Brain Protection by Erythropoietin: A Manifold Task

Many hematopoietic growth factors are produced locally in the brain. Among these, erythropoietin (Epo) has a dominant role for neuroprotection, neurogenesis, and acting as a neurotrophic factor in the central nervous system. These functions make erythropoietin a good candidate for treating diseases associated with neuronal cell death.

The hormone erythropoietin (Epo) is a 165-amino acid (~30 kDa) glycoprotein that belongs to the cytokine type I superfamily. Originally, it was believed that the only role of Epo was the regulation of erythropoiesis. This role is attributed to the ability of Epo to inhibit programmed cell death (apoptosis) in erythroid cells and thus allow the maturation of erythrocytes. Since blood oxygen availability is the main regulator of erythropoiesis, hypoxia induces the gene expression of Epo in the kidney, the main site for Epo production, and in the liver (53) in a negative feedback system between the kidney and the bone marrow. Research performed in the last decade has shown that Epo and its receptor (EpoR) are expressed in tissues other than those involved in erythropoiesis. These include the brain, the reproductive tract, the lung, the spleen, and the heart (89). Accordingly, a novel cytoprotective effect of Epo was established in several organs. For example, Epo reduced injury and dysfunction after ischemia-reperfusion in the mouse brain (86), and it showed protection in various myocardial ischemia models (13, 18, 85). In the following review, we will concentrate on the effects of Epo in the brain.

**Epo/EpoR Expression and Regulation in the Brain**

Epo is mainly produced in the interstitial fibroblasts in the adult kidney and the hepatocytes of the fetus, whereas EpoR is normally expressed in erythroid precursor cells in the bone marrow (71). However, recent data have shown that the expression of Epo and its receptor, EpoR (both mRNA and protein), coincides in the same organ and even within the same cell. Epo and EpoR expression are widely distributed in the mammalian brain (42, 71), albeit at lower levels than in the kidney (14). Epo thus has to be added to the growing list of hematopoietic growth factors found to be expressed and act in the central nervous system (CNS).

**Expression of Epo/EpoR in the brain**

Epo and EpoR mRNA and protein were detected in several regions of the murine and primate brain, including cortex, hippocampus and amygdala, cerebellum, hypothalamus, and caudate nucleus (74, 103). With respect to the type of cells in the brain that express Epo, astrocytes are the main source of Epo in the brain (74, 77). Moreover, it has been shown in vitro and in vivo that neurons express Epo (11, 12, 103). Similarly, Epo is expressed on neurons and astrocytes (11, 12, 103). In addition, primary cultures of human neurons, astrocytes, and microglia express Epo mRNA (82), and EpoR expression was also detected in primary cultures of rat oligodendrocytes (41). In addition to neurons, oligodendrocytes, and glial cells, a strong immunoreactivity for EpoR was found to be associated with brain vascular endothelial cells, showing that these cells also express EpoR (15) (FIGURE 1). These findings implicate a broad spectrum of actions of Epo in the brain.

**Regulation of Epo/EpoR expression**

As mentioned above, Epo is upregulated in response to hypoxia. As, for many of the hypoxic adaptation processes in the body, the regulation of Epo expression is based on the transcriptional regulation of two hypoxia-inducible factors HIF-1 and HIF-2 (117). HIFs are heterodimers composed of an α- and a β-subunit. Two forms of the oxygen-labile α exist, 1αs and 2αs. The α-subunit is stabilized under hypoxic conditions leading to the binding of the heterodimer HIF-1 or HIF-2 to specific DNA sequences located in the hypoxia-response elements of target genes such as Epo or vascular endothelial growth factor (VEGF) (118). Although HIF-1α was originally identified as the transcription factor responsible for Epo expression (97), more recent evidence suggests that Epo is a target of HIF-2 (33). The stability of HIF-α is regulated by enzymatic hydroxylation of specific amino acids on the α subunit by a group of oxygenases (FIGURE 2). Under normoxic conditions, a specific prolyl hydroxylase within the oxygen-dependent degradation domain of HIF-α takes place. This prolyl hydroxylation allows binding of the von Hippel-Lindau protein (pVHL), leading to ubiquitination and proteasomal degradation of the HIF-α subunit (50, 51). The enzymes responsible for this hydroxylation are termed prolyl hydroxylase domain enzymes (PHD1-3) (16, 37) and are widely expressed. Furthermore, in the presence of oxygen, another hydroxylation reaction takes place on

---

**REFERENCES**

Many hematopoietic growth factors are produced locally in the brain. Among these, erythropoietin (Epo) has a dominant role for neuroprotection, neurogenesis, and acting as a neurotrophic factor in the central nervous system. These functions make erythropoietin a good candidate for treating diseases associated with neuronal cell death.

The hormone erythropoietin (Epo) is a 165-amino acid (~30 kDa) glycoprotein that belongs to the cytokine type I superfamily. Originally, it was believed that the only role of Epo was the regulation of erythropoiesis. This role is attributed to the ability of Epo to inhibit programmed cell death (apoptosis) in erythroid cells and thus allow the maturation of erythrocytes. Since blood oxygen availability is the main regulator of erythropoiesis, hypoxia induces the gene expression of Epo in the kidney, the main site for Epo production, and in the liver (53) in a negative feedback system between the kidney and the bone marrow. Research performed in the last decade has shown that Epo and its receptor (EpoR) are expressed in tissues other than those involved in erythropoiesis. These include the brain, the reproductive tract, the lung, the spleen, and the heart (89). Accordingly, a novel cytoprotective effect of Epo was established in several organs. For example, Epo reduced injury and dysfunction after ischemia-reperfusion in the mouse brain (86), and it showed protection in various myocardial ischemia models (13, 18, 85). In the following review, we will concentrate on the effects of Epo in the brain.

**Epo/EpoR Expression and Regulation in the Brain**

Epo is mainly produced in the interstitial fibroblasts in the adult kidney and the hepatocytes of the fetus, whereas EpoR is normally expressed in erythroid precursor cells in the bone marrow (71). However, recent data have shown that the expression of Epo and its receptor, EpoR (both mRNA and protein), coincides in the same organ and even within the same cell. Epo and EpoR expression are widely distributed in the mammalian brain (42, 71), albeit at lower levels than in the kidney (14). Epo thus has to be added to the growing list of hematopoietic growth factors found to be expressed and act in the central nervous system (CNS).

**Expression of Epo/EpoR in the brain**

Epo and EpoR mRNA and protein were detected in several regions of the murine and primate brain, including cortex, hippocampus and amygdala, cerebellum, hypothalamus, and caudate nucleus (74, 103). With respect to the type of cells in the brain that express Epo, astrocytes are the main source of Epo in the brain (74, 77). Moreover, it has been shown in vitro and in vivo that neurons express Epo (11, 12, 103). Similarly, Epo is expressed on neurons and astrocytes (11, 12, 103). In addition, primary cultures of human neurons, astrocytes, and microglia express Epo mRNA (82), and EpoR expression was also detected in primary cultures of rat oligodendrocytes (41). In addition to neurons, oligodendrocytes, and glial cells, a strong immunoreactivity for EpoR was found to be associated with brain vascular endothelial cells, showing that these cells also express EpoR (15) (FIGURE 1). These findings implicate a broad spectrum of actions of Epo in the brain.

**Regulation of Epo/EpoR expression**

As mentioned above, Epo is upregulated in response to hypoxia. As, for many of the hypoxic adaptation processes in the body, the regulation of Epo expression is based on the transcriptional regulation of two hypoxia-inducible factors HIF-1 and HIF-2 (117). HIFs are heterodimers composed of an α- and a β-subunit. Two forms of the oxygen-labile α exist, 1αs and 2αs. The α-subunit is stabilized under hypoxic conditions leading to the binding of the heterodimer HIF-1 or HIF-2 to specific DNA sequences located in the hypoxia-response elements of target genes such as Epo or vascular endothelial growth factor (VEGF) (118). Although HIF-1α was originally identified as the transcription factor responsible for Epo expression (97), more recent evidence suggests that Epo is a target of HIF-2 (33). The stability of HIF-α is regulated by enzymatic hydroxylation of specific amino acids on the α subunit by a group of oxygenases (FIGURE 2). Under normoxic conditions, a specific prolyl hydroxylase within the oxygen-dependent degradation domain of HIF-α takes place. This prolyl hydroxylation allows binding of the von Hippel-Lindau protein (pVHL), leading to ubiquitination and proteasomal degradation of the HIF-α subunit (50, 51). The enzymes responsible for this hydroxylation are termed prolyl hydroxylase domain enzymes (PHD1-3) (16, 37) and are widely expressed. Furthermore, in the presence of oxygen, another hydroxylation reaction takes place on
an asparaginyl group in the COOH-terminal transactivating domain of HIF-α, blocking its binding to the transcriptional coactivators (65). This process is governed by a specific asparaginyl hydroxylase termed factor-inhibiting HIF (FIH) (40, 64). So, under normoxia, FIH and PHD(s) are active, leading to transcriptional inactivation and degradation of HIF-α, whereas under hypoxic conditions both enzymes are inactive. HIF is then stabilized and able to induce the expression of target genes, including Epo. This basic mechanism of regulation seems to be of relevance for brain-expressed Epo, since in several experimental systems Epo was upregulated under hypoxic conditions in the brain of several mammalian species including mouse, rat, monkey, and human (42, 72, 74, 103). However, depending on the severity of hypoxia, Epo mRNA level can increase 3- to 20-fold in the brain in contrast to 200-fold in the kidney (74). Moreover, although the increase in Epo expression in the kidney seems to be transient with a decrease after 8 h of continuous hypoxia, the level of Epo in the brain remains high for at least 24 h (23). This indicates a tissue-specific degree of regulation. Indeed, although HIF-1α levels in the kidney under systemic hypoxia peak after 1 h and again reach basal levels 4 h thereafter, in the brain the HIF-1α peak level is reached after only 5 h and returns to the basal level not before 12 h (106). A possible explanation for the different time course in the brain might be an altered composition of the various PHD forms. It has to be noted that hypoxia is not the only factor activating HIF. Several studies have shown that pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) or lipopolysaccharide (LPS) induce the expression of HIF (40). With regard to the EpoR, it is regulated by pro-inflammatory cytokines (82), such as TNF-α, IL-1β, and Epo itself (24). The role of hypoxia in the regulation of Epo expression is controversial. Whereas we did not observe hypoxic induction of Epo expression in neurons or astrocytes (11), anemic stress induced up-regulation of Epo expression in the brain of human Epo receptor gene knock-in mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic Epo regulation remains to be established, since Epo has not been identified as HIF target gene so far.

Epo Signaling

Epo promotes cell survival through inhibiting apoptosis (FIGURE 3). In erythroid cells, after binding of Epo to its receptor (EpoR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus activated. This leads to activation of secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), followed by the activation of Ras mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt (22). Moreover, Epo induced the up-regulation of the anti-apoptotic protein BCL-XL (59). The role of STAT5 in Epo-induced neuroprotection is controversial. Whereas we showed that pro-inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and Epo itself (24). The role of hypoxia in the regulation of Epo expression is controversial. Whereas we did not observe hypoxic induction of Epo expression in neurons or astrocytes (11), anemic stress induced up-regulation of Epo expression in the brain of human Epo receptor gene knock-in mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic Epo regulation remains to be established, since Epo has not been identified as HIF target gene so far.

Epo promotes cell survival through inhibiting apoptosis (FIGURE 3). In erythroid cells, after binding of Epo to its receptor (EpoR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus activated. This leads to activation of secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), followed by the activation of Ras mitogen-activated protein kinase (MAPK), ERK-1/-2, and PI3K/Akt (22). Moreover, Epo induced the up-regulation of the anti-apoptotic protein BCL-XL (59). The functional significance of these signaling molecules in erythropoiesis is not absolutely clear though. For instance, whereas in one study STAT5 knockout adult mice were largely unaffected in their erythroid lineage (108), in another study STAT5 knockout embryos suffered from severe anemia, showed a reduced number of erythroid progenitors, and had higher numbers of apoptotic cells (104). Most of these pathways seem also to be functional in the brain (14, 59). In vitro, inhibition of MAPK and PI3K blocked Epo-mediated protection of rat hippocampal neurons against hypoxia (102). Moreover, using ERK-1/-2 and Akt inhibitors, Kilic et al. showed that activation of these proteins is essential for Epo-mediated neuroprotection in an animal model of focal cerebral ischemia (59). The role of STAT5 in Epo-induced neuroprotection is, however, controversial. STAT5 phosphorylation has been shown to occur in hippocampal CA1 neurons after transient global cerebral ischemia in rats (126). Therefore, the authors concluded that STAT5 plays a role in Epo-mediated neuroprotection. However, in a very recent study, in an in vitro model of glutamate toxicity using hippocampal neuronal culture from STAT5 knockout mouse fetuses, STAT5 was not required for Epo-mediated neuroprotection (157). However, STAT5 was indispensable for the neurotrophic function of Epo (17). A unique pathway for the brain seems to be that activation of EpoR induces nuclear factor-κB (NF-κB) translocation and transcription of NF-κB target genes (36). Although EpoR is expressed in many cell types and not only in erythropoietic cells, it is currently not clear that NF-κB is involved in Epo’s neuroprotective effects. However, both p38 and extracellular signal-regulated kinase (ERK-1/-2) have been found to be involved in Epo-mediated neuroprotection (46, 128). Additionally, because of their role in the immune system, Fcγ receptors were also investigated in Epo-induced neuroprotection (58, 111). In the absence of EpoR, Fcγ receptors appear to have no functional significance in Epo-induced neuroprotection (58, 111). For almost all of the proposed neuroprotective mechanisms, the involvement of EpoR has not been demonstrated yet. In vitro, Epo could directly target neurons and astrocytes, or indirectly act on glial cells and not target neurons directly. In vivo, the situation is more complex. In some studies, EpoR has been found to be expressed in neurons (11), whereas in other studies EpoR expression in the brain of human EpoR transgenic mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic EpoR regulation remains to be established, since EpoR has not been identified as HIF target gene so far.

Epo Promotes Cell Survival through Inhibiting Apoptosis (FIGURE 3). In erythroid cells, after binding of Epo to its receptor (EpoR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus activated. This leads to activation of secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), followed by the activation of Ras mitogen-activated protein kinase (MAPK), ERK-1/-2, and PI3K/Akt (22). Moreover, Epo induced the up-regulation of the anti-apoptotic protein BCL-XL (59). The role of STAT5 in Epo-induced neuroprotection is controversial. Whereas we showed that pro-inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and Epo itself (24). The role of hypoxia in the regulation of Epo expression is controversial. Whereas we did not observe hypoxic induction of Epo expression in neurons or astrocytes (11), anemic stress induced up-regulation of Epo expression in the brain of human Epo receptor gene knock-in mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic Epo regulation remains to be established, since Epo has not been identified as HIF target gene so far.

Epo Promotes Cell Survival through Inhibiting Apoptosis. In erythroid cells, after binding of Epo to its receptor (EpoR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus activated. This leads to activation of secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), followed by the activation of Ras mitogen-activated protein kinase (MAPK), ERK-1/-2, and PI3K/Akt (22). Moreover, Epo induced the up-regulation of the anti-apoptotic protein BCL-XL (59). The role of STAT5 in Epo-induced neuroprotection is controversial. Whereas we showed that pro-inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and Epo itself (24). The role of hypoxia in the regulation of Epo expression is controversial. Whereas we did not observe hypoxic induction of Epo expression in neurons or astrocytes (11), anemic stress induced up-regulation of Epo expression in the brain of human Epo receptor gene knock-in mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic EpoR regulation remains to be established, since EpoR has not been identified as HIF target gene so far.

Epo Promotes Cell Survival through Inhibiting Apoptosis. In erythroid cells, after binding of Epo to its receptor (EpoR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus activated. This leads to activation of secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), followed by the activation of Ras mitogen-activated protein kinase (MAPK), ERK-1/-2, and PI3K/Akt (22). Moreover, Epo induced the up-regulation of the anti-apoptotic protein BCL-XL (59). The role of STAT5 in Epo-induced neuroprotection is controversial. Whereas we showed that pro-inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and Epo itself (24). The role of hypoxia in the regulation of Epo expression is controversial. Whereas we did not observe hypoxic induction of Epo expression in neurons or astrocytes (11), anemic stress induced up-regulation of Epo expression in the brain of human Epo receptor gene knock-in mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic EpoR regulation remains to be established, since EpoR has not been identified as HIF target gene so far.

Epo Promotes Cell Survival through Inhibiting Apoptosis. In erythroid cells, after binding of Epo to its receptor (EpoR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus activated. This leads to activation of secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), followed by the activation of Ras mitogen-activated protein kinase (MAPK), ERK-1/-2, and PI3K/Akt (22). Moreover, Epo induced the up-regulation of the anti-apoptotic protein BCL-XL (59). The role of STAT5 in Epo-induced neuroprotection is controversial. Whereas we showed that pro-inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and Epo itself (24). The role of hypoxia in the regulation of Epo expression is controversial. Whereas we did not observe hypoxic induction of Epo expression in neurons or astrocytes (11), anemic stress induced up-regulation of Epo expression in the brain of human Epo receptor gene knock-in mice (24). Moreover, in the same study, hypoxia increased Epo expression in neuronal cells in vitro (24). The mechanism of hypoxic EpoR regulation remains to be established, since EpoR has not been identified as HIF target gene so far.
hypoxia is not studies have tions such as receptors; Epo is re- the expression is regula- as TNF-α, IL-8, and is in the regu- lated number resulted in the regu- lators such as ned Epo-medi- L-XL (59). The al. Whereas we oft expression stress induced co-rans-estudy, hypoxia in cells in vitro ol regulation has not been hating apopto- binding of Epo use 2 (JAK2) is This leads to such as sig- lation 5 ras mitogen- er-K/1-2, and the upregula- cartridge-X, (29). The mEpo knockout adult thyroid lineage embryos suf- uced number hese pathways were involved in (14, 59). In ded Epo-medi- kons against 1/2 and Akt ation of these neuroprotec- rnal ischemia neuroprotec- topothesis in the rat and that STAT5 pro- tection. In the in vitro model of neuronal cul- tures, STATs was protection (17). For the neu- ral pathway for EpoR induces nuclear factor-κB (NF-κB) translocation into the nucleus and that this effect is important for Epo-mediated neuroprotection (28). Interestingly, Epo-induced NF-κB translocation was observed only in neuronal cells and not in astrocytes (29). Thus it appears likely that NF-κB, in the nucleus, induces the expression of neuroprotective and anti-apoptotic proteins. However, some differences exist between the signaling cascade activated by Epo in the CNS and in ery- throid cells. For instance, in one study, BCL-XL has been found to be important in Epo-mediated protec- tion of erythroid but not neuronal cells (93). Additionally, Epo has been found to activate phospho- lase kinase-γ (PLC-γ) (70) and thus can directly influ- ence neuronal activity (62) and neurotransmitter release (57) by modulating intracellular calcium concen- trations in neurons.

**Epo Function in the CNS**

For almost a century, Epo was thought to be involved in the process of erythropoiesis only. Through its anti- apoptotic action, it enables committed erythroid progenitor cells to survive and mature (52). However, during the last decade, it became evident that Epo is implicated in other processes such as neuroprotection, neurogenesis, and angiogenesis, and plays an important role as neurotrophic as well as immunomodulatory factor (Table 1). The important role of Epo in the CNS is also evident from studies with EpoR knockout mice. As a result of EpoR deficiency, these mice show massive apoptosis and a reduction in the number of neuronal progenitor cells (125). A comprehensive description of the role of the Epo-EpoR system in development is found elsewhere (7, 26).

**Neuroprotection**

For a long time, Epo has been used clinically in patients suffering from anemia due to end-stage renal failure. In addition to the correction of anemia, these patients showed improved cognitive abilities (101). Initially, it was believed that systemic Epo cannot pass through the blood-brain-barrier due to its large size (88) and brain-derived Epo production and expression of EpoR in the CNS were not yet discov- ered. The positive effect on cognition was attributed to the improved oxygen-carrying capacity of the blood after Epo-induced erythropoiesis. However, since later studies have shown that both Epo and its receptor are expressed in different regions of the brain by different cell types (42, 71), the hypothesis was established that locally produced as well as exogenously added Epo could directly influence cognitive function. Interestingly, the expression level of Epo and EpoR is especially high in regions of the brain known to be particularly sensitive to acute hypoxia (68), the hip- pocampus and the tectal ephysal cell (28), suggesting that Epo might act as a protective agent against hypoxia. Indeed, infusion of soluble EpoR into the brain of ger- bils, which were subjected to a mild form of ischemia that normally does not cause neuronal damage, resulted in neuronal death in the hippocampus, clearly showing that endogenous Epo has a neuro- protective effect (95).

**Neurotrophic function and neurogenesis**

Besides neuroprotection, Epo also has a neurotrophic function. This was first demonstrated by Konishi and co-workers showing that Epo augments the activity of choline acetyltransferase in primary cultured mouse septal neurons (61). Epo promoted the regeneration of septal cholinergic neurons in adult rats that had undergone limbia-fornix transections. In addition and similar to its anti-apoptotic role in erythropoiesis, Epo promoted the survival and differentiation of dopaminergic precursor neurons in vitro (107). Moreover, hypoxia-induced Epo production appeared to directly act on neuronal stem cells in the forebrain, showing that Epo plays a direct role in neurogenesis.

![FIGURE 2. Regulation of Epo expression is based on the transcriptional regulation of HIF](http://physiologyonline.physiology.org/Downloaded from http://physiologyonline.physiology.org/). Under normoxic conditions, specific prolyl hydroxylation within the oxygen-dependent degradation domain of HIF-α takes place. This prolyl hydroxylation allows binding of the von Hippel-Lindau protein (pVHL), leading to ubiquitylation and proteasomal degradation of the HIF-α subunit. The enzymes responsible for this hydroxylation are termed prolyl hydroxylase domain enzymes (PHD1-3). Furthermore, in the presence of oxygen, another hydroxylation reaction takes place on an asparaginyl group in the COOH-terminal transactivation domain (TAD) of HIF-α, blinding its binding to the transcrip- tional coactivators. This process is governed by a specific asparaginyl hydroxylase termed factor-inhibiting HIF (FIH). So, even when HIF-α molecules that escape proteasomal degradation enter the nucleus, they remain transcriptionally inactive, and target gene expression is blocked. By contrast, under hypoxic conditions, FIH and PHD1 are both inactive, and HIF is stabilized and able to induce the expression of target genes including Epo.
after hypoxia (99). In addition, Epo also acts indirectly by inducing brain-derived neurotrophic factor (BDNF) expression (113), which in turn augmented the effect of Epo on neurogenesis. These data show that Epo is not only involved in neuroprotection, but also in neuronal survival, differentiation, and neurogenesis.

**Angiogenesis and vascular permeability**

Besides its direct effects on neurons, Epo-induced neuroprotection may be attributed to an improvement in brain perfusion by promoting new vessel growth. Anagnostou et al demonstrated mitogenic and chemotactic effects of Epo on human umbilical vein and bovine adrenal capillary endothelial cells (4). Moreover, Epo stimulated vessel outgrowth of rat aortic rings (19), suggesting that Epo has angiogenic effects. This was further supported by the observation that Epo injection into the mouse uterine cavity stimulated neovascularization in the endometrium (123). Similarly, neovascularization was stimulated in the chick embryo chorionicallantoic membrane upon Epo administration (93). The angiogenic effect of Epo was also found in the brain, since capillary endothelial cells express two forms of EpoR mRNA and Epo showed a dose-dependent mitogenic activity on brain capillary endothelial cells (121). This angiogenic effect was finally confirmed in mice genetically engineered to lack either Epo or its receptor (EpoR) where the effect was finally confirmed in mice genetically engineered to lack either Epo or its receptor (EpoR) where the effect was finally confirmed in mice genetically engineered to lack either Epo or its receptor (EpoR).

Table 1. Functions of Epo

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroprotection</td>
<td>Infusion of soluble EpoR into the brain of gerbils, subjected to a mild form of ischemia, caused neuronal death in the hippocampus.</td>
<td>95</td>
</tr>
<tr>
<td>Neurotrophic factor</td>
<td>Regeneration of septal cholinergic neurons in adult rats, which had undergone fimbria-fornix transections. Promotion of the survival and differentiation of dopaminergic precursor neurons in vitro.</td>
<td>107</td>
</tr>
<tr>
<td>Neurogenesis</td>
<td>Hypoxia-induced Epo production acts directly on neuronal stem cells in the forebrain. Indirectly by inducing BDNF expression.</td>
<td>99</td>
</tr>
<tr>
<td>Anti-inflammation</td>
<td>Reduced production of inflammatory mediators leading to Cerebral ischemia: smaller infarcts. Multiple sclerosis: protection. Optic neuritis: improved survival of retinal ganglion cells.</td>
<td>112</td>
</tr>
<tr>
<td>Vascular permeability</td>
<td>In vitro: BBB protection against VEGF-induced increase in vascular permeability.</td>
<td>75</td>
</tr>
</tbody>
</table>

BDNF, brain-derived neurotrophic factor; BBB, blood-brain barrier; VEGF, vascular endothelial growth factor.

In addition to its angiogenic effect, Epo is involved in the regulation of vascular permeability. In an in vitro model of the blood-brain barrier (BBB), Epo treatment protected bovine brain endothelial cells against VEGF-induced increase in vascular permeability (75). This suggests that the protective effect of Epo on the brain could be mediated by stimulating angiogenesis as well as by protecting the BBB.

**Anti-inflammation**

Inflammatory processes play a major role in the pathogenesis of cerebral ischemia, where Epo is protective (see below). Inflammation results in influx of leukocytes from the blood into the brain and in activation of resident microglial cells (30). These cells produce inflammatory mediators and cytokines leading to barrier damage, microvascular occlusion, and thus the aggravation of the injury (119). In an animal model of cerebral ischemia, administration of Epo resulted in the reduction of the local production of TNF, IL-6, and the chemokine MCP-1, all markers of inflammation, subsequently leading to a marked reduction of infarct size. These results indicate that Epo has an anti-inflammatory effect that contributes to its direct neuroprotective effect during cerebral ischemia (112). Since Epo did not reduce cytokine production in response to LPS applied directly in vitro and in vivo, the authors concluded that the observed anti-inflammatory effect is due to inhibiting neuronal apoptosis and not to a direct effect on inflammatory cells (112). Inflammation is also central to the pathogenesis of autoimmune diseases and Epo significantly reduced the exacerbation of an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (37). In a rat model of cerebral ischemia, Epo significantly reduced the exacerbation of an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (37). The exact effects are unknown, but transmigration of leukocytes from the blood into the brain and inactivation of resident microglial cells enhances the production of inflammatory mediators, which leads to the aggravation of the injury (119).

**Table 2. Epo Effects in Case of CNS, Autoimmune, and Neurodegenerative Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epo Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Stroke</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Potential Clinical Applications</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Stroke</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
<tr>
<td>Potential Clinical Applications</td>
<td>Enhances the survival of oligodendrocytes in autoimmune encephalomyelitis.</td>
</tr>
</tbody>
</table>
of autoimmune diseases. In an experimental model of multiple sclerosis (MS), an autoimmune disease of the CNS, administration of Epo delayed the onset of the disease and decreased its severity (2). Similarly, in a rat model of optic neuritis, systemic administration of Epo significantly increased the survival of the retinal ganglion cells (96).

The exact mechanisms of these anti-inflammatory effects are unknown. Epo might reduce leukocyte transmigration through endothelial cells, since Epo enhances the resistance of endothelial cells toward ischemia (25). The protective effect of Epo on oligodendrocytes against cytotoxicity induced by inflammatory stimuli (41) could explain the beneficial effect of Epo in case of MS where oligodendrocytes play a crucial role in the pathogenesis of the disease.

Epo as a Therapeutic Agent for CNS Disease

Transport through BBB

An important prerequisite for considering Epo as a therapeutic agent in CNS diseases is to answer the question as of whether Epo, administered systemically, is able to cross the BBB. Brines et al. (15) injected mice with biotinylated Epo and subsequently visualized brain section with peroxidase-labeled streptavidin. Indeed, a signal for biotin was detected in a region surrounding the capillaries extending into brain parenchyma. The authors concluded that Epo crosses the BBB. However, biotin might not be an ideal tool to study BBB permeability since it is rapidly transported across the BBB (98, 105), and, therefore, even a small amount of free biotin in the plasma would contribute to a marked influx of Epo into brain parenchyma.

potential Clinical Indications

<table>
<thead>
<tr>
<th>Disease</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke</td>
<td>Various animal models: reduced infarct size.</td>
</tr>
<tr>
<td></td>
<td>Multicenter phase 2/3 trial is being conducted.</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Model of acute EAE (rat): prophylactic administration of Epo delayed the disease onset and reduced its severity.</td>
</tr>
<tr>
<td></td>
<td>Model of chronic EAE (mouse): Epo, administered several weeks after the onset of the clinical signs of MS, reduced the inflammatory response in the spinal cord.</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>Pre-clinical: Epo improved cognitive functions measured in an aversion task involving cortical and subcortical pathways (affected in schizophrenia).</td>
</tr>
<tr>
<td></td>
<td>Clinical: 3 mo Epo add-on medicament improved schizophrenia-related cognitive performance of chronic schizophrenic patients (phase II trial). Epo improved the executive functions of healthy volunteers.</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Models of glaucoma: Transient global ischemia of the retina: systemic administration of Epo reduced the neuronal damage and improved the functional recovery.</td>
</tr>
<tr>
<td></td>
<td>Transaction of the optic nerve: intraocular injection of Epo enhanced the survival of the axotomized RGCs.</td>
</tr>
<tr>
<td></td>
<td>Model of AMD and retinitis pigmentosa: Exposure to high-intensity visible light: systemic administration of Epo protects photoreceptor cells from light-induced apoptosis.</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>6-OHDA model: Epo is protective in vivo and in vitro, enhances the survival and function of grafted dopamine neuron.</td>
</tr>
<tr>
<td></td>
<td>MPTP model: Epo is protective.</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Epo is protective in different animal models (kainate, pilocarpine, and pentylenetetrazol).</td>
</tr>
<tr>
<td>Brain trauma</td>
<td>In TBI models Epo reduces necrosis, reduces inflammation and improved functional recovery.</td>
</tr>
<tr>
<td></td>
<td>Improves neurogenesis in the dentate gyrus as well as the performance in spatial memory tasks.</td>
</tr>
<tr>
<td></td>
<td>Reduces brain edema.</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>Protective effect of Epo in different animal models.</td>
</tr>
</tbody>
</table>

EAE, experimental autoimmune encephalomyelitis; RGC, retinal ganglion cells; AMD, age-related macular degeneration; 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine; TBI, traumatic brain injury.
strophenoxin signaling

Upon binding of Epo to its receptor (EpoR), Janus kinase 2 (JAK2) is phosphorylated and thus activated. This leads to engaging secondary signaling molecules such as signal transducer and activator of transcription 5 (STAT5), the mitogen-activated protein kinases ERK-1/-2, PI3K/Akt, and the activation/nuclear translocation of nuclear factor-κB (NF-κB). It has been shown that ERK-1/2 proteins are essential for Epo-mediated neuroprotection. Moreover, strong evidence from other growth factors suggests ERK-1/2 to be important in Epo-mediated angiogenesis. Controversial data suggest a role of STAT5 in Epo-mediated NF-κB nuclear translocation, although its direct role in Epo-mediated neuroprotection is questionable. PI3K, via Akt phosphorylation, is involved in Epo-mediated anti-inflammation, vascular protection, as well as angiogenesis. Bcl-XL is involved in Epo-mediated vascular protection and neuroprotection. Nuclear translocation of NF-κB reduces expression of genes that are involved in Epo-mediated neurogenesis and neuroprotection.

**Stroke**

The most common cause of stroke is cerebral ischemia where blood flow to the brain is interrupted due to a thrombus in a major cerebral artery, in most cases the middle cerebral artery (MCA). Currently, the only therapeutic approach available is to dissolve the thrombus by injection of tissue plasminogen activator (TPA). A more recent approach that has started to gain attention is neuroprotection, the ability to prevent neuronal death and enhance endogenous protective mechanisms. We will now discuss evidence supporting the potential of Epo in neuroprotection for the treatment of stroke. The first hint came from the observation that the expression of Epo and its receptor in the brain is upregulated upon cerebral ischemia (12, 103). Several in vivo experiments confirmed this hypothesis. Intracerebroventricular injection of Epo 24 h before permanent occlusion of the MCA in mice reduced infarct volume significantly (12). Similarly, infusion of Epo in the lateral ventricles of gerbils in a global ischemia model rescued hippocampal CA1 neurons and increased the number of synapses in the same region (95). Moreover, in another experimental rodent model of cerebral ischemia where the MCA is occluded transiently or also reduced protective effects were applied to ischemic stroke (15). In addition, reduced infarct volume in a cerebral ischemia model has been shown that thalamic hemorrhage reduced cerebral blood flow and hemorrhage was prompted the ischemic stroke patients. The Göttingen Safe and Independent Stroke trial is being conducted for direct neuroprotection observed brain hemorrhage attributed to ischemic brain hemorrhage and prompted the ischemic stroke. However, independent of this, the therapeutic potential of Epo in many CNS diseases (Table 2).

**MS**

MS is a chronic disease where the myelin is attacked by the immune system. The hallmark of MS patients have shown enhanced myelination for MS), including immunosuppressive drugs. These drugs showed a reduction in the number of new lesion in patients. Thus, the potential of Epo on the use of Epo as a treatment for MS is promising. However, independent of this, the therapeutic potential of Epo in many CNS diseases (Table 2).
transiently occluded, systemic administration of Epo also reduced the infarct size (15). Significantly, this protective effect of Epo was retained even when Epo was applied 6 h after the onset of the cerebral ischemia (15). In addition, brain-specific overexpression of Epo reduced infarct size in mice subjected to transient cerebral ischemia (39). Other studies, where the functional outcome of Epo treatment was investigated, have shown that Epo not only reduces infarct volume but also improves the learning ability in gerbils and reduces the navigation disability in rats (94, 95). Epo has also shown to be protective in models of hemorrhagic stroke where the interruption of the cerebral blood flow is due to subarachnoid or cerebral hemorrhage (3, 45). The above-mentioned studies prompted the initiation of clinical trials in stroke patients. The safety and proof-of-concept phases of the Göttingen-Epo-Stroke Study have shown Epo to be safe and to improve the patient functional outcome after stroke (35). Currently, a multicenter phase 2/3 trial is being conducted (48). Although good evidence for direct neuroprotection exists (see above), the observed brain-protective effect of Epo could also be attributed to its effect on astrocytes. Astrocytes protect neurons from oxidative stress by neutralizing reactive oxygen species (31). It has been reported that activated astrocytes in ischemic human brain express increased levels of EpoR (103). Since Epo enhances brain glutathione peroxidase activity (63), Epo, by binding to EpoR on the surface of activated astrocytes, might contribute to the astrocyte-mediated neuroprotective effect against ischemia-induced free-radical formation.

MS

MS is a chronic disease of the brain and the spinal cord where the myelin sheath of the neuronal axons is attacked by the body’s own immune system. A hallmark of MS pathogenesis is inflammation. This has been shown in several experimental autoimmune encephalomyelitis (EAE) models (the animal model for MS), including studies with knockout mice or inhibitors (or antibodies) of inflammatory cytokines that showed a protective effect (92). Since Epo inhibits anti-inflammatory effects (see above), a possible therapeutic benefit of Epo in this neuroinflammatory disease has been tested. In a rat model of acute EAE, administration of Epo in an early phase delayed the disease onset and reduced its severity (2). This study suggested that Epo might be used as a prophylactic agent for MS but said nothing about its therapeutic potential. This question was addressed using a chronic mouse model of EAE. Administration of Epo several weeks after the onset of the clinical signs of MS reduced the inflammatory response in the spinal cord, showing that Epo has a therapeutic effect (66). The underlying mechanisms have not been elucidated so far. However, the effect of Epo in both models is independent on its erythropoietic function since non-erythropoietic carboxylated Epo (ClEpo) had similar effects (66).

Schizophrenia

Schizophrenia is a psychiatric disorder characterized by impairment of perception, dissociation from reality mainly manifested by hallucinations and disorganized speech and thinking. The affected persons are thus socially and occupationally dysfunctional. It has been recognized in the last decade that schizophrenia is associated with neurodegeneration and gradual decline in cognitive capacities (6, 10, 67, 80, 87). Although current neuroleptics used tend to reduce or eliminate psychotic symptoms, allowing the patients to return to “normal” with regard to their social life (34), they have no influence on the silently ongoing neurodegeneration. These drugs can even worsen neurodegeneration due to neurotoxicity of classical neuroleptics (9). Based on the neuroprotective effect, the potential of Epo as an add-on therapy in schizophrenia was therefore investigated. In a rodent study, administration of rhEpo improved cognitive functions of mice as measured in an aversion task involving cortical and subcortical pathways, believed to be affected in schizophrenia (34). In the same study, the authors showed that rhEpo attenuated the neurotoxic effect of the classical neuroleptic drug haloperidol on cultured hippocampal neurons. Very recently, in a follow-up multicenter “proof-of-principle” clinical trial (phase II), the same authors showed that chronic schizophr enic patients treated over a period of 3 months with rhEpo as an add-on medication had an improvement in their schizophrenia-related cognitive performance (36). This was further supported by the observation that a single administration of Epo improved the executive functions of healthy volunteers (79). These results demonstrate that Epo could represent a novel approach for the treatment of schizophrenia.

Retinopathy

The therapeutic potential of Epo also extends to diseases that affect the retina. In glaucoma, one of the most common causes of vision loss and blindness, the intraocular pressure is increased leading to retinal ischemia with subsequent retinal degeneration. In an in vivo model of transient global retinal ischemia, where the intraocular pressure is experimentally elevated, EpoR expression was shown to increase, suggesting that the Epo/EpoR system could play a neuroprotective role in this process. This was confirmed by the observation that exacerbation of ischemia-induced retinal damage upon injection of soluble EpoR. Moreover, systemic administration of Epo shortly before or immediately after the ischemic insult reduced the neuronal damage and improved the functional recovery. Moreover, reduced numbers of TUNEL positive, and thus apoptotic, neurons in the retina were observed (34). Death of retinal ganglion
Cells (RGCs) observed in glaucoma is believed to be mainly mediated by apoptosis (78, 83). Another experimental model to induce apoptosis of RGCs is the transection of the optic nerve, which leads to the apoptosis of the axotomized RGCs (115). Using this model, Weishaupt et al. showed that repeated intraocular injection of Epo after transection of the optic nerve enhanced the survival of the axotomized RGCs (115). Similar to the retinal ganglion cells in glaucoma (78), apoptosis is believed to be the major mechanism of death of the photoreceptor cells in the age-related macular degeneration (AMD) (1) and retinitis pigmentosa (110). A well-established model to study photoreceptor cell apoptosis is the exposure of animals to high-intensity visible light (89). Using that model, Grimm et al. showed that systemic administration of Epo protects photoreceptor cells from light-induced apoptosis (46). However, it is noteworthy that Epo protects only induced, but not inherited, retinal degeneration such as retinitis pigmentosa (47).

Parkinson’s disease
Parkinson’s disease (PD) is a neurodegenerative disease affecting the dopamine neurons in the substantia nigra pars compacta of the nigrostriatal system. Although the etiology of the PD is not yet clear, recent reports suggest apoptosis of dopamine neuron and inflammation to be important for the pathogenesis of PD (5, 27). Using the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA), Signore et al. showed that Epo is neuroprotective in immortalized and primary dopaminergic neurons as well as in mice unilaterally lesioned by 6-OHDA (100). This effect was mainly mediated by the inhibition of Akt/PKB and caspase-3 activation and subsequent DNA laddering. A similar protective effect against 6-OHDA neurotoxicity was observed by Xie et al. (120). However, the authors attributed this effect to Epo-mediated anti-inflammation. Epo has also been shown to be neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD (43) and to enhance the survival and function of grafted dopamine neurons in a PD animal model (35).

Epilepsy
A neuroprotective function for Epo in epilepsy has also been investigated. In a kainate model of status epilepticus (SE), Brines et al. showed that treatment with Epo 24 h before kainate injection both prolonged the latency and reduced the severity of the seizures (15). Very recently, Nadam et al. showed a similar neuroprotective effect of Epo in pilocarpine-induced SE, where the administration of Epo significantly protected hippocampal neurons (81). Although the exact mechanism is not yet fully elucidated, evidence for anti-apoptotic effects of Epo during SE exists, since Epo preconditioning inhibited caspase-3 activation and upregulated the anti-apoptotic protein Bcl-2 (116, 122). Epo has also been reported to prevent an increase in BBB permeability and thus edema formation in pentyleneetrazol-induced seizures, providing another mechanism of the neuroprotection (119).

Brain trauma
The pathophysiology of traumatic brain injury (TBI) is complex, and so far only symptomatic treatments are available. In recent years, a possible neuroprotective effect of Epo in TBI has been investigated in different animal models. Brines et al. showed that mice treated with rhEpo before or after receiving a blow delivered to the cortex developed less necrosis at 10 days postinjury (15). Similarly, reduction of inflammation, apoptosis, and an improvement in functional recovery has been observed in mice subjected to focal TBI when they were treated with rhEpo 1 and 24 h after the insult (124). In addition, treatment of mice on a daily basis for 14 days after a focal TBI improved neurogenesis in the dentate gyrus as well as the performance in spatial memory tasks (89). Very recently it has been shown in a rat model of diffuse TBI that rhEpo treatment after TBI significantly reduces the development of brain edema (111).

Spinal cord injury
Spinal cord injury (SCI) is caused by damage in the nerves of the spinal canal, which results in the inability of the spinal cord to transmitafferent/efferent signals controlling sensory, motor, or autonomic activities. It is usually caused by trauma. The main determinant of the clinical outcome of SCI is the extent of spreading of the injury away from its epicenter where cell death, caused by a combination of inflammation, ischemia, and apoptosis, takes place (20, 32, 84). Since Epo has anti-inflammatory and anti-apoptotic functions as well as a protective role against ischemia, a potential protective/therapeutic role of Epo in SCI has been investigated in several animal models. In a rabbit model of transient spinal ischemia, where the infrarenal aorta is transiently occluded, treating animals with Epo immediately after the release of the occlusion resulted in an improvement in the neurological score compared with saline-treated animals (21). Moreover, Epo has also been shown to be protective in rodent compression and contusion models (44, 56).
This page contains a discussion on the use of erythropoietin (Epo) in neurological research, particularly in the context of neuroprotection and neurodegenerative diseases. The text covers various aspects including the role of Epo in immune modulation, its therapeutic potential in neurological disorders, and recent developments in its use.

**Summary and Outlook**

Epo treatment is a promising strategy not only for erythropoiesis but also for cell survival in various organisms including heart and brain. In addition, Epo might also be effective as a modulator of inflammation. Outgoing from basic research where the protective role of Epo has been demonstrated in many experimental models of neuropathology, Epo has been shown to be a promising molecule. In the current review, the authors discuss the underlying mechanisms and summarize the current knowledge of its role in various neurological diseases.

**References**


...
Magnesium

Mg²⁺ is the main intracellular cation. It is present at concentrations of 15–25 mM (39, 53, 55). Mg²⁺ is bound to ATP at multiple enzymatic sites, and as a second messenger, it occupies a pocket of a multitude of enzymes and plays a role in various biological processes. The hydrated radius of Mg²⁺ is half of that of Na⁺. Mg²⁺, unlike Na⁺, prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³. Mg²⁺ also prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³. Mg²⁺, unlike Na⁺, always prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³.

In addition, Mg²⁺, unlike Na⁺, always prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³. Mg²⁺, unlike Na⁺, always prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³.

Considering the lack of information by which the chelator has been characterized, Mg²⁺ is the main intracellular cation. It is present at concentrations of 15–25 mM (39, 53, 55). Mg²⁺ is bound to ATP at multiple enzymatic sites, and as a second messenger, it occupies a pocket of a multitude of enzymes and plays a role in various biological processes. The hydrated radius of Mg²⁺ is half of that of Na⁺. Mg²⁺, unlike Na⁺, prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³. Mg²⁺, unlike Na⁺, always prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³. Mg²⁺, unlike Na⁺, always prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³.

Regarding this lack of information, Mg²⁺ is the main intracellular cation. It is present at concentrations of 15–25 mM (39, 53, 55). Mg²⁺ is bound to ATP at multiple enzymatic sites, and as a second messenger, it occupies a pocket of a multitude of enzymes and plays a role in various biological processes. The hydrated radius of Mg²⁺ is half of that of Na⁺. Mg²⁺, unlike Na⁺, prefers hydrated Mg²⁺ cations over Na⁺, holding the water molecules with a factor of 10³.