Effect of propofol on brain-derived neurotrophic factor and tyrosine kinase receptor B in the hippocampus of aged rats with chronic cerebral ischemia

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Abstract
We intraperitoneally injected 10 and 50 mg/kg of propofol for 7 consecutive days to treat a rat model of chronic cerebral ischemia. A low-dose of propofol promoted the expression of brain-derived neurotrophic factor, tyrosine kinase receptor B, phosphorylated cAMP response element binding protein, and cAMP in the hippocampus of aged rats with chronic cerebral ischemia, but a high-dose of propofol inhibited their expression. Results indicated that the protective effect of propofol against cerebral ischemia in aged rats is related to changes in the expression of brain-derived neurotrophic factor and tyrosine kinase receptor B in the hippocampus, and that the cAMP-cAMP responsive element binding protein pathway is involved in the regulatory effect of propofol on brain-derived neurotrophic factor expression.

Key Words
propofol; chronic cerebral ischemia; aged; brain-derived neurotrophic factor; tyrosine kinase receptor B; cAMP-cAMP responsive element binding protein; neural regeneration

INTRODUCTION
Propofol is a short-acting, intravenously administered anesthetic agent, and has been extensively used in the induction and maintenance of clinical anesthesia, and sedation for patients in severe pain[1-2]. Li et al[3] injected propofol in rats with cerebral ischemia/reperfusion injury and found that hippocampal caspase-3 expression was decreased, Bcl-2 expression increased, and nerve cell apoptosis reduced. A previous study indicated that during anesthesia, propofol may influence the function of brain regions, in particular the hippocampus, through neurotransmitter release and receptor function changes[4]. Brain-derived neurotrophic factor is induced at early stages of cerebral ischemia and binds to its specific high-affinity receptor tyrosine kinase receptor B to inhibit lipid peroxidation, stabilize membrane structure, and maintain blood-brain barrier function, thereby protecting the brain[5-7]. cAMP response element binding protein (CREB) can regulate brain-derived neurotrophic factor expression, and phosphorylate CREB.
tyrosine kinase receptor B expression in the hippocampus of aged rats were included in the final analysis.

RESULTS

Quantitative analysis of experimental animals
A total of 20 aged Sprague-Dawley rats were randomly assigned to sham-surgery, model, and low- and high-dose propofol groups (n = 4 for each). The rat model of chronic cerebral ischemia was established in the model, low- and high-dose propofol groups; the sham-surgery group was free of occlusion of the common carotid artery. Following model establishment, the sham-surgery and model groups were intraperitoneally injected with 2.5 mL normal saline, while the low- and high-dose propofol groups were intraperitoneally injected with 10 and 50 mg/kg propofol, respectively. All 20 rats were included in the final analysis.

Propofol influenced brain-derived neurotrophic factor, tyrosine kinase receptor B and pCREB expression in the hippocampus of aged rats (Figures 1–3)

Western blot revealed that hippocampal brain-derived neurotrophic factor, tyrosine kinase receptor B and pCREB expression was significantly increased in the model group when compared with the sham-surgery group (P < 0.05 or P < 0.01); hippocampal brain-derived neurotrophic factor, tyrosine kinase receptor B and pCREB expression was significantly increased in the low-dose propofol group compared with the model group (P < 0.05), but brain-derived neurotrophic factor expression was significantly reduced in the hippocampus of the high-dose propofol group (P < 0.05; Figures 1–3).
Propofol influenced cAMP expression in the hippocampus of aged rats

Radioimmunoassay showed that hippocampal cAMP expression was significantly increased in the model group when compared with the sham-surgery group ($P < 0.01$); while compared with the model group, hippocampal cAMP expression was significantly increased in the low-dose propofol group ($P < 0.05$), but significantly decreased in the high-dose propofol group ($P < 0.05$; Figure 4).

**DISCUSSION**

Recent evidence indicates that cAMP-CREB pathway-mediated brain-derived neurotrophic factor expression changes may be involved in anti-noxious stimulation and the promotion of neuronal regeneration post injury[11]. As CREB is an upstream signaling molecule, cAMP plays a key role in the cAMP-CREB pathway[12-13]. Transcriptional activation of CREB is based on pCREB. pCREB binds to cAMP responsive elements to induce gene transcription[14], CREB exerts important regulatory effects on cell proliferation, differentiation and survival during vertebrate development[15]. The present study assessed expression changes of brain-derived neurotrophic factor, pCREB and cAMP in the hippocampus, and found that brain-derived neurotrophic factor, pCREB, and cAMP expression was significantly increased in the hippocampus of model rats; low-dose propofol also increased hippocampal brain-derived neurotrophic factor, pCREB, and cAMP expression, however, high-dose propofol decreased their expression, indicating that the cerebroprotective effect of propofol in aged rats with chronic cerebral ischemia may be associated with brain-derived neurotrophic factor expression changes. Brain-derived neurotrophic factor plays an important role in the central nervous system and in neural stem cell survival, proliferation, differentiation and migration after brain injury. Brain-derived neurotrophic factor nourishes nerves and promotes axonal growth through binding to its receptor tyrosine kinase receptor B[16-18]. Results from studies regarding brain-derived neurotrophic factor and tyrosine kinase receptor B mRNA expression changes in hippocampal neurons of aged animals remain controversial. Some studies reported that old age did not influence brain-derived neurotrophic factor and tyrosine kinase receptor B mRNA expression in hippocampal neurons[19-20], however, other studies proposed that old age could downregulate tyrosine kinase receptor B mRNA expression in hippocampal neurons[21]. Results from the present study showed that after chronic cerebral ischemia, brain-derived neurotrophic factor and tyrosine kinase receptor B protein expression was significantly increased in the hippocampus of aged rats. Low-dose propofol further increased brain-derived neurotrophic factor and tyrosine kinase receptor B protein expression, but high-dose propofol inhibited protein expression. The opposing effects of low- and high-dose propofol may result from a number of pathways. Low-dose propofol can attenuate ischemia-induced synaptic structural injury, ameliorate brain tissue metabolic disorders, significantly improve neurological function after ischemia, reduce cerebral infarction and edema volume, and decrease brain cell death surrounding the infarct region. However, high-dose propofol worsens ischemic brain injury[22-24].

In conclusion, low-dose propofol promoted brain-derived neurotrophic factor, pCREB, tyrosine kinase receptor B and cAMP expression in the hippocampus of aged rats with ischemic brain injury, but high-dose propofol suppressed their expression.

**MATERIALS AND METHODS**

**Design**

A randomized, controlled, animal study.

**Time and setting**

The experiment was performed at the Laboratory of PLA General Hospital, China, from May 2007 to May 2009.

**Materials**

A total of 20 male Sprague-Dawley rats, aged 18 months, weighing 450 ± 46 g, were provided by the Animal Institute of Sichuan Academy of Medical Sciences (license No. SCXK (Chuan) 2006-0023). They were housed in the Laboratory Animal Center of PLA General Hospital, China, at 20–26°C with a humidity of 40–70% and natural illumination. Experimental procedures were performed in...
accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, issued by the Ministry of Science and Technology of China[29].

Methods

Establishment of chronic cerebral ischemia model
Rats were anesthetized with 2% (v/v) pentobarbital sodium, and a median incision was made at the neck to separate the bilateral common carotid artery, followed by permanent ligation using a double thread[26]. The bilateral common carotid artery of the sham-surgery group was permeated using the thread, but not ligated.

Intraperitoneal injection of propofol
From the first day after model establishment, low- and high-dose propofol groups were intraperitoneally injected with 10 and 50 mg/kg propofol, respectively, twice a day for 7 consecutive days.

Sampling
Rats were sacrificed within 4–6 hours after the final administration. The entire brain was harvested on ice and the intact hippocampus was isolated[27]. Homogenate was prepared using a ratio of 50 mg tissue: 500 μL lysis, followed by sonication for 10 seconds after 30 minutes. Samples were centrifuged at 13 000 × g at 4°C for 20 minutes. The supernatant was harvested to determine protein content. Sample buffer (0.3 M Tris-HCl, 12% (w/v) SDS, 0.6% (w/v) bromophenol blue, 60% (v/v) glycerol and 9.25% (w/v) DTT) was added, and lysates were boiled for 3–5 minutes and stored at -70°C.

Western blot for hippocampal brain-derived neurotrophic factor, tyrosine kinase receptor B and pCREB expression
Hippocampal homogenate was prepared, electrophoresed at constant voltage, electrically transferred, stained with Coomassie brilliant blue, blocked with blocking solution for 2 hours, washed with Tris-buffered saline-Tween-20, and incubated with rabbit anti-brain-derived neurotrophic factor polyclonal (1:1 000; Upstate, NY, USA), goat anti-tyrosine kinase receptor B polyclonal (1:1 000; Upstate) and rabbit anti-rat pCREB monoclonal antibody (1:1 000; Upstate) at 4°C for 15 hours. The products were washed with Tris-buffered saline-Tween-20 three times for 10 minutes each, and mixed with horseradish peroxidase-labeled goat anti-rabbit IgG (1:5 000; Beijing Zhongshan, Beijing, China) and rabbit anti-goat IgG (1:2 000; Beijing Zhongshan) at 26°C for 1 hour. Membranes were washed with Tris-buffered saline-Tween-20 twice, and twice with Tris-buffered saline for 10 minutes each, followed by enhanced chemiluminescence. After scanning, band absorbance was measured using ScionImage 4.02 Beta software (Scion, Hagerstown, USA). Low molecular weight standard protein, 14.4–97 kDa, provided by the Shanghai Institute of Chinese Academy of Sciences, was used as an internal reference. Results from the sham-surgery group were regarded as 100%, the ratio of each group to sham-surgery was calculated.

Radioimmunoassay for hippocampal cAMP content
Hippocampal homogenate (about 50 mg) was placed in 2 mL cold acetic acid buffer solution (50 mM; ice bath), homogenized, transferred to 10-mL tubes, and rinsed with 2 mL absolute alcohol. The rinse solution was placed in 10-mL tubes, mixed, left for 5 minutes, and centrifuged at 3 500 × g for 15 minutes (Eppendorf, Hamburg, Germany). The supernatant was collected, washed with 1 mL 75% (v/v) alcohol, centrifuged at 3 500 × g for 15 minutes, mixed with the supernatant, and dried in an oven at 60°C. Curves were mapped with the cAMP concentration as the X-axis and the Co/Cx ratio as the Y-axis. The standard curvilinear equation was calculated using linear regression. cAMP concentration was calculated using the equation, and results were represented as pmol/50 mg protein.

Statistical analysis
Results were analyzed using SPSS version 12.0 (SPSS, Chicago, IL, USA) and measurement data were expressed as the mean ± SD. Intergroup differences were compared by one-way analysis of variance. A value of P < 0.05 was considered statistically significant.

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Author contributions: Gang Chen conceived and designed the study, provided, integrated and analyzed experimental data, and wrote the manuscript. Qiang Fu and Jiangbei Cao reviewed the manuscript, guided the study and was in charge of funds.

Conflicts of interest: None declared.

Ethical approval: This study received permission from the Animal Ethics Committee of PLA General Hospital, China.

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