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ERYTHROPOIETIN (EPO): ROLE IN NEUROPROTECTION/ NEUROREGENRATION AND COGNITION

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ABSTRACT

The discovery of the broad neuroprotective potential of erythropoietin (EPO), an endogenous hematopoietic growth factor, leaded to the new therapeutic avenues in the treatment of brain diseases. EPO has direct effects on cells of the nervous system that make it a highly attractive candidate drug for neuroprotection/neuroregeneration. EPO expression in the brain is induced by hypoxia. Practically all brain cells are capable of production and release of EPO and expression of its receptor. EPO exerts multifarious protective effects on brain cells. It protects neuronal cells from noxious stimuli such as hypoxia, excess glutamate, serum deprivation or kainic acid exposure in vitro by targeting a variety of mechanisms and involves neuronal, glial and endothelial cell functions. In rodent models of ischemic stroke, EPO reduces infarct volume and improves functional outcome, but beneficial effects have also been observed in animal models of subarachnoid hemorrhage, intracerebral hemorrhage, traumatic brain injury, and spinal cord injury. EPO has a convenient therapeutic window upon ischemic stroke and favorable pharmacokinetics. EPO has been found by many investigators to be protective or regenerative and to improve cognitive performance in various rodent models of neurological and psychiatric disease. Results from first therapeutic trials in humans are promising, but will need to be validated in larger trials. This article reviews on the preclinical and clinical work on EPO for the indications neuroprotection/neuroregeneration and cognition.

Keywords: Erythropoietin, cognition, neuroprotection clinical study, preclinical study.

INTRODUCTION

Most diseases of the nervous system are etiologically unclear, extremely heterogeneous, and non-curable, with chances being very low that within the next decades a cure will be available for any of them. Facing this disillusioning reality, and considering the enormous human and socio-economic burden to be expected with an increasingly aging society in industrialized countries, the urgent demand of neuroprotective/neuroregenerative treatment approaches becomes even more plausible. Neuroprotective treatments aim at an enduring improvement of symptoms and/ or slowing of an ongoing disease process. Essentially all compounds that have been associated with a potential neuroprotective/neuroregenerative capacity target determinants of the final common pathway of many different diseases of the nervous system, e.g. apoptosis, oxidative stress, inflammation, metabolic dysfunction or compromised neuroplasticity.[1]

Erythropoietin (often shortened to EPO) is a naturally occurring hormone, secreted by the kidneys, whose function is to regulate red blood cell production. The principal growth factor that regulates erythropoiesis is EPO. Its main function is stimulation of red blood cell production and its systemic overexpression results in erythrocytosis.EPO has now over more than a decade attracted so much attention as candidate for neuroprotection/neuroregeneration .Not many other drug candidates have triggered so many preclinical studies on entirely different disease models, investigated by multiple independent research groups worldwide, than EPO. This overwhelming amount of data on EPO, showing mostly positive results

regarding neuroprotection and neuroregeneration, has also stimulated clinical research as reflected by a substantial number of ongoing clinical trials on EPO in nervous system indications [2].The few thus far published clinical studies all yielded positive results or at least positive signals to be further pursued.
The neuroprotective actions of EPO can be separated from its hematopoietic actions, a fact that is of value for therapeutic applications where the increase in red cell mass is not desired. EPO and EPO derivatives are directly neuroprotective in cell culture models and after direct application into the brain [3]. Expression of EPO and the classical EPOR in brain cells is induced by hypoxic-ischemic stress and contributes to ischemic tolerance while neutralization of the brain endogenous EPO augments ischemic damage [4, 5, 6]. Brain-specific genetic ablation of the classical EPOR impairs post-stroke neurogenesis and neuronal survival [7, 8] whereas transgenic brain specific over expression of human EPO is associated with reductions in post ischemic infarct volume, brain swelling and functional deficits in a transient stroke model [9].

Beneficial effects of EPO on cognitive functioning have been shown in different animal models of neuropsychiatric diseases, e.g. on place navigation after global ischemia or neurotrauma [10]. EPO turned out to be the first compound to exert a selective and lasting beneficial effect on cognition in schizophrenia [11]. Similarly, an increase in cognitive performance upon EPO in patients with chronic progressive multiple sclerosis occurred independently of changes in hemoglobin levels, and persisted for months after termination of EPO treatment[12,13]. The application of a single high intravenous dose of EPO in healthy human volunteers was reported to enhance the functional MRI-detectable hippocampus response during memory retrieval 1 week later, before any effect on hemoglobin was measured [14]. Hengemihle et al [15] reported that 19 weeks of low-dose EPO treatment increased spatial memory performance, and a conditioned learning task, taste aversion, was enhanced by a single high-dose injection of EPO in healthy mice [16].

Many pre-clinical and clinical studies dealing with EPO treatment of neuropsychiatric diseases on healthy individuals, rodents and humans, set up to gain more mechanistic insight into the potent effects of EPO on its role on neuroprotection/ neuroregeneration and cognitive performance [1] .The present review, will summarize the overall positive outcomes of preclinical as well as of clinical work, done over the years on EPO treatment of brain diseases.

PHYSIOLOGY OF ERYTHROPOIETIN (EPO)

The hematopoietic growth factor erythropoietin (EPO) circulates in plasma and controls the oxygen carrying capacity of the blood.EPO is essential for red blood cell (RBC) production. The relationship between the O_2 content of the blood and erythropoiesis was first described by the French anatomist Francois-Gilbert Viault in 1890 [17]who observed a rise in RBC numbers on a journey to the highland of Peru (Morococha, about 4500 m).Indeed, the specific stimulus for EPO expression is a fall in tissue O_2 pressure (pO_2). EPO production increases under hypoxic conditions in the kidneys and, in minor amounts, in distinct other organs such as the liver and the brain [18].

To maintain the increase in red-cell volume associated with fetal growth, it is estimated that approximately 50×10^9 erythrocytes per day must be produced. Compared to adult EPO concentrations present at the time of acute anemia, measured fetal EPO concentrations seem low in the face of such production requirements. It has therefore been proposed that EPO is either more efficient in the stimulation of the erythropoiesis during fetal development, that it acts as a paracrine factor during hepatic hematopoiesis, and/or that other growth factors synergize with EPO. Likely candidates include hepatic growth factor (HGF), thrombopoietin (TPO), and insulin-like growth factor-1 (IGF-1). Fetal EPO production is clearly regulated by requirements for tissue oxygenation, as elevated EPO concentrations (up to 8000 mU/mL) have been reported in several pathologic states, such as fetal hypoxia, anemia, placental insufficiency, and in infants of diabetic mothers [19].

In humans, the EPO mRNA encodes a protein with 193 amino acids .The cleavage of 27 N-terminal amino acids (signal peptide) and loss of the C-terminal arginine during post translation modification result in a 165-amino acid structure that comprises the mature protein (Fig. 1). The EPO molecule contains two structure-stabilizing disulfide bonds between amino acids 7 and 161 and 29 and 33, the reduction of which results in loss of bioactivity and also the EPO molecule possesses three N-linked sugars, at positions 24, 38, and 83, and one O-linked sugar at position 126. The O-linked sugar has no important function, but the N-linked sugars are necessary for stability of the EPO molecule in the circulation [20]. [Fig 1] EPO is produced primarily in the adult kidney and fetal liver and was originally believed to play a role restricted to stimulation of early erythroid precursor proliferation, inhibition of apoptosis, and differentiation of the erythroid lineage

.EPO mRNA is also detectable in brain, liver, spleen, lung and testis, but these organs are not able to substitute for renal EPO in chronic kidney disease (CKD). Brain-derived EPO acts locally as a neuroprotective factor [21 , 22].

EPO acts primarily to stimulate erythroid cell production by supporting the survival, proliferation and differentiation of erythroid progenitor cells. In addition to hematopoietic cells, expression of the EPO receptor (EPO-R) and EPO response are observed in other cell types including endothelial and neural cells [23].

The human hematopoietic EPO receptor (EPO-R) is a 484 amino acid glycoprotein of about 60 kDa, which belongs to the cytokine class I receptor family and forms homodimers. On binding of EPO to the EPO-R dimer, cytoplasmic Janus kinases 2 (JAK2) catalyze the phosphorylation of tyrosine residues of the EPO-R and of various intracellular proteins (enzymes and transcription factors). Erythropoiesis is a slow-acting process. The primary mechanism of EPO clearance is by EPO-R binding, internalization and degradation. During development, the EPO-R is widespread, involving erythrocyte precursors not only in the marrow, but also in liver stromal cells, smooth muscle cells, myocardiocytes, endothelial cells, enterocytes, renal tubular cells, epithelial cells in the lung, retinal cells, placental tissues, Leydig cells, and cells specific to the central nervous system [19]. Following a rise in plasma EPO it takes 3 to 4 days before reticulocytosis becomes apparent [18]. Receptors in this family share several distinct features, including an extracellular ligand binding domain with two pairs of conserved cysteine residues and a conserved motif, WSXWS, located close to the transmembrane domain; a single transmembrane domain; and an intracellular domain lacking catalytic activity (Fig. 2) [20].

EPO is not only expressed in the adult kidney and fetal liver but also in many other organs in the body. In general, its expression can be upregulated via hypoxia inducible factor (HIF) which in turn is stimulated predominantly by low oxygen levels. In addition, other mechanisms might tissue dependently is involved in regulation of EPO gene expression [19, 23, 25]. The EPO/EPO-R system in the brain apparently plays an important role during fetal development and displays a strong reduction of expression levels towards postnatal life and adulthood [25, 26].
In the event of hypoxia/ischemia, inflammation or neurodegenerative processes, an upregulation of the endogenous EPO/EPOR system is observed. Experimental reduction of the available EPO molecules in such situations by e.g. application of soluble EPO-R, leads to a dramatic increase in the model-specific tissue damage [27]. Reduced concentrations of EPO in the cerebrospinal fluid in amyotrophic lateral sclerosis may point to a relative deficiency of endogenous EPO versus EPOR production in neurodegenerative disease [28].

Another important aspect of EPO biology, and perhaps of future pharmacological developments building on the EPO system, is the existence of 'brain specific' EPO variants - both endogenous molecules and exogenously modified compounds - potentially devoid of hematopoietic and other peripheral effects [29].

BRAIN EPO/EPO-R SYSTEM

The mRNA and protein of EPO and EPOR are detected in brain (hippocampus, internal capsule, cortex, midbrain), as well as in vitro in neurons, astrocytes, oligodendrocytes, microglia and cerebral endothelial cells [30] suggesting that this factor can function in the brain in a paracrine and/or autocrine manner. In the developing mouse brain expression of EPO and EPO-R peaks during midgestation and decreases to adult levels in late gestation [31, 32, 33].

Expression of EPO and EPOR in the adult brain is stress-responsive and is regulated by oxygen supply. Both receptor and ligand expression is upregulated after hypoxia or ischemia [34,35,36].Other stimuli such as hypoglycemia, insulin release, reactive oxygen species and insulin-like growth factor activate hypoxia-inducible factor and lead to increased expression of EPO [37, 38]. Proinflammatory cytokines down regulate expression of EPO mRNA but increase that of EPO-R in astrocytes [39].

EPO: A MULTIFARIOUS NEUROPROTECTIVE

EPO has been reported to induce a broad range of cellular responses in the brain directed to protect and repair tissue damage [40]. EPO is neuroprotective in a variety of hypoxic, hypoglycemic, and excitotoxic in vitro models. A fundamental mechanism of EPO-induced neuroprotection in cultured neurons is its ability to inhibit apoptosis reducing both DNA damage and cell membrane asymmetry [41, 42]. Another tissue-protective mechanism of EPO is its ability to protect cells against oxidative damage [43]. EPO inhibits lipid peroxidation by increasing the activities of cytosolic antioxidant enzymes such as superoxide dismutase and glutathione peroxidase [44]. EPO attenuates inflammation by reducing reactive astrocytosis and microglia activation and by inhibiting immune cells recruitment into the injured

area [45]. In Cerebrovascular endothelial cell cultures EPO down-regulates TNF-α-induced gene expression of interleukin-6 (IL-6), IL-1beta, CXCR4, and IL-1alpha. It also directly counteracts interferon-γ- and lipopolyssaccharide-induced cytotoxicity in oligodendrocytes, preserves white matter and reduces TNF-α release and its effects in cultured Schwann cells [46, 47].

EPO protects vascular integrity and stimulates angiogenesis [48]. It preserves blood-brain barrier integrity during injury by restoring expression of tight junction proteins, by reducing vascular inflammation and reactive free radical expression. In vasculogenesis EPO stimulates proliferation of endothelial precursor cells, production of matrix metalloproteinase- 2, migration of endothelial cells into vascular sites and formation of capillary tubes [49].

EPO displays direct antiapoptotic activity in cerebral endothelial cells during oxidative stress and ischemic injury as well [50]. Stimulation of endothelial nitric oxide synthase (eNOS) activity has been shown to contribute to the improvements by EPO after experimental cerebral hemorrhage [51]. EPO increases proliferation of oligodendrocyte progenitors and promotes differentiation of oligodendrocytes in culture [30].

EPOR-/- fetuses exhibit increased apoptosis in the brain and a reduction in the number of neural progenitor cells, as well as increased sensitivity to hypoxia prior to significant anemia or heart defects in the embryo proper .Moreover, adult mice that lack EPOR in the brain have significantly reduced neurogenesis in the sub ventricular zone and demonstrate impaired migration of precursors into infracted cortex [41, 42]. The reported neurotrophic effects of EPO include the ability to stimulate axonal regrowth, neurite formation, dendritic sprouting, electrical activity and modulate intracellular calcium and neurotransmitter synthesis and release .In rat hippocampal slices, EPO improved synaptic transmission during and following oxygen and glucose deprivation [52].

PRECLINICAL STUDIES

The preclinical data in support of the use of EPO in human brain disease have explosively increased since the first discovery of its neuroprotective action.

EPO and Ischemic Preconditioning: Prior exposure to sublethal ischemia or hypoxia can provide tolerance to cerebral ischemia or preconditioning. For example, mice exposed to sublethal low levels of oxygen tension 24 h prior to focal permanent ischemia in mice resulted in a reduction in infarct volumes [53]. Preconditioning was associated with cerebral expression of HIF-1α and HIF target genes such as EPO and VEGF. The importance of endogenous EPO in hypoxia preconditioning for cerebral infarct in mice was demonstrated by administration of soluble EPO-R in the cerebral ventricle that reduced the protective effect in the rage of 40–88% [54, 55].

In an in vitro model of cerebral ischemia, EPO mediated ischemic tolerance in primary cortical neurons, and soluble EPO-R, anti-EPO-R antibody and a JAK2 inhibitor blocked protection. In the retina, hypoxic preconditioning protects against light-induced apoptosis and interferes with caspase-1 activation, and this activity could be mimicked by EPO injected intraperitoneally to provide neuroprotection to retinal photoreceptors [56].

Cerebral ischemia: Studies in animal models show the potential for EPO neuroprotection in brain ischemia or trauma. Evidence that endogenous EPO may provide neuroprotection was first demonstrated in gerbils with mild brain ischemic treatment after infusion into the lateral ventricle of soluble EPO-R capable of binding EPO resulting in neuronal degeneration and impaired learning ability [57].

Infusion of exogenous EPO was neuroprotective against ischemic damage of hippocampal CA1 neurons and ischemia-induced learning disability. In mice after cerebral infarct induced by focal permanent ischemia, induction of EPO-R in brain is observed in endothelial cells, microglia and astrocytes in a temporal manner followed by induction of EPO expression [58].

Intracerebral injection of exogenous EPO 24 h prior to challenge reduces infarct volume by about 50%. In rats, permanent ischemia of the left cortex resulted in upregulation of EPO-R expression in the periphery or ischemic penumbra [59]. Infusion of EPO into the cerebroventricle just after middle cerebral artery occlusion (MCAO) was neuroprotective, reduced secondary thalamic degeneration and reduced ischemia-induced place navigation disability. Induction of EPO-R is also observed in cerebral ischemia in the neonatal rat brain in ischemic areas. These studies provide evidence for increase of EPO-R after infarct to facilitate EPO signaling and provide protection to minimize the degree and extent of damage, and that administration of exogenous EPO directly to brain significantly reduces damage and

infarct size in rodent models [60]. These animal models suggest the potential for direct administration of EPO in brain for treatment of brain ischemia/trauma or disease.

Traumatic brain and spinal cord injury: Administration of EPO and EPO-analogs in experimental models of traumatic brain and spinal cord injury leads to morphological, functional and cognitive recovery that can be attributed to a multitude of cytoprotective mechanisms including inhibition of apoptosis, anti-inflammatory and anti-oxidant actions, restoration of blood-brain barrier integrity, stimulation of neurogenesis and angiogenesis [61, 62].

Brain edema after experimental brain injury can effectively be attenuated by post-treatment with EPO [49]. A reduction of cytotoxic and vasogenic edema may be anticipated based on the direct actions of EPO on glutamate release and on the endothelial barrier function. It is not clear to date which from the panoply of neurorestorative effects of EPO are responsible for the long term prevention of trauma-induced brain atrophy, cognitive and neurobehavioral dysfunction [63]. In this context it is interesting to note that chronic peripheral administration of EPO has been reported to improve spatial memory function and cognitive functioning in the context of an aversion task also in healthy mice [64, 65].

Cerebral hemorrhage: Post-treatment with EPO starting at 2 h after induction of intracerebral hemorrhage (ICH) by intraparenchymal injections of collagenase or autologous blood dose dependently reduced volume of hemorrhage, brain edema, perihematomal apoptosis and inflammation in a rat model. Functional recovery was faster and more efficient in the EPO-treated group and was associated with reduction in hemispheric brain atrophy 5 weeks after the induction of ICH [66].Cerebral vasospasm and ischemic brain damage after subarachnoid hemorrhage (SAH) by autologous blood injections into the cisterna magna in rabbits are reduced by EPO administered either by intraperitoneal injections of rhEPO or by delivery of adenoviral vectors encoding the human EPO into cisterna magna immediately after induction of SAH . Mortality and functional deficits 3 days after induction of SAH were reduced in EPO treated rabbits [67, 68]. In a rat model of SAH, the impaired autoregulatory response of cerebral blood flow to intravenous noradrenaline was restored by a single subcutaneous bolus of EPO [69].

Degeneration & neuroinflammation: EPO and its analogs offer protection also in models of neurodegenerative and neuroinflammatory disease. In experimental autoimmunencephalitis (EAE), an animal model for multiple sclerosis (MS), treatment with EPO and EPO analogs can improve functional recovery, reduce tissue damage, inflammatory responses and blood-brain barrier leakage [45] Beneficial effects of EPO have also been reported in models of peripheral axonal nerve injury, injury-induced Wallerian degeneration and HIV-associated sensory neuropathy [45, 46, 47, 70]. Here, the anti-cytokine, anti-apoptotic, anti-oxidative and tropic effects on both neurons and oligodendrocyte progenitor cells by EPO seem to play an important role in reducing inflammation and preserving myelination and neuronal function[45, 46, 70] Chronic neurodegeneration might also be a target for EPO therapy as EPO and its analogs can counteract degenerative processes in experimental models of Parkinson disease and amyotrophic lateral sclerosis (ALS) by inducing anti-oxidant enzymes, inhibiting apoptosis and stimulating axonal regeneration .EPO improved graft survival of embryonic ventral mesencephalic dopamine neurons when transplanted into the striatum of 6-hydroxy-dopamine lesioned rats [71].

CLINICAL STUDIES

Clinical studies on EPO in neurological and psychiatric indications are still rare even though some studies have been done and some are still ongoing worldwide.

Cerebrovascular diseases: The first study investigating the potential neuroprotective/ neuroregenerative capacities of EPO in humans was the Göttingen EPO Stroke Study, started as early as 1998.This double-blind placebo controlled monocentric study yielded a positive clinical outcome of EPO as compared to placebo treated patients, together with promising results on evolution of lesion size and levels of the circulating damage marker S100B [72]. Encouraged by these findings, a German multicenter study on EPO in stroke patients was started in 2003 and concluded in 2008. From the first to the second EPO study, the 'stroke landscape' had considerably changed with the regulatory approval of thrombolytic treatment using rtPA (recombinant tissue plasminogen activator) for stroke [73]. Predictions by advising stroke experts at study start regarding percentage of rtPA treatments ranged between 3% and maximally 10%. Unexpectedly, an incredible number of nearly 64% of the 522 patients included in this study received thrombolysis. This unforeseen development certainly explains the overall failure of the second EPO stroke study which

formally ended up as a negative trial. Whereas the subpopulation of per-protocol treated patients non-qualifying for rtPA treatment (the actual comparator of the first study) again benefited from EPO treatment with respect to clinical recovery, the rtPA patients did not have any clear profit from EPO treatment. Those patients who had received thrombolysis despite contraindications to rtPA even showed a deterioration of their outcome upon combination of rtPA and EPO [74, 75].At this point, EPO should only be considered for further development in ischemic stroke patients non-qualifying for rtPA. For these patients, however, it may ultimately provide a true alternative [75].

Hopefully, the overall results of the German multicenter EPO stroke trial, forcing to call it a formally negative study, will not discourage researchers and funding agencies from further pursuing EPO/EPO variants for this indication. It should be emphasized that at present, there is no better neuroprotective/neuroregenerative compound available anywhere for treatment of ischemic stroke. This indication is additionally supported by recent clinical studies showing beneficial effects of EPO in patients after subarachnoid hemorrhage where ischemia is a frequent consequence as well as in patients suffering from cardiac arrest. Even though cerebrovascular events in neonates are certainly different from cerebrovascular events in adults, a just published study on late neurodevelopmental parameters of extremely pre-term infants with cerebral hemorrhage shows a dramatic improvement of the intellectual outcome of former high risk infants at the age of ten years [76].

Recent trials on asphyctic newborns support the protective effect of EPO against hypoxic-ischemic encephalopathy.[77] In the context of cerebrovascular disease and EPO, it is important to mention that a recent clinical trial testing the effect of the EPO variant darbepoetin in patients with diabetes, chronic kidney disease, and moderate anemia who were not undergoing dialysis did not reduce the risk of death or a cardiovascular or renal event, and was associated with an increased risk of stroke [78].

Chronic brain diseases: In addition to acute cerebrovascular brain diseases, chronic, degenerative and inflammatory brain diseases may be an interesting field for EPO application in humans. These certainly also include the regenerative/rehabilitative phase post stroke where EPO can be expected to promote and consolidate functional recovery and should be tested in a clinical study.

In a double-blind, placebo-controlled multicenter trial, chronic schizophrenic patients showed upon high-dose weekly EPO add-on treatment over 12 weeks a significant improvement of higher cognitive functions and a reduction of cortical gray matter loss in discrete disease-relevant brain areas [79] .The basis of their scattered distribution, including the lateralized (left-sided) preference of EPO-mediated gray matter protection, is still unclear but likely indicates areas with most progressive neurodegeneration inherent to the disease process.

Also patients suffering from chronic-progressive multiple sclerosis displayed improvement in motor functions and cognitive performance upon high dose EPO treatment without considerable increase in hemoglobin [13]. Since no treatment options are available for patients with chronic-progressive multiple sclerosis or chronic schizophrenia that effectively target motor functions and/or cognition, EPO and EPO variants should be considered as candidate drugs to address these severely disabling symptoms exemplifies the improvement in attention/concentration upon high-dose EPO treatment in a patient suffering from multiple sclerosis.

A clinical study illustrates the clinical course before and during a 16-week placebo/EPO treatment period of a patient suffering over years from severe, therapy-resistant depression. This patient was unaware of the time point of EPO or placebo infusions (single-blind design). He did not improve upon placebo but clearly responded to EPO injection with reduced depressive symptoms, as evaluated by a trained clinician using the Hamilton rating scale for depression [80, 81].

Similarly to the multiple sclerosis case, the patient displayed an only weak reaction of hemoglobin to EPO. Just towards the end of his treatment period (in the 10th week), bloodletting (450 ml) was necessary. A potential new EPO indication ‘major depression’ is further supported by a functional magnetic resonance imaging study on depressed patients responding to EPO in a fashion comparable to that observed upon antidepressive medication [82]. Based on these grounds, a clinical trial has been started in Denmark, testing the effect of high-dose EPO treatment on affective and cognitive symptoms of major depression [83].

EPO AND COGNITION

Effects of EPO on cognition have been observed as early as around the time of its introduction to the clinic for the treatment of anemia in chronic kidney disease [80]. At that time, improvement of cognitive

performance was attributed to the EPO-induced increase in red blood cells/ hemoglobin with subsequently enhanced tissue oxygenation. In fact, artificial reduction of circulating red blood cells in human volunteers to anemic levels leads to compromised cognitive performance, which can be corrected again by re-transfusion of the blood [84].

The first animal study testing cognitive functions in healthy mice upon chronic EPO treatment was also conducted on these grounds. This study found a slight improvement in hippocampal learning and memory of mice, as measured using Morris water maze, following chronic EPO treatment, and attributed it to the increase in hemoglobin [85]. The similar results were obtained in healthy mice following chronic high-dose EPO treatment over a period of 3 weeks [1]. Particularly the last days of the learning curve in the hidden platform task reveal superiority of the EPO treated group, underlined by the results of the probe trial. The fact that EPO acts in the nervous system, has specific binding sites in neurons, and crosses even an intact blood–brain barrier [25, 26, 85] makes it very likely that at least some of these effects on cognition are direct effects on the brain.

In fact, human studies demonstrating improvements in cognitive performance failed to show a correlation between changes of blood values and cognitive enhancement.[79] An even more convincing argument for direct cognitive effects of EPO is the observation that non hematopoietic EPO variants (e.g. CEPO ¼ carbamoylated erythropoietin) were found to exert specific actions on the nervous system. High-dose EPO treatment of young mice every other day for 3 weeks leads not only to an improvement in hippocampus associated learning and memory processes, but also to a highly significant enhancement of long-term potentiation in the hippocampus[86]. The same treatment schedule lastingly enhances higher brain functions in mice, ranging from various types of learning and memory processes to attention performance [87]. Interestingly, after only one single high intravenous dose of EPO, before any change in hematological readouts, healthy human subjects display alterations in functional MRI studies investigating the hippocampal response [88,89] Inspired by all these findings, we created a mouse model, over expressing continuously active EPOR (i.e. an EPOR not requiring endogenous ligand) under the a calcium/ calmodulin-dependent protein kinase II (a-CaMKII) promoter. In this model, we found a highly significant improvement of hippocampus-associated higher cognitive performance (manuscript in preparation). EPO effects on cognition, as delineated in the chapters above, are also evident in human disease states like schizophrenia and multiple sclerosis [79]. Taken together, in the nervous system, EPO targets cellular/molecular mechanisms involved in cognitive functions. These mechanisms apparently range from fast responding actions, [82, 83] to more delayed and longer lasting effects that persist despite discontinuation of EPO treatment, both in human brain disease [80] as well as in mouse models, and most likely influence readouts of neuroplasticity, e.g. synapse formation[86, 88, 89].

CONCLUSION

After multiple positive findings of EPO as a neuroprotective/ neuroregenerative agent for the treatment of human brain diseases, it appears mandatory to further pursue these indications. Therefore it can be concluded that further clinical development of EPO or EPO variants, the doses, application routes and treatment schedules would have to be varied and re-tested in different clinical studies for the most promising indications ischemic stroke, neurotrauma, multiple sclerosis and as decline in cognitive function is one of the leading symptoms of diseases in an aging society, strategies employing EPO as a cognition improving compound have to be further pursued. Also more basic research has to be performed to investigate potential pharmacological interactions of EPO with drugs used routinely for treatment of the above listed conditions in order to predict potential beneficial or detrimental interactions.

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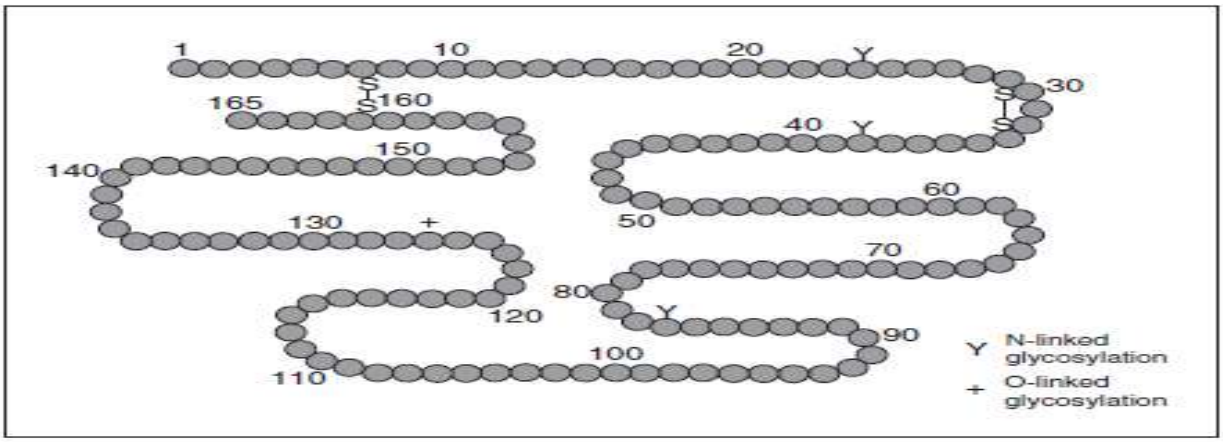

Fig 1: **Structure of Erythropoietin [20].**

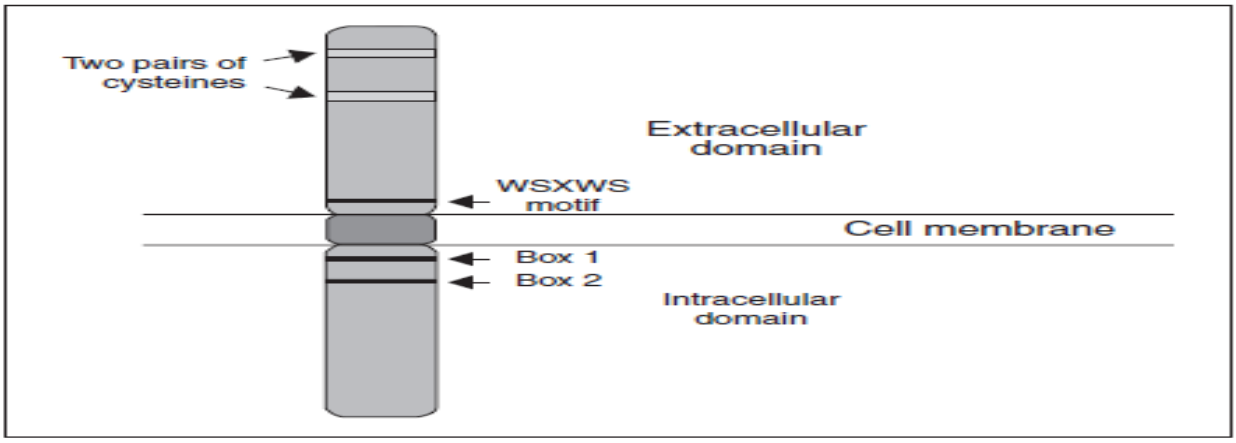

Fig 2: Schematic representation of EPO receptor [20].

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