrine precursor, improved hemodynamic and renal alterations of portal hypertensive rats, Droxidopa-treated rats showed a decreased ratio of p-AKT/AKT in superior mesenteric artery (Coll et al. 2012). In our experiment, we also found that the expression of p-AKT was significantly downregulated in decidualized ESCs induced by cAMP and MPA, but 10 µM NE couldn’t increase the downregulated expression of p-AKT.

Using a normal pregnancy model, the size and weight of the embryo implantation site in the NE-treated group were found to be significantly lower than those in the control group on GD 8.5. Moreover, the weights of embryos and placentas in the NE-treated group were significantly lower than that in the control group on GD 13.5. These results suggested that NE exposure inhibited the growth and development of embryos and fetuses. This study provides new ideas for the clinical prevention and treatment of endometrial decidualization and intrauterine growth restriction caused by excessive stress. It is well-known that decidualization of the mouse endometrium occurs after embryo implantation (GD 4.5). In our experiment, we injected NE intraperitoneally after embryo implantation (GD 5.5–7.5) to avoid the direct impact of NE on embryo implantation. That is why there is no significant difference of embryo implantation numbers between NE treatment and the control group.
Figure 6
Schema of how NE/α1b-AR/PKC signaling impairs uterine decidualization. In decidualization, NE activated α1b-AR on the cell membrane of ESCs, and then the intracellular PKC was phosphorylated, inhibiting the expression of IGFBP1, PRL, Dtprp, etc. related to cell differentiation. A full color version of this figure is available at https://doi.org/10.1530/JOE-20-0479.

Excessive stress leads to a series of abnormal reproductive events, mechanisms that have not been elucidated. This study analyzed the effects of NE (one of the main stress hormones) on endometrial decidualization. However, identifying the precise mechanisms of long-term chronic or acute stress on decidualization in early pregnancy requires further experimental research.

In conclusion, NE inhibited endometrial decidualization of the endometrium through the activation of the PKC signaling pathway by upregulating α1b-AR (Fig. 6). Our results partially revealed potential molecular stress pathways that affect endometrial decidualization.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Meirong Du conceived the study; Dajin Li, Meirong Du and Jiju Wang designed experiments; Jiju Wang performed research, acquired data and drafted the manuscript; Songcun Wang helped to perform experiments and analyze data; Yunhui Tang and Liyuan Cui helped collecting the samples and interpreted data; Dajin Li and Meirong Du revised the manuscript; all authors gave the final approval of the version to be published.

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