Original Research

Antiapoptotic effects of velvet antler polypeptides on damaged neurons through the hypothalamic-pituitary-adrenal axis

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We investigated the effects of velvet antler polypeptide on cognitive impairment and the underlying mechanisms. Hydrogen peroxide-induced cell injury was used to establish an *in vitro* model of SH-SY5Y cells. In addition, we established an *in vivo* mouse model of cognitive impairment using intraperitoneal injections of scopolamine hydrobromide in strain mice. We administered three different doses of velvet antler polypeptide in this mouse model and assessed the influence of velvet antler polypeptide on the morphology of hippocampal neurons, hippocampal neuronal apoptosis, adrenocorticotropic hormone, and corticosterone activities in brain tissue samples, and the molecular and biochemical regulation of B-cell lymphoma-2, B-cell lymphoma-2 Associated X-protein, Cysteine-aspartic acid protease-3, glucocorticoid receptor, mineralocorticoid receptor, and corticotropin-releasing hormone in murine hippocampal neurons. Our data suggest that velvet antler polypeptide decreases glucocorticoid receptor, mineralocorticoid receptor, and corticotropin-releasing hormone levels and regulates the hormones released by the hypothalamic-pituitary-adrenal axis, thus suppressing neuronal apoptosis.

Keywords

Apoptosis; hypothalamic-pituitary-adrenal axis; neuronal injury; velvet antler polypeptide

1. Introduction

With the aging of the world's population, the incidence of neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, and Parkinson's disease is increasing (Jodeiri Farshbaf and Ghaedi, 2017). Neurodegenerative diseases induce neuronal dysfunction and damage, leading to neuronal apoptosis and loss (Ising and Heneka, 2018). The hypothalamic-pituitary-adrenal (HPA) axis is associated with neurodegenerative diseases and affects the central nervous system function (Aziz et al., 2009; Herrero et al., 2015; Raber et al., 2000).

The HPA axis is an important neuroendocrine system (Spencer and Deak, 2017). Abnormal changes in the levels of hormones released by the HPA axis results in its dysfunction (Paragliola et al., 2017; Spencer and Deak, 2017), leading to its impaired suppression via the hippocampal neurons (Godoy et al., 2018). Decreased function of the hippocampal neurons causes an increased secretion of corticosterone, resulting in the positive feedback of the HPA axis dysfunction (Herman, 2018; Sampedro-Piquero et al., 2018); this leads to further loss of hippocampal function, ultimately inducing the development of cognitive impairment (Ahmad et al., 2019; Galts et al., 2019).

Traditional Chinese medicine (TCM) has unique advantages, showing great promise in the prevention and treatment of neurodegenerative diseases (Jarrell et al., 2018). Deer antler velvet (DAV)is a unique tissue predominantly collagen widely used in TCM, was first described in the Shen Nong Ben Cao Jing as a warming tonic with bone-strengthening, muscle strengthening, and anti-aging effects (Wu et al., 2013). Since then, many studies have demonstrated its antioxidative and anti-injury properties (Huo et al., 2014; Zang et al., 2016). Velvet antler polypeptide (VAP) in particular, extracted from DAV, has been extensively investigated as an important active ingredient (Lin et al., 2011; Ma et al., 2017); it has been shown to reduce oxidation levels and exert anti-inflammatory effects (Xin et al., 2017; Zhang et al., 2013; Zhu et al., 2017). However, reports on the protective neuronal effects of VAP in both cell culture and animal models are relatively rare.

Thus, the purpose of the present study was to investigate the role of VAP in the prevention and treatment of neuronal damage and to elucidate the mechanisms by which VAP regulates the HPA axis using both *in vitro* and *in vivo* approaches. In the *in vitro* experiments, Hydrogen peroxide (H_2O_2) was used to induce injury in SH-SY5Y cells, thereby establishing a neural injury model that allows the assessment of the influence of VAP on cell viability, apoptosis, and apoptosis-related proteins in this cell line. Additionally, intraperitoneal injection of scopolamine hydrobromide (SCOP) in ICR strain mouse. The Institute of Cancer Research (ICR) mouse is a strain of albino mice used to generate an *in vivo*

mouse model of neural damage. This model allowed to evaluate the influence of VAP on murine hippocampal neurons regarding morphology, apoptosis, and expression of apoptosis-related proteins, as well as the expression and release of hormones related to the HPA axis.

2. Materials and methods

2.1 Source and preparation of velvet antler polypeptide (VAP)

DAV was purchased from Zhenyuan Deer Co., Ltd. (Jilin Province, P. R. China). VAP extracts were prepared by the Molecular Pharmacology Laboratory of the Research and Development Center at the Changchun University of Chinese Medicine.

2.2 Cell culture and treatment

The human neuroblastoma cell line SH-SY5Y was provided by the Pharmacology Laboratory of the College of Basic Medical Sciences at Jilin University, Jilin, P. R. China.

SH-SY5Y cells were cultured in RPMI 1640 medium containing 12% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin under standard culture conditions of 37 °C and 5% CO_2. The cells were divided into the following groups: control group, model group, low-dose VAP group (VAP1), medium-dose VAP group (VAP2), and high-dose VAP group (VAP3). The VAP1, VAP2, and VAP3 groups were pretreated with 100, 250, and 500 μg/mL of VAP for 24 h, respectively, while the control and model groups were only exposed to the new culture medium for 24 h. Subsequently, 400 μmol/L H_2O_2 was added to all treatment groups for 4 h, while the control group was treated with an equivalent volume of culture medium for 4 h.

2.3 3-(4,5-Dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) assay to assess cell viability

SH-SY5Y cells were seeded into 96-well plates at a density of 20,000 cells/well, and MTT was added after treating the cells as described in 2.2. Following a reaction period of 4 h, 150 μL of dimethyl sulfoxide was added to each well, the well contents were mixed, and the optical densities were measured at 495 nm using a microplate reader (Bio-Rad, USA).

2.4 Measurement of apoptosis by flow cytometry

Treated SH-SY5Y cells, as described in 2.2, were washed twice with cold phosphate-buffered saline (PBS). Apoptosis was detected using Annexin V-FITC/PI (BestBio, P. R. China) staining. A volume of 400 μL PBS binding buffer was added to re-suspend the cells at a concentration of approximately 1×10^6 cells/mL, and 5 μL of Annexin V-FITC staining solution was added to the cell suspension. The solution was gently mixed and incubated at room temperature in darkness for 15 min. Subsequently, 10 μL of PI staining solution was added; the solution was again gently mixed and incubated at room temperature in darkness for 5 min. The apoptosis rate was detected using a flow cytometer (BD, USA).

2.5 Mice and their treatments

A total of 60 ICR mice (30 male and 30 female; 2-3 months of age; weight 25 ± 2 g) were purchased from Changchun Yisi Experiment Animal Technology Co., Ltd.

Mice were kept at a constant temperature (22-25 °C) and relative humidity (40-50%) with standard feed and water. The 60 ICR mice were randomly allocated into the following groups: control group, model group, positive group (3 mg/kg memantine), low-dose VAP group (VAP1, 100 mg/kg VAP), medium-dose VAP group (VAP2, 200 mg/kg VAP), and high-dose VAP group (VAP3, 400 mg/kg VAP).

Mice of the positive group and the VAP groups received intragastrically memantine or VAP for 21 days, whereas saline was intragastrically administered to mice of the control and model groups. Apart from the control group, mice in all groups received from the seventh day intraperitoneal SCOP injections 1 h after memantine or VAP treatment.

2.6 Preparation of the brain tissue

After the drug treatment period of 21 days, each mouse was decapitated, and the brain was rapidly removed. Brain tissue samples of seven mice per group were stored at -80 °C for enzyme-linked immunosorbent assay (ELISA), western blot, and PCR experiments. Brain tissue samples of three mice per group were placed in 4% paraformaldehyde for 24 h and then processed into paraffin sections for subsequent tissue staining.

2.7 Hematoxylin-eosin (HE) staining

Paraffin wax sections were dewaxed and washed. Subsequently, they were placed in hematoxylin staining solution for 5 min, washed with tap water, differentiated using 1% hydrochloric acid in alcohol for 1 min, washed with distilled water, blued in weak ammonia water for 30 s, washed with distilled water, and stained with eosin for 15 min. Finally, they were rinsed with distilled water and ethanol at different concentrations. The morphology and arrangement of hippocampal neurons in each experimental group were observed using an optical microscope (Olympus, Japan).

### 2.8 Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) Staining

The sections were washed using PBS, and the TUNEL assay (Beyotime Biotechnology, P. R. China) was performed according to the manufacturer's instructions. To each sample, 50 μL of the TUNEL staining solution was added, and the mixture was incubated at 37 °C in darkness for 60 min, followed by three washing steps with PBS. Apoptosis of hippocampal neurons in the various groups was observed with a confocal laser scanning microscope (Olympus, Japan) using an excitation wavelength range of 450-500 nm and an emission wavelength range of 515-565 nm.

2.9 Measurements of ACTH and CORT levels

Murine brain tissue samples of each group were placed in saline at a 1: 9 ratio, homogenized and centrifuged. Adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels were determined using the appropriate ELISA kits (Nanjing Jiancheng Bioengineering Institute, P. R. China) according to the manufacturer's instructions.

2.10 Western blotting analysis

After adding Radio-Immunoprecipitation Assay lysis buffer, brain tissues of mice were adequately lysed, and all samples were centrifuged at 14,000× g for 5 min to collect supernatants subsequently. Protein concentrations were quantified using the Coomassie brilliant blue assay (Sigma-Aldrich, USA). Processed samples were separated by 12% SDS-PAGE, and proteins were transferred to polyvinylidene fluoride (PVDF) membranes. The
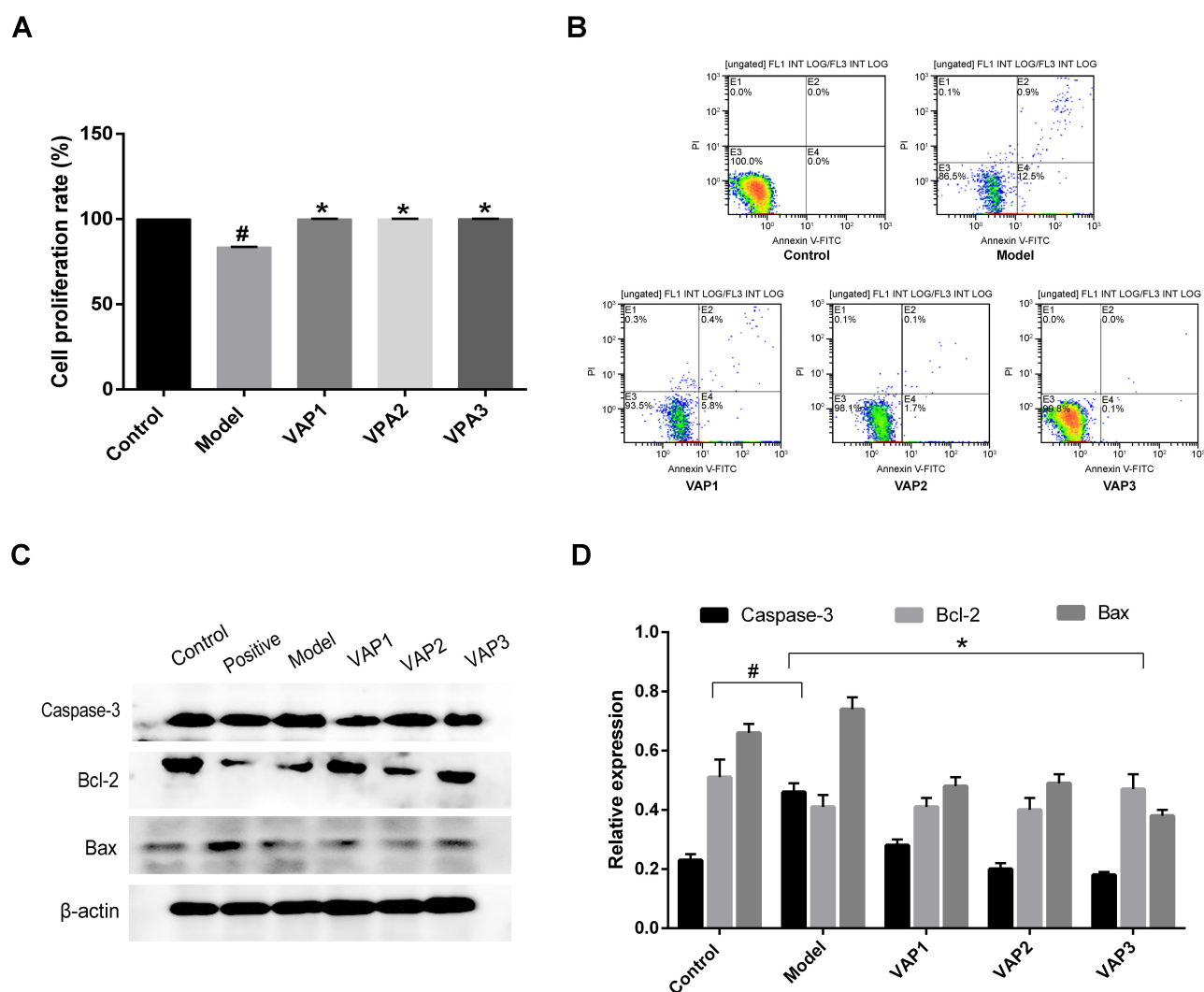
Fig. 1. Protective effects of VAP on H_2O_2-induced SH-SY5Y cell injury. (A) Effect of VAP on SH-SY5Y cell proliferation rate (n = 8). (B) Effect of VAP on SY5Y cell apoptosis (n = 3). (C) Effect of VAP on apoptosis proteins in damaged SH-SY5Y cells. (D) Effect of VAP on the relative expression of apoptotic proteins in damaged SH-SY5Y cells (n = 3). All data were analyzed by Student's t-test and are presented as the mean $\pm$ SEM. $^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. model.

membranes were then blocked in skimmed milk powder for 2 h and blotted with β-actin antibody (1 : 20000), glucocorticoid receptor (GR) antibody (1 : 1000), mineralocorticoid receptor (MR) antibody (1 : 400), corticotropin-releasing hormone (CRH) antibody (1 : 1000), B-cell lymphoma-2 Associated X-protein (Bax) antibody (1 : 1000), B-cell lymphoma-2 (Bcl-2) antibody (1 : 1000), or Cysteine-aspartic acid protease-3 (Caspase-3) antibody (1 : 1000) (all Proteintech, P. R. China). After washing with Tris-buffered saline/Tween buffer, PVDF membranes were incubated at room temperature for 2 h with a secondary peroxidase-conjugated antibody (Proteintech, P. R. China). The antibody signals were detected using the enhanced chemiluminescent substrate kit (Proteintech, P. R. China), and bands were visualized using a chemiluminescence imager (Aplegen, USA). The gray values of the bands were analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA) and calculated according to the following equation: relative expression = sample expression value/β-actin expression value.

2.11 RNA isolation and RT-PCR analysis

Total RNA was isolated using Trizol reagent. cDNA was synthesized from total RNA (0.2-2 μg) using the Bioteke Super RT Kit (BioTeke Corporation, P. R. China). Real-time quantitative PCR (qPCR) was performed using DNA-binding dye SYBR Green (Roche, P. R. China). Amplification was performed using the following primers: β-actin forward primer 5'-CCAGCCGAGCCACATCGCTC-3', reverse primer 5'-ATGAGCCCCAGCCTTCTCCAT-3'; Bcl-2 forward primer 5'-CAGCTGCACCTGACGCCCTT-3', reverse primer 5'-CCCAGCCTCCGTTATCCTGGA-3'; Bax forward primer 5'-GATCAGCTCGGGCACTTTAG-3', reverse primer 5'-TTGCTGATGGCAACTTCAAC-3'; Caspase-3 forward primer 5'-CTGACTGGAAAGCCGAAACTC-3', reverse primer 5'-CGACCCGTCCTTTGAATTTCT-3'; GR forward primer 5'-GTGAAATGGGCAAAGGCCATAC-3', reverse primer 5'-GAAGAGAAACGAGCAAGCATAG-3'; MR forward primer 5'-GGCTACCACAGTCTCCCTGA-3', reverse primer 5'-

AGAACGCTCCAAGGTCTGA-3'; and CRH forward primer 5'-CAGAACAACAGTGCGGGCTCA-3', reverse primer 5'-GGAAAAAGTTAGCCGCAGCCT-3'. Using the $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001), relative expression levels of Bcl-2, Bax, Caspase-3, GR, MR, and CHR mRNA were calculated for each sample after normalization to the housekeeping gene β-actin.

2.12 Statistical analysis

All statistical analyses were performed using the GraphPad Prism software (Graph-Pad Software Inc). All data are expressed as mean ± SEM. Statistical comparisons between two groups were performed using Student's t-tests; $P < 0.05$ was considered statistically significant.

3. Results

3.1 Protective effect of VAP on H_2O_2-induced SH-SY5Y cell injury

H_2O_2 was added to establish a neuron damage model and to assess the protective effect of pretreatment using different doses of VAP on damaged SH-SY5Y cells. The cell survival rate of SH-SY5Y cells was significantly lower in the model group when compared to the control group (83.58% ± 0.07%, $P < 0.05$), indicating the successful establishment of the cell injury model. The cell survival rates of the VAP1, VAP2, and VAP3 groups were significantly higher than the model group (all $P < 0.05$; Fig. 1A). Compared to the control group, the total apoptosis rate of the model group was significantly increased ($P < 0.05$); the total apoptosis rate of the VAP3 group was significantly decreased in comparison to the model group ($P < 0.05$, Fig. 1B).

The expression levels of Caspase-3 and Bax had also increased ($P < 0.05$), whereas the expression of Bcl-2 had reduced ($P < 0.05$). By contrast, VAP administration inhibited the expression of Caspase-3 and Bax ($P < 0.05$), and promoted the expression of Bcl-2 ($P < 0.05$, Fig. 1C-D). This indicated that H_2O_2-induced injury caused increased apoptosis in SH-SY5Y cells and that VAP rescued this apoptotic process.

3.2 Protective effects of VAP on hippocampal neurons in SCOP-injured mice

We observed the morphology of hippocampal neurons in each group of mice by HE staining, as well as the pre-protective effect of VAP on neurons. In the control group, the hippocampal regions of the mice comprised a large number of neurons with dense and neat arrangements, uniform distribution, intact structures, normal morphology, and distinct cell and nuclear membranes. In the model group, a decrease in the number of hippocampal neurons was observed, and cells exhibited a loose and disorderly arrangement of neurons, uneven staining, and distribution, and widened extracellular spaces, where the majority of cells exhibited abnormal morphology and showed obvious pyknosis. In comparison to the model group, the hippocampal neurons of the positive, VAP1, VAP2, and VAP3 groups exhibited denser and neater arrangements and normal cell morphology, with a small minority of cells exhibiting pyknosis (Fig. 2A).

TUNEL fluorescence staining revealed that compared to the control group, the numbers of apoptotic hippocampal neurons marked by green fluorescence were significantly increased in the model group. Compared to the model group, significant decreases in the numbers of apoptotic hippocampal neurons were observed in the positive, VAP1, VAP2, and VAP3 groups (Fig. 2B).

To further examine the effect of VAP on neuronal apoptosis, we detected the protein and gene products of Caspase-3, Bax, and Bcl-2 in mouse brain tissue. After SCOP injury, the expression of Bcl-2 decreased, while the expression of Caspase-3 and Bax increased at the gene and protein level ($P < 0.05$). By contrast, after pretreatment with VAP, the expression of Bcl-2 increased, while the expression of Caspase-3 and Bax decreased at the gene and protein level ($P < 0.05$); the ability of VAP to regulate apoptosis was better than that of memantine (Fig. 2C-E).

3.3 Influence of VAP on hormone levels related to the HPA axis

We detected the hormone regulation ability of VAP on the HPA axis release. ACTH and CORT concentrations increased in the brains of mice after SCOP injury ($P < 0.05$), whereas they significantly reduced after pretreatment with VAP and memantine ($P < 0.05$). The ability of 400 mg/kg VAP to regulate the two hormones was better than both 100 mg/kg and 200 mg/kg VAP (Fig. 3A and 3B).

3.4 Levels of proteins and mRNAs related to the HPA axis in the murine brain

CORT, secreted by the HPA axis, directly affects the expression of GR and MR on hippocampal neurons, thereby affecting the secretion of CRH on the HPA axis. The GR, MR, and CRH related proteins and genes in the brain tissue of mice were detected; results indicated that their expression increased in SCOP model mice ($P < 0.05$), and decreased in the brains of mice pretreated with memantine and VAP ($P < 0.05$) (Fig. 4). VAP may, therefore, regulate the expression of GR and MR in the hippocampus, whereas negative feedback regulates the conduction of the HPA axis and secretion of related hormones.

4. Discussion

With increasing age, aged brains become highly prone to neurodegenerative diseases (Wyss-Coray, 2016). This mainly causes both neuronal loss and death of the central nervous system, further resulting in cognitive dysfunction and bradykinesia (Abeliovich and Gitler, 2016; Chi et al., 2018). This severely affects the normal life and health of the elderly (Cho, 2010). Additionally, related drugs for the treatment of neurodegenerative diseases are not able to alter the disease progression, some with adverse reactions (Colović et al., 2013; Fredericks et al., 2018).

DAV is traditional Chinese medicine and tonic with a variety of pharmacological effects (Jiang et al., 2018; Zhang et al., 2020). It contains a variety of bioactive components, such as mineral elements, amino acids, peptides, and proteins (Sui et al., 2014; Wu et al., 2013). Modern pharmacological studies have indicated that VAPs derived from DAV promote the growth of nerve cells. VAPs are also known to play a vital role in regulatory and metabolic pathways of the immune system and antioxidant processes (Xiao et al., 2017; Zha et al., 2016; Zhang et al., 2017); however, in this paper, we explored the effect of VAPs on inhibiting neuronal damage through the HPA axis.

H_2O_2 induces neuronal damage and causes neurotoxicity, thereby inhibiting the activity of neurons (Lee et al., 2019). VAP inhibits H_2O_2-induced SH-SY5Y cell damage and improves the
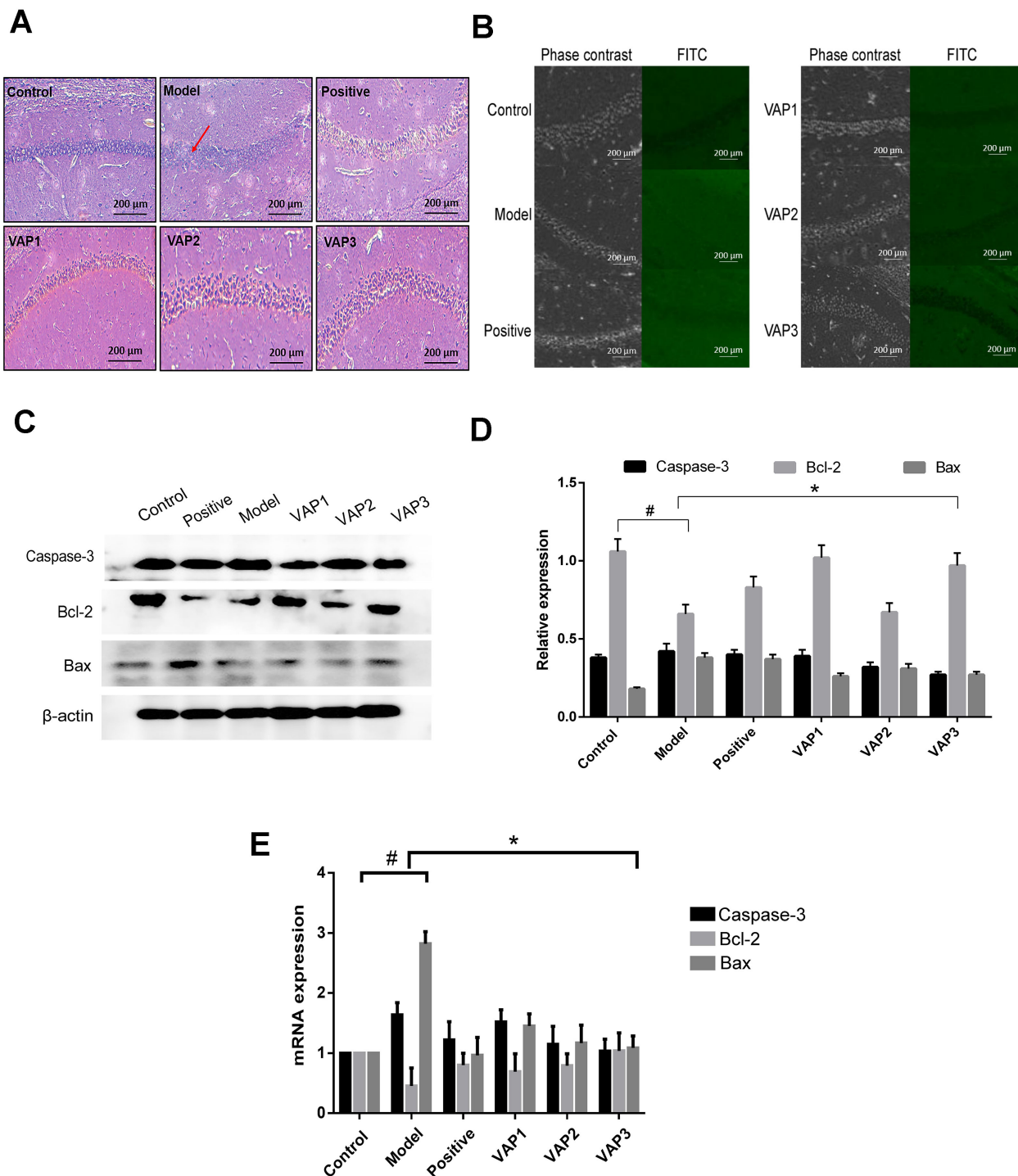
Fig. 2. Protective effects of VAP on hippocampal neurons in SCOP-injured mice. (A) Morphology of mouse hippocampal neurons. The red arrow points to the damaged neuron. (B) Effect of VAP on the apoptosis of hippocampal neurons in mice. (C) VAP regulates apoptosis proteins in the mouse brain. (D) Quantification of the relative expression of apoptosis proteins (n = 3). (E) Regulation of apoptosis genes in murine brain tissue by VAP administration (n = 8). All data were analyzed by Student's t-test and are presented as the mean $\pm$ SEM. $^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. model.

vitality of damaged SH-SY5Y cells. The intraperitoneal injection of SCOP to replicate cognitive impairment and neural damage is a commonly used and effective experimental model (Zhang et al., 2017). After intraperitoneal injection of SCOP, HE staining showed that the number of hippocampal neurons in the mouse brain was decreased. In contrast, the intercellular space was increased, suggesting SCOP-induced neuronal damage in these mice. VAP treatment effectively inhibited the decrease in the number of hippocampal neurons and reduced the degree of neuronal damage in the mouse brain. Thus, *in vitro* and *in vivo* experiments verified that VAP administration reduced neuronal damage and protected neurons.
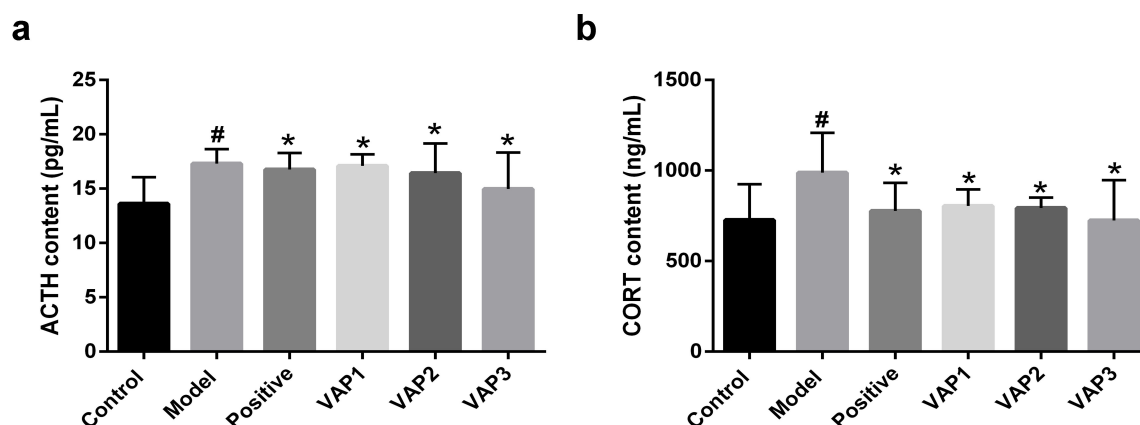
Fig. 3. Influence of VAP on hormone levels related to the HPA axis. (A) Regulation of the ACTH content in the mouse brain by VAP treatment (n = 8). (B) Effect of VAP on the CORT content in the mouse brain (n = 8). All data were analyzed by Student's t-test and are presented as the mean $\pm$ SEM. $^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. model.

Neuronal apoptosis is related to the progression of neurodegenerative diseases and affects the function and morphology of neurons (Mattson, 2000; Radi et al., 2014). Bcl-2, which has been shown to suppress cell death caused by various types of cytotoxins, is currently the primary target molecule in research regarding the molecular mechanisms of apoptosis (Ashkenazi et al., 2017). Thus, studying the regulation of Bcl-2 in the body has the potential to increase our understanding of these phenomena and improve our diagnostic standards and treatment of diseases related to apoptosis (Liu et al., 2019). Bax is a pivotal apoptosis-promoting gene of the Bcl-2 gene family, and the overexpression of Bax may lead to cell death (Chagtoo et al., 2018). Caspase-3, a protease, is typically regarded as the most important terminal cleavage enzyme in the process of apoptosis and is thought to play an indispensable role in apoptotic processes (Yu et al., 2017). Therefore, the combined investigation of Bcl-2, Bax, and Caspase-3 provides a better understanding of the apoptotic status of cells (Kaczanowski, 2016). In the present work, the administration of VAP upregulated the expression of Bcl-2. It downregulated the expression of Bax and Caspase-3, which indicates that VAP has the potential to suppress apoptosis, thereby delaying neuronal damage.

Neurodegeneration is closely related to an imbalance in the neuroendocrine-immuno-modulation network (Höschl and Hajek, 2001; Pedersen et al., 2001). As the regulation of the HPA axis plays a vital role in the function and morphology of hippocampal neurons (Kim and Diamond, 2002; Rothman and Mattson, 2010), the dysregulation of the HPA axis causes decreased neuronal activity, inducing neuronal damage and apoptosis (Joëls et al., 2004; Schloesser et al., 2009). Furthermore, abnormal hyperactivity of the HPA axis occurs when the hippocampal suppression of the HPA axis decreases in mice with dementia (ArunSundar et al., 2018). In the HPA axis, the hypothalamus synthesizes and secretes CRH. This promotes ACTH release by the pituitary gland (Huo et al., 2017; Ruan et al., 2019), thus stimulating the synthesis and secretion of glucocorticoids (Gjerstad et al., 2018), with cortisol (CORT) being the most representative glucocorticoid (Hill et al., 2015). The excessive secretion of glucocorticoids weakens the negative regulatory feedback of the pituitary gland and the hypothalamus, leading to the regulatory dysfunction of the HPA axis (Nichols et al., 2001). This series of regulatory dysfunctions ultimately results in uncontrolled feedback (Xu et al., 2018). The MR and GR are nuclear transcription factors expressed in the hippocampus that exert various biological effects upon activation (Dai et al., 2018; Keller-Wood, 2015). GR and MR exist in the hippocampal neurons and are the main receptors mediating glucocorticoid stress; they can also directly adjust the HPA axis via negative feedback. This affects the secretion of CRH in the hypothalamus of the HPA axis, thereby causing increased CORT secretion (Nguyen et al., 2017), and reduced hippocampal function. The results of the present study also indicate that the administration of VAP reduced the levels of CRH, ACTH, CORT, GR, and MR in mice. This suggests that VAP effectively maintains the steady-state equilibrium of the HPA axis, thereby ensuring the proper hippocampal regulation of the HPA axis. VAP can reduce ACTH and CORT contents by regulating the levels of hormones released by the HPA. These changes in hormones released by the HPA axis trigger dysfunction of the HPA axis itself, decrease GR, MR, and CRH expression, thus impairing the inhibition of the HPA axis by hippocampal neurons. This inhibits neuronal apoptosis and protects neurons.

5. Conclusions

Our results indicate that VAP may reduce neuronal damage and apoptosis of neurons *in vitro* and *in vivo*. Furthermore, VAP regulates the HPA axis to restore homeostatic functions, thus promoting the restoration of physiological, metabolic mechanisms in the brain and reducing neuronal apoptosis. Altogether, these beneficial effects contribute to the improvement of neurodegeneration and neuronal damage in mice. The results have laid the foundation for the development of VAP as an alternative medicine for the prevention and treatment of neurodegenerative diseases such as Alzheimer's disease.

Abbreviations

ACTH: Adrenocorticotropic hormone; Bax: Bcl-2 associated X protein; Bcl-2: B-cell lymphoma-2; Caspase-3: Cysteine-aspartic acid protease-3; CORT: Corticosterone; CRH:
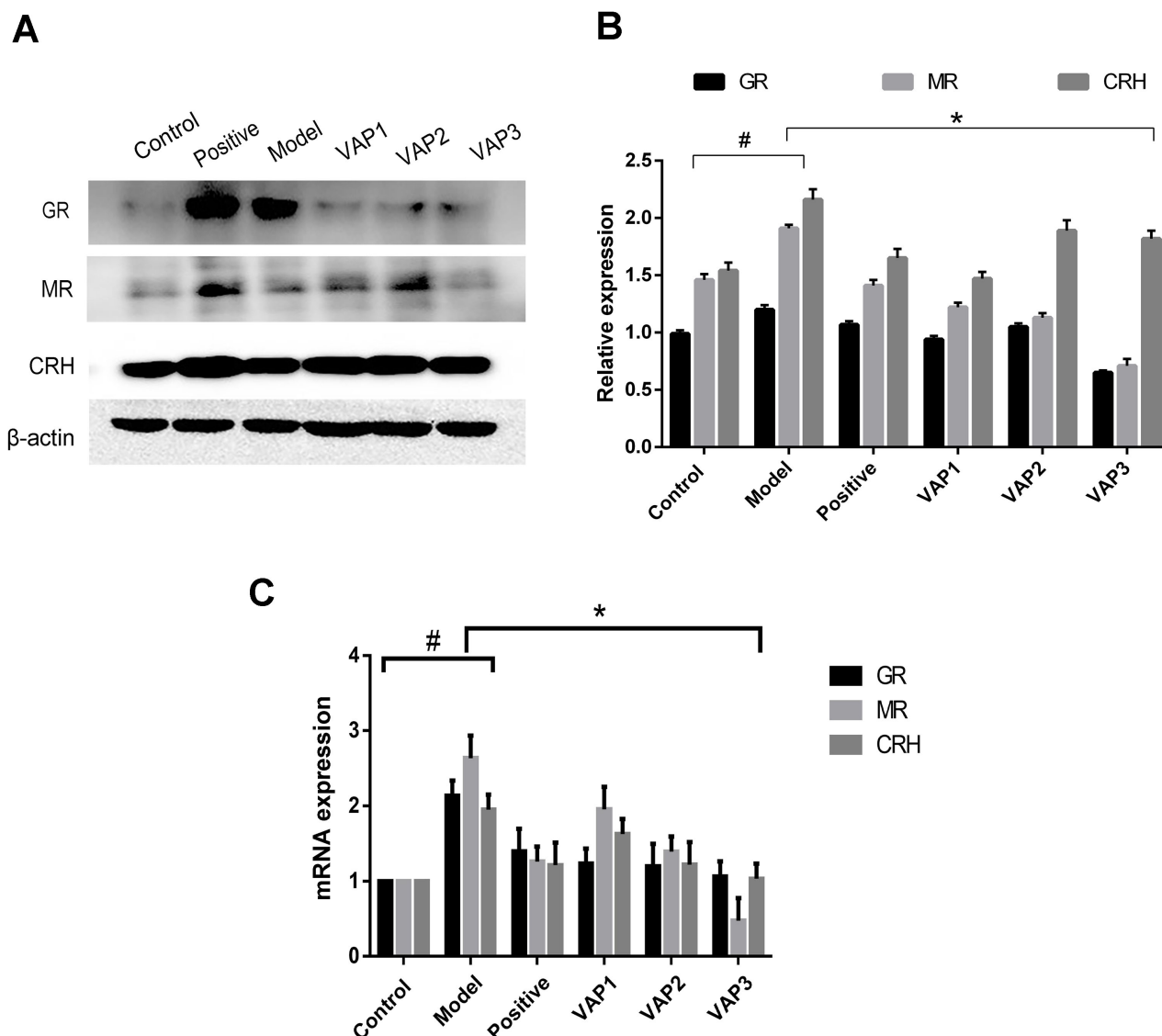
Fig. 4. Levels of proteins and mRNAs related to the HPA axis in the mouse brain. (A) VAP regulates the expression of HPA axis-related proteins in the mouse brain. (B) Quantification of the relative expression of HPA axis-related proteins (n = 3). (C) Effect of VAP on HPA axis-related genes in the mouse brain (n = 8). All data were analyzed by Student's t-test and are presented as the mean $\pm$ SEM. $^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. model.

Corticotropin-releasing hormone; DAV: Deer antler velvet; GR: Glucocorticoid receptor; HPA axis: Hypothalamic-pituitary-adrenal axis; MR: Mineralocorticoid receptor; VAP: Velvet antler polypeptide.

Author contributions

XXQ and NL conceived and designed the experiments; MK, JFW, XS, and JL performed the experiments; XRL collected the data; ZZ and STM analyzed the data; HL contributed reagents and materials; QY and JNL wrote the paper.

Ethics approval and consent to participate

The Laboratory Animal Ethics Committee approved all experimental procedures at the Changchun University of Chinese Medicine (20180121).

Conflict of Interest

The authors declare no conflict of interest.

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