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Effects of L-655,708 on expression changes of GABA, glutamate, and beta-endorphin induced by propofol anesthesia in rats

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Abstract

Anesthetics are considered to be one of the important inducing factors of postoperative cognitive dysfunction (POCD). The hippocampal region of the rat is one of the action sites of general anesthesia drugs. L 655,708, a reverse agonist of gamma aminobutyric acid (GABA) receptor, can significantly improve short-term memory dysfunction in mice after anesthetized with isoflurane. So the purpose of this study is to investigate the effects of L-655,708 on expression of GABA, glutamate (GLU), and beta-endorphin (β-EP) in the dentate gyrus region of the hippocampus and cognition of rats anesthetized with propofol. In all, 30 male Sprague–Dawley (SD) rats were randomly allocated into the control group, sham group, and L-655,708 group, with 10 in each group. The cognitive function of rats was measured by Morris water maze before and 1 h after administration. Then the rats were sacrificed for brain tissues. Immunohistochemistry was used to study the expression of GABA, GLU, and β-EP in the hippocampus of anesthetized rats in each group. Compared with the control group, the latency of the sham group and L-655,708 group were significantly prolonged after administration ($P < 0.05$). However, L-655,708 could shorten the prolonged latency ($P < 0.05$). There was no significant difference in times of accessing original platform area between the three groups before and after medication ($P > 0.05$). The expression level of GABA in the dentate gyrus region of hippocampus of rats in the sham group was significantly higher than that in the control group ($P < 0.05$), while the expression level in the L-655,708 group was significantly lower than that in the sham group ($P < 0.05$). No significant difference was found in the expression of GLU in the dentate gyrus region of hippocampus of rats in each group ($P > 0.05$). Compared with the control group, the expression of β-EP was significantly lower in the dentate gyrus region of the hippocampus of sham group rats ($P < 0.05$). However, the expression of β-EP in the L-655,708 group was significantly higher than that in the sham group ($P < 0.05$). Cognitive dysfunction in rats anesthetized with propofol may be related to high expression of GABA and low expression of β-EP in the hippocampus. The mechanism of L-655,708 in reducing the cognitive impairment in propofol anesthetized rats may be bound up with down-regulating the expression of GABA and increasing the expression of β-EP in the hippocampus.

Keywords

anesthesia, GABA, GLU, L-655,708, propofol, β-EP

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Postoperative cognitive dysfunction (POCD) refers to an acute neurological syndrome characterized by memory, orientation, and abstract thinking disorders, which are caused by many factors after anesthesia.[1] The release of cytokines due to the systemic stress response caused by anesthesia and surgical procedures might induce changes in brain function and be involved in the development of POCD.[2] Anesthetics are considered to be one of the important inducing factors of POCD.[1] In recent

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years, studies have shown that continuous application of certain systemic intravenous anesthetics can have adverse effects on the learning, memory, and cognitive function of animals, and as a result, postoperative POCD occurs.[3] O'Gorman et al.[4] found that in passive avoidance experiment, low-dose propofol induced anterograde amnesia in rats, and retrograde amnesia was produced when the anesthetic dose increased. Other clinical studies have reported that in patients who underwent propofol anesthesia for short-time surgery still had varying degrees of learning, language, logical judgment, and other cognitive dysfunctions within a few hours, despite regaining consciousness after withdrawal.[5] Previous study showed that L 655,708, a reverse agonist of gamma aminobutyric acid (GABA) receptor, could significantly improve short-term memory dysfunction in mice after anesthetized with isoflurane, but the mechanisms remained unclear.[6] The hippocampal region of the rat is one of the action sites of general anesthesia drugs. GABA, glutamate (GLU), beta endorphin (β-EP), and other neural active substances, the material basis of many physiological functions of the hippocampus, are abundant in this area. Under general anesthetics, changes in the brain function are accompanied with changes in neurotransmitter functions.[7] Previous studies have shown that anesthetics can exert a pivotal inhibitory effect through a variety of neurotransmitters and their receptors.[8] In our study, the rats were anesthetized with propofol to establish the model of consciousness disorder without injury to investigate the effects of L 655,708 on brain neurotransmitters, such as GABA, GLU, and β-EP, and to reveal the possible mechanism of L 655,708 in improving cognitive impairment.

Materials and methods

Animals

Thirty male SPF Sprague–Dawley rats (200 ± 30 g) were obtained from the Xi'an Jiao Tong University Laboratory Animal Center.

Reagents and materials

The following were the materials used: propofol (Xi'an Libang Pharmaceutical Co. Ltd.), GABA polyclonal antibody, GLU polyclonal antibody, a SABC kit, DAB chromogenic agent (Wuhan boshide Company Limited Company); paraformaldehyde, sodium dihydrogen phosphate, sodium hydrogen phosphate (Sinopharm Chemical Reagent Co. Ltd.); Morris water maze (Chengdu taimeng Science & Technology Co Ltd); GABAA receptor inverse agonist L 655,708 (Sigma).

Grouping, modeling, and sampling of animals

All rats were randomly allocated into the control group, the sham group, and the L-655,708 group, with 10 rats in each group. Propofol was injected slowly through the caudal vein at 15 mg/kg until the righting reflex disappeared, and then propofol was injected slowly at 30 mg/kg • h through the caudal vein for 30 min to establish the rat model of propofol anesthesia in the sham group and the L-655,708 group. While in the control group, normal saline was injected instead of propofol. Then, in the L-655,708 group, L-655,708 was injected subcutaneously at 0.7 mg/kg, while normal saline was injected instead in the sham and the control groups. Spontaneous respiration of rats was maintained during administration. After regaining consciousness, the rats were placed in cages individually and with free access to water and food. The cognitive function of rats was measured by Morris water maze before and 1 h after administration. Then the rats were sacrificed for brain tissues. The tissues were placed in 4% paraformaldehyde and fixed at 4 °C for 3–4 h, followed by dehydration, transparency, immersion wax, embedding, and other steps to make paraffin blocks.

Morris water maze

The water maze is a black round cylinder with a diameter of 150 cm and a height of 54 cm. During training, the height of the cylinder surface is 38 cm, and the water temperature is maintained at 26 °C, which is opaque with starch. A circular 9 cm with a diameter of 2 cm is hidden at the bottom of the cylinder. Because the water is dyed opaque, the platform is invisible. Rats began water maze training 6 days before administration in all the three groups. In the first 5 days, navigation training was performed four times a day. The latency was defined as the time to find the platform. If the platform was not found within 60 s, the latency was recorded as 60 s. The 20th latency was taken as the test value before medication. On day 6, the platform was removed, space exploration training was carried

Table 1. Comparison of results in Morris water maze test between groups.

Groups	Number	Latency (s)		Times of accessing original platform area	
		Before administration	After administration	Before administration	After administration
Control group	10	8.2±1.4	7.3±1.1	6.5±1.1	7.1±1.3
Sham group	10	7.5±1.2	28.5±2.6	5.4±1.3	5.6±1.5
L-655,708 group	10	9.5±1.5	20.8±1.7	6.8±1.6	6.8±1.7
F		35.32	41.16	3.207	3.495
P		<0.05	<0.05	>0.05	>0.05

out, and the times of rats entering the original platform area within 90 s was recorded. Navigation training and space exploration were carried out in the three groups 1 h after medication. Before and after administration, the latency and the times of accessing original platform area were recorded.

Methods of detecting GABA, GLU, and β-EP

Immunohistochemistry method was used to detect protein expression levels of GABA, GLU, and β-EP according to the instructions of reagents. Image analysis of sections was carried out by Lecia microscope high-definition color image analysis system. All sections were analyzed at the same intensity and at the same magnification (40 × 10). Image-Pro Plus 6.0 software was used to detect the integral optical density (IOD) of positive signals. Three microscopic fields were selected in each section to calculate the mean value of IOD of immunohistochemistry positive signals to evaluate the protein expression levels of GABA, GLU, and β-EP.

Statistical analysis

SPSS 21 statistical software was used for statistical analysis, and single factor analysis of variance (one-way ANOVA) was used to compare between the groups. If the variance test is homogeneous, data were analyzed by least significant difference (LSD) test, or else Games–Howell test was adopted. *P* values < 0.05 were considered significant.

Results

Comparison of results in Morris water maze test between groups

After administration, the latency of the sham and L-655, 708 groups were both significantly prolonged. Compared with the control group, the latency of the sham and L-655,708 groups were significantly prolonged after administration. However, L-655,708 could shorten the prolonged latency. There was no significant difference in times of accessing the original platform area between the three groups before and after medication, as shown in Table 1.

Comparison of the expression of GABA, GLU, and β-EP in the dentate gyrus region of the hippocampus between the groups

The distributions of GABA, GLU, and β-EP immunoreactivity substances in brain of rats were widespread. Cell localization of GABA, GLU, and β-EP in the dentate gyrus region of the hippocampus in each group are shown in Figure 1. The expression level of GABA in the dentate gyrus region of the hippocampus of rats in the sham group was significantly higher than that in the control group, while the expression level in the L-655,708 group was significantly lower than that in the sham group. No significant difference was found in the expression of GLU in the dentate gyrus region of the hippocampus of rats in each group. Compared with the control group, the expression of β-EP was significantly lower in the dentate gyrus region of the hippocampus of the sham group rats. However, the expression of β-EP in the L-655,708 group was significantly higher than that in the sham group; see Table 2.

Discussion

The inhibitory neurotransmitter, excitatory neurotransmitter, and many endorphins play important roles in the process of coma and arousal. GABA, GLU, and β-EP are important neurotransmitters in the brain. GABA is produced by decarboxylation of GLU by glutamic acid decarboxylase and is widely distributed in the brain. GABA can inhibit the opening of ion channels coupled with

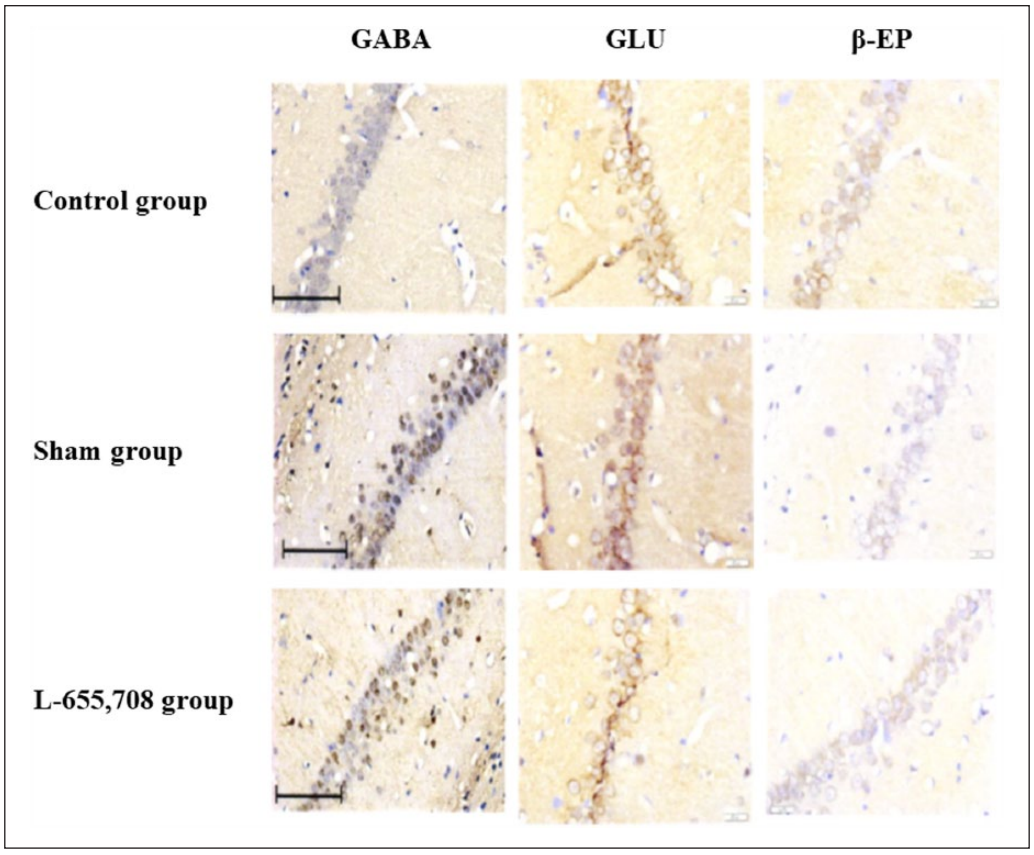

Figure 1. Cell localization of GABA, GLU, and β-EP in the dentate gyrus region of the hippocampus in each group.

Table 2. Comparison of the expression of GABA, GLU, and β-EP in the dentate gyrus region of the hippocampus between the groups.

Groups	Number	IOD value		
		GABA	GLU	β-EP
Control group	10	52.7 ± 6.2	40.1 ± 7.8	25.3 ± 3.4
Sham group	10	65.3 ± 7.1	39.5 ± 6.2	20.9 ± 2.6
L-655,708 group	10	55.6 ± 5.5	39.3 ± 4.1	24.4 ± 2.5
F		30.93	2.064	34.52
P		<0.05	>0.05	<0.05

GABA: gamma aminobutyric acid; GLU: glutamate; β-EP: beta endorphin; IOD: integral optical density.

N-methyl-D-aspartate (NMDA) receptors and voltage-dependent calcium channels, and can ultimately make neurons be "homeostatic" and balance abnormal neuronal excitability induced by hypoxia damage.[9] After binding of GABA-to-GABA receptor, the chloride conductance of the cell membrane is enhanced, which opens the chloride channel and causes chloride ions to transfer into the cell. This produces hyperpolarization potential, reduces the excitability of neurons, inhibits the discharge of neurons, inhibits the excitatory response to depolarizing stimulation, and finally results in postsynaptic inhibitory effects. In our study, the positive expression of GABA was decreased in the L-655,708 group compared with the sham and control groups. The expression level of GABA in the dentate gyrus region of the hippocampus of rats in the sham group was significantly higher than that in the control group, while the expression level in the L-655,708 group was significantly lower than that in the sham group. These results suggest that L-655708 could down-regulate the expression of GABA in the hippocampus of propofol anesthetized rats.

GLU is the major neurotransmitter in hippocampal neurons. GLU can activate and blind to the NMDA receptors in the hippocampal CA1 region and dentate gyrus, which makes the calcium channel open and then the calcium concentration in the synaptic membrane increases.[10] In theory, it will increase with the effect of wake promoting drugs, showing the opposite trend with GABA. The combination of GABA and GABA receptors can inhibit

the activation of the hypothalamic pituitary adrenal (HPA) axis, and then inhibit the increase of excitatory amino acid content. When the content of GABA decreases, the inhibitory effect on the HPA axis activity is weakened, and the activity of HPA axis is enhanced, which results in the increase of GLU and other excitatory amino acids. In this study, there was no significant difference in the positive expression of GLU between the L-655,708 and control groups. Previous studies have shown that acute stress (first day) had no significant effect on the excitatory amino acid glutamate and aspartate content in the hippocampus, but decreased the GABA content significantly.[11] However, 3 days after stress, the contents of glutamic acid and aspartic acid increased significantly and maintained a higher level with the increase in days. This suggested that the changes of GLU may be slow, resulting in no increase of GLU at the end of our experiment.

β-EP is one of the endogenous opioid peptides, derived from the thalamic arcuate nucleus POMC system. It enters the blood through the hypothalamus pituitary portal system and stress is an important factor to promote the secretion and release of β-EP.[12] At the same time, it is a strong opioid receptor agonist, which can inhibit the release of central and peripheral neurotransmitters and the electrophysiological activity of neurons, resulting in a series of pathophysiological changes. In this study, compared with the control group, the expression of β-EP was significantly lower in the dentate gyrus region of the hippocampus of the sham group rats. However, the expression of β-EP in the L-655,708 group was significantly higher than that in the sham group. These results indicate that L-655708 could increase the expression of β-EP. The reason may be that L-655,708 could inhibit the release of β-EP in the blood, resulting in increased expression of β-EP in the brain, which remains to be further studied.

In conclusion, cognitive dysfunction in rats anesthetized with propofol may be related to high expression of GABA and low expression of β-EP in the hippocampus. The mechanism of L-655,708 in reducing the cognitive impairment in propofol anesthetized rats may be bound up with down-regulating the expression of GABA and increasing the expression of β-EP in the hippocampus. Further studies should be carried out to verify these assumptions through knockout rats.

Declaration of conflicting interests

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