Expression and distribution of neuroglobin and hypoxia-inducible factor-1α in the diencephalon of young yaks*

JAMES BLACKAR MAWOLO1, XIAOHUA DU2, XIA LIU1, XIAOYU MI1, QIAO LI1, YONGQIANG WEN1

1College of Life Science and Technology, 2College of Veterinary Medicine, Gansu Agricultural University, Lanzhou City, Gansu Province, 730070, People’s Republic of China

Received 28.09.2020 Accepted 20.01.2021

Mawolo J. B., Du X., Liu X., Mi X., Li Q., Wen Y.
Expression and distribution of neuroglobin and hypoxia-inducible factor-1α in the diencephalon of young yaks

Summary

The diencephalon, or interbrain, is a primary relay and processing center for sensory information and autonomic control. It connects structures of the endocrine system with the nervous system and works with the limbic system to generate and manage emotions and memories. Neuroglobin (Ngb) is a member of the vertebrate globin family involved in cellular oxygen homeostasis and reactive oxygen/nitrogen scavenging, while hypoxia-inducible factor (Hif-1α) is a transcription factors that respond to decreases in available oxygen in the cellular environment or hypoxia. The study explored the expression of Ngb and Hif-1α in the diencephalon of young yaks and examined factors that influenced the levels of Ngb and Hif-1α. Immunohistochemistry (IHC), quantitative real-time PCR (qRT-PCR), and Western blot (WB) were employed to investigate the expression of Ngb and Hif-1α in the diencephalon of young yaks. Ngb and Hif-1α are significantly expressed in all tissues of the diencephalon except the hypothalamus and epithalamus. The thalamus, cerebellar cortex, the white matter of the cerebellum, basal ganglia, and cerebrum showed significant levels of expression, and each plays an important role in the diencephalon. The overall expression of Ngb was higher than that of Hif-1α. Both Ngb and Hif-1α participate in oxygen transport throughout the telencephalon and have functions in neuroprotection. The results suggest that Ngb and Hif-1α influence the mechanism of adaptation of young yaks to their high altitude environment. Further studies on the mechanism of adaptation are recommended.

Keywords: neuroglobin, hypoxia-inducible factor-1α, yak, diencephalon, oxygen

The diencephalon is a region of the vertebrate neural tube that gives rise to posterior forebrain structures and contains, among others, the epithalamus, thalamus, and hypothalamus. High-altitude is a component of the physical environment to which animals adapt (10). High-altitude environments present many physiological challenges for mammals because the increasing altitude is characterized by decreasing oxygen availability in the environment without major changes in tissue requirements. In 2000, Burmester et al. (1) observed that Ngb is expressed in the vertebrate nervous system. Zhao et al. (21) reported that Ngb is highly expressed in the brain of mice with traumatic brain injury, whereas in humans Ngb is significantly expressed in the hypothalamus, amygdala, and pontine tegmental nuclei (14). In the hypothalamus and cerebrum of sheep, Ngb shows a lower expression (8). Oxygen (O2) is vital for respiration, which is a process that transfers energy from glucose to cells. To adapt to this condition, evolutionarily conserved responses must be engaged. In mammals and other non-vertebrate species, the primary transcriptional factor that responds to hypoxic stress is mediated by a dimeric protein called the hypoxia-inducible factor (Hif-1α). Hif-1α is an important transcriptional factor that regulates oxygen consumption and morphological changes in response to varying oxygen concentrations. Frank et al. (9) reported that Hif-1α targets genes in the adult rat brain and promotes cell survival in hypoxic tissues. A study conducted by Joana et al. ascertained that Hif-1α expression in the hypothalamus regulates cellular responses to inflam-
mation and hypoxia, which is essential for normal cell function and survival (16). Despite these reports, a connection between Ngb and Hif-1α has not been demonstrated, and evidence argues against such a connection because the Ngb promoter region appears to lack consensus Hif-1α-binding hypoxia-response elements (19). Although researchers have recorded data on Ngb and Hif-1α expression, none of them focus on the connection between Ngb and Hif-1α, their exact function, expression levels, and mechanisms’ pattern, quantities of expression, and the mechanism, which are debated among scientists. Therefore, the present study aims to provide data on the connection between Ngb and Hif-1α in the diencephalon of young yaks and further investigate factors influencing their diverse patterns and levels of expression. The yak is a long-haired domesticated bovid found throughout the Himalayan region of the Indian subcontinent, north Mongolia, and the Tibetan Plateau. The research provided essential morphological, physiological, and biochemical data on the expression of Ngb and Hif-1α.

**Material and methods**

**Animals and setting.** The Animal Ethics and Welfare Committee of Gansu Agricultural University (AEWC-GAU-2019-045) reviewed and approved all experimental procedures in this study in October 2019. All animals were housed at the Hezuo Xingfa Yak and Sheep Breeding Cooperative Center in the Gannan Tibetan Autonomous Prefecture in Gansu Province of China. Three (3) healthy young yaks (aged 6 months) were purchased from the center. The animals were housed and monitored by trained personnel, and they fed on grasses and sedges, such as Carex, Stipa, and Kobresia. In the plateau environment of Gannan Tibetan Autonomous Prefecture, the altitude is 3000 m. Experiments were carried out with young yaks weighing 200-230 kg. The animals were maintained at a temperature between −7°C and −8°C and had free access to food and water. Every effort was made to reduce the number of animals used and minimize animal suffering during the sampling process.

**Treatment and specimen techniques.** The animals were retrieved one at a time from their living areas and minimally immobilized to facilitate sacrificing and extraction of the brain. This was done under the guidance of resident veterinarians to reduce harm and pain to the animals. Upon sacrificing each animal, the whole brain was quickly extracted by craniotomy. Subsequently, the thalamus, hypothalamus, and the other tissues were extracted. Tissue samples prepared for immunohistochemistry were fixed in 4% paraformaldehyde (PH 7.4, w/v), and samples for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blotting were stored at −80°C.

**Reagents and instrumentation.** Quantitative real-time polymerase chain reaction (qRT-PCR) reagents and supplies were AG RNase Pro RNA kit, SYBR Green Pro Taq HS kit, Evo M-MLV reverse-transcription kit (removal gDNA reagent), and Rox. They were purchased from Accurate Biotechnology (Hunan) Co. Ltd. P.R. China. Western-blotting reagents and supplies, including Rabbit Anti-Ngb, Polyclonal Antibody (bs-1859R), Rabbit Anti-HIF-1, Alpha Polyclonal Antibody (bs-0737R), Rabbit Anti-Beta-Actin (Loading Control), Polyclonal antibody (bs-0737R), and goat anti-rabbit IgG/HRP(bs-0295G-HP), were purchased from Bioss Co. Ltd. P.R. China. RIPA tissue/cell rapid lystate was purchased from Bio topped, whereas 0.22 µm PVDF membranes, 4 × protein loading buffer (DTT), Rainbow 245 broad-spectrum protein marker (11-245KD), and ECL hypersensitivity luminescent solution were purchased from Solarbio Co. Ltd. P.R. China. Immunohistochemical reagents and supplies consisted of an immunohistochemical staining kit and an HRP-DAB kit, and they were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd. P.R. China.

**Total RNA isolation and qRT-PCR.** Total RNA was isolated using the TRizol reagent (Accurate Biotechnology, China). Eight hundred nanograms of total RNA was reverse transcribed using the Evo M-MLV cDNA synthesis kit (Accurate Biotechnology, China). Real-time PCR was performed using Quant Studio 5. The qRT-PCR primer sequences and accession numbers are shown in Table 1. Reaction mixtures (20 µL) consisted of 10 µL SYBR Green Pro Taq (Accurate Biotechnology, China), 0.8 µL forward and reverse primers (0.2 µmol/mL), 0.4 µL Rox, 2 µL cDNA, 6 µL dH₂O. The thermocycler was set at 50°C for 2 min, 95°C for 2 min, 40 cycles at 95°C for 10 s, annealing for 34 s (annealing temperatures are shown in Table 1), with melting temperatures examined from 65°C to 95°C, increments of 0.5°C every 5 s. The 2^-ΔΔCt method was used to analyze the expression of Ngb and Hif-1α mRNA relative to the expression of β-actin mRNA according to a system-generated Ct value.

**Western blotting.** For Western blotting analyses (18), frozen tissue samples from different regions were weighed. After that, the tissues were homogenized using a glass rod in lysis buffer (1 ml RIPA + 10 µL PMSF) at ice-cold temperature, shaken in an ice bath for 2 h (120 r/min), and centrifuged at 12,000 rpm at 4°C for 10 min to collect the supernatant. The protein was subjected to SDS polyacrylamide gel electrophoresis (PAGE). Separated proteins were transferred to a polyvinylidene difluoride filter (PVDF) membrane via a transfer apparatus at 110 V for 60 min. The membranes were then blocked via 5% milk/PBST at 4°C.

| Primer name | Accession numbers | Sequence (5’ to 3’) | Tm/°C | Amplicon size | Note |
|-------------|------------------|---------------------|------|--------------|------|
| Ngb         | J0241373.1       | F: CTTTCGGCCAGGCTGGTTGGA | 60.0 | 134          | qRT-PCR |
|             |                  | R: CTGATGGTGGTCAGGGACTG|     |              |      |
| HIF-1α      | NM_174339.3      | F: CTACATACATGGGCTGGAAACTCC | 59.8 | 146          | qRT-PCR |
|             |                  | R: AGGCTTTCTGGTGGCTTCC |     |              |      |
| β-actin     | NM_173979.3      | F: ATATTCGGCGGCGTGTGTT | 60.2 | 158          | qRT-PCR |
|             |                  | R: TCACTCCACAGTACAGTCC |     |              |      |

**Tab. 1. Primer sequences of target and house-keeping genes**
overnight and then incubated with primary antibody against Ngb, Hif-1α, and β-actin for 3 hours at room temperature. The antibody concentrations (v/v) of Ngb, Hif-1α, and β-actin were 1: 800, 1: 500, and 1: 3000, respectively. The membranes were washed thrice (10 min each) with PBST and incubated with secondary antibody (HRP-conjugated goat anti-rabbit IgG, 1: 4000) for 1 h at room temperature. After triple washing in PBST (10 min each), the membranes were scanned with an ECL Western-blotting machine (GE Al600, USA) Western blotting for each group of proteins was repeated 3 times. The signals were analyzed with the Image J software (NIH, Bethesda, MD, USA) to determine the relative expression levels of Ngb and Hif-1α.

**Immunohistochemical staining.** Tissue samples from the diencephalon of the yaks’ brains were fixed (4% paraformaldehyde) and trimmed (2 cm × 2 cm). The researchers used conventional gradient alcohol dehydration, and made tissue wax blocks with paraffin embedding, cutting tissues into serial sections (thickness 4 µm), exhibiting, patching, baking sheet processing, hematoxylin-eosin (HE) routine staining, and microscopy. The paraffin-embedded tissue sections were deparaffinized in xylene and then rehydrated in graded alcohol. PBS (0.01 mol/L, pH = 7.2) was rinsed 3 times, each time for 5 minutes. 0.125% trypsin antigen was repaired for 30 minutes and rinsed in PBS for 2 times.

Endogenous peroxidase activity was blocked by incubating the sections for 10 minutes in 30 mL/L hydrogen peroxide blocking solution, followed by rinsing 3 times with PBS for 5 minutes each time to reduce non-specific binding of the first antibody. Normal sheep serum was added for blocking and incubated at room temperature for 15 minutes. The corresponding primary antibody was added to the sections, incubated at 37°C for 2 hours, and rinsed in PBS 3 times. The appropriate secondary antibody was added after having been removed from PBS and incubated at 37°C for 15 minutes. Streptomyces avidin-peroxidase solution was added to the sections and incubated at 37°C for 15 minutes; PBS was rinsed 3 times for 5 minutes each time. The immunoperoxidase color reaction was developed with HRP-DAB substrate chromogen solution after removing PBS. Distilled water stopped the reaction, and the sections were lightly counterstained with hematoxylin, dehydrated in increasing concentrations of ethanol, cleared, and covered with mounting medium and coverslips (at 4°C). Then, the sections were stored at 20°C until used for taking photographs and microscopic analysis.

**Animal housing conditions.** The Hezuo Xingfa Yak and Sheep Breeding Cooperative is located in Hezuo city, Gansu Province, the People’s Republic of China. With an elevation of nearly 3,000 meters (9,800 ft), Hezuo has an alpine subarctic climate, with long, very cold, dry winters and short, mild summers. The monthly daily mean temperature is –9.3°C (15.3°F) in January, the coldest month, and 13.3°C (55.9°F) in July, the warmest month, whereas the annual mean is 2.82°C (37.1°F). Most of the annual precipitation is delivered from May to September. With monthly percent possible sunshine ranging from 44% in June and September to 71% in December, the city receives 2,370 hours of bright sunshine annually. The animals live together (male and female) to enable reproduction. Yaks are moved to a calming area before calving. Veterinarians monitor the feeding habitat and health status of the animals at the center. The animals are trained to cooperate with the veterinarians and investigative personnel. When it rains, the animals enter chutes or cages with the aid of guards. The center is surrounded by a fence, and security measures are employed to safeguard the animals.

**Sacrifice.** A jugular intravenous injection was made to perform the procedure, which required at least two individuals: one to restrain the animal and one to make the injection. An appropriately sized needle was used. The syringe was drawn up with the required dose, and all air bubbles were expelled. Fleece from the side of the animal’s neck which showed the jugular vein was removed, and the needle or catheter was inserted at an angle of approximately 10-20° into the animal’s neck. Blood was gently drawn into the hub of the needle and flowed freely into the syringe. Subsequently, the required dose was injected. The personnel withdrew the needle and applied pressure until bleeding ceased.

**Anesthesia procedures.** As per the regulations of the Animal Ethics and Welfare Committee of Gansu Agricultural University, all animals involved in the study were housed separately until they were confirmed to be healthy. The animals were observed for two weeks before further procedures were performed according to the committee regulations. The animals were free while under observation, and the observation confirmed that they were free of specified infectious diseases that could harm the experimental procedures. The animals’ diet was provided while under observation, and no search for food, chewing, gnawing, or gnawing was allowed during the activity periods. To avoid or minimize pain, the animals were treated gently by trained personnel. Dealing with these large animals requires more personnel, so additional trained personnel were employed to assist during the sacrifice. The animals were spoken to by the personnel, and loud sounds were avoided so that the animals would not escape. At the same time, more food was given to the yaks to enable interaction between them and the personnel and support a developing relationship with the personnel. The animals were made to lie on their side by scratching their back and flanks. When they were calm, injections were administrated and sacrifice took place. The injections were performed under slow pressure to reduce pain and animals suffering. The injection needles were appropriate for the size of the animals. The environment was well lit, and sharp or damaged objects were removed.

**Data analysis.** Statistical analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL, USA). The data for Ngb and Hif-1α protein levels were subjected to analysis of variance (ANOVA), and treatment means were separated by Duncan’s multiple range test at p < 0.05 using SPSS version 22. Data were presented as mean and standard deviation (SD). Statistical significance was defined as P < 0.05.

**Results and discussion**

The descriptive statistics for the expression of Ngb and Hif-1α in the young yaks are presented in a table and figures. The immunohistochemical results are reported in images. Table 1 shows the primer sequence,

---

**Animal housing conditions.** The Hezuo Xingfa Yak and Sheep Breeding Cooperative is located in Hezuo city, Gansu Province, the People’s Republic of China. With an elevation of nearly 3,000 meters (9,800 ft), Hezuo has an alpine subarctic climate, with long, very cold, dry winters and short, mild summers. The monthly daily mean temperature is –9.3°C (15.3°F) in January, the coldest month, and 13.3°C (55.9°F) in July, the warmest month, whereas the annual mean is 2.82°C (37.1°F). Most of the annual precipitation is delivered from May to September. With monthly percent possible sunshine ranging from 44% in June and September to 71% in December, the city receives 2,370 hours of bright sunshine annually. The animals live together (male and female) to enable reproduction. Yaks are moved to a calming area before calving. Veterinarians monitor the feeding habitat and health status of the animals at the center. The animals are trained to cooperate with the veterinarians and investigative personnel. When it rains, the animals enter chutes or cages with the aid of guards. The center is surrounded by a fence, and security measures are employed to safeguard the animals.

**Sacrifice.** A jugular intravenous injection was made to perform the procedure, which required at least two individuals: one to restrain the animal and one to make the injection. An appropriately sized needle was used. The syringe was drawn up with the required dose, and all air bubbles were expelled. Fleece from the side of the animal’s neck which showed the jugular vein was removed, and the needle or catheter was inserted at an angle of approximately 10-20° into the animal’s neck. Blood was gently drawn into the hub of the needle and flowed freely into the syringe. Subsequently, the required dose was injected. The personnel withdrew the needle and applied pressure until bleeding ceased.

**Anesthesia procedures.** As per the regulations of the Animal Ethics and Welfare Committee of Gansu Agricultural University, all animals involved in the study were housed separately until they were confirmed to be healthy. The animals were observed for two weeks before further procedures were performed according to the committee regulations. The animals were free while under observation, and the observation confirmed that they were free of specified infectious diseases that could harm the experimental procedures. The animals’ diet was provided while under observation, and no search for food, chewing, gnawing, or gnawing was allowed during the activity periods. To avoid or minimize pain, the animals were treated gently by trained personnel. Dealing with these large animals requires more personnel, so additional trained personnel were employed to assist during the sacrifice. The animals were spoken to by the personnel, and loud sounds were avoided so that the animals would not escape. At the same time, more food was given to the yaks to enable interaction between them and the personnel and support a developing relationship with the personnel. The animals were made to lie on their side by scratching their back and flanks. When they were calm, injections were administrated and sacrifice took place. The injections were performed under slow pressure to reduce pain and animals suffering. The injection needles were appropriate for the size of the animals. The environment was well lit, and sharp or damaged objects were removed.

**Data analysis.** Statistical analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL, USA). The data for Ngb and Hif-1α protein levels were subjected to analysis of variance (ANOVA), and treatment means were separated by Duncan’s multiple range test at p < 0.05 using SPSS version 22. Data were presented as mean and standard deviation (SD). Statistical significance was defined as P < 0.05.

**Results and discussion**

The descriptive statistics for the expression of Ngb and Hif-1α in the young yaks are presented in a table and figures. The immunohistochemical results are reported in images. Table 1 shows the primer sequence,
Table 2 presents a full analysis of the expression of Ngb and Hif-1α in the yaks’ brain tissues, and figure 1 compares the expression of Ngb and Hif-1α. The results reveal that Ngb and Hif-1α were significantly expressed in some brain tissues of the young yaks as compared to other tissues. The expression plays an important role in the adaptation of young yaks to a high altitude environment. Figure 2 reports the immunohistochemical expression of Ngb and Hif-1α, and the Western blot results. Ngb and Hif-1α were widely distributed in the diencephalon of the young yaks. They were significantly expressed in the thalamus, cerebellar cortex, white matter of the cerebellum, basal ganglia, and cerebrum, while other tissues showed lower expression. However, the expression of Ngb in the brain tissues of the young yaks was higher than that of Hif-1α.

The expression pattern of Ngb and Hif-1α varied in different tissues of the diencephalon, but factors leading to these differences remain unknown. In addition, the results provide a clear explanation of oxygen function in the neuronal tissues related to the adaptive mechanism of young yaks.

**Thalamus.** The current research found that the expression of both Ngb and Hif-1α in the thalamus of young yaks was significant. However, the expression of Ngb was higher than that of Hif-1α. During sensory signals, Ngb regulates oxygen expression and plays a neuroprotective function. The level of Hif-1α increases the expression of Ngb, as it regulates oxygen in neuronal tissues. When encountering predators, young yaks respond by huddling closely together with adult yaks. Adult yaks scare predators away with their horns and protect calves. Ngb not only responds to oxygen demand, but also protects neuronal tissues from damage. The Ngb expression reported in the current study is similar to that found in other mammals (6, 13).

**Hypothalamus.** The present results show a higher expression of Ngb and Hif-1α in the hypothalamus, but it is not significant. The expression of Ngb in the hypothalamus promotes neuronal survival (7). Christian et al. reported that Ngb was highly expressed in the hypothalamus of humans (4). In the adult mouse brain, Ngb expression was higher than other tissues (8), and, as reported by Joana et al. (16), Hif-1α was predominantly expressed in the human hypothalamus. It is suggested that the high expression of Ngb in the hypothalamus of young yaks facilitates oxygen supply to the blood flow in the body and acts as an endogenous protector in nerve cells, while Hif-1α in the hypothalamus can have an oxygen-independent regulation, such as oxidative stress (3, 5). Ngb expression in the hypothalamus of adult yaks may also be involved in preventing an imbalance.

| Tissues                  | Factors | Mean ± SD | Minimum | Maximum | %    | Significant rate |
|-------------------------|---------|-----------|---------|---------|------|-----------------|
| Thalamus                | Ngb     | 10.884 ± 0.108 | 10.729  | 10.968  | 66.2%| 0.008**         |
|                         | Hif-1α  | 5.551 ± 0.094  | 5.461   | 5.640   | 33.8%| 0.008**         |
| Hypothalamus            | Ngb     | 11.134 ± 0.043 | 11.097  | 11.182  | 64.1%| 0.210           |
|                         | Hif-1α  | 6.238 ± 0.013  | 6.229   | 6.254   | 35.9%| 0.210           |
| Epithalamus             | Ngb     | 12.150 ± 0.070 | 11.975  | 12.325  | 69.6%| 0.638           |
|                         | Hif-1α  | 6.710 ± 0.073  | 6.529   | 6.893   | 30.4%| 0.638           |
| Cerebellar cortex       | Ngb     | 12.179 ± 0.150 | 11.805  | 12.553  | 63.1%| 0.000***        |
|                         | Hif-1α  | 6.276 ± 0.015  | 5.238   | 5.314   | 36.9%| 0.000***        |
| White matter of the cerebellum | Ngb     | 5.308 ± 0.212 | 5.084   | 5.379   | 35.3%| 0.000**         |
|                         | Hif-1α  | 8.301 ± 0.059  | 8.329   | 8.787   | 71.2%| 0.000**         |
| Basal ganglia           | Ngb     | 11.022 ± 0.152 | 10.644  | 11.400  | 66.3%| 0.015**         |
|                         | Hif-1α  | 5.595 ± 0.118  | 5.300   | 5.891   | 33.3%| 0.015**         |
| Cerebrum               | Ngb     | 11.805 ± 0.212 | 11.278  | 12.331  | 67.2%| 0.000***        |
|                         | Hif-1α  | 5.232 ± 0.059  | 5.084   | 5.379   | 35.3%| 0.000***        |

Explanations: Ngb and Hif-1α were significantly expressed in the thalamus, cerebellar cortex, the white matter of the cerebellum, and basal ganglia, while other regions showed smaller expressions.
in the blood flow and nutrients, such as glucose and lactate, leading to biochemical and molecular changes that cause neuronal damage, whereas Hif-1α might attenuate Ngb functions in the hypothalamus. Brunori et al. (2) confirmed that Ngb is involved in NO metabolism by detoxification of harmful NO under normoxic conditions.

**Epithalamus.** There are few studies on the expression of Ngb and Hif-1α in several neuronal tissues of mammals, and this study is the first to report the expression of Ngb and Hif-1α in the epithalamus of young yaks. The expression patterns of Ngb and Hif-1α were different. The Ngb and Hif-1α expression intensity in the young yak epithalamus displayed higher as compare to other tissues. The different patterns of expression may be related to the secretion of melatonin in young yaks. The level of Ngb expression was higher than that of Hif-1α, and their expression might play a role in the protection of neuronal tissues.

**Cerebellar cortex.** According to Reuss et al. (17), Ngb is expressed solely in the cerebellar cortex of the rodent brain. Purkinje cells of the cerebellar cortex also showed a certain level of Ngb mRNA expression. A study performed by Christian et al. (4) also confirmed a significant expression of Ngb in the cerebellar cortex of the adult mouse brain, but Fabrizius et al. (8) interestingly revealed a lower expression of Ngb in the cerebellar cortex during fetal development of the mouse brain, which has a tendency to increase as the mouse approaches adulthood. The current study

---

**Fig. 2A-G. Ngb and Hif-1α expression in different tissues of the brain of young yaks**

- **Fig. 2A.** The expression of Ngb and Hif-1α in the thalamus of the diencephalon of young yaks. Positive and negative controls are indicated by arrows.
- **Fig. 2B.** Ngb and Hif-1α are found in the upper regions of the hypothalamus.
- **Fig. 2C.** Ngb is observed in the middle region of the epithalamus, while Hif-1α occurs in the upper right extreme of the epithalamus.
- **Fig. 2D.** Ngb and Hif-1α can be seen in the middle region of the cerebral cortex.
- **Fig. 2E.** Ngb is located in the lower region of the white matter while Hif-1α can be found in the upper region of the white matter.
- **Fig. 2F.** Ngb is found in the entire region of the basal ganglia, and Hif-1α occurs in the upper and lower regions.
- **Fig. 2G.** Ngb and Hif-1α are found in the middle region of the cerebrum.
revealed significant levels of Ngb and Hif-1α in the cerebellar cortex of young yaks, but the expression of Ngb was higher than that of Hif-1α. Despite its higher expression, Hif-1α regulates the expression of Ngb in channels of information from other tissues of the diencephalon and activates Ngb while responding to signals. Ngb plays a protective role in the control movement and influences many other functions in the cerebellar cortex, whereas Hif-1α activates or modifies the expression of Ngb. In the human brain, a low Ngb expression was reported (13), while Huquing et al. (15) reported that the expression of Hif-1α increased in the cerebellar cortex between the 3rd and the 18th month of age.

**White matter of the cerebellum.** The white matter of the cerebellum showed significant levels of Ngb and Hif-1α in the young yaks. The expression intensity of Ngb and Hif-1α in the young yaks was significantly similar. The similarity may be involved in the neuro-protection of information channels between neuronal tissues of the CNS in young yaks. The thickness of skin might be another factor for the similarity. These attributes protect animals from the effects of solar radiation. Previous researches revealed the expression of Ngb and Hif-1α in the brains of rodents and other mammals (1, 13). The expression of Ngb in the white matter of the rodent brain suggests that Ngb mRNA is transported in nerve cell processes to provide protein synthesis in distal regions of axons and dendrites (15). This might also be an indication that Ngb mRNA is expressed in varicosities and synaptic terminals, as well as in dendritic spines. The distribution and expression of Ngb transcripts might have significant functional consequences for synaptic plasticity, an active metabolic process that presumably requires an enormous amount of oxygen.

**Basal ganglia.** In a 26-year-old man Ngb was highly expressed in the basal nuclei, while a woman reported to be 42 years old showed low expression (12). Age factors may influence the expression of Ngb in neuronal tissues, especially in the basal ganglia. The current findings show a significant expression of Ngb and Hif-1α in the basal nuclei of the young yaks. The expression pattern may play a role in protecting neuronal tissues during transportation or movement in a high altitude environment. During transportation, the yak’s breathing often intensifies, and oxygen is paramount when breathing. The significant expression of Ngb is involved in protecting the movement and coordination of neuronal tissues in the basal ganglia. The expression of Hif-1α protects the basal ganglia from hypoxic or ischemic conditions, potentially limiting brain damage. Ngb lacks conserved hypoxia-responsive elements (HREs) for transcriptional activation (Hif-1α contains HREs), but contains conserved hypoxia-inducible mRNA stabilization signals (8). Thus, in the basal ganglia of young yaks, Hif-1α expression activates Ngb functions during movement and reacts against tumors. Hawa et al. reported that the strong expression of Hif-1α in the basal nuclei and other neuronal tissues reacts against tumors (11).

**Cerebrum.** A significant expression of Ngb and Hif-1α was found in the cerebrum of the young yaks, but the expression of Ngb was higher than that of Hif-1α. Ngb helps increase the oxygen rate in young yaks during movement, while Hif-1α regulates Ngb and protects neuronal tissues. The expression of Ngb and Hif-1α in the cerebrum may also be involved in the adaptation of yaks to the precipitous terrain. According to a study by Hu et al. (12), the expression of Ngb was high in the cerebrum of 39-year-old adults and lower in 33-year-old adults. Yang et al. (20) used enzyme-linked immunosorbent assay (ELISA) and Western blot to determine the levels of Hif-1α in the cerebrum of epileptic rats. That study revealed that Hif-1α significantly increased in the cerebrum after the induction of status epilepticus (SE) and decreased when an amplified TNF-α expression was evoked by the status epilepticus. The current study also reports a significant expression of Hif-1α in the cerebrum.

Ngb has an important physiological role in oxygen absorption, usage, and transportation in neuronal cells and serves as an oxygen sensor to regulate signal transmission according to changes in oxygen concentration. Ngb also facilitates oxygen movement between neuronal tissues and provides a secondary level of neuronal protection from hypoxia. Hif-1α regulates and increases Ngb expression in the diencephalon and contains hypoxia-responsive elements (HREs) and Ngb lacks hypoxia-responsive elements (HREs). The authors of the present study suggest that the expression levels of Ngb and Hif-1α can influence the adaptive potential of young yaks to a high-altitude environment. The current report provides data relevant for understanding the influence of Ngb and Hif-1α on the adaptive mechanism. Further studies to explore the adaptive mechanism are needed.

**References**

1. Burmester T., Weich B., Reinhardt S., Hankeln T.: A vertebrate globin expressed in the brain. Nature 2000, 407, 520-523, doi: 10.1038/35035093.
2. Bruniomi M., Giuffre A., Nienhaus K., Nienhaus G. U., Scandurra F. M., Vallone B.: Neuroglobin, nitric oxide, and oxygen: functional pathways and conformational changes. Proc. Natl. Acad. Sci. USA 2005, 102, 8483-8488, doi: 10.1073/pnas.0408766102.
3. Catrina S. B.: Impaired hypoxia-inducible factor (HIF) regulation by hyperglycemia. J. Mol. Med. 2014, 92, 1025-1034, doi: 10.1007/s00109-014-1166-x.
4. Christian A. H., Kelsen J., Hay-Schmidt A.: Neuroglobin and Cytoglobin expression in the human brain. Brain Struct Funct. 2013, 218, 603-609, doi: 10.1007/s00381-012-0800-0.
5. Crane S. T., Yamanishi Y., Clausen B. E., Forster I., Pawlinski R., Mackman N.: HIF-1alpha is essential for myeloid cell-mediated inflammation. Proc. Natl. Acad. Sci. USA 2005, 102, 8483-8488, doi: 10.1073/pnas.1008602102.
6. Della-Valle B., Hempel C., Kuckhaas J. A., Penkowa M.: In vivo expression of neuroglobin in reactive astrocytes during neuroprotection in murine models of traumatic brain injury, cerebral malaria, and autoimmune encephalitis. Glia. 2010, 10, 1220-1227, doi: 10.1002/glia.21002.
7. Eilana B., Valentina E., Ricardo C., Marco Á. R., Luis M. G.: Protection by Neuroglobin Expression in Brain Pathologies. Front. Neurol. 2016, 7, 146-149, doi: 10.3389/fneur.2016.00146.
8. Fabrizius A., Andre D., Lasfs T., Bicker T., Reuss S., Burmester T., Hankeln T.: A critical re-evaluation of neuroglobin expression reveals conserved patterns among mammals. Neuroscience 2016, S0306-4522 16, 30357-30358, doi: 10.1016/j.neuroscience.2016.07.042.

9. Frank R. S., Bergeron M., Bernaudin M.: Hypoxia-inducible factor in the brain. Springer Nature 2001, p. 273-279.

10. Hall F., Dill G. D. B., Barron G. E. S.: Comparative physiology at high altitude. J. Cell Comp. Physiol. 1936, 8, 301-313.

11. Hawa N., Lavina A., Jarle B., Arnes H. W., Lars A. A.: Strong Expression of Hypoxia-Inducible Factor-1α (HIF-1α) is Associated with Axial Expression and Features of Aggressive Tumors in African Breast Cancer. Plos One 2016, 11, e0146823, doi: 10.1371/journal.pone.0146823.

12. Hu J., Xiyan C., Dejiang P., Qihui L., Yuanfeng Z., Bin F.: Tumor grade related expression of neuroglobin is negatively regulated by PPARγ and confers antioxidant activity in glioma progression. Redox Biol. 2017, 12, 682-689, doi: 10.1016/j.redox.2017.03.023.

13. Hundahl C. A., Fahrenkrug J., Luuk H., Hay-Schmidt A., Hannibal J.: Restricted expression of Neuroglobin in the mouse retina and co-localization with Melanopsin and Tyrosine Hydroxylase. Biochem. Biophys. Res. Commun. 2012, 425, 100-106, doi: 10.1016/j.bbrc.2012.07.061.

14. Hundahl C. A., Hay-Schmidt A., Kelsen J.: Neuroglobin and Cytoglobin expression in the human brain. Brain Struct. Funct. 2013, 218, 603-609, doi: 10.1007/s00429-012-0480-8.

15. Huaing W., Haiqin W., Hena G., Guilian Z., Ru Z., Shuqin Z.: Increased hypoxia-inducible factor 1 alpha expression in rat brain tissues in response to aging. Neural regen. res. 2012, 10, 778-782, doi: 10.3969/j.issn.1673-5374.2012.10.010.

16. Joana M., Licio G., Vellioso A.: Hypoxia-Inducible Factor as a Central Regulator of Metabolism – Implications for the Development of Obesity. Front. Neurosci. 2018, doi: 10.3389/fnins.2018.00813.

17. Reuss S., Saaler-Reinhardt S., Weich B., Wystub S., Reuss M., Burmester T., Hankeln T.: Expression analysis of neuroglobin mRNA in rodent tissues. Neuroscience 2002, 115, 645-656, doi: 10.1016/S0306-4522(02)00536-5.

18. Song L. L., Cui Y., Yu S. J., Liu P. G., Liu J., Yang X., He J. F., Zhang Q.: Expression characteristics of BMP2, BMPR-IA, and Noggin in different stages of the hair follicle in yak skin. Gen. Comp. Endocr. 2018, 260, 18-24, doi: 10.1016/j.ygcen.2017.11.016.

19. Wystub S., Ehner B., Fuchs C., Weich B., Burmester T., Hankeln T.: Interspecies comparison of neuroglobin, cytoglobin, and myoglobin: sequence evolution and candidate regulatory elements, Cytogenet. Genome Res. 2004, 105, 65-78.

20. Yang J., He F., Meng Q., Sun Y., Wang W., Wang C.: Inhibiting HIF-1α Decreases Expression of TNF-α and Caspase-3 in Specific Brain Regions Exposed Kainic Acid-Induced Status Epilepticus. Cell Physiol. Biochem. 2016, 38, 75-82, doi: 10.1159/000438610.

21. Zhao S., Yu Z., Zhao G., Xing C., Hayakawa K.: Protection by Neuroglobin Expression in Brain Pathologies. Neuroscience 2012, 13, 67-69, doi: 10.1371/ journal.pone.0076565.

Corresponding author: Dr. Xia Liu, Anning District, Lanzhou City, Gansu Province, 730070, People’s Republic of China; e-mail: drdugau2019@gmail.com (XL)