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Research article

Expression of erythropoietin receptor protein in the mouse hippocampus in response to normobaric hypoxia

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ABSTRACT

Background: Over the past decades, accumulating research on erythropoietin (EPO) and its receptor (EPOR) has revealed various neuroprotective actions and upregulation in hypoxic conditions. To our knowledge, EPOR protein levels in the hippocampus and isocortex have never been measured. Therefore, the aim of this study was to measure EPOR protein in the hippocampus (HPC) and prefrontal cortex (PFC). Further objectives were to examine the effects of exposure to normobaric hypoxia of various degrees and durations on EPOR protein and to explore how long-lasting these effects were.

Method: Adult C57BL/6 mice were randomized into a control group (N = 12) or various hypoxia groups (N = 5–11). Mice were exposed to three different O_2 concentrations (10 %, 12 %, or 18 %) for 8 h a day for 5 days and sacrificed immediately after the last exposure. The effect of exposure to 12 % O_2 for 1 day and 4 weeks (8 h per day) at this survival time was also examined. Additionally, groups of mice were exposed to 12 % O_2 for 1 or 5 days (8 h per day) and euthanized at various times (up to 3 weeks) thereafter to examine the duration of EPOR protein regulation in the HPC and the PFC. EPOR protein was detected with a sandwich-ELISA method.

Results: EPOR protein was present in the HPC and PFC, at 206.64 ± 43.98 pg/mg and 184.25 ± 48.21 pg/mg, respectively. The highest increase in EPOR protein was observed in the HPC after 5 days of 8 h exposure to 12 % O_2 and was most pronounced 24 h after last exposure. The effect of hypoxia normalized within one week after the last exposure.

Conclusion: This study successfully measured hippocampal EPOR protein and showed a significant association between normobaric hypoxia and acute EPOR elevation. It is our hope that this study can provide guidance to future research on the neuroprotective effects of EPO.

1. Introduction

Erythropoietin (EPO) is an endogenous glycoprotein known for its primary role in the hematopoietic system, stimulating the production of erythrocytes, in response to hypoxic conditions. More importantly, over the past two decades research has revealed a secondary neuroprotective role for EPO [1]. Expression of the EPO receptor (EPOR) has been observed in astrocytes, neurons,

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Abbreviation explanation

ANOVA	*Analysis of variance*
ARRIVE	*Animal Research: Reporting of In Vivo Experiments*
EPO	*Erythropoietin*
EPOR	*Erythropoietin receptor*
ELISA	*Enzyme-linked-immunoassay*
H	*hour*
HPC	*Hippocampus*
HRP	*Horseradish peroxidase*
Ip	*Intraperitoneal*
PFC	*Prefrontal cortex*
rhEPO	*Recombinant-human-erythropoietin*

microglia, brain vascular endothelial cells, and oligodendrocytes. Essentially, EPO and EPOR are most abundant in brain regions susceptible to hypoxia, including the hippocampus (HPC) [1–5]. EPOR is a type I cytokine receptor, which upon EPO binding activates several downstream pathways involved in cell proliferation, cell differentiation, apoptosis, inflammation, and calcium homeostasis. Encouragingly, numerous studies of EPO and EPOR in the brain have revealed various neuroprotective actions, including enhanced neurogenesis, synaptic plasticity, and cognitive performance [4–10]. Furthermore, recombinant human EPO (rhEPO), has been administered to millions of patients, many with severe medical conditions, and has been used as a highly effective, safe, and widely accepted treatment for anemia [11].

It is well established, that the expression of EPO is under the influence of hypoxia. The EPO gene has specifically been identified as a hypoxia-inducible transcription factor target and several studies have confirmed increased levels of neuronal EPO following hypoxia [1,4,5,12,13].

Furthermore, a recent study demonstrated EPO mediated neuroprotective actions such as neuroplasticity and neurogenesis during functional hypoxia [5]. However, only a few studies have investigated the expression of the neuronal EPOR focusing on gene expression and its relative increase (messenger-RNA) under conditions of hypoxia [5,13–18].

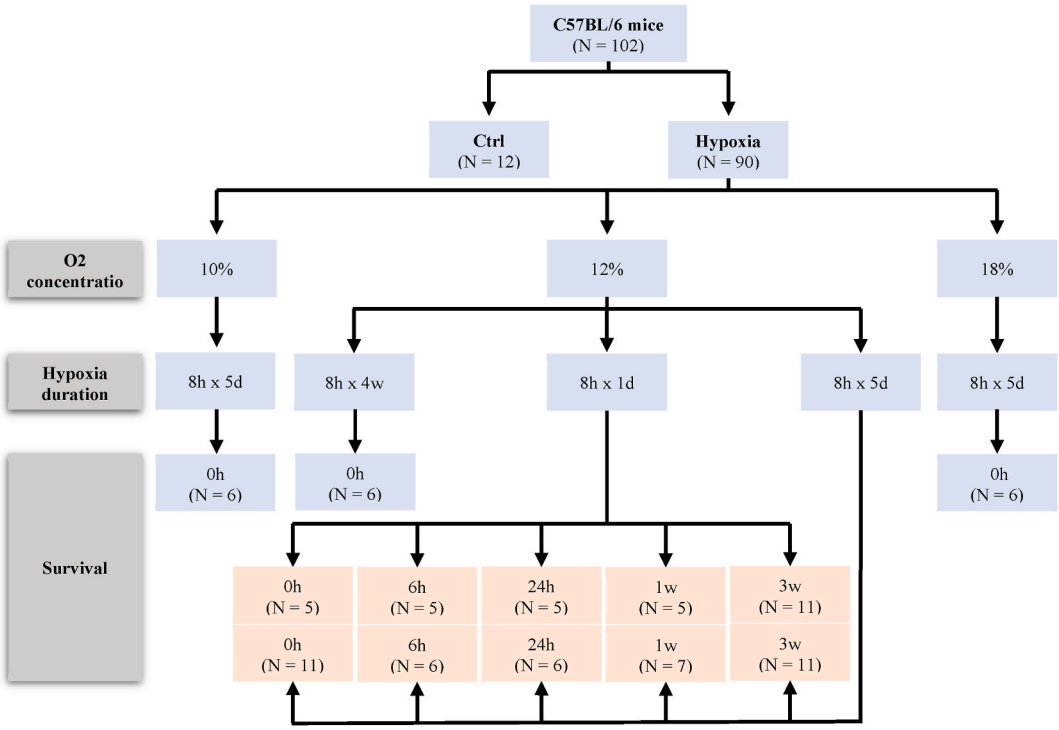

Fig. 1. Flowchart of experimental groups. A total of 102 male C57BL/6 mice were used in the experiment including a control group of 12 animals. The animals receiving hypoxia were randomly divided into experimental groups according to three variables: oxygen concentration, hypoxia dose and survival. Blue box: the HPC was analyzed. Orange box: the HPC and PFC were analyzed. h: hours, d: day, w: weeks, %: concentration of oxygen in the air.

To the best of our knowledge, EPOR protein levels in the HPC and prefrontal cortex (PFC) have never been measured and compared in an ELISA model. Thus, the primary aim of this study was to measure EPOR protein in the HPC and PFC. Further objectives were to examine the effects of exposure to normobaric hypoxia of various degrees and durations on EPOR protein in the hippocampus and to explore how long lasting these effects were. We hypothesized that EPOR is detectable in the hippocampus and prefrontal cortex and increases in response to normobaric hypoxia.

2. Methods

Experimental model. The experiment was performed using adult male C57BL/6 mice (10–22 weeks old). All mice were housed in a temperature- and humidity-controlled environment (22 ± 2 °C, 55 %) on a 12/12 h light-dark cycle with food and water available *ad libitum*. Humane endpoints were evaluated daily and included: abnormal behavior changes or more than 10 % body weight loss. The experiment was approved by the Animal Experiments Inspectorate under the Danish Ministry of Food, Agriculture and Fisheries (license number 2017-15-0201-01334). All procedures were performed in accordance with the EU directive 2010/63/EU under the supervision of a local animal welfare committee. All efforts were made to minimize pain or discomfort as well as the number of animals used during the experiment.

Experimental groups. A total of 102 animals were randomized by an online random number generator into a control group (n = 12) or hypoxia groups (n = 5–11). The hypoxia groups were exposed to different O_2 concentrations (10 %, 12 %, or 18 %) and hypoxia durations. Hypoxia duration was either a single exposure (8 h), repeated exposure (8 h for 5 days) or chronic exposure (8 h for 4 weeks). Animals exposed to a single or repeated dose of hypoxia at 12 % O_2 were euthanized at the times: 0 h, 6 h, 24 h, 1w or 3w after the last hypoxia exposure.

For more information see Fig. 1.

Hypoxia. Normobaric hypoxia was created by coupling a ventilated cabinet (Scantainer, SCANBUR A/S, Karlslunde, Denmark) to an Everest Summit II hypoxia generator (cat. 5571, Hypoxico, Bickenbach, Germany). The desired oxygen concentration was obtained within 1 h and logged every 15 min with a PASCO Wireless Oxygen Gas Sensor (cat. PS-3217, Frederiksen Scientific, Ølgod, Denmark).

Tissue processing. The mice were sedated with a mixture of Fentanyl (50 mg/ml), Midazolam (5 mg/ml), and Droperidol (2,5 mg/ml) administered intraperitoneally (ip) at a volume of 10 ml/kg 5 min before terminal anesthesia with Pentobarbital (5 mg/ml, 10 ml/kg, ip). Sedation was used to limit the side effects of high-dose Pentobarbital [19]. Transcardial perfusion was performed using ice-cold PBS (0.01 M) to remove blood and followed by decapitation and brain extraction. The PFC and both HPC were dissected on ice and immediately weighed and frozen in dry ice. The samples were stored at −80 °C until analysis. On the day of analysis, the samples were homogenized with a Potter Elvehjem PTFE pestle and glass tube (4 mL) in PBS at a dilution of 1:9. The homogenates were centrifuged at 5000×*g* for 5 min and the supernatants collected, diluted 1:1 with sample buffer and immediately transferred to the ELISA plate.

Detection of mouse EPOR in the HPC and PFC was performed using a sandwich-ELISA (cat. NBP2-67948, Novus Biologics, CO, USA) according to the manufacturer's instructions. In brief, the samples were diluted 1:1 in sample buffer and loaded in doublets onto the ELISA plate followed by biotinylated detection antibodies, horseradish peroxidase (HRP) conjugate, substrate reagent, and a stop solution. Between steps the wells were washed extensively with washing buffer. The absorbance was read at 450 nm and the concentration of EPOR (ng/mL) was calculated from the standard curve and converted to pg/mg tissue. All samples were homogenized and analyzed within one week from perfusion.

ARRIVE guideline**.** This experiment is in line with the 2020 essential ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments) to improve transparency and reproducibility.

Statistics. The sample size was estimated by power-calculation from previous literature (exercise induced hypoxia in mice, N = 10, d = 1.26, power = ∼ 86 %) [5] and expert knowledge (hypoxic hypoxia has greater effect than exercise) to ∼ 7. Unsuccessful transcardial perfusion, dissection, and ELISA was noted and the datapoint was removed in case of an extreme outlier (3rd quartile plus

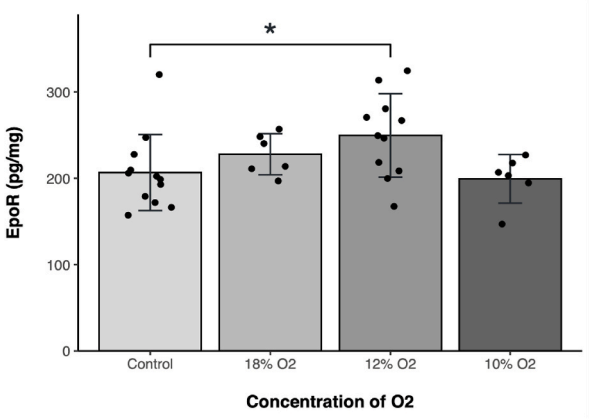

Fig. 2. EPOR protein at t = 0 after 5 days of 8 h hypoxia. *N(control) = 12, N(18 %) = 6, N(12 %) = 11, N(10 %) = 6.*

three times interquartile range or 1st quartile minus three times interquartile range). Statistical analysis included: evaluation for normality graphically, Leven's test of variance, and analysis of variance (ANOVA) followed by *post hoc* Dunnett's test on the fixed factors: O_2 concentration, survival, and duration of hypoxia. A *post hoc* Bonferroni was also used on the survival variable. Data are expressed as mean values (mean ± SD) and a $P < 0.05$ was considered statistically significant. IBM® SPSS® version 27 and R version 4.1.2 were used for statistical analysis.

3. Results

EPOR protein was present in the HPC (206.64 ± 43.98 pg/mg) and the PFC (184.25 ± 48.21 pg/mg). No extreme outliers were detected in the statistical analysis and no animals suffered any unexpected adverse events throughout the experiment. The effect of O_2 concentration and duration of hypoxia on EPOR protein levels were examined first. Subsequently, the time course of EPOR changes after exposure to 12 % O_2 for 1 or 5 days (8 h per day) were investigated. The PFC was only investigated after exposure to 12 % O_2 at different durations and survival times and no alterations were found, hence the data are not included. For data and figures regarding the PFC see supplemental material.

O_2 concentration and hypoxia duration. The effect of O_2 concentration on EPOR protein levels in the HPC was significant ($F_{(3,31)} = 2.916$, $p = 0.0498$, see Fig. 2). Post-hoc Dunnett's test showed that the animals that had been exposed to 12 % O_2 for 5 days (8 h per day) had significantly elevated levels of EPOR protein in the hippocampus ($p = 0.0458$). The effect of hypoxia duration on EPOR protein levels in the HPC was also significant ($F_{(3,30)} = 3.228$, $p = 0.0363$, see Fig. 3). However, post-hoc Dunnett's test showed that no individual group differed significantly from the control group (see Figs. 2 and 3). With respect to both variables, exposure to moderate levels of hypoxia increased EPOR, while the most severe level of hypoxia exposure reversed this effect, which is in agreement with the literature [20].

Time course of hypoxia effects. EPOR protein levels in the HPC changed significantly over time after exposure to a single dose of 12 % O_2 ($F_{(5,36)} = 2.991$, $p = 0.0233$). Post-hoc Dunnett's test showed that the animals surviving for three weeks had significantly lower EPOR protein levels in the HPC ($p = 0.0165$, see Fig. 4). Also after exposure to 5 days of 8 h with 12 % O_2, EPOR protein levels in the HPC changed significantly over time ($F_{(5,46)} = 8.799$, $p = 0.0001$). Post-hoc Dunnett's test showed that 24 h after exposure to 5 days of 8 h with 12 % O_2 EPOR protein levels in the HPC were significantly increased ($p = 0.0104$) and this normalized within one week. In a post-hoc Bonferroni test, animals euthanized three weeks after exposure to repeated hypoxia had significant reduced levels of hippocampal EPOR compared to the animals euthanized immediately after the last exposure to hypoxia ($p = 0.0002$), and those euthanized at 6 h ($p = 0.0005$) and 24 h ($p = 0.0000$) post hypoxia (see Fig. 5). For mean values and standard deviations of all groups, see Table 1.

4. Discussion

This study found that EPOR protein levels were approximately 10 % higher in the HPC as compared to the PFC. This could be attributed to various factors, including biological, functional, and regulatory mechanisms. The HPC and PFC are distinct brain regions with different functions. The HPC is primarily associated with memory formation and consolidation, while the PFC is involved in higher cognitive functions such as decision-making and executive control. Higher EPOR levels in the HPC could be related to its role in supporting neuroplasticity and memory processes [5]. Erythropoietin (EPO) and its receptor, EPOR, have been shown to have neuroprotective properties. Elevated EPOR levels in the HPC may be a response to the region's greater vulnerability to various forms of stress and damage [2,21]. Thus increased EPOR expression in HPC relative to PFC could be a protective mechanism to enhance cell survival and repair in the HPC.

Our initial dose finding study showed that exposure to hypoxia at 12 % O2 for 8 h a day for 5 consecutive days resulted in significant upregulation of EPOR protein in the HPC when measured immediately after the last exposure while 18 % O2 and 10 % O2 were without effect. This agrees with the literature, which suggests that moderate levels of hypoxia are functionally beneficial, while strong

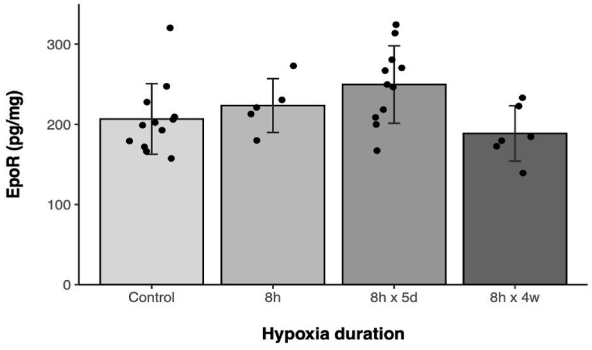

Fig. 3. EPOR protein at t = 0 after different durations of 12 % hypoxia. *N(control) = 12, N(8h) = 5, N(8hx5d) = 11, N(8hx4w) = 6. Error bars show mean±1 SD.*

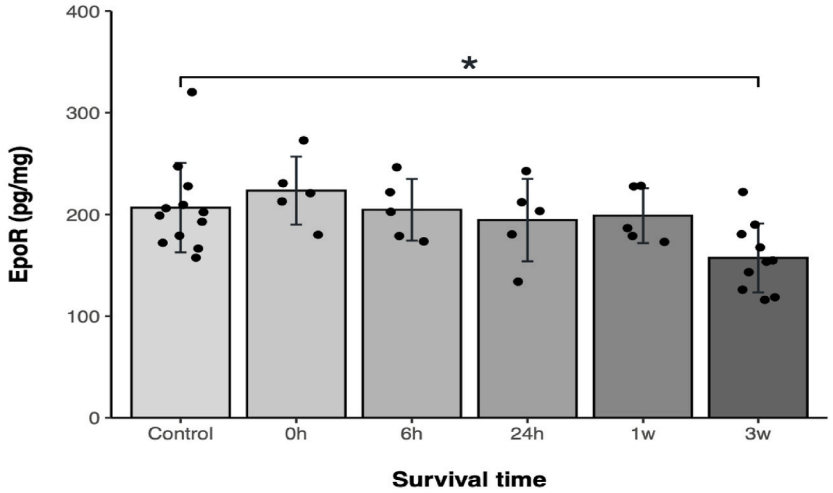

Fig. 4. EPOR protein in the HPC at various time points after 8 h with 12 % O_2.. $N_{control} = 12$, $N_{0h} = 11$, $N_{6h} = 6$, $N_{24h} = 6$, $N_{1w} = 6$, $N_{3w} = 11$. Error bars show mean±1 SD.

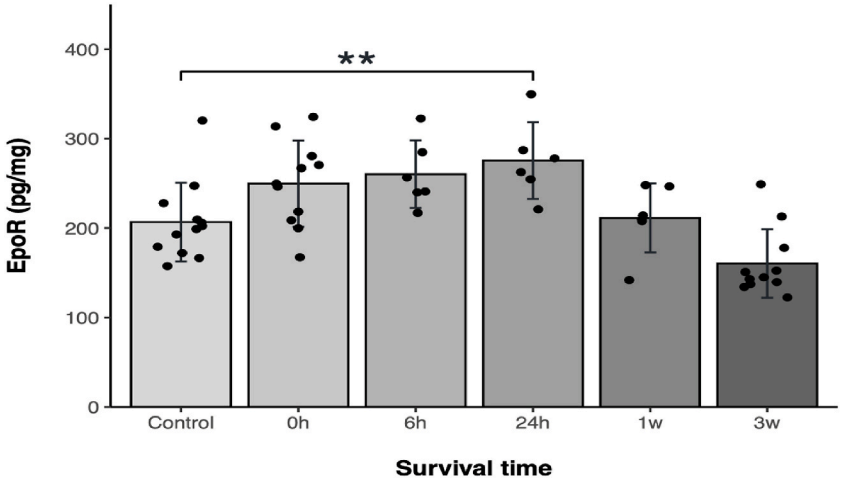

Fig. 5. EPOR protein in the HPC at various time points after 5 days of 8 h with 12 % O_2.. $N_{control} = 12$, $N_{0h} = 11$, $N_{6h} = 5$, $N_{24h} = 5$, $N_{1w} = 5$, $N_{3w} = 10$. Error bars show mean±1 SD.

Table 1
Mean and standard deviation of EPOR protein levels in the hippocampus of the different groups.

	Hypoxia: 8 h, 12 % O_2			Hypoxia: 8 h × 5 d, 12 % O_2		
	Mean: EPOR (pg/mg)	SD: EPOR (pg/mg)	N	Mean: EPOR (pg/mg)	SD: EPOR (pg/mg)	N
Control	206.64	33.52	12	206.64	33.52	12
0 h	223.42	33.52	11	249.63	48.29	11
6 h	204.6	30.36	6	260.25	37.83	5
24 h	194.43	40.58	6	275.43	42	5
1w	198.8	26.98	6	211.28	38.57	5
3w	157.2	33.84	11	160.39	38.32	10

intermittent hypoxic conditions can have detrimental effects [1].

The increase in hippocampal EPOR protein levels caused by repeated exposure to moderate levels of normobaric hypoxia was transient, peaking 24 h after 5 daily exposures to 8 h of 12 % O_2 with an approximately 30 % elevation and normalizing within one

week. Our findings agree with previous reports, indicating that EPOR mRNA is upregulated under the conditions of ischemia, especially on endothelial cells, astrocytes, and neurons [7,22–26]. The fact that EPOR returns to control levels some time after exposure to hypoxia has ended is not surprising but can be attributed to regular protein turnover. However, the apparent rebound effect, we observed 3 weeks after termination of exposure to either a single or repeated dose of hypoxia at 12 % O_2 is more unusual. It might be ascribed to a change in degradation rate of the protein, but this requires further experimentation and is beyond the focus of the present paper [27].

Method. When interpreting assay results, the critical factor of antibody validity cannot be overlooked. In our study, the selected antibody's validation was limited to testing for cross-reactivity with relevant mouse proteins, such as EPO, IGF-1, RANTES, and ICAM-1, as performed by the supplier. Constraints like time, budget, and sample size influenced this decision.

Given the well-documented sensitivity of EPO and EPOR to hypoxia, and their established upregulation during acute hypoxia in the brain [1,5], our pilot study aimed to validate these responses using the chosen antibody. The outcome of this validation closely aligned with our expectations. As a result, any potential issues related to the antibody's validity would likely manifest as unexpected or conflicting results. However, it is essential to exercise caution when interpreting the findings, considering the reliance on supplier claims in the absence of comprehensive, in-house antibody validation procedures.

To the best of our knowledge, EPOR protein levels in the HPC and PFC have never been compared before. This is of interest as, compared to RNA, protein is generally considered more stable, active at the site of interest, and directly related to effect [28,29]. Since the goal of this study was to investigate the effect of an experimental manipulation (normobaric hypoxia) on EPOR protein levels, it was decided to use a commercially available ELISA rather than flow cytometry or mass spectrometry, due to time and cost constraints. Flowcytometry is considered more suitable for detecting and quantifying transmembrane proteins. However, isolation of neurons in flowcytometry is also problematic because of the tight adhesion of cell somas, axons, and thousands of synapses [30]. Neurons are easily damaged by enzymatic dissociation and mechanical trituration during the isolation step for single-cell suspension which increases the risk of cell death [31], theoretically affecting the concentration of EPOR [32–34].

Sample size. The results are possibly affected by one deviating animal in the control group, increasing the risk for committing a type II error (false negative). In broad strokes, there are three causes for outliers: data entry or measurement errors, sampling problems, or natural variation. Readdressing the method, analysis, and data did not reveal any error(s). Furthermore, the experiment was repeated without new deviating values, making a methodological or sampling error unlikely. In this context, it was unfortunately reasonable to assume that this value was the result of natural variation, hence it was included in the data set analyzed statistically.

Bias. Selection biases are of particular concern because the results depend on the assumption that groups are identical and handled in an uniform manner. If data available for analysis are not representative, the validity of the conclusion is threatened.

The lack of blinding and pooling of data could potentially create a selection bias (e.g., handling based on data). However, when designing the experiment, handling the animals was considered to have limited effects on the concentration of EPOR, since EPOR is known to respond as tissue-protective factor during brain injury (hypoxia) [32–35], independent of external stimuli (e.g., environmental enrichment [13]). In addition, post-experimental subgroup analysis of pooled data showed no trace of selection bias.

Prospects. The aim of this study was to compare EPOR protein in the HPC and PFC, to investigate the effects of exposure to normobaric hypoxia of various degrees and durations on EPOR protein and to explore how long-lasting these effects were. This with a view on informing future research on the neuroprotective effects of EPO. We would argue that EPOR signaling is a well conserved cellular survival mechanism and therefor the results of the present study would also be relevant in the context of investigations in other species, including humans [36]. Furthermore, EPO has been administered to millions of patients, many with serious conditions, without any severe complications, giving EPO a positive cost-benefit profile if considering new indications, such as neuroprotection.

During the execution of this experiment, it became evident that a study with larger sample size would have been beneficial, as one deviating animal in the control group may have given rise to type II error. Furthermore, simultaneous analyses such as EPOR messenger-RNA in the brain or hemoglobin levels in the blood could be of interest. Subsequent investigations should examine the impact of hypobaric hypoxia on EPOR, conduct a comprehensive analysis of hypoxia's effects on various brain structures, and investigate its influence on EPOR turnover.

5. Conclusion

To conclude, this study successfully measured and compared hippocampal and prefrontal cortex EPOR protein. Elevation of EPOR protein was most pronounced when measured 24 h after repeated exposure to 12 % O2 and normalized within one week. Our findings are in agreement with previous reports, indicating that EPOR mRNA is upregulated under acute ischemic conditions [32–35]. It is our hope that this study can provide guidance to future research on the neuroprotective effects of EPO.

Funding

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Data availability statement

The data for this study has not been deposited into a publicly available repository. However, upon request, we will provide access to the data, analysis, and protocols associated with this research.

CRediT authorship contribution statement

J. Christensson: Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Frederik Fussing:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gitta Wörtwein:** Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:F. Fussing reports financial support was provided by The Lundbeck Foundation. J. Christensson reports financial support was provided by The Lundbeck Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25051.

References

[1] H. Ehrenreich, et al., Introducing the brain erythropoietin circle to explain adaptive brain hardware upgrade and improved performance, Mol. Psychiatr. 27 (5) (2022) 2372–2379.
[2] D. Coimbra-Costa, et al., Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain, Redox Biol. 12 (2017) 216–225.
[3] C. Ott, et al., Widespread expression of erythropoietin receptor in brain and its induction by injury, Mol. Med. 21 (1) (2015) 803–815.
[4] I. Hassouna, et al., Revisiting adult neurogenesis and the role of erythropoietin for neuronal and oligodendroglial differentiation in the hippocampus, Mol. Psychiatr. 21 (12) (2016) 1752–1767.
[5] D. Wakhloo, et al., Functional hypoxia drives neuroplasticity and neurogenesis via brain erythropoietin, Nat. Commun. 11 (1) (2020) 1313.
[6] B. Adamcio, et al., Erythropoietin enhances hippocampal long-term potentiation and memory, BMC Biol. 6 (2008) 37.
[7] D. Sargin, et al., Expression of constitutively active erythropoietin receptor in pyramidal neurons of cortex and hippocampus boosts higher cognitive functions in mice, BMC Biol. 9 (2011) 27.
[8] A. Millet, et al., Erythropoietin and its derivates modulate mitochondrial dysfunction after diffuse traumatic brain injury, J. Neurotrauma 33 (17) (2016) 1625–1633.
[9] Y.C. Shang, et al., Prevention of beta-amyloid degeneration of microglia by erythropoietin depends on Wnt1, the PI 3-K/mTOR pathway, Bad, and Bcl-xL, Aging (Albany NY) 4 (3) (2012) 187–201.
[10] D. Agnello, et al., Erythropoietin exerts an anti-inflammatory effect on the CNS in a model of experimental autoimmune encephalomyelitis, Brain Res. 952 (1) (2002) 128–134.
[11] K. Kalantar-Zadeh, History of erythropoiesis-stimulating agents, the development of biosimilars, and the future of anemia treatment in nephrology, Am. J. Nephrol. 45 (3) (2017) 235–247.
[12] J.C. Chavez, et al., The transcriptional activator hypoxia inducible factor 2 (HIF-2/EPAS-1) regulates the oxygen-dependent expression of erythropoietin in cortical astrocytes, J. Neurosci. 26 (37) (2006) 9471–9481.
[13] P.E. Sanchez, et al., Optimal neuroprotection by erythropoietin requires elevated expression of its receptor in neurons, Proc. Natl. Acad. Sci. U. S. A. 106 (24) (2009) 9848–9853.
[14] L. Terraneo, et al., Brain adaptation to hypoxia and hyperoxia in mice, Redox Biol. 11 (2017) 12–20.
[15] M. Digicaylioglu, et al., Localization of specific erythropoietin binding sites in defined areas of the mouse brain, Proc. Natl. Acad. Sci. U. S. A. 92 (9) (1995) 3717–3720.
[16] E. Spandou, et al., Hypoxia-ischemia affects erythropoietin and erythropoietin receptor expression pattern in the neonatal rat brain, Brain Res. 1021 (2) (2004) 167–172.
[17] D.J. Manalo, et al., Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1, Blood 105 (2) (2005) 659–669.
[18] S. Kato, et al., Endogenous erythropoietin from astrocyte protects the oligodendrocyte precursor cell against hypoxic and reoxygenation injury, J. Neurosci. Res. 89 (10) (2011) 1566–1574.
[19] J.W. Dutton 3rd, et al., Assessment of pain associated with the injection of sodium pentobarbital in laboratory mice (Mus musculus), J Am Assoc Lab Anim Sci 58 (3) (2019) 373–379.
[20] J.M. Al-Qahtani, B.A. Abdel-Wahab, S.M. Abd El-Aziz, Long-term moderate dose exogenous erythropoietin treatment protects from intermittent hypoxia-induced spatial learning deficits and hippocampal oxidative stress in young rats, Neurochem. Res. 39 (1) (2014) 161–171.
[21] C.D. Conrad, Chronic stress-induced hippocampal vulnerability: the glucocorticoid vulnerability hypothesis, Rev. Neurosci. 19 (6) (2008) 395–411.
[22] R. Castaneda-Arellano, A.I. Feria-Velasco, M.C. Rivera-Cervantes, Activity increase in EpoR and Epo expression by intranasal recombinant human erythropoietin (rhEpo) administration in ischemic hippocampi of adult rats, Neurosci. Lett. 583 (2014) 16–20.
[23] B.O. Ratilal, et al., Neuroprotective effects of erythropoietin pretreatment in a rodent model of transient middle cerebral artery occlusion, J. Neurosurg. 121 (1) (2014) 55–62.
[24] A.L. Parra, J.C. Rodriguez, Nasal neuro EPO could be a reliable choice for neuroprotective stroke treatment, Cent. Nerv. Syst. Agents Med. Chem. 12 (1) (2012) 60–68.
[25] G. Zhang, et al., Erythropoietin enhances nerve repair in anti-ganglioside antibody-mediated models of immune neuropathy, PLoS One 6 (10) (2011) e27067.
[26] D. Yu, et al., Effects of erythropoietin preconditioning on rat cerebral ischemia-reperfusion injury and the GLT-1/GLAST pathway, Exp. Ther. Med. 11 (2) (2016) 513–518.
[27] A.B. Ross, J.D. Langer, M. Jovanovic, Proteome turnover in the spotlight: approaches, applications, and perspectives, Mol. Cell. Proteomics 20 (2021) 100016.

[28] P. Heir, et al., Oxygen-dependent regulation of erythropoietin receptor turnover and signaling, J. Biol. Chem. 291 (14) (2016) 7357–7372.
[29] B. Schwanhausser, et al., Global quantification of mammalian gene expression control, Nature 473 (7347) (2011) 337–342.
[30] G.J. Brewer, J.R. Torricelli, Isolation and culture of adult neurons and neurospheres, Nat. Protoc. 2 (6) (2007) 1490–1498.
[31] A. Saxena, et al., Trehalose-enhanced isolation of neuronal sub-types from adult mouse brain, Biotechniques 52 (6) (2012) 381–385.
[32] K. Maiese, et al., Erythropoietin: elucidating new cellular targets that broaden therapeutic strategies, Prog. Neurobiol. 85 (2) (2008) 194–213.
[33] J. Nadam, et al., Neuroprotective effects of erythropoietin in the rat hippocampus after pilocarpine-induced status epilepticus, Neurobiol. Dis. 25 (2) (2007) 412–426.
[34] I. Wallach, et al., Erythropoietin-receptor gene regulation in neuronal cells, Pediatr. Res. 65 (6) (2009) 619–624.
[35] G. Grasso, et al., Erythropoietin and erythropoietin receptor expression after experimental spinal cord injury encourages therapy by exogenous erythropoietin, Neurosurgery 56 (4) (2005) 821–827. ; discussion 821-7.
[36] J.C. Ameisen, On the origin, evolution, and nature of programmed cell death: a timeline of four billion years, Cell Death Differ. 9 (4) (2002) 367–393.