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Original Article

Ginsenoside Rd improves behavioral impairment of rats with acute plateau status by modulating synaptic plasticity

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ABSTRACT

Objective: To investigate the protective effect of ginsenoside Rd on the improvement of the behavior and synaptic plasticity in rats with acute plateau status.
Methods: A total of 60 Wistar rats were randomly divided into the control group, the model group, and the intervention group, with 20 rats in each group. The model was established in low-pressure oxygen chamber simulating the plateau, and the intervention group was administered with ginsenoside. Electron microscope was used to observe synaptic ultrastructure of hippocampal CA1 area, and analyze the structural parameters on the Gray I synaptic interface. Morris water maze and Y electric maze experiment were used for behavioral detection.
Results: Compared with the control group, the number of electrical stimulation required for rat to avoid was increased in the model group, the latency in the Morris water maze was prolonged, the swimming distance was increased, and the frequency of crossing the platform was decreased. Under the electron microscope, the synaptic cleft was increased, the length of the synaptic active area was shorter, the postsynaptic density (PSD) was thinner, the flat synapse was increased, and the concave and perforated types were significantly reduced. Compared with the model group, the number of electrical stimulation required for rat to avoid was decreased in the intervention group, the latency in the Morris water maze was shortened, the swimming distance was decreased, and the frequency of crossing the platform was increased. Under the electron microscope, the synaptic cleft was decreased, PSD was thicker, the flat synapse was decreased, and the concave and perforated types were increased.
Conclusion: Low pressure and low oxygen environment of plateau damages the plasticity changes of the synaptic structure and function. And to a certain extent, ginsenoside Rd reverses Gray I synaptic interface structure parameters, so as to improve the behavior performance of model rats at high altitude condition.

1. Introduction

With the economic development and increasing security affairs in the surrounding areas of Qinghai-Tibet region, the number of people entering the high-altitude area has increased dramatically. Brain dysfunction under high-altitude condition not only has a great influence on physical activity of people, but also causes cognitive function impairments such as memory, thinking judgment, attention, and computing problems, affecting the work and living ability of workers on plateaus; But the specific molecular mechanism is unclear, and short of effective interventions (Neumayr, 2013; Ren et al., 2010). Ginseng, the root of *Panax ginseng* C. A. MEYER (Araliaceae), is reputedly known for its nootropic and anti-aging functions and has been widely used to treat various diseases and enhance health for thousands of years in Asia. Recent studies revealed that ginsenoside Rd, responsible for the pharmacological effects of ginseng, can prevent memory loss and improve spatial learning in mice and show protective effects on cardiovascular, cerebrovascular, nervous, and immune systems (Zhou & Zhou, 2009), but underlying mechanisms are still largely unknown. The hippocampus is an important part of the limbic system and plays an important role in learning and memory (Wang & Cui, 2018). One of the pathogenesis of learning and memory impairment is related to the plastic of synapse (Lisman, Cooper, Sehgal, & Silva, 2018). The plateau environment of 3500 meters was simulated in this study, rats were intervened with ginsenoside Rd, and the influences of ginsenoside Rd on rats' learning and

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memory function and hippocampal synaptic plasticity under the plateau were observed to further reveal the pathogenesis of brain cognitive impairment under the plateau.

2. Materials and methods

2.1. Animal grouping and model establishment

A total of 60 healthy adult Wistar rats (clean grade, half male and half female), weighting 230−250 g, were provided by the animal experimental center of Medical College of Qinghai University, and randomly divided into the control group, model group, and intervention group (model + ginsenoside Rd group), with 20 rats in each group. Experimental rats of the model group and the intervention group were implanted into the hypobaric oxygen cabin to simulate plateau environment of an altitude of 3500 m for 3 d.

2.2. Main reagents and drug intervention

Ginsenoside Rd (purity of 99.8%) was purchased from Guangdong Taihe Bio-pharmaceutical Co., Ltd. Rats of the blank control group and the model group were given lavage of physiological saline, while the intervention group was given lavage of ginsenoside Rd suspension (2 mg/kg), once daily.

2.3. Ethological detection

2.3.1. Y-electric maze experiment

The frequency of electric shocks indicated the ability of learning and memorizing, and the times of each rat learning to avoid the electrical stimulation was recorded, with 30 times as the maximum.

2.3.2. Morris water maze positioning navigation test (PNT)

Rats were put into the water for freely swimming for 2 min one day before the experiment; After the start of the formal experiment, the procedures were repeated for a certain time, and the platform quadrant was not selected as the entry point, where the rats were placed into the water facing the pool wall in a clockwise direction, and the latency time from when the rats entering into the water to when the rats climbing up the platform, the swimming distance and trajectory were observed and recorded.

2.3.3. Morris water maze space exploration test (SPT)

The platform was removed after the last PNT for rats of each group, then the rats were put into the water facing the pool wall from the entry point of the last time to continuously swim in the water maze, and the times of rats penetrating through the original platform within 120 s.

2.4. Ultrastructural detection

The hippocampus was separated, washed by the phosphate buffer solution (PBS) twice, fixed with 4% glutaraldehyde and sectioned after cooling; then it was fixed with 2% osmic acid, gradiently dehydrated with acetone, soaked, embedded, double stained with uranyl acetate–citric acid, and observed by transmission electron microscope. Three copper networks were observed in each case, and five photos were taken randomly on each copper wire mesh, which finally magnified for 40 000 times.

2.5. Observation and quantitative analysis of synaptic interface structure parameters in hippocampal CA1 area

A total of 30 pieces (five pieces each) were randomly selected from each group under the electron microscope for measurement and statistics. Image analyzer was used to measure the width of synaptic cleft, thickness of postsynaptic density (PSD), chord length and arc length of the postsynaptic membrane and the length of the synaptic activity. The length of active area and the thickness of PSD were measured according to the methods of Güdner (Güdner & Ingham, 1980). The measurement of the curvature of synaptic interface referred to the methods of Jones (Jones & Devon, 1978). The width of synaptic cleft was determined by multipoint average method. The percentage of concave, flat, convex, and perforated synapses was calculated, and the observation and measurement of this experiment were double-blind.

2.6. Statistical treatment

SPSS 12.0 software was used for statistical analysis. The measurement data was represented with mean ± SD. *t*-test was used for comparison between the two groups, and multiple groups of independent samples were compared by one-way ANOVA. $P < 0.05$ referred for the statistically significant differences.

3. Results

3.1. Y-electric maze experiment

Compared with the control group, the number of times to avoid electrical stimulation was significantly increased in the model group ($t = 12.0134$, $P < 0.01$) and the intervention group ($t = 2.0943$, $P < 0.05$). However, compared with the model group, the number of times to avoid electrical stimulation was significantly decreased in the intervention group ($t = 11.2633$, $P < 0.01$) (Table 1).

Table 1
Effect of ginsenoside Rd on learning and memory in Y-maze test.

Groups	Before modeling	3 d after intervention
Control	15.87 ± 2.12	15.77 ± 2.09
Model	16.02 ± 3.03	26.15 ± 2.17*
Intervention	15.91 ± 2.14	20.21 ± 1.98*△

*$P < 0.05$ *vs* control group.
△$P < 0.05$ *vs* model group, same as below.

3.2. Morris water maze detection

Compared with the control group, both rats' latency time ($t = 13.4628$, $P < 0.01$) and swimming distance ($t = 5.0890$, $P < 0.01$) in Morris water maze were increased in the model group, while the number of times to across the platform was significantly decreased ($t = 15.3765$, $P < 0.01$); Compared with the model group, both rats' latency time ($t = 5.4012$, $P < 0.01$) and swimming distance ($t = 2.0374$, $P < 0.05$) in Morris water maze were decreased in the intervention group, while the number of times to across the platform was significantly increased ($t = 4.9252$, $P < 0.01$) (Tables 2 and 3).

Table 2
Comparison on rats' behaviors in water maze (mean ± SD).

Groups	Cases/*n*	Escape latencies/ms	Swimming speed/(ms^{-1})
Control	20	36 411.31 ± 12 580.34	28.13 ± 1.29
Model	20	73 619.62 ± 13 089.43*	16.42 ± 4.09*
Intervention	20	50 891.72 ± 22 164.57*△	22.06 ± 4.15*△

Table 3
Comparison on rats' behaviors in water maze (mean ± SD).

Groups	Cases/*n*	Latency test/s	Distance test/m	Platform cross test/times
Control	20	16.14 ± 1.34	3.89 ± 1.14	16.03 ± 3.04
Model	20	34.06 ± 3.81*	9.03 ± 2.51*	3.01 ± 1.06*
Intervention	20	26.06 ± 2.97*△	6.38 ± 2.14*△	5.48 ± 1.12*△

3.3. Observation of synaptic ultrastructure and interface structure parameters in hippocampal CA1 area

Control group: The structure of the tissue is clear and complete, presynaptic mitochondrial structure was normal, the crest was complete without swelling, and Gray I type synapsis had typical asymmetric interface. There were thick dense materials in the postsynaptic membrane, which was obviously larger than the presynaptic membrane. The synaptic cleft was small, and there were concave, convex, flat, and perforated synapses, given priority to the concave synapsis (Tables 4 and 5).

Model group: Compared with the control group, presynaptic mitochondria cristae were fuzzy, the width of synaptic cleft was significantly increased, the length of synaptic active area was shorter, the postsynaptic density (PSD) was thinner, the flat synapsis was significantly increased, while concave and perforated synapses were significantly reduced (Tables 4 and 5).

Intervention group: Compared with the model group, presynaptic mitochondria cristae were fuzzy, the width of synaptic cleft was smaller, and the PSD was increased; Flat synapsis was decreased, while concave and perforated synapses were significantly increased. Compared with the control group, the width of synaptic cleft was slightly larger, and the PSD was slightly thinner in the postsynaptic membrane; the flat synapsis was significantly increased, while concave and perforated synapses were significantly reduced (Tables 4 and 5).

4. Discussion and conclusion

Currently, it is believed that the neurotoxicity of hypobaric hypoxia is the key factor for acute plateau status. The synaptic plasticity damage of neurons is considered to be one of the pathogenesis of learning and memory impairment caused by hypobaric hypoxia (Kedzierewicz & Cabane, 2013; Gonggalanzi et al., 2016). Therefore, in the experiment, the model was established in low-pressure oxygen chamber simulating the plateau to observe the change of synaptic plasticity and the intervention effect of ginsenoside Rd1 in rats.

The expression of synapsis-related protein appears in the early stage of hypobaric hypoxia, which was the most serious in molecular layer of hippocampal dentate, while in a lesser degree in other area of the hippocampus and neocortex area, leading to the abnormal synaptic structure and transfer (Suvrathan, Payne, & Raymond, 2018). Studies have shown that hypobaric hypoxia is an early inducing factor of synaptic transmission abnormality and synaptic injury in the hippocampus ring (Berberich, Pohle, Pollard, Barroso-Flores, & Köhr, 2017). As a result, synaptic transmission abnormality and synaptic injury due to hypobaric hypoxia is one of the main causes of progressive decline in learning and memory ability of acute plateau status. The process of learning and memory requires plasticity of synaptic connections on the structure and function. The plasticity of structure mainly includes the morphology of presynaptic terminal, the curvature of synaptic interface and changes of PSD; Functional plasticity includes the synthesis and release of neurotransmitter, and phosphorylation and dephosphorylation of second messenger, G protein, and regulatory proteins, etc. after the activation of receptors (Zovkic, Guzman-Karlsson, & Sweatt, 2013). The plasticity of synapses plays an important role in the formation of learning and memory processes (Gafarov, 2018).

In the behavior experiment of this study, Y-electric maze mainly reviewed the learning ability of mice; Morris water maze PNT experiment mainly investigated the spatial resolution of mice, while SPT mainly measured working memory ability in mice. According to the experiment results, the learning ability, spatial orientation, and working memory of mice in model group were obviously decreased, characterized by the increased number of times to avoid electrical stimulation in Y-electric maze, the prolonged latency of Morris water maze test, the increased swimming distance and less number of times to across the platform, showing that hypobaric hypoxia damaged the learning memory, spatial memory, and working memory of mice.

Table 4
Parameters for synaptic interface structure in CA1 region (mean ± SD).

Groups	Synaptic number	Length of active zone /nm	Curvature of synaptic interface /D	Width of synaptic cleft /nm	Thickness of PSD /nm
Control	98	367.19 ± 14.08	1.3073 ± 0.0506	11.67 ± 1.14	90.32 ± 6.13
Model	98	297.05 ± 6.51*	1.0274 ± 0.0213*	22.81 ± 1.87*	46.07 ± 4.87*
Intervention	98	314.27 ± 7.89*△	1.1870 ± 0.0204*△	15.77 ± 2.03*△	71.26 ± 4.19*△

Table 5
Average percentage of three types of synapse in CA1 region (mean ± SD).

Group synaptic number	Concave /%	Flat /%	Convex	Perforated
Control ($n = 96$)	76.4 ± 3.04	18.5 ± 0.76	4.96 ± 0.21	6.43 ± 0.14
Model ($n = 94$)	60.3 ± 2.35*	37.1 ± 1.32*	2.55 ± 0.13*	2.13 ± 0.09*
Intervention ($n = 99$)	69.6 ± 2.63*△	26.7 ± 0.85*△	3.62 ± 0.17*△	4.57 ± 0.11*△

Synaptic ultrastructure results showed that compared with the control group, presynaptic mitochondria patchy cristae in hippocampal CA1 area were fuzzy, PSD was thinner, the width of synaptic cleft was significantly increased, the length of synaptic active area was shorter, and the synaptic interface curvature was significantly decreased in the model group. The PSD region is known to contain a variety of protein, including microtubulin, actin, neurofilament proteins, NMDA receptors, and so on. When the synaptic function and activity changes, the phosphorylation of some enzymes and their substrates (such as NMDA receptor, and Src etc.) cause the changes of their molecular conformation, which are characterized by the increase or decrease of dense zone (Cohen, 1977). In case of thicker PSD and smaller synaptic cleft, the nerve impulse conduction easily passes; While in case of wider synaptic cleft and thinner PSD, the nerve impulse conduction is not easy to pass. This is consistent with the significant thinning of PSD in the hippocampal CA1 area and the widening of the synaptic cleft in the experimental model. Jones and Devon observed and demonstrated that the curvature of synaptic interface was related to the different functional states of synapses under different conditions (Jones & Devon, 1978). The increased curvature of the synaptic interface indicated that the synapse transmission function is better, whereas the curvature of the synaptic interface is decreased, indicating that its synaptic transmission function is poor, which is not conducive to the formation of Long-term potentiation (LTP). Transgenic mice, therefore, likely because of the significantly thinner PSD, significantly wider synaptic cleft, significantly shorter synaptic active area and decreased curvature of the interface of synaptic transmission caused by the toxic effect of hypobaric hypoxia, the nerve impulse is not prone to transduction and the formation of the inhibited LTP, affecting the requirement of learning memory. In addition, the proportional changes of the flat and concave synapsis among three types of synapses are also important factors that affect synaptic transmission efficiency; the released neurotransmitters reach the target and improve the effectiveness of the neurotransmitter information transfer because the concave synaptic interface is concentrated. Synaptic perforation also plays an important role in synaptic plasticity (Jones & Calvery, 1991; Zhang & Hu, 1999). It is believed that synaptic perforation can increase the contact area of PSD and the neurotransmitters and enhance the transmission efficiency of synapses. The experiment results showed that compared with the control group, the flat synapsis was increased while the concave type synapses and perforated synapse were decreased in the hippocampal CA1 area of mice in the model group ($P < 0.05$), and affected the synaptic transmission efficiency, severely affected and blocked the neurotransmitter information transfer; As a result, hypobaric hypoxia model mice have obvious obstacles in learning and memory.

By Rd1 intervention, the latency and swimming distance were decreased while the number of times to across the platform was increased. The presynaptic mitochondrial patchy crest of the hippocampal CA1 area was clearer, the PSD was significantly thickened, the synaptic cleft was significantly narrowed, the synaptic activity area was significantly increased, and the curvature of synaptic interface was enhanced; The flat synapsis was increased, and the distribution of concavity synapses and perforated synapses was increased. It is indicated that Rd1 can improve the learning memory, spatial memory and working memory capacity damage caused by hypobaric hypoxia, with the function of brain protection (Li et al., 2016; Liu et al., 2015; Nabavi, Sureda, Habtemariam, & Nabavi, 2015). It suggests that Rd1 can reverse the distribution of structural parameters such as concavo synapses and perforated synapses, and further improve the effectiveness of neurotransmitter transmission, thereby significantly improving the learning and memory impairment caused by hypobaric hypoxia.

In conclusion, plateau low pressure and low oxygen environment damages learning disabilities, and the pathogenesis involve the plasticity changes in the structure and function of the synapsis. To a certain extent, ginsenoside Rd reverses Gray I synaptic interface structure parameters to improve the behavior performance of model rats at high altitude condition. Therefore, it has the prospect of brain protection and potential for cognition impairment of high altitude stress.

Conflict of interest

The authors declare no conflict of interest.

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