VIA MEDICA

ORIGINAL PAPER

Folia Histochem Cytobiol
Vol. 64, No. 1, 2026, pp. 39–47

DOI: 10.5603/fhc.110409
ISSN 0239-8508, e-ISSN 1897-5631

Expression of BDNF–TrkB–AKT1 pathway components and apoptosis-related factors across yak brain regions at low and high altitudes

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ABSTRACT

Introduction. This study investigated the expression of brain-derived neurotrophic factor (BDNF) signaling components (BDNF–TrkB–AKT1) and apoptosis-related factors (Bcl-2 and Bax) in yak brain regions at different altitudes.

Material and methods. The cerebral cortex, cerebellum, hippocampus, thalamus, and medulla oblongata were collected from 3-year-old yaks living at low and high altitudes. The relative mRNA expression of *BDNF*, *TrkB*, *AKT1*, *Bcl-2*, and *Bax* was assessed by qRT-PCR. Protein abundance and cellular localization of BDNF, TrkB, AKT1, Bcl-2, and Bax were evaluated by Western blotting and immunohistochemistry, with immunoreactivity quantified by optical density analysis.

Results. Within each altitude group, *BDNF*, *TrkB*, *AKT1*, and *Bcl-2* mRNA expression and the corresponding protein levels (BDNF, TrkB, AKT1, and Bcl-2) were significantly higher in the cerebral cortex and hippocampus than in the cerebellum, thalamus, and medulla oblongata ($P < 0.05$). In contrast, *Bax* mRNA and Bax protein levels did not differ significantly among the five regions. Compared with low-altitude yaks, high-altitude yaks showed significantly higher *BDNF*, *TrkB*, *AKT1*, and *Bcl-2* mRNA expression and higher BDNF, TrkB, AKT1, and Bcl-2 protein levels in brain tissues ($P < 0.05$), whereas Bax protein expression did not differ between altitude groups. Immunohistochemistry revealed immunoreactivity for BDNF, TrkB, AKT1, Bcl-2, and Bax in both altitude groups, with prominent labeling in cortical pyramidal neurons and across the pyramidal cell layer in the hippocampal CA region. Immunoreactivity was also detected in large neurons of the thalamus and medulla oblongata. In the cerebellum, labeling was strongest in Purkinje cells, with weaker signals in the granule cell layer and molecular layer.

Conclusions. BDNF–TrkB–AKT1 pathway components and Bcl-2 showed relatively higher expression in the cerebral cortex and hippocampus within each altitude group, whereas Bax expression did not vary across regions. These patterns are consistent with an association between BDNF–TrkB–AKT1 signaling and increased Bcl-2 expression without a corresponding increase in Bax, which may support neuronal adaptation in the cerebral cortex and hippocampus. Elevated expression of BDNF, TrkB, AKT1, and Bcl-2 at high altitude suggests enhanced adaptation to hypoxia in high-altitude yaks; the underlying mechanisms require further investigation.

Keywords: BDNF–TrkB–AKT1; yak; brain regions; hypoxia adaptation; altitude

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Submitted:
December 30, 2025
Accepted after reviews:
March 2, 2026
Available as Online first:
March 11, 2026

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is among the most widely distributed and extensively studied neurotrophic factors in the central nervous system (CNS) [1]. Its abundance in the CNS is substantially higher than in peripheral tissues, including the heart, kidney, and testis. Secreted predominantly by neurons, BDNF supports neuronal survival, proliferation, differentiation, and synaptic plasticity [2, 3]. BDNF expression can be upregulated in neonatal rat neurons in response to hypoxic stimulation [4]. BDNF primarily signals through its high-affinity receptor, tropomyosin receptor kinase B (TrkB), which is widely expressed

in multiple brain regions, including the cerebral cortex, hippocampus, and cerebellum. Yang *et al*. [5] reported that, under hypoxic conditions, BDNF binding to TrkB activates the downstream effector AKT serine/threonine kinase 1 (AKT1). AKT1 activation modulates apoptosis--related proteins by increasing the anti-apoptotic factor Bcl-2 and decreasing the pro-apoptotic factor BAX. This signaling cascade inhibits neuronal apoptosis after hypoxic--ischemic injury, promotes neuronal survival, and protects brain tissue [6].

The brain underpins high neural activity yet has high metabolic demand and oxygen dependence. At high altitude, the brain is particularly vulnerable to hypoxia-associated neurological injury [7]. Hou *et al.* [8] established a stable hypobaric hypoxia brain injury model in SD rats. Their results revealed significant neuronal loss, cellular swelling, widened pericellular spaces, and the presence of shrunken neurons with darkly stained pyknotic nuclei in the hippocampus and cerebral cortex of the hypoxic group.

The yak (*Bos grunniens*) is an economically important species endemic to the Qinghai-Tibet Plateau and exhibits exceptional tolerance to high-altitude hypoxic environments [9–11]. Adaptation involves not only organ-level morphological features but also coordinated cellular and molecular regulatory mechanisms [12, 13]. Previous studies suggest that yak brain tissue displays a degree of hypoxia tolerance [14–16]. Our team investigated the expression of the HIF1α/BNIP3/Beclin-1 and HIF2α/BNIP3L signaling factors in yak brain tissue. Morphological examination revealed no significant neuronal apoptosis in these sections [14, 16]. This finding suggests a potential neuroprotective role for the BDNF/TrkB signaling pathway. However, the regional distribution and expression patterns of BDNF/TrkB signaling components and related factors in the yak brain remain poorly defined.

Here, we used qRT-PCR, Western blotting, and immunohistochemistry to assess the regional distribution and expression of *BDNF*, *TrkB*, *AKT1*, *Bcl-2*, and *Bax* transcripts, together with the expression of the corresponding proteins (BDNF, TrkB, AKT1, Bcl-2, and Bax), in different brain regions from yaks living at high and low altitudes. This work aims to clarify the putative neuroprotective role of BDNF/TrkB signaling in the yak brain and provide a foundation for mechanistic studies of hypoxia adaptation in plateau mammals.

MATERIALS AND METHODS

Animals and tissue collection

All procedures involving animals were conducted in accordance with the animal ethics guidelines of the People's Republic of China and were approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, Gansu Agricultural University. Healthy adult male yaks (3 years old; 258.24 ± 58.13 kg; n = 5 *per* altitude group) residing at 2500 m and 4500 m in the Qinghai region were selected based on physical examination and serum biochemical indices. Yaks were maintained at low-altitude (2500 m above sea level, with an approximate partial pressure of oxygen (PO_2) of 14.4 kPa) and high-altitude (4500 m above sea level, with an approximate PO_2 of 10.3 kPa) sites in the Qinghai region. They were housed under standardized conditions of temperature and lighting and were provided with free access to food and water from birth until the moment of euthanasia. Animals were euthanized by intravenous administration of pentobarbital sodium (180 mg/kg).

As we previously reported [14, 16], brain regions including the cerebral cortex, hippocampus, cerebellum, thalamus, and medulla oblongata were collected from each yak. Tissues were fixed in 4% paraformaldehyde for immunohistochemistry or snap-frozen in liquid nitrogen for Western blotting (WB) and quantitative real-time PCR (qRT-PCR) analyses.

Antibodies

The following primary antibodies were used: polyclonal rabbit anti-BDNF (Bioss, Beijing, China; bs-4989R), polyclonal rabbit anti-TrkB (Bioss; bs-5526R), polyclonal rabbit anti-AKT1 (Bioss; bs-0115R), polyclonal rabbit anti-Bcl-2 (Bioss; bs-0032R), polyclonal rabbit anti-Bax (Bioss; bs-0015R), and anti-β-actin (Bioss; bs-0061R).

Quantitative real-time PCR

Total RNA was extracted from frozen tissue samples using TRIzol (Invitrogen, Waltham, MA, USA). First-strand cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. cDNA was stored at –80°C until analysis.

Gene-specific primers were designed from *B. grunniens* nucleotide sequences [17] using Primer 5 (Premier Biosoft, USA) and synthesized by Beijing Genomics Institute (BGI, Shenzhen, China). Primer sequences and optimal annealing temperatures are provided in Table 1. qRT-PCR was performed on a LightCycler 96 system (Roche, Basel, Switzerland) in 20 μL reactions containing 1.5 μL cDNA, 0.75 μL each primer (10 μM), 10 μL SYBR Green II master mix [Takara Biomedical Technology (Beijing) Co., Ltd., Beijing, China], and 7 μL nuclease-free water. Cycling conditions were 95°C for 300 s, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 15 s. Each sample was analyzed in quadruplicate. β-A*ctin* served as the endogenous reference, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Total protein was extracted from frozen tissues using RIPA lysis buffer (Beyotime, Shanghai, China). and denatured at 100°C for 8 min. Equal amounts of protein (20 μg *per* lane)

Table 1. Primer sequences

Genes	Primer sequences (5′-3′)	Length (bp)	Annealing (°C)
BDNF	F:ATGAAAGAAGCCAACCTCCG R:TCAAAAGTGTCAGCCAACGAC	140	58
TrkB	F:TCGTGCCCCGATATGTAGAG R:TGAGTGGGATGTCATTGAGGAT	169	59
AKT1	F:CCTAAAGAAGGAGGTCATCGTG R:GGGACAGGTGGAAGAAAAGC	182	54.5
Bcl-2	F:GATGACCGAGTACCTGAACCG R:GACAGCCAGGAGAAATCAAACA	120	60
Bax	F:CCTTTTGCTTCAGGGTTTCAT R:CGCTCAGCTTCTTGGTGGAT	110	58
β-actin	F:CCGTGACATCAAGGAGAAG R:AGGAAGGAAGGCTGGAAG	207	56

were separated by 10% SDS–PAGE and transferred to PVDF membranes (Amersham, Marlborough, MA, USA). Membranes were blocked in 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1.5 h at room temperature and incubated overnight at 4°C with primary antibodies against BDNF (1:1000), TrkB (1:1000), AKT1 (1:1000), Bcl-2 (1:1000), and Bax (1:1000). β-actin (1:1000) was used as a loading control. After washing in TBST, membranes were incubated for 2 h with HRP-conjugated goat anti-rabbit IgG (Bioss; bs-0295G-HRP, 1:2000). Bands were detected using enhanced chemiluminescence and quantified by densitometry in ImageJ.

Immunohistochemistry

The distribution of BDNF-, TrkB-, AKT1-, Bcl-2-, and Bax-positive cells in yak brain tissues was assessed by immunohistochemistry. Paraffin-embedded tissues were sectioned at 4 μm, mounted on slides, and processed using standard procedures. Sections were incubated with primary antibodies against BDNF (1:50), TrkB (1:50), AKT1 (1:50), Bcl-2 (1:50), and Bax (1:50) at 37°C for 2 h in a humidified chamber. Sections were then incubated with a biotinylated anti-rabbit secondary antibody (Bioss; SP-0023) for 10 min, followed by streptavidin–peroxidase for 10 min. Immunoreactivity was visualized using 3,3'-diaminobenzidine tetrahydrochloride (Bioss; c-0010) and counterstained lightly with hematoxylin. Negative controls were processed in parallel, with the primary antibody replaced by rabbit serum albumin. Images were acquired using a DP71 microscope (Olympus, Tokyo, Japan), and optical density was quantified using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). The integrated optical density (IOD) of immunohistochemical staining for BDNF–TrkB–AKT1 was quantified in five randomly selected fields of view (original magnification ×400) in the section of a given brain region. Five sections were randomly chosen for each brain tissue [18].

Statistical analysis

Statistical analyses were performed using IBM SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard error. Group comparisons were performed by one-way ANOVA, and $P < 0.05$ was considered statistically significant.

RESULTS

BDNF, *NTRK2*, *AKT1*, *Bcl-2*, and *Bax* mRNA expression in yak brain tissues

RT-qPCR showed that, within each altitude group, *BDNF*, *TrkB*, *AKT1*, and *Bcl-2* mRNA levels were highest in the cerebral cortex and hippocampus and were significantly greater than those in the cerebellum, thalamus, and medulla oblongata ($P < 0.05$; Fig. 1). Across all regions examined (cerebral cortex, cerebellum, hippocampus, thalamus, and medulla oblongata), *BDNF*, *TrkB*, *AKT1*, and *Bcl-2* mRNA expression was significantly lower in low-altitude yaks than in high-altitude yaks ($P < 0.05$).

In contrast, *Bax* mRNA expression did not differ significantly among brain regions within either altitude group ($P > 0.05$), and *Bax* mRNA levels were not significantly different between low- and high-altitude yaks within the same brain region ($P > 0.05$).

BDNF, TrkB, AKT1, Bcl-2, and Bax protein expression in yak brain tissues

Protein abundance of BDNF, TrkB, AKT1, Bcl-2, and Bax was assessed by Western blotting (Fig. 2) and immunohistochemistry (Fig. 3). Within each altitude group, BDNF, TrkB, AKT1, and Bcl-2 showed region-dependent expression, with the highest levels in the cerebral cortex and hippocampus, followed by the cerebellum, thalamus, and medulla oblongata ($P < 0.05$). In each brain region analyzed, BDNF, TrkB, AKT1, and Bcl-2 protein levels were significantly lower in low-altitude yaks than in high-altitude yaks ($P < 0.05$).

Bax protein expression showed no significant regional differences within either altitude group ($P > 0.05$). Consistently, Bax protein levels did not differ significantly between low- and high-altitude yaks in any of the regions examined ($P > 0.05$).

Immunohistochemical localization

Immunohistochemistry indicated that BDNF (Fig. 4), TrkB (Fig. 5), AKT1 (Fig. 6), Bcl-2 (Fig. 7), and Bax (Fig. 8) immunoreactivity was predominantly cytoplasmic in neurons. Positive staining was observed in neurons of the cerebral cortex (polymorphic cell layer), the pyramidal cell layer of the hippocampal CA region, the Purkinje cell layer of the cerebellum, and large neurons in the thalamus and medulla oblongata.

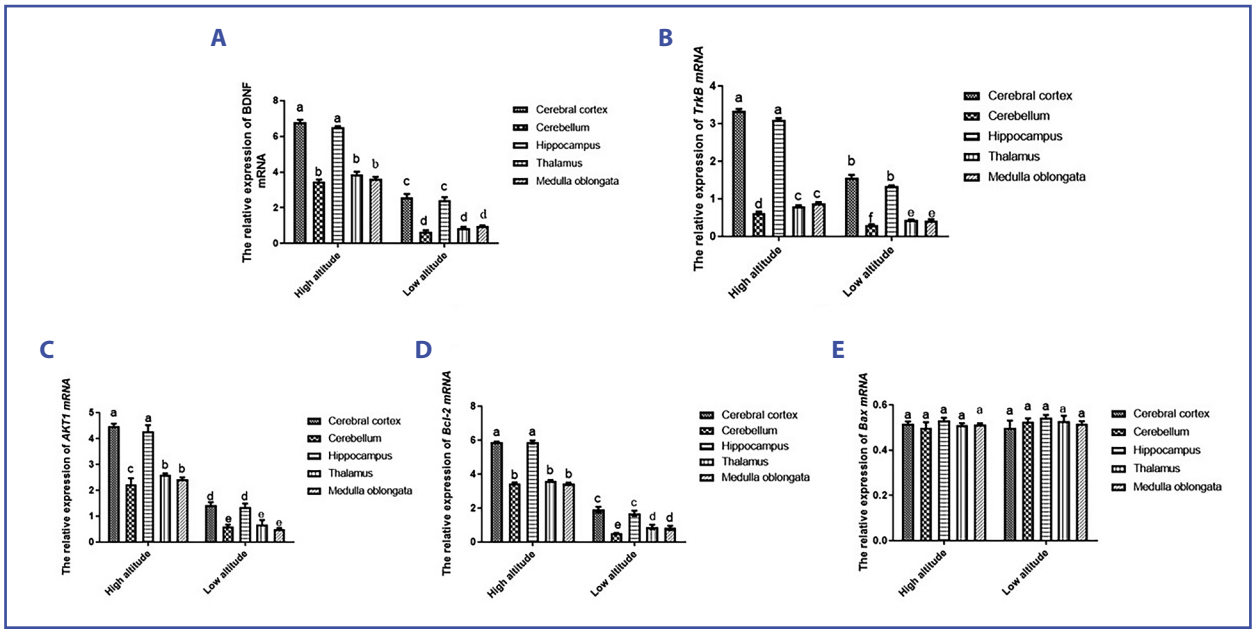

Figure 1. The gene expressions of *BDNF* (**A**), *TrkB* (**B**), *AKT1* (**C**), *Bcl-2* (**D**), and *Bax* (**E**) in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. The gene expression levels represent the mRNA levels in relation to the mRNA expression of the control gene (β-actin). The data are expressed as the means ± standard error of $2^{-\Delta\Delta Ct}$. Bars with different superscripts are significantly different ($P < 0.05$). n = 5.

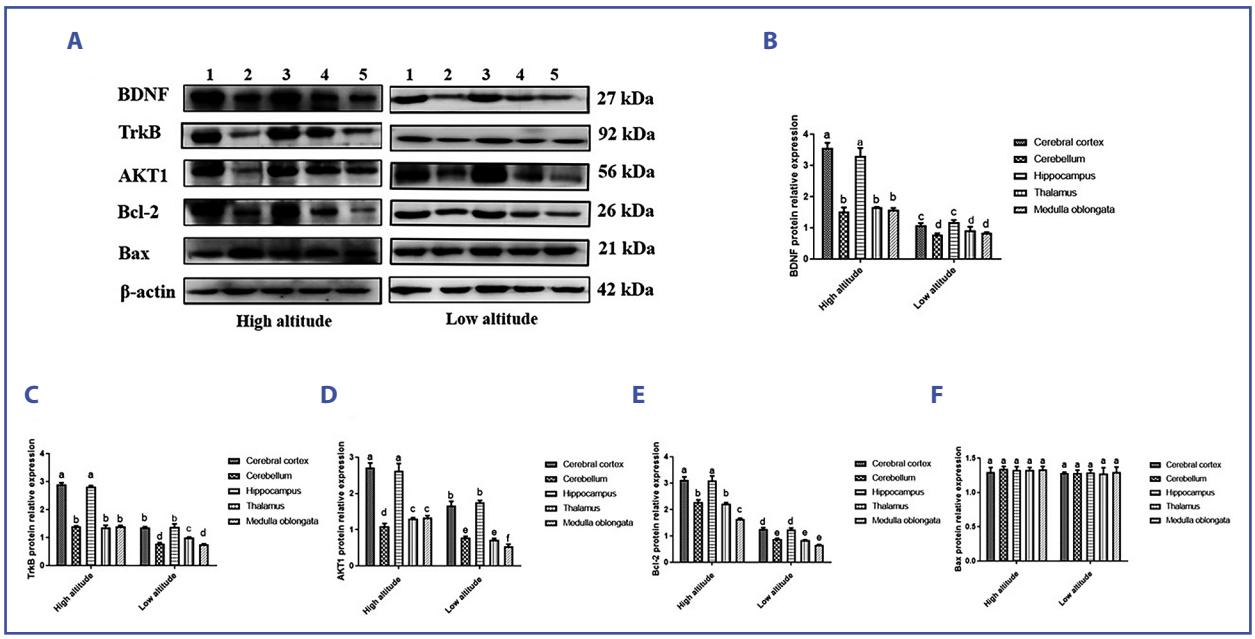

Figure 2. Relative content of the studied proteins in the brain of high- and low-altitude yaks. **A.** Representative Western blots of BDNF, TrkB, AKT1, Bcl-2, Bax and β-actin protein levels in the yaks' brain regions. 1 — cerebral cortex; 2 — cerebellum; 3 — hippocampus; 4 — thalamus; 5 — medulla oblongata; **B–F.** The relative expression levels of BDNF (**B**), TrkB (**C**), AKT1 (**D**), Bcl-2 (**E**), and Bax (**F**) in the brain regions of high- and low-altitude yaks. The values indicate the mean ± standard error. Bars with different superscripts are significantly different ($P < 0.05$). N = 5.

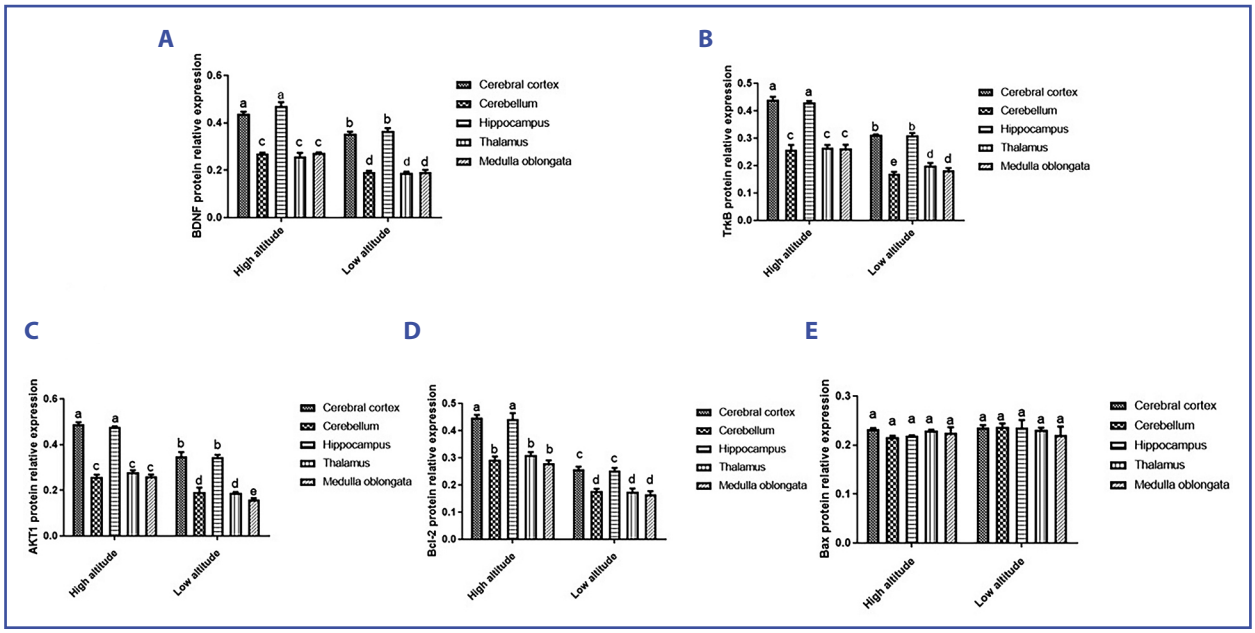

Figure 3. The optical density analysis values of immunohistochemically-stained BDNF (**A**), TrkB (**B**), AKT1 (**C**), Bcl-2 (**D**), and Bax (**E**) in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. The data are expressed as the mean ± standard error (SE). Bars with different superscripts are significantly different ($P < 0.05$).

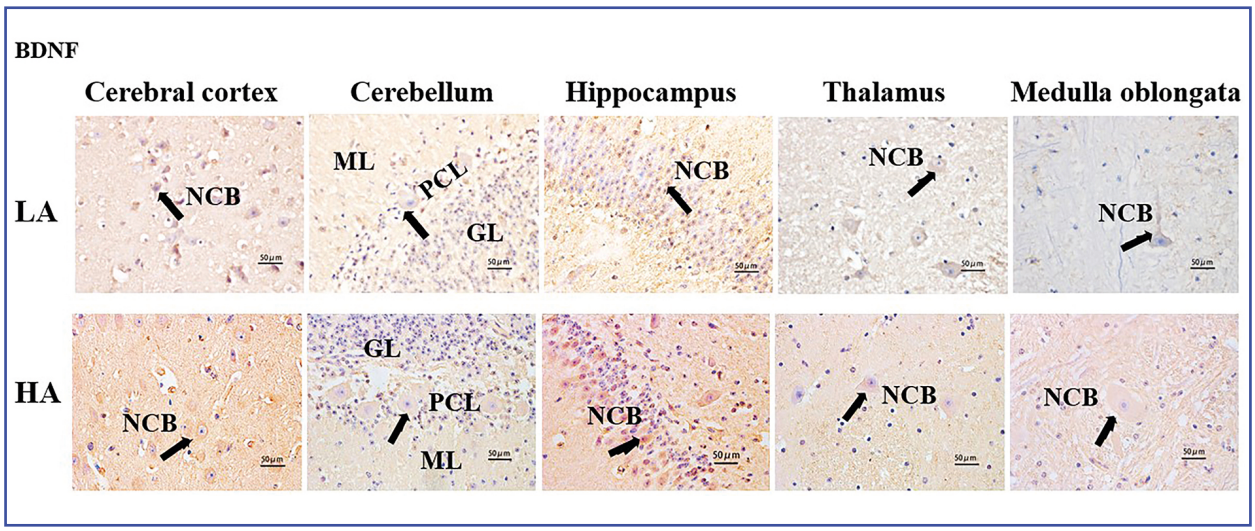

Figure 4. Immunohistochemical staining of BDNF in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. Arrowheads indicate examples of positive cells. Bar = 50 μm. Abbreviations: GL — granular layer; HA — high altitude yak; LA — low altitude yak; ML — molecular layer; NCB — neuronal cell body; PCL — Purkinje cell layer.

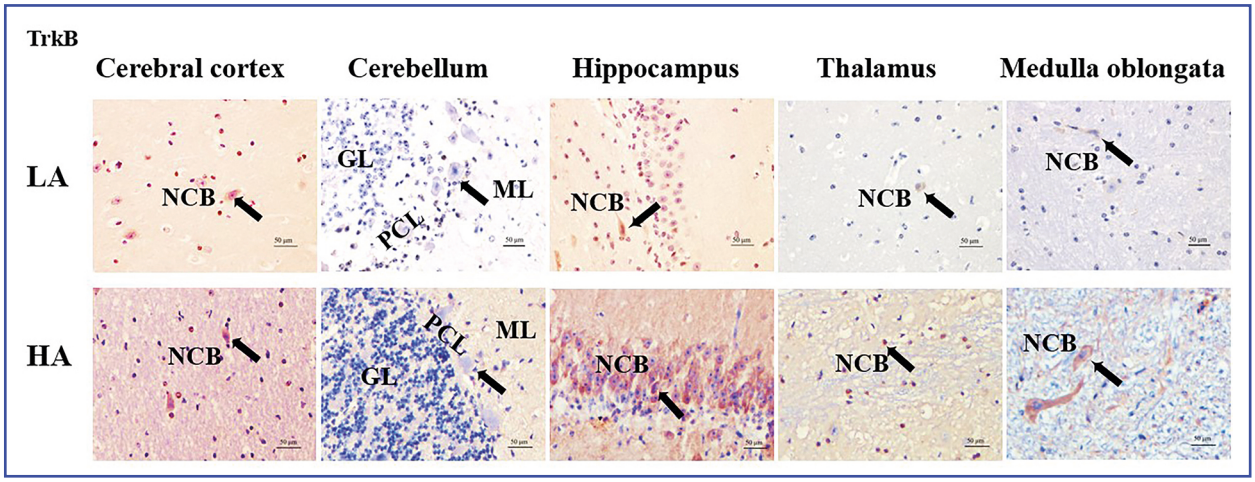

Figure 5. Immunohistochemical staining of TrkB in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. Arrowheads indicate examples of positive cells. Bar = 50 μm. Abbreviations: GL — granular layer; HA — high altitude yak; LA — low altitude yak; ML — molecular layer; NCB — neuronal cell body; PCL — Purkinje cell layer.

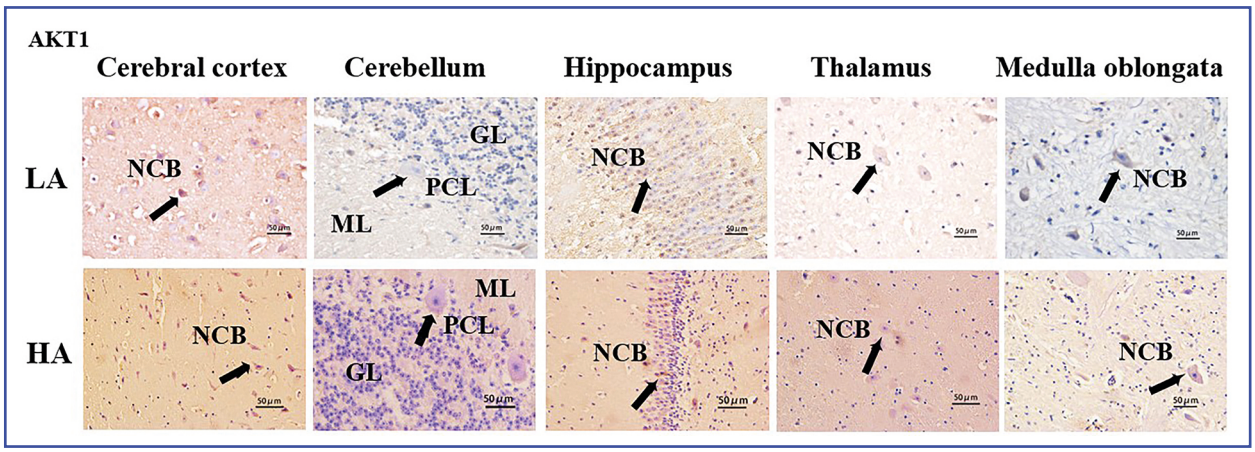

Figure 6. Immunohistochemical staining of AKT1 in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. Arrowheads indicate examples of positive cells. Bar = 50 μm. Abbreviations: GL — granular layer; HA — high altitude yak; LA — low altitude yak; ML — molecular layer; NCB — neuronal cell body; PCL — Purkinje cell layer.

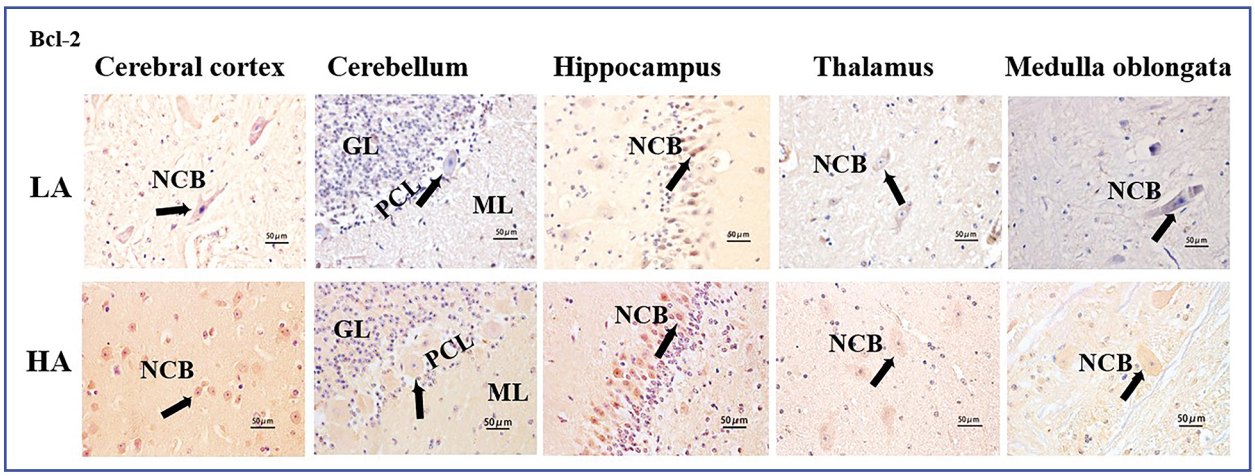

Figure 7. Immunohistochemical staining of Bcl-2 in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. Arrowheads indicate examples of positive cells. Bar = 50 μm. Abbreviations: GL — granular layer; HA — high altitude yak; LA — low altitude yak; ML — molecular layer; NCB — neuronal cell body; PCL — Purkinje cell layer.

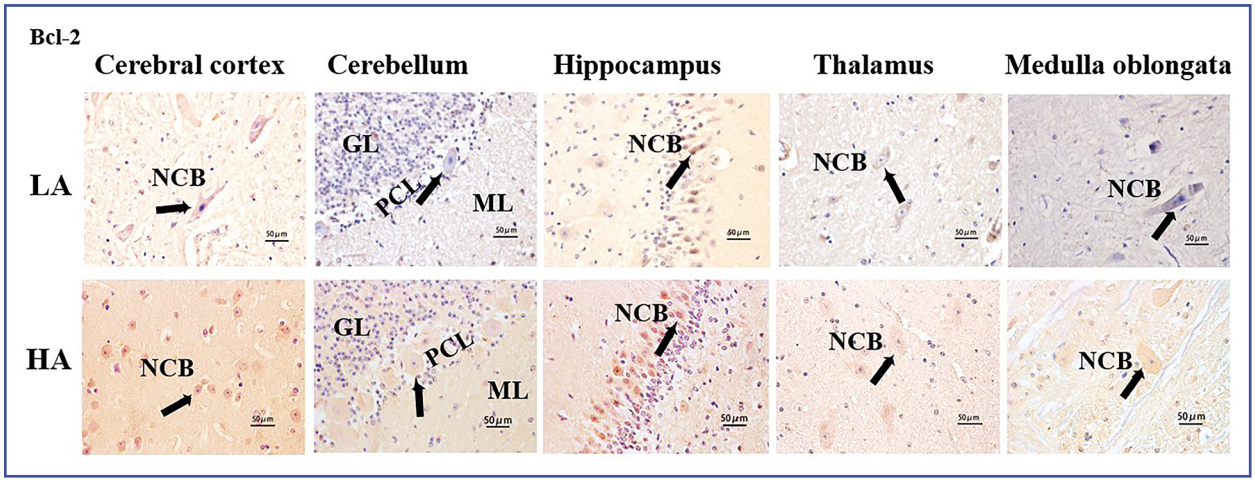

Figure 8. Immunohistochemical staining of Bax in the cerebral cortex, cerebellum, hippocampus, thalamus and medulla oblongata of high- and low-altitude yaks. Arrowheads indicate examples of positive cells. Bar = 50 μm. Abbreviations: GL — granular layer; HA — high altitude yak; LA — low altitude yak; ML — molecular layer; NCB — neuronal cell body; PCL — Purkinje cell layer.

DISCUSSION

This is the first report describing the distribution and expression of the BDNF–TrkB–AKT1 signaling pathway and apoptosis-related factors in yak brain tissues at different altitudes. BDNF and its high-affinity receptor TrkB are widely expressed in the central nervous system. Prior studies indicate that BDNF is predominantly produced and secreted by neurons, where it regulates neuronal growth and development, suppresses apoptosis, promotes neuronal survival, and contributes to adaptation under hypoxic conditions [19–23]. Here, within each altitude group, *BDNF* and *TrkB* mRNA expression and the corresponding protein levels (BDNF and TrkB) were highest in the cerebral cortex and hippocampus, followed by the thalamus, medulla oblongata, and cerebellum. This regional pattern is consistent with previous reports in mouse [24] and sheep [25]. Elevated BDNF/TrkB expression under hypoxic conditions has been linked to reduced neuronal apoptosis and protection of the brain from hypoxic-ischemic injury [26]. Additionally, Huang et al used hypoxia–hypoglycemia stimulation to establish an ischemia and hypoxia model of mouse primary hippocampal neurons in vitro and reported exogenous BDNF inhibited hippocampal neuron apoptosis caused by hypoxic–ischemic conditions [27]. In addition, our prior work showed that HIF1α and HIF2α mRNA and protein levels are enriched in the yak cerebral cortex and hippocampus [14, 16], suggesting that these regions may be particularly sensitive to reduced oxygen availability. Thus, the regional enrichment of BDNF and TrkB may reflect differential susceptibility of brain regions to hypoxia, with the cerebral cortex and hippocampus likely among the most vulnerable regions.

AKT1 is a key downstream effector of BDNF/TrkB signaling and regulates cellular survival [5]. In the present study, within each altitude group, *AKT1* mRNA expression and AKT1 protein levels were highest in the cerebral cortex and hippocampus, followed by the thalamus, medulla oblongata, and cerebellum. Notably, AKT1 expression mirrored the regional patterns observed for BDNF and TrkB. Consistent with this relationship, Yuan *et al*. [28] reported in neonatal rat pups that isoflurane exposure promoted BDNF binding to TrkB in the hippocampus, leading to AKT1 activation, which in turn engaged downstream protective signaling to support neuronal survival. Together, these findings in non-ruminant mammals support the interpretation that BDNF/TrkB signaling may contribute to AKT1 upregulation and activation in the cerebral cortex and hippocampus of yaks.

Neuronal apoptosis is a prominent consequence of hypoxic stress. Bcl-2 exerts anti-apoptotic effects by stabilizing mitochondrial membrane permeability and limiting cytochrome c release, thereby protecting cells under hypoxic conditions [29]. Prior studies have reported that BDNF/TrkB signaling can modulate AKT1 expression and thereby influence apoptosis-related pathways in neurons of rats exposed to oxygen–glucose deprivation [30]. In this study, we found within each altitude group, *Bcl-2* mRNA expression and Bcl-2 protein levels were highest in the cerebral cortex and hippocampus, paralleling the expression trends of BDNF, TrkB, and AKT1. These findings suggest that higher BDNF–TrkB–AKT1 signaling activity in the cerebral cortex and hippocampus may promote Bcl-2 expression and thereby suppress neuronal apoptosis. By contrast, the pro-apoptotic factor Bax did not differ significantly

among brain regions. This observation is notable given evidence that BDNF/TrkB signaling increases AKT1 activity, which can elevate Bcl-2 and reduce Bax expression, thereby limiting hypoxic brain injury in cultured cortical networks [31] and middle cerebral artery occlusion/reperfusion (MCAO/R) model in adult rats [32]. The absence of regional differences in Bax in yaks may reflect effective anti-apoptotic regulation in the more hypoxia-sensitive regions such as cerebral cortex and hippocampus, where BDNF–TrkB–AKT1 signaling and Bcl-2 expression are comparatively higher.

By comparing yaks adapted to distinct altitudes, we found that high-altitude yaks exhibited higher levels of BDNF, TrkB, AKT1, and Bcl-2 mRNA and protein expression than low-altitude yaks. In contrast, *Bax* mRNA and Bax protein levels did not differ significantly between altitude groups. Hypoxia can damage brain tissue and trigger neuronal apoptosis. Qi *et al*. [33] reported that after cerebral ischemia, hypoxia increased BDNF and TrkB expression and promoted AKT1 activation, which reduced hippocampal cell apoptosis. Consistent with this framework, the lower oxygen availability (with an approximate PO_2 of 10.3 kPa) at higher altitude may induce upregulation of BDNF–TrkB–AKT1 signaling and Bcl-2 expression in yak brain tissue, thereby enhancing tolerance to hypoxia. Additionally, Zhang *et al.* [34] reported that in neonatal rats subjected to hypoxia-ischemia, the protein expression of BDNF and Bcl-2 was increased, while the expression of the Bax was decreased in the hippocampus. In our study, although Bax did not differ significantly between altitude groups, the altitude-associated increases in BDNF signaling may still shift the overall balance toward neuronal protection.

Consistent with the mRNA changes, immunohistochemistry further showed that the distribution of BDNF, TrkB, AKT1, Bcl-2, and Bax across the cerebral cortex, cerebellum, hippocampus, thalamus, and medulla oblongata was broadly similar, with immunoreactivity primarily localized to the neuronal cytoplasm. In a cellular model of hypoxia/reoxygenation injury, Zhai *et al*. [35] demonstrated that BDNF, upon binding to its receptor TrkB, promoted Bcl-2 expression and reduced Bax expression, thereby inhibiting apoptosis. Collectively, our findings support the conclusion that BDNF/TrkB signaling and its downstream effectors primarily contribute to neuronal protection in yak brain tissue, particularly under high-altitude hypoxic conditions.

Within each altitude group, BDNF, TrkB, AKT1, and Bcl-2 mRNA and protein levels were significantly higher in the cerebral cortex and hippocampus than in the cerebellum, thalamus, and medulla oblongata, whereas Bax expression did not differ between the cerebral cortex and hippocampus and the other regions. These findings suggest that elevated BDNF–TrkB–AKT1 signaling in the cerebral cortex and hippocampus may confer anti-apoptotic protection. In addition, BDNF–TrkB–AKT1 signaling-related factors were expressed at higher levels in brain tissues from high-altitude yaks than in those from low-altitude yaks, consistent with a potential role in hypoxia adaptation. Immunolabeling indicated that these factors were primarily localized to the neuronal cytoplasm, supporting the conclusion that their protective effects occur mainly in neurons. Collectively, these data provide a basis for further investigation of hypoxia-adaptive mechanisms in the plateau yak brain.

Article information and declarations

Data availability statement

All original contributions generated and analyzed during this study are included in the manuscript. Further inquiries can be directed to the corresponding author.

Ethics statement

All the experimental animals were treated in accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China (2006-398, 30 September 2006), and the study received approval from the Institutional Animal Care and Use Committee at the College of Veterinary Medicine, Gansu Agricultural University (GAU-LC-2020-32, Apr 2020).

Author contributions

Conception and study design: QZ, YC. Data analysis: QZ, JF H, HL J. Histological examination: QZ. Data interpretation: QZ. Immunohistochemistry, WB and PCR experiments: QZ, HLJ. Data acquisition: QZ, YYP. Manuscript drafting: QZ, MW. All authors reviewed and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 32002241).

Conflict of interest

The authors declare that they have no competing interests related to this manuscript.

Supplementary material

None.

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