Tissue-specific regulation of erythropoietin production in the murine kidney, brain, and uterus

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Chikuma, Mariko, Seiji Masuda, Toshihiro Kobayashi, Masaya Nagao, and Ryuzo Sasaki. Tissue-specific regulation of erythropoietin production in the murine kidney, brain, and uterus. Am J Physiol Endocrinol Metab 279:E1242–E1248, 2000.—Erythropoietin (Epo) produced by the kidney regulates erythropoiesis. Recent evidence suggests that Epo in the cerebrum prevents neuron death and Epo in the uterus induces estrogen (E2)-dependent uterine angiogenesis. To elucidate how Epo expression is regulated in these tissues, ovariectomized mice were given E2 and/or exposed to hypoxia, and the temporal patterns of Epo mRNA levels were examined. Epo mRNA levels in the kidney and cerebrum were elevated markedly within 4 h after exposure to hypoxia. Although the elevated level of Epo mRNA in the kidney decreased markedly within 8 h despite continuous hypoxia, the high level in the cerebrum was sustained for ≥24 h, indicating that downregulation operates in the kidney but not in the brain. E2 transiently induced Epo mRNA in the uterus but not in the kidney and cerebrum. Interestingly, the uterine Epo mRNA was hypoxia inducible only in the presence of E2. Thus Epo expression appears to be regulated in a tissue-specific manner, endorsing the tissue-specific functions of Epo.

OXYGEN IS THE MAJOR FACTOR that regulates production of erythropoietin (Epo) (reviewed in Refs. 15 and 19). The hypoxic stimulation of Epo production is largely due to the transcriptional activation of the Epo gene, although the prolongation of mRNA half-life may also play a partial role. The Epo gene contains the hypoxia-responsive enhancer in the 3′ flanking region, and hypoxia-inducible factor-1 (HIF-1) binds to this enhancer under hypoxia, thereby activating the Epo promoter. HIF-1 is a basic-helix-loop-helix-PAS heterodimeric transcription factor consisting of HIF-1α and aryl hydrocarbon receptor nuclear translocator (ARNT). Whereas ARNT is relatively stable, HIF-1α is rapidly degraded via the ubiquitin-proteasome pathway under normoxia. Under hypoxia, HIF-1α is stabilized to form an active heterodimer with ARNT. Details of these findings can be found in recent reviews (4, 7, 33, 34, 37, 41) and references therein.

At least four production sites of Epo have been found: kidney, liver, brain, and uterus. The kidney is a major production site in adults, and the kidney-derived Epo is responsible for the stimulation of erythropoiesis (15, 19). The liver produces Epo essentially for fetal erythropoiesis (42). In the brain, there is a paracrine Epo/Epo receptor (EpoR) system that is independent of the endocrine system in erythropoiesis (5, 21–25, 27); neurons express EpoR (5, 24, 27), and astrocytes produce Epo (22, 23, 25). Epo protects the primary cultured cerebrocortical and hippocampal neurons from glutamate toxicity, which is believed to be a major cause of ischemia-induced neuron death (27). Epo prevents the ischemia-induced death of cerebrocortical and hippocampal neurons in vivo (2, 35, 36). The Epo production by the cultured astrocytes (22, 25) and the brain Epo mRNA level (39) are also enhanced by low oxygen tension. In the uterus, expression of Epo is stimulated by 17β-estradiol (E2), and Epo is implicated in the uterine angiogenesis that occurs cyclically in the estrous cycle (43). The effect of oxygen on the production of Epo in the uterus is not known.

The existence of multiple Epo-producing sites with tissue-specific physiological functions raises important questions. Is the production of Epo stimulated by hypoxia in the uterus? Is the production of Epo in the kidney and brain stimulated by E2? Is there an interplay between two stimuli (E2 and hypoxia) for Epo production? Are the temporal patterns of stimuli-induced changes in the Epo mRNA level relevant to the assigned functions of Epo in these tissues? To answer these questions, we measured Epo mRNA quantitatively in the kidney, cerebrum, and uterus by using real-time reverse transcription-polymerase chain reaction (RT-PCR). This method has made it possible to quantify the basal levels of Epo mRNA, as well as the enhanced levels, in individual tissues. Here we report the effects of E2 treatment and exposure to hypoxia on the Epo mRNA contents in the ovariectomized (OVX) mouse.
MATERIALS AND METHODS

Animals. Animals were maintained and handled in accordance with the guidelines for the care and use of laboratory animals at Kyoto University. Outbred mice of the ICR strain (Clea) ovariectomized at 3 wk of age were used for experiments at 7–9 wk of age (35–40 g).

E2 administration and hypoxic exposure. E2 (Research Biochemicals International) was dissolved in olive oil. Each mouse was given 100 μl of E2 intraperitoneally. The control groups were given 100 μl of olive oil. For hypoxic stimulation, we used an air-tight cabinet into which the premixed gas was introduced. The gas flow rate was adjusted so that 10% O2 was achieved at ~30 min after the animals were placed into the cabinet. To examine the effects of E2 and hypoxia on Epo mRNA levels in the uterus, kidney, and cerebrum, OVX mice were treated with the following three conditions. The first group of animals was given E2 and was left under normoxia. The second group was exposed to normobaric hypoxia (7% O2/N2) without E2 administration. The third group was exposed to hypoxia immediately after E2 administration. At different time points after E2 administration and/or hypoxic exposure, the animals were anesthetized with ether, and blood was collected for the determination of serum Epo concentrations, and then the tissues were quickly removed and frozen in liquid nitrogen until used for RNA extraction.

Standard plasmid containing cDNA fragment of Epo or β-actin. Sequence coordinates of mouse Epo cDNA are based on the definition of the transcription start site as +1 (38). A 451-bp fragment encompassing 272–722 of the mouse Epo cDNA was ligated into a vector pCR3.1-Uni using a Eukaryotic TA Cloning Kit (Invitrogen). The resulting plasmid was used as a standard for PCR of Epo cDNA. As a standard plasmid for β-actin cDNA, pAL41 (accession number X03765) was used. These cDNA fragments contained the 112-bp (Epo) and 261-bp (β-actin) nucleotide sequences, which correspond to the PCR products amplified from the mRNA-derived cDNAs using the primers described in the next section.

RT and real-time PCR. Total RNA was prepared from the frozen tissues according to the protocol of the RNA Isolation System kit (Promega). RT was carried out at 45°C for 60 min in 20 μl RT mixture containing 1 μg total RNA, 200 U reverse transcriptase (GIBCO-BRL), 20 U RNase inhibitor (Takara), 0.5 mM each dNTPs, and 2.5 μM random nonamer primer. One microliter of the RT mixture was used for real-time PCR.

The PCR product of Epo mRNA-derived cDNA was quantified in real time, using a double dye-labeled fluorogenic oligonucleotide probe (12) and an automated fluorescence-based system for detection of PCR products. The probe was labeled at its 5′ end with a fluorogenic reporter dye, 6-carboxy-fluoresceine (FAM), and at its 3′ end with a quencher dye, 6-carboxy-tetramethylrhodamine (TAMRA). The nucleotide sequence in the probe 5′-(FAM)-TGCGAAGGTCCCA-GACTGACTGAAATATA-3′-(TAMRA) corresponds to 397–425 of the mouse Epo cDNA. This double-labeled probe was obtained from PE Applied Biosystems. The Epo-specific sequences used for the mouse PCR were the forward primer 371F, 5′-GAGGCAGAAATGTCAGATG-3′, and the reverse primer 482R, 5′-CTTCCACCTCCATTTTCC-3′. The forward and reverse primers correspond to the nucleotides 371–391 and 462–482 in Epo cDNA, respectively. They span exon/intron boundaries (exons II and III in the 371F, and exons III and IV in the 482R); thus amplification of contaminating genomic DNA is prevented. For PCR, we used TaqMan Universal PCR Master Mix containing dUTP instead of dTTP (PE Applied Biosystems, cat. no. 4304437). This master mixture also contained uracil-N-glycosylase (UNG), which destroys any carryover PCR products. Before PCR was started, the complete PCR mixture containing reverse-transcribed cDNA was treated at 50°C for 2 min for the action of UNG and then at 95°C for 10 min to inactivate UNG and activate DNA polymerase. Then PCR, consisting of 50 cycles at 95°C for 15 s and 60°C for 1 min, was performed. When DNA polymerase engaged in extension of the primer reaches the quencher-labeled nucleotide of the probe hybridized to cDNA, the exonuclease activity of DNA polymerase excises the labeled nucleotide, resulting in the emission of fluorescence. All procedures, including data analysis, were performed on the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems) using the software provided with the instrument. Messenger RNA of β-actin was also measured, and its level was used for normalization of Epo mRNA level. The β-actin sequence was amplified and detected using the primers 5′-CTAGGGCACCAAGGTGTGAT-3′ and 5′-CAAAAGCATGCTGGGGTCACT-3′ and the probe 5′-(FAM)-TGGCAC-CACACCTTCTCAATGAG-3′ (TAMRA).

The reaction mixture for PCR contains 1 μl RT mixture, 200 nM forward primer, 200 nM reverse primer, 100 nM probe, and 25 μl TaqMan Universal PCR Master Mix in a total volume of 50 μl.

When the samples lacking RNA or reverse transcriptase in the reverse transcriptase reactions were subjected to PCR, each of these control reactions yielded no detectable fluorescence, if we exclude the possibility that any contaminating DNA was amplified. The copy values of mRNAs for Epo and β-actin were calculated from the standard PCR curves drawn by the use of standard plasmids containing the cDNA fragments of Epo and β-actin.

Culture of the uterus from OVX mouse. Bilateral horns of the uterus from an OVX mouse were cut into two separate horns. One horn was cultured in medium containing a test substance, and the contralateral horn was cultured without the substance as a control. They were incubated for 6 h in a humid 5% CO2 atmosphere at 37°C in phenol red-free DMEM supplemented with 10% charcoal-treated FCS. Epo protein in the culture media and sera was measured with an enzyme-linked immunosorbent assay by use of two monoclonal antibodies that bind Epo at different epitopes (31). Recombinant human Epo, produced and isolated as described previously (10, 11), was used as a standard. This assay measures Epo as low as 1 pg/ml.

RESULTS

Measurement of Epo mRNA. mRNA of Epo was measured by detecting its reverse-transcribed cDNA with the real-time PCR. To draw the standard curve of Epo cDNA, twofold serial dilutions of the standard plasmid containing the Epo cDNA fragment were subjected to PCR. By plotting the cycle threshold values (Ct) (12) against the log of the copy number of the plasmid, we obtained a linear curve (Fig. 1). The lower limit of quantitative detection was ~10 copies of cDNA. Figure 1 also shows the results of quantitative measurement of Epo mRNA in the uterus, where Epo mRNA is markedly induced upon E2 administration, as described later. The extracts prepared from the uterus of E2-treated and E2-untreated mice were subjected to RT. Epo cDNA in three preparations (nondiluted, 2-fold diluted, and 4-fold diluted) of each RT sample was amplified, and the Ct values were plotted on the standard curve. The Ct values increased in proportion...
The liver is responsible for Epo production in the fetal stage. Under normoxia, the adult liver expresses Epo mRNA at a level below the threshold of detection by the RNase protection assay (18, 39). We could detect Epo mRNA in the liver under normoxia by the real-time PCR technique, but the interindividual variability was so large that we did not examine the Epo mRNA in the liver.

**Hypoxia induces a marked increase of Epo mRNA in the kidney and cerebrum, and downregulation operates in the kidney but not in the cerebrum.** To examine the effect of E2 on the Epo mRNA level in the kidney and cerebrum, OVX mice were given E2 or olive oil, and they were immediately exposed to hypoxia (7% O2) or kept under normoxia. At various time points, the tissues were removed to measure the amount of Epo mRNA. As Fig. 2A shows, hypoxia markedly induced Epo mRNA in the kidney, which is in agreement with previous results (reviewed in Refs. 15 and 19). However, the Epo mRNA level was quickly lowered despite continuous hypoxia: the level at 8 h after hypoxic

to the dilution both with and without E2 treatment. The copy value of Epo mRNA in the uterus from OVX mice \( n = 6 \) without E2 treatment was calculated to be \( 3.8 \pm 0.8 \times 10^4 \). To check the reproducibility of the PCR, we did an intra-assay study in which four samples containing Epo or β-actin cDNA at different concentrations were assayed by six runs at one time. We also performed an interassay study in which the four samples were assayed on six different days. In both studies, the coefficients of variation were satisfactory (<15%). The ratio of basal levels of Epo mRNA in the kidney, cerebrum, and uterus from OVX mice without stimulation was ~370:3:1. Hereafter, the Epo mRNA levels induced by E2 and hypoxia are expressed as values relative to the basal levels; Epo mRNA copy number per microgram of total RNA in each tissue from the control animals without treatment was defined as 1.

It has been reported that β-actin mRNA in the immature rat uterus is induced by E2 but that the level peaked at 4 h after E2 administration with a 1.4-fold increase (14). As shown below, the change in Epo mRNA by E2 was much greater than that in β-actin mRNA. Moreover, we found that the E2-induced increase in β-actin mRNA was at most 1.2-fold in the mouse uterus, and no significant changes were induced by either E2 or hypoxia in other tissues (kidney and cerebrum). Thus we used β-actin mRNA as an internal control for normalization of Epo mRNA.

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**Hypoxia induces a marked increase of Epo mRNA in the kidney and cerebrum, and downregulation operates in the kidney but not in the cerebrum.** To examine the effect of E2 on the Epo mRNA level in the kidney and cerebrum, OVX mice were given E2 or olive oil, and they were immediately exposed to hypoxia (7% O2) or kept under normoxia. At various time points, the tissues were removed to measure the amount of Epo mRNA. As Fig. 2A shows, hypoxia markedly induced Epo mRNA in the kidney, which is in agreement with previous results (reviewed in Refs. 15 and 19). However, the Epo mRNA level was quickly lowered despite continuous hypoxia: the level at 8 h after hypoxic
stimulation was reduced to 30% of the maximum induction found at 2 h. E2 showed no significant effect on the Epo mRNA level in the kidney under normoxia and hypoxia.

Figure 2B shows the effects of E2 and hypoxia on Epo mRNA levels in the cerebrum. Exposure to hypoxia markedly elevated the Epo mRNA level. Under normoxia, the Epo mRNA level was unaffected by the administration of E2. E2 slightly increased the Epo mRNA level under hypoxia, although not significantly. The hypoxic enhancement of Epo mRNA in the cerebrum reached a peak at 4 h and was still high at 8 h, which is in contrast to the rapid decrease in the Epo mRNA in the kidney. This notable difference in temporal patterns of hypoxia inducibility of Epo mRNA between kidney and brain was also confirmed in non-OVX mice (Fig. 3). The high level of Epo mRNA in the cerebrum was sustained for >24 h under hypoxia. In contrast, the level of the Epo mRNA in the kidney and the serum Epo level were quickly reduced despite continuous hypoxia. Male mice showed similar temporal patterns (data not shown). Hematocrit values were unchanged under all conditions used here, indicating that the reduction of Epo mRNA in the kidney and Epo in the serum by the prolonged exposure to hypoxia is not due to the operation of the classical negative-feedback loop, but that the stimulated erythrocyte formation represses expression of the Epo gene through the improved oxygen delivery.

*E2 induces a transient increase of Epo mRNA in the uterus, and the uterus responds to hypoxia only in the presence of E2.* In contrast to the kidney and cerebrum, administration of E2 increased Epo mRNA in the uterus. The E2-derived increase was dose dependent; 5 and 15 μg (E2/kg body wt) caused 10- and 14-fold increases of Epo mRNA at 3 h after administration, respectively, and the enhancement reached a plateau at 50–100 μg E2/kg with a 20-fold increase. Figure 4 shows the time-dependent changes of Epo mRNA level in the uterus. To ensure maximal induction, a dose of 500 μg/kg was used. There was a marked increase in Epo mRNA level at 2 h after E2 administration, but the level markedly decreased at 8 h. At 24 h after E2 administration, the Epo mRNA level was similar to that in the control mice given olive oil (not shown). The Epo mRNA level in the uterus of animals without E2 treatment was unchanged by exposure to hypoxia. Surprisingly, the Epo mRNA level in the uterus of animals given E2 was increased under hypoxia. Although this hypoxia-induced increase (2.5-fold) was small compared with the dramatic increase (30-fold) of Epo mRNA in the kidney and brain by hypoxia (see Fig. 2), the combination of E2 and hypoxia caused a 50-fold increase of Epo mRNA in the uterus. The Epo mRNA level in the uterus of animals exposed to hypoxia also markedly decreased at 8 h after E2 administration, like that in animals under normoxia. Such a transient increase of Epo mRNA in the uterus was also seen at the low dose of 5 μg E2/kg (data not shown).

**Induction of Epo mRNA in the uterus by E2 is transient.** To determine whether the rapid reduction in the level of Epo mRNA in the uterus after E2 administration is attributable to the metabolic depletion of E2 or to downregulation of the cellular response to E2, the effects of readministration of E2 on the induction were examined. Figure 5 shows the results. A high increase in the level of Epo mRNA was seen at 4 h after treatment with 50 μg E2/kg, but at 8 h the level markedly

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**Fig. 3.** Levels of Epo in the serum and Epo mRNA in the kidney and cerebrum of non-OVX mice under hypoxia. Eight-week-old mice were exposed to hypoxia (7% O2). At the indicated time points, the kidney and cerebrum were obtained for measurement of Epo mRNA and the serum for Epo protein. C, Cerebrum Epo mRNA; K, kidney Epo mRNA; S, serum Epo. The left ordinate shows degree of induction of Epo mRNA over the basal level, defined as 1. Basal levels of Epo mRNA in the kidney and cerebrum were similar to those in the OVX mice. The right ordinate shows serum Epo concentration. Each bar represents mean ± SE (of 3 mice). Statistical analysis was as in Fig. 2. *P < 0.01 vs. basal.

**Fig. 4.** Temporal pattern of E2-induced Epo mRNA in the uterus and effect of hypoxia. OVX mice were exposed to hypoxia (7% O2) immediately after administration of 500 μg E2/kg body wt (E2/Hx). OVX mice were exposed to hypoxia after administration of olive oil (Hx). OVX mice were left under normoxia after administration of 500 μg E2/kg body wt (E2). At the indicated time points, the uterus was obtained for measurement of Epo mRNA. The ordinate shows the degree of induction over the basal Epo mRNA level in the uterus of mice given olive oil. Each bar represents mean ± SE (of 5 mice). The statistical significance of differences was determined with ANOVA followed by Tukey-Kramer’s test. *P < 0.01 and **P < 0.05 vs. basal. †P < 0.01 and ‡P < 0.05 vs. E2.
decreased (experiments 2 and 3). E2 was readministered at 4 h after the first treatment, and Epo mRNA in the uterus was measured at 4 h after readministration (experiment 4). Readministration of E2 did not increase the Epo mRNA; the level was similar to that at 8 h after the first administration (compare experiments 3 and 4), if we exclude the possibility that the rapid reduction of the Epo mRNA level is caused by the deficiency of E2. At 120 h after the first treatment with E2, the level of Epo mRNA in the uterus was low, similar to that of the control mice (experiment 5). When E2 was readministered at 120 h after the first treatment and Epo mRNA was assayed at 4 h after readministration, a high level of Epo mRNA was induced (experiment 6). These results suggest that the response of Epo-producing cells in the uterus to E2 is quickly downregulated after administration of E2 and that these cells have restored their responsiveness to E2 at 120 h.

E2 and tamoxifen induce Epo production by the in vitro cultured uterus. In addition to oxygen concentration, some substances have been shown to influence Epo production; for example, thyroid hormones enhance hypoxia-induced Epo production by the perfused rat kidney and HepG2 cells (9), and Epo production by cultured astrocytes is stimulated by insulin, insulin-like growth factor (IGF) I, and IGF-II (23). To examine whether or not various nuclear receptor ligands and these growth factors modulate Epo production by the in vitro cultured uterus, Epo was measured in the medium after culture of the uterus with or without test substances. There was significant Epo production in the culture with E2, but the production was undetectable without E2 (Fig. 6). Tamoxifen, which is believed to function as an antiestrogen in breast tissue but acts as an E2-like ligand in uterine tissue (17), induced Epo production. E2- or tamoxifen-induced production of Epo was inhibited by ICI-182780, a specific E2 receptor (ER) antagonist (40), which alone showed no effect. Epo production was not induced by culture with progesterone, testosterone, 3,3',5-triiodo-L-thyronine, L-thyroxin, all-trans retinoic acid, vitamin D3, insulin (all at 1 μM), IGF-I (100 nM), and IGF-II (100 nM) (data not shown). These results suggest that E2 induces Epo production specifically in the uterus and that the production is mediated by ER.

**DISCUSSION**

In adults, the kidney is the major production site of Epo, stimulating erythropoiesis (15, 19). Recently the brain and uterus have also been shown to produce Epo. Epo in the brain functions as a neurotrophic factor (2, 35, 36), and Epo in the uterus is implicated in angiogenesis, which is under the control of E2 (43). Epo production in the kidney (15, 19) and brain (22, 25, 39) is hypoxia inducible, whereas that in the uterus is E2 inducible (43). Taken together with our findings of regulatory properties of Epo production in the kidney, cerebrum, and uterus, we propose that the regulation of Epo production is tissue specific, conformable to the

![Graph](image)

**Table 1. Functions of Epo and tissue-specific regulation of its production**

<table>
<thead>
<tr>
<th>Production Site</th>
<th>Possible Function</th>
<th>Increase of Epo mRNA By</th>
<th>Hypoxia (7% O2)</th>
<th>Estradiol</th>
</tr>
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<tbody>
<tr>
<td>Kidney</td>
<td>Erythropoietic</td>
<td>++ + + +</td>
<td>(Transient)</td>
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<tr>
<td>Cerebrum</td>
<td>Neurotrophic</td>
<td>++ + + +</td>
<td>(Long term)</td>
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<tr>
<td>Uterus</td>
<td>Angiogenic</td>
<td>+ + + +</td>
<td>(Transient)</td>
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Epo, erythropoietin. *In the presence of E2 (see Fig. 4).
specific functions of Epo produced by the individual tissues (Table 1).

E2 had no significant effect on the Epo mRNA level in the kidney (Fig. 2A) or on the Epo level in the serum (data not shown). Contradictory results of the effects of E2 on serum Epo have been reported; one laboratory reported that E2 repressed (26), and another reported that E2 potentiated the hypoxia-induced elevation of serum Epo (1). In these studies, mice were exposed to hypoxia before assay to elevate the serum Epo to an assayable level. Thus the previous results cannot be directly compared with our results.

Downregulation in the hypoxic induction of the Epo mRNA in the kidney and the Epo level in the serum, despite continued hypoxia, has been well documented (8, 20, 39). The direct feedback inhibition (Epo represses its production) has been excluded as a mechanism of downregulation (8). Although the mechanism remains to be studied, the different temporal patterns of the induction of Epo mRNA by hypoxia in the kidney and cerebrum provide physiologically important implications. In the brain, Epo supports neuronal survival under hypoxia, and therefore Epo expression needs to be sustained at a high level as long as hypoxia continues. In contrast, continuous activation of Epo gene expression in the kidney overproduces erythrocytes, causing various disorders.

E2-induced accumulation of the Epo mRNA in the uterus does not require de novo protein synthesis, and the accumulation is inhibited by actinomycin D, suggesting that this accumulation is due to transcriptional activation of the Epo gene by E2 (43). The palindromic consensus sequence of the E2 response element (AGGTCAAXXTGACCT) is not present, but its half-site exists in the 5′ flanking region of both mouse and human Epo genes (3). Imperfect E2 response elements, including the half-site, have been shown to confer E2 responsiveness to target genes (16, 28). Expression of the reporter gene flank a 5′ flanking region of Epo gene was activated by E2, and this activation required the ER (unpublished observations), indicating that the E2-induced increase of Epo mRNA in the uterus is at least partly attributable to transcriptional activation of the Epo gene.

Blood vessel formation and the concomitant remodeling of uterine endometrium take place every 3–5 days in the murine estrus cycle. The downregulation of the Epo mRNA level in the uterus that occurred shortly after administration of E2 is due to the loss of the cellular response to E2. At 5 days after treatment with E2, however, the Epo-producing cells regained their response to E2. Thus the downregulation of the responsiveness to E2 of the Epo-producing cells in the uterus may be very important for preventing uterine angiogenesis in an estrous cycle stage where it should not occur. The cellular degradation of ER is stimulated by E2 (13). The half-life of ER is ~5 days in the absence of E2, but in the presence of E2 it is degraded to a half-life of 3–4 h (30, 32). The ubiquitin-proteasome pathway has been shown to mediate E2-induced degradation of ER (29, 30). It is possible that the ligand-induced ER degradation serves to downregulate the expression of E2-responsive genes, including the Epo gene.

Although the magnitude (2.5-fold) of hypoxic induction of Epo mRNA in the uterus in the presence of E2 was much smaller than that (30-fold) in the kidney and brain, this hypoxic induction in the uterus may be physiologically significant, because the uterine endometrium would be hypoxic in the proestrus stage, when the ovary produces E2 actively and the endometrium grows rapidly. Interestingly, little hypoxic induction was found in the absence of E2. A multiprotein complex including HIF-1, hepatocyte nuclear factor-4, and p300/cAMP-responsive element binding protein is involved in the hypoxic induction of the Epo gene expression (6). The hypoxic inducibility may depend on the cellular availability of these components.

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