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Research on the role of GLP-2 in the central nervous system EPK signal transduction pathway of mice with vascular dementia

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Abstract. – OBJECTIVE: To investigate the role of glucagon-like peptide-2 (GLP-2) in the central nervous system eukaryotic protein kinase (EPK) signal transduction pathway of mice with vascular dementia.

MATERIALS AND METHODS: We take the 3-week-old mice raised in the laboratory as the object of study in this research and then divide them into four groups in random, including sham operation group, control group, GLP-2 gr[illegible] and GLP-2+ERK (extracellular-signal-regu[illegible] kinase) inhibitor intervention group, with [illegible] each group. The step-down test, water-m[illegible] test, electron microscopy observation, immun[illegible] histochemical method, and Western-blotting a[illegible] adopted to investigate the role of [illegible]2 in the central nervous system EPK sig[illegible]uction pathway of mice with vascular [illegible]menti[illegible]

RESULTS: The step-down [illegible], as we[illegible]as the water-maze learning and [illegible]ry tes[illegible] that the mice injected with [illegible]in the [illegible] iment group have their [illegible]arnin[illegible] memory ability improved signi[illegible]ntly whe[illegible]pared with other three gro[illegible] which is gre[illegible]ffer-ent from that of ot[illegible]ee groups (p [illegible]05); the electron micr[illegible]p[illegible]ervation shows that the injection of [illegible]P-2 ca[illegible]ially reverse the reduction of [illegible]esicles while [illegible] inhibitor re-moves the [illegible] protection; t[illegible]unohisto-chemical r[illegible]t and image analys[illegible] result show that the E[illegible] expres[illegible]ion quantity in GLP-2 group has no [illegible]lificant[illegible]ference from that of other three [illegible] (p [illegible]05). Ho[illegible]ever, the content of *pi*-EPK i[illegible]-2 grou[illegible] significantly high-er than tha[illegible]D gro[illegible]nd GLP-2+PD98095 g[illegible]nd is [illegible]ca[illegible]y different from them [illegible] and su[illegible]ult is in line with the re-ult of [illegible]stern-blot[illegible]g.

CON[illegible]USIONS: GLP-2 can influence the chang[illegible]mpus cells extensions and fi-[illegible]y influe[illegible]ir cognitive function by activat-[illegible] EPK signal transduction pathway of hip-poc[illegible] neuron in the central nervous system.

Key W[illegible]
C[illegible], Vascular dementia, [illegible]ntral nervous system, EP[illegible]gnal transduction.

[illegible]roduction

Vascular dementia (Vascular Dementia, VD) [illegible] kind of disease produced based on a [illegible]riety [illegible]vascular diseases and resulting from [illegible]erebrovascular disease, and such disease is mainly featured by neurocognitive impairment in the clinic[1]. Tingting et al[2] show that the number [illegible]f patients with dementia has gradually increased [illegible] recent years due to all kinds of factors, among which the number of patients with vascular dementia also increases gradually[3], and the patients with vascular dementia have accounted for 48.5% of total patients with dementia in China as of 2014, and such percentage will increase gradually with the increasing population aging. Thus, all sectors of the society increasingly pay their attention to the research on the pathogenesis of vascular dementia and prevention of such disease at present[4]. Hu et al[5] found that the injury of the central cholinergic system, excessive release of excitatory amino acid, oxygen free radical and inflammatory mediators will lead to memory dysfunction in VD patients.

However, the mechanism remains unclear. The results of researches carried out by Setlow et al[6] show that the extracellular signal-regulated kinase (extracellular signal-regulated kinase, ERK) is considered to be in relation with learning and memory as an important member of mitogen-activated protein kinase family. Lv et al[7] demonstrated that

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decreased expression of ERK2 and p-ERK in the hippocampus of VD mice may be related to dysfunction of study and memory. Based on this, therapeutics, which increased ERK level, may have potential effects in treating VD. Previous evidence[8] showed that glucagon like peptide-2 (glucagon like peptide-2, GLP-2) not only plays a role in activating ERK signal pathway and stimulating intestinal multiplication, but also plays a certain function in improving learning and memory function. However, we still have no idea whether GLP-2 can activate ERK signal pathway of hippocampal neuron in the central nervous system, whether GLP-2 can influence synaptic changes in hippocampus cells and finally influence the cognitive function of mice with dementia. We investigated the role of GLP-2 in ERK signal transduction pathway in the central nervous system of mice with vascular dementia to provide a certain theoretical and experimental basis for the treatment of vascular dementia.

Materials and Methods

General Materials

The 3-week-old mice raised in our labor... are chosen by us as the object of study i... research, and we will build a model based... the said study object. The mice are random... divided into the sham operation group, contr... group, GLP-2 group, and GLP-2... inhibitor intervention group, with 30... ca... roup. The common carotid arteries... peated ...rebral ischemia-reperfusion metho... d back... ex-sanguination method are ado... prepa... model. The mice in the ...P-2 g... have been injected with 250 pg/... GLP-2 in t... in for 2 times a day for co... ...tive 30 days. ...ice in the control gro... ...en injected w...h an equal dose of n... ...al sa... the brain for 2 times a day. The ...ice in the ... inhibitor intervention grou... ...ave been inject... ...h 50 μmol ERK inhibi... in abdominal cavity ...0 min before the ische..., at the ...me time, the mice in this latter ... have ...en injected with 250 pg/kg GLP-2 i... ... for 2 ti... a day.

Praxi... Test

The s... test and water-maze test have ... carried out on the survival mice, respectively ... 29th and 30th day after the treatment of each group. In this way, it can test the ... and memory scores of mice.

Step-Down Test

The step-down test referred t... ...is research has been carried out according to the ... ...iments conducted by Velazquez et ... and a litt... ...ge shall be applied to it.

Water-Maze Test

The water-maze tes... ...ed ... in this r... ...arch has been carried ... accor... the ex... ...iments conducted by T... Christens... [10] ...d a little change shall l... ...plied to it.

Electron... cros... Observation

The me...ods abou... ...istribution and expression ... RK2 and p-ER... ...tein in hippocampus ...3 area of mice have ...been carried out according to the experiment conducted by Lovshin e..., and a little change shall be applied to it.

Im... ...nohistoch... ...ical Test

I... ...the streptomycin and peroxidase (S... ...od is adopted by us to carry out ...onventional antibody incubation and staining ... samples in hippocampus CA3 area ...me... ...e standard for the judgment of immunohistochemistry is as below: the immunohistochemistry is judged to be negative when capsule staining <10% or shows negative after Giemsa staining; the immunohistochemistry is judged to be positive when the only cell membrane is stained or it is >10% and the Giemsa staining can be observed.

Western-Blotting Test

In this research, we use Roche's animal cells protein to extract kit and total protein in samples (see the specification for the specific operation) and make a little change to it. Then, we carry out operation according to the product specification provided by the Roche Company. The antibody dilution has been carried out according to the specification, and its final dilution ratio is 1:5000; besides, relevant operations shall also be carried out according to *Molecular Cloning:* A Laboratory Manual - 3rd ed. Vols 1,2 and 3. Cold Spring Harbor Laboratory Press. 2001 (JF Sambrook and DW Russel, ed.).

Statistical Analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. The results have

been analyzed by the computer pictures, and relevant data is expressed by mean ± standard deviation. The comparison between groups was done using one-way ANOVA test followed by post hoc test (LSD). $p < 0.05$ shows that the results have statistical significance.

Results

Results of Step-Down Test and Water-Maze Test

From the water-maze test of mice (Figure 1A), we may see that the escape latency time of mice injected with GLP-2 in experiment group is shorter than that of miceF in VD group and GLP-2+PD98095 group, and the experiment group is significantly different from the VD group and GLP-2+PD98095 group ($p < 0.05$), which shows that the injection of GLP-2 can improve the memory ability of mice to some extent; in contrast, from the step-down test of mice (Figure 1B), we may see that the learning ability of mice injected with GLP-2 is higher than that of mice in VD group and GLP-2+PD98095 group. The research results show that the injection of G can improve the memory ability, learning a y, and other cognitive ability of mice in VD g to some extent.

Electron Microscopy Observa f Nerve Cell Extensions in Hi us CA3 Area of Mice

Results of nerve cell exte s in hi pus CA3 area of mice by tra sion e microscope revealed that the number synaptic vesicles reduces significantl er mic model (the synaptic vesicles are insi e red circle). In addition, the number of m ondria also reduces significantly (the yello w shows the mitochondria). We also found th reased neuron synaptic vesicles ca partiall sed by injection with GLP-2. H ever, ERK in could significantly red the sy tic vesic These results showed t GLP- ould increase the synaptic vesicles i ip campus ome extent and impro e ex ty of th rvous system of mice w D group.

Immunoh mical Results

Immun stoc l staining of ERK (Figure 3A) an pi-ERK (3B) in hippocampus CA3 suggested tha expression in the GL test group was not ignificantly different fr other groups (sham, control, and GLP-2 98095) ($p > 0.05$). However, p-ERK in the G 2 test group s significantly increased co ed with rol and GLP-2+PD98095 grou l above results demonstrated that GL d activate ERK signal pathway f hippocampal neuron in the central nervous

Expression Level of ERK2 and p-ERK Protein in Hippocampus CA3 Area of Mice

Western-Blot found that ERK in the GLP-2 test group was not significantly different om other groups (sham, control, and GLP-2+PD98095) ($p > 0.05$) (Figure 4A). However, p-ERK level in the GLP-2 test group

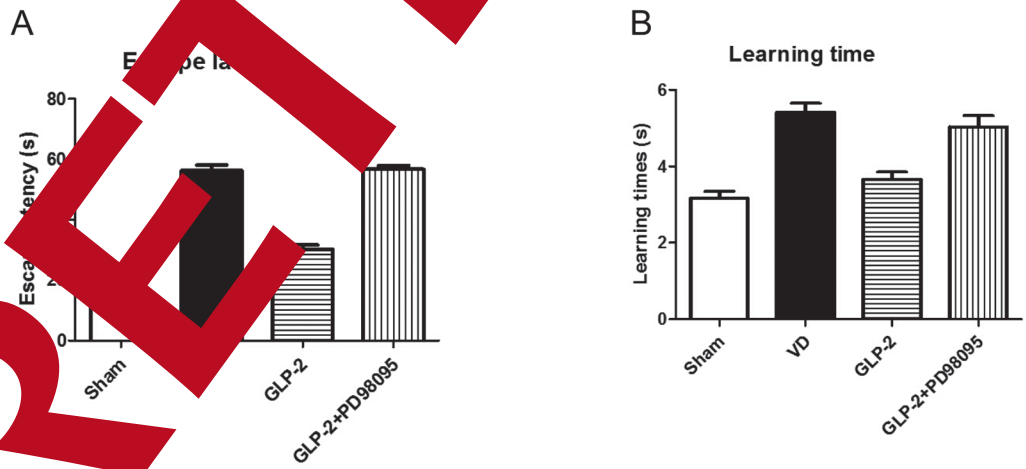


Figure 1. Results of water-maze test *(A)* and Step-down test *(B)* of mice.

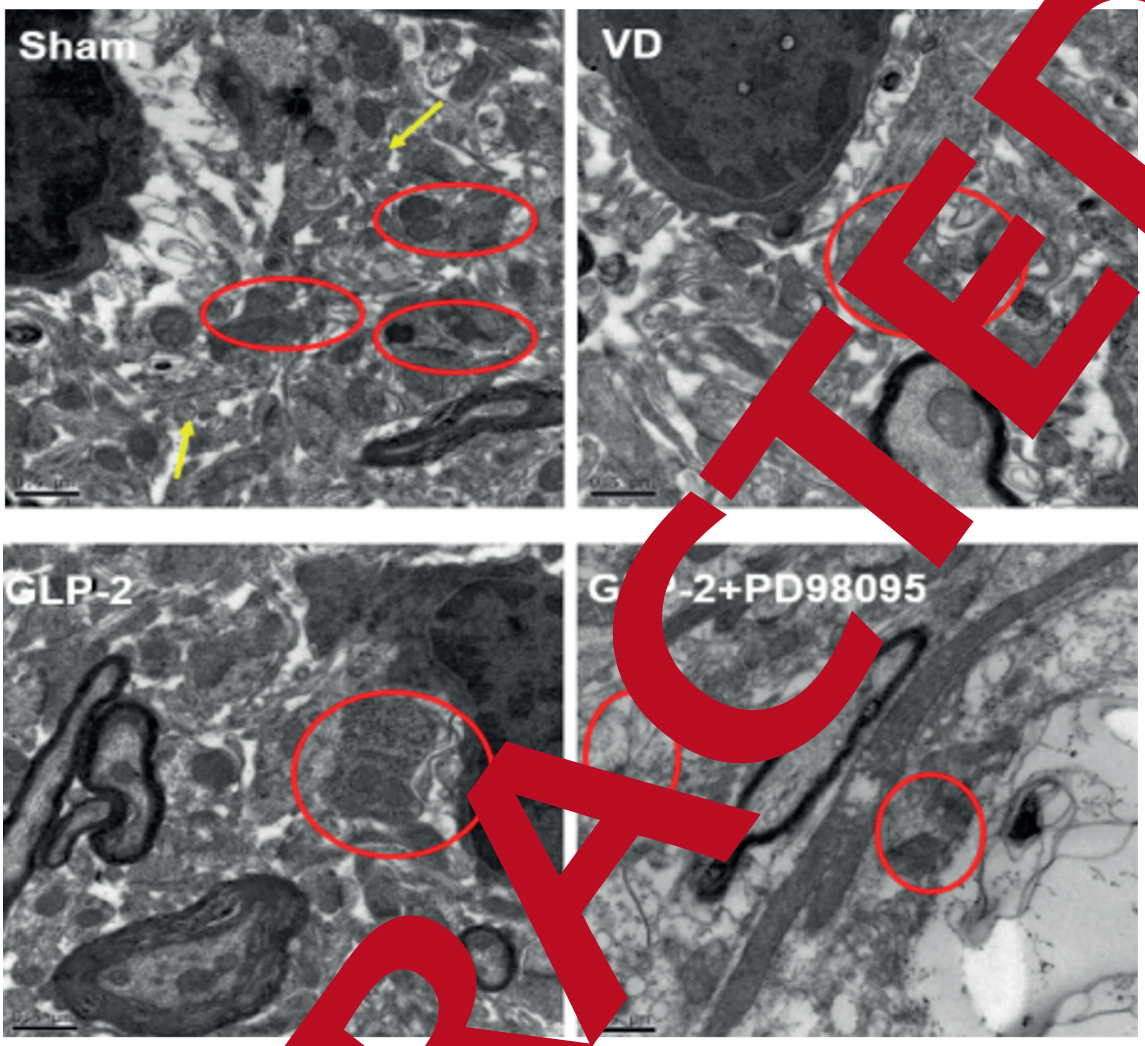
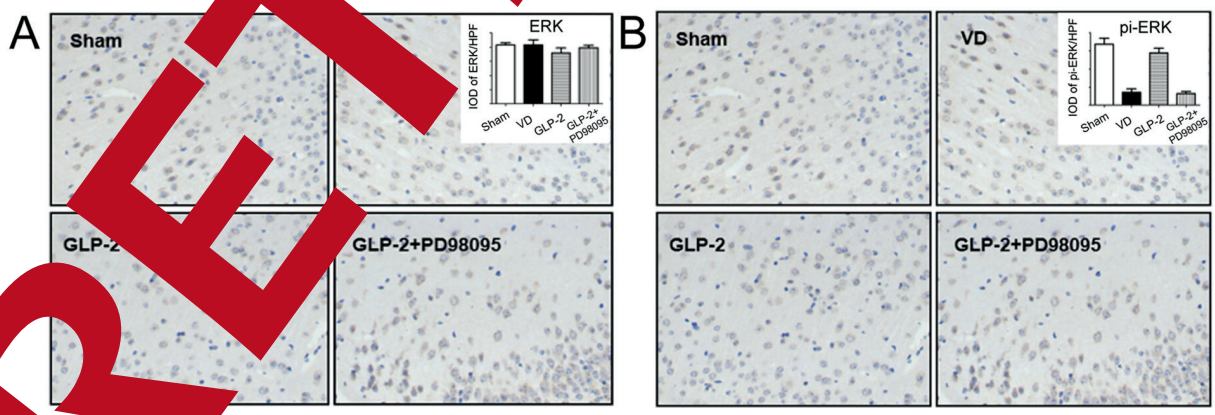


Figure 2. Electron microscopy and ructural n of nerve cell extensions in hippocampus CA3 area of mice.



3. Immunohistochemical results of neuron in hippocampus CA3 area of mice. ***A*,** Results of ERK protein immunohist al and positive position; ***B*,** *pi*-ERK Protein immunohistochemical results.

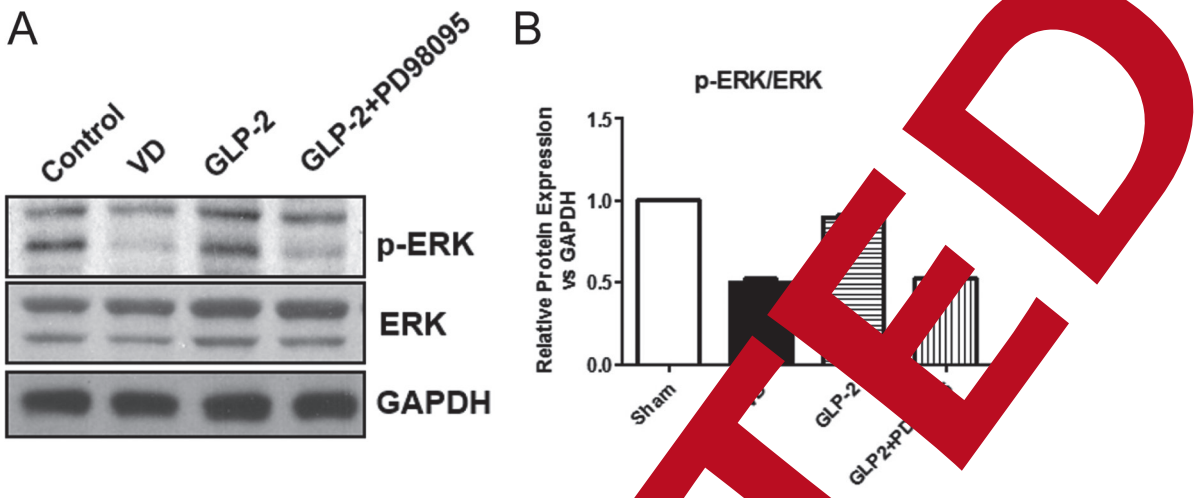


Figure 4. Western-blotting results of ERK2 and *p*-ERK protein expression in hippocampus of mice.

was significantly higher than that of VD and GLP-2+PD98095 group ($p < 0.05$). Also, the quantitative analysis found that *pi*-ERK/ERK level in the GLP-2 treatment group was significantly higher than VD and GLP-2+PD98095 group ($p < 0.05$) (Figure 4B). These results showed that GLP-2 could activate ERK signal pathway of hippocampal neuron in the central nervous system.

Discussion

Previous researches[9-13] have shown that GLP-2 protein belonging to the family of proglucagon-derived peptides is one of gene expression products of proglucagon (PG). It is a kind of single-chain polypeptide composed of 33 amino acid residues, and its acid sequence ranks the 126th-158th place in PG with about 3900 Da molecular weight. The amino acid sequence of human beings and mammals is highly conservative. In the field of medicine, it is extensively applied in the treatment experiments for repairing intestinal injury caused for a variety of reasons because it has special function to promote the growth of intestinal mucosa and after-damage repair[14]. Yusta et al[15] showed that the receptor of GLP-2 (GLP-2R) is widely distributed, and it is distributed not only in the digestive system but also in cerebral cortex, cerebellum, hypothalamus, amygdaloid nucleus and other central nervous systems according to Wu et al[16] and Yusha et al[17] through immunohistochemistry, hybridization *in situ*, RT-PCR, Western blot and Southern blotting analysis. However, the reports on the researches to investigate the role of GLP-2 in the nervous system are very few. Jung et al showed that as an important protein in signal pathway of the body in relation to learning and memory, ERK protein plays an important role in improving vascular dementia[19]. Erkinjuntti et al[20] observed that the substrates of ERK in animal cells contain other important kinase, transcription factors, histone and K+pathway in cells. Also, ERK may participate in the formation process of the morphologic plasticity of neurons. *p*-ERK can lead to a morphologic change in dendrites of hippocampus cells and promote the growth of new filopodia on dendrite handle and spine. It also participates in the formation of the morphologic plasticity of neurons, which was helpful for the transmission and reception of information[21]. However, whether there is an interaction between *p*-ERK and GLP-2 protein still remains unknown. Through the step-down test and water-maze test of mice in the present study, we found that the learning and memory of the mice in GLP-2 test group improved significantly compared with VD group. These results showed that injection of GLP-2 could improve the learning and memory ability of mice to a large extent. Afterwards, through the immunohistochemistry, electron microscope observation, Western-blotting and other tests, we find that the wiring hippocampal neuron extensions in the group treated with GLP-2 increase significantly as well as the content of

activated ERK protein (*pi*-ERK protein), which shows that GLP-2 can activate ERK signal pathway to some extent.

Conclusions

We showed that ERK signal pathway is closely related to the learning ability, memory ability, and other cognitive ability and also plays a certain role for the treatment of vascular dementia, which shows that GLP-2 can cure vascular dementia by activating ERK signal transduction pathway in the central nervous system. However, there is still no research on the interactive mechanism and action mode between GLP-2 and ERK protein, and the said interactive mechanism and action mode will be the focus of research in the future.

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Conflict of interest

The authors declare no conflicts of interest.

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