Erythropoietin (EPO) is produced in the brain, uterus, and oviduct. Brain EPO plays a neuroprotective role, and uterine EPO is likely involved in estrogen-dependent angiogenesis. Hypoxic induction of brain EPO markedly differs from that in the kidney. EPO in the uterus and oviduct is estrogen inducible.

Expression of EPO and its receptor in the brain

A survey of rat organs has revealed hypoxia-inducible expression of EPO mRNA in the testis, brain, liver, and kidney (19). Subsequently, astrocytes have been shown to be responsible for production of brain EPO (11, 14). More recently, it has been shown that neurons also produce EPO (2).

Expression of the functional EPO receptor (EPOR) has been found in neuronal cell lines such as PC12. Binding of EPO causes a rapid and transient Ca\(^{2+}\) influx via plasma membrane Ca\(^{2+}\) channels (1, 8, 13). EPO also increases the intracellular acetyltransferase in mouse embryonic primary septal neurons without serum and nerve growth factor (8). EPO augments choline acetyltransferase in mouse embryonic primary septal neurons (7). EPOR is expressed in primary cultured neurons prepared from hippocampus and cerebral cortex of rat embryos (15). Specific binding sites of radioiodinated EPO are present in some defined areas of the adult mouse brain, including hippocampus and cerebral cortex, in which neurons highly vulnerable to ischemia exist (5). EPOR mRNA is abundantly expressed in the brain of mouse early embryos, and its level is dramatically reduced during development (9), suggesting that EPO might play an unidentified role in brain development. The CNS of primates, including humans, also expresses EPO and EPOR (6, 10, 11). It is unlikely that the renal EPO crosses the blood-brain barrier under physiological conditions. Thus CNS has a paracrine EPO/EPOR system that is independent of the endocrine system (kidney/bone marrow and spleen) for erythropoiesis.

Neuroprotective role of EPO

In vivo neuroprotective action. In a gerbil forebrain ischemia model, intracerebroventricular infusion of EPO ameliorates the ischemia-induced loss of synapses in the hippocampal CA1 field, which contains neurons highly vulnerable to ischemic insult, and eventually alleviates neuron death and learning disability (Fig. 1A; see Ref. 17). The use of soluble EPOR (sEPOR), an extracellular domain capable of binding with EPO, has provided evidence that the endogenous brain EPO is critical for neuronal survival. Infusion of sEPOR yielded neurons containing fragmented DNA (TUNEL positive) in brain subjected to a mild ischemia, under which brain damage is undetectable. Infusion of sEPOR also caused neuron death and impaired learning ability, whereas infusion of the heat-denatured sEPOR was not detrimental (Fig. 1B; see Ref. 17). Focal cerebral ischemia by permanent middle cerebral artery occlusion is thought to be a better model for stroke. Infusion of EPO into the cerebroventricles attenuates navigation disability and cortical infarction induced by permanent occlusion of middle cerebral artery (2, 16).

In addition to ischemia, the exogenous EPO has been shown to be neuroprotective in the brain exposed to a variety of brain insults. EPO injected into the lateral ventricle supports the survival of septal cholinergic neurons in adult rats that have been subjected to fimbria-fornix transection (7). Interestingly, intraperitoneal administration of EPO diminishes the extent of ischemic neuron death, concussive brain injury, immune damage in experimental autoimmune encephalomyelitis, and kainate-induced seizures (3). Brines et al. (3) proposed that translocation of peripheral EPO across the blood-brain barrier might take place through transcytosis by using EPOR expressed abundantly in brain capillaries. Since a massive dose of EPO (5,000 U/kg) was used, however, their findings do not indicate that the renal EPO crosses the blood-brain barrier under normal physiological conditions and thereby exhibit a neuroprotective effect in the CNS. Nevertheless, the neuroprotective
effect of systemically administered EPO encourages a novel therapeutic approach.

**In vitro neuroprotective action.** Glutamate is a principal excitatory amino acid neurotransmitter in the mammalian CNS and also mediates pathological neuronal injury (see citations in Ref. 18). Activation of the N-methyl-D-aspartate (NMDA) receptor, a glutamate receptor expressed in neurons, opens a channel permeable to both Na⁺ and Ca²⁺. Prolonged NMDA receptor activation because of insufficient recovery of glutamate released as a neurotransmitter has been thought to be mainly responsible for ischemia-induced neuronal death. A massive increase of intracellular Ca²⁺ by NMDA receptor activation leads to neuron death. EPO protected primary cultured neurons of the hippocampus and cerebral cortex from glutamate toxicity (2, 15).

A glutamate-mediated increase in intracellular Ca²⁺ activates neuronal nitric oxide synthase that requires the Ca²⁺-calmodulin complex, resulting in increased nitric oxide that is rapidly converted to highly toxic compounds such as peroxynitrite. Addition of EPO in neuron cultures rescued neurons from death induced by nitroprusside, a nitric oxide generator (17). Thus EPO may prevent neuronal death by mitigating the toxicity of nitric oxide-derived compounds. EPO supports the survival of erythroid precursor cells by inducing the expression of Bcl-X, a member of the Bcl-2 family that acts as an anti-apoptotic protein. The mechanism by which EPO supports neuron survival is unknown.

**Angiogenic role of EPO**

A number of investigators have reported that EPOR is expressed in cultured endothelial cells and that EPO exerts angiogenic activity on these cells (see citations in Ref. 20). However, whether endothelial EPOR is physiologically functional or is only a vestige reflecting a common developmental lineage between endothelial cells and hematopoietic cells remained unknown. Angiogenesis occurs actively in embryos, but it is repressed in healthy adults. An exception in adults is the female reproductive organs. In the uterine endometrium, angiogenesis takes place to support the endometrial growth that occurs during transition from the diestrus to estrous stage. Injection of sEPOR into the mouse uterine cavity in the diestrus stage inhibited endometrial growth (20). Activation of the uterine angiogenesis is triggered by 17β-estradiol (E₂), an ovarian hormone, and therefore this activation can be mimicked by injection of E₂ to ovariectomized (OVX) mouse. EPO injected into the OVX mouse uterine cavity stimulated endometrial growth and angiogenesis, although the alterations induced by EPO differed somewhat from those induced by E₂ (20). These results, combined with the finding that endothelial cells in the uterine endometrium express EPOR, suggest that EPO is one of the E₂-regulated angiogenic factors, including vascular endothelial growth factor, that are required for completion of the cyclic uterine angiogenesis in the estrus cycle.

**Regulation of EPO production**

**Brain and kidney.** Exposure of mice to severe hypoxia (7% O₂) markedly elevates serum EPO and EPO mRNA in the kidney and cerebrum. However, the levels of serum EPO and renal EPO mRNA are quickly lowered despite continuous hypoxia. Surprisingly, brain EPO mRNA is sustained at a high level for at least 24 h (Fig. 2A). Since hematocrit values are unchanged during experiments, the rapid decline of renal EPO mRNA is not due to the operation of the classic negative-feedback inhibition (EPO gene expression is repressed through the improved O₂ delivery by the increased erythrocytes). Although the mechanism for the rapid decrease in the kidney remains
unknown, this notable difference in the temporal patterns of hypoxia inducibility of EPO mRNA between kidney and brain seems to well reflect the tissue-specific functions of EPO. In the brain, EPO supports neuron survival under ischemia, and therefore a high level of EPO expression is required as long as hypoxia continues, whereas the continuous activation of EPO gene expression in the kidney would result in overproduction of erythrocytes, causing various disorders. Therefore, the downregulation of EPO gene expression must operate even under hypoxia in the kidney but not in the brain. EPO mRNA in the cerebellum is also hypoxia inducible, with a temporal pattern similar to that in the cerebrum, but its role in the cerebellum is not known (18). E2 induces EPO production in the female reproductive organs as described below, but E2 shows little effect on the kidney and brain (4).

**Female reproductive organs.** The role of EPO in uterine angiogenesis raises a possibility that the uterus produces EPO in an E2-dependent manner, because it is hard to speculate that the renal EPO is responsible for the periodic angiogenesis in the uterus. In vitro cultured uterus from OVX mouse expressed EPO protein and its mRNA in an E2-dependent manner (20). Administration of E2 to the OVX mouse induced a rapid and transient increase in EPO mRNA in the uterus (Fig. 2B). Interestingly, the uterine EPO mRNA is hypoxia inducible only in the presence of E2. Furthermore, the hypoxia-induced increase of EPO mRNA is far smaller in the uterus (2.5-fold) than in the kidney or brain (30-fold) (Fig. 2A). The rapid reduction in the uterine EPO mRNA level after E2 administration is not due to the metabolic depletion of E2 but due to the loss of responsiveness of the uterine EPO-producing cells to E2 (4). Although the molecular mechanism is not known, this downregulation may be very important for preventing uterine angiogenesis in an estrous cycle stage in which it should not occur.

The oviduct also produces EPO in an E2-inducible manner (12). Administration of E2 to a 3-wk-old non-OVX mouse before commencement of cyclic E2 synthesis in the ovary causes a transient increase in the oviductal EPO mRNA (Fig. 2C). Notably, hypoxia induces oviductal EPO mRNA not only in mouse given E2 but also in mouse without E2 administration. As in the uterus of the OVX mouse (see Fig. 2B), exposure of a 3-wk-old non-OVX mouse to hypoxia did not induce uterine

**TABLE 1. Possible roles of EPO and tissue-specific regulation of expression**

<table>
<thead>
<tr>
<th>Production</th>
<th>Functions</th>
<th>Induction by</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Erythropoetic</td>
<td>yes*</td>
<td>4,19</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>Neurotrophic</td>
<td>yes†</td>
<td>2,3,7,16,17,19</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>?</td>
<td>yes†</td>
<td>18</td>
</tr>
<tr>
<td>Uterus</td>
<td>Angiogenic</td>
<td>yes*</td>
<td>4,20</td>
</tr>
<tr>
<td>Oviduct</td>
<td>?</td>
<td>yes*</td>
<td>12</td>
</tr>
<tr>
<td>Testis</td>
<td>?</td>
<td>?</td>
<td>19</td>
</tr>
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Induction of erythropoietin (EPO) mRNA can be transient (*), being rapidly downregulated despite the presence of stimuli, or long term (†), remaining at high levels. †, In the presence of estrogen.

![FIGURE 2. Temporal patterns of stimuli (hypoxia and 17β-estradiol (E2)-induced EPO and EPO mRNA. (A) EPO in the serum and EPO mRNA in the kidney and cerebrum of mice under hypoxia. Eight-week-old mice were exposed to hypoxia (7% O2). ○, Cerebrum EPO mRNA; ●, kidney EPO mRNA; △, serum EPO. The left ordinate shows the fold induction of EPO mRNA over the basal level, defined as 1. Values are means ± SE (n = 3). (B) EPO mRNA in the uterus of ovariectomized (OVX) mouse. ●, Mice exposed to hypoxia (7% O2) immediately after E2 injection; ○, mice exposed to hypoxia after olive oil (solvent for E2); △, mice left under normoxia after E2. Values are means ± SE (n = 3). C: EPO mRNA in the oviduct. ●, 3-wk-old mice exposed to hypoxia (7% O2) immediately after E2; ○, mice exposed to hypoxia after olive oil; △, mice left under normoxia after E2. Values are means ± SE (n = 4). Modified from Refs. 4 and 12.](http://physiologyonline.physiology.org/)

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EPO mRNA when E2 was not given (12). Thus the EPO-producing cells in the uterus and oviduct differ in requirement of E2 for hypoxia inducibility of EPO mRNA. The possible physiological functions of EPO and tissue-specific regulatory features of EPO production are summarized in Table 1.

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References


