Modulation of γ -aminobutyric acid on painful sense in central nervous system of morphine-dependent rats

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Abstract: Objective To observe the effects of γ -aminobutyric acid (GABA) on the electric activities of pain-excited neurons (PEN) in nucleus accumbens (NAc) in central nervous system (CNS) of morphine-dependent rats. **Methods** After GABA or the GABA_A-receptor antagonist, bicuculline (Bic), was injected into cerebral ventricles or NAc, right sciatic nerve was stimulated by electrical pulses, which was considered as traumatic pain stimulation. Extracellular recordings methods were used to record the electric activities of PEN in NAc. **Results** When GABA was injected into intracerebroventricle (ICV) as well as NAc, it could decrease the pain-evoked discharge frequency and prolong the latency of PEN. Bic could interdict the above effects of GABA on the electric activities of PEN. **Conclusion** Exogenous GABA might have an inhibitory effect on the central pain adjustment. Furthermore, GABA and GABA_A receptor participate and mediate the traumatic information transmission process in CNS.

Keywords: γ -aminobutyric acid; pain-excited neurons; nucleus accumbens; bicuculline; morphine-dependent rats

1 Introduction

γ -Aminobutyric acid (GABA), an important inhibitory neurotransmitter of central nervous system (CNS) in mammals, acts at inhibitory synapses in the brain by binding with specific transmembrane receptors in the pre- and postsynaptic plasma membranes^[1]. Many brain cells can secrete GABA and inhibit pain signal transduction in pain regulation pathway^[2]. Recent research has pointed that GABA receptor, dopamine (DA) receptor, together with other transmitters, are involved in regulating the activity of nucleus accumbens (NAc)^[3,4]. Both GABA and opioid exist in NAc, which hints that they have a close correlation in function. Previously, we have confirmed that the exogenous GABA could weaken the responses of pain-excited neurons (PEN)

of NAc in the normal rats to the noxious stimulation and have an analgesic effect which was mediated by GABA_A receptors^[5]. These results revealed that GABA and NAc played an important role in the modulation of algnesia. But no electrophysiological research about GABA effect on morphine analgesia has been reported. In the present study, we give nociceptive stimulus to morphine-dependent rats to observe the influence of GABA on the electric activities of PEN in NAc, in order to discuss the effect and mechanism of GABA and receptors on pain regulation.

2 Materials and methods

2.1 Animals and instruments Eighty-two Wistar rats in either gender, weighing (240-280) g, were obtained from the Center for Experimental Animals of the Second Affiliated Hospital of Harbin Medical University. Instruments comprise the following parts: cerebral solid orientation apparatus (SN-2, Narishige), microelectrode operator SM-11 and SM-12 (Narishige), amplifier (JSD-731-F, Radio Factory of Guangdong Teacher's College), oscillogram (VC-10, Nihon Kohden), bi-track tape recorder (ST-CH707X, Panasonic), data

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analyzer (Powerlab/8s, ADInstruments), and electronic stimulator (SEN-3301, Nihon Kohden).

2.2 Model establishment The morphine-dependent models were established by subcutaneous injection of morphine on the back with gradually increasing dosages of 5, 10, 20, 40 and 50 mg/kg, three times a day at 8:00, 12:00 and 16:00 respectively, for 5 continuous days. The experiment began at 8:00 on the sixth day, 30 min after the symptoms disappeared naturally.

2.3 Surgery Routine surgery was performed under anaesthesia using a mixture of 500 mg/kg of ethyl carbamate and 50 mg/kg of chloralose. Chloridized curarine (1 mg/kg) was injected into the abdominal cavities after consciousness of the animals, and artificial respiration rate was set at 80 times/min. The heads of the rats were fixed on the cerebral solid orientation apparatus and orientated according to the B coordinate system of Pellegrino atlas^[6]. A stainless steel tube with an external diameter of 0.8 mm was inserted into lateral ventricle (AP: -0.2 mm, ML: 1.5 mm, DV: 3.0 mm) for GABA (50 μ g/ μ L) or Bic (1 μ g/ μ L) or saline (0.9%) injection. A glass microelectrode filled with GABA (50 μ g/ μ L) was fixed on microelectrode operator SM-21 and inserted into right NAc (AP: 3.2-4.0 mm, ML: 1.0-1.8 mm; DV: 6.0-7.0 mm) for medication (24° angle with sagittal plane). Another glass microelectrode (a direct current resistance of 10-30 M Ω) filled with 3 mol/L of KCL solution was fixed on microelectrode operator SM-11 and inserted into left NAc for recording electric activity. The signal of electric activity was displayed on the oscilloscope through amplifier and recorded by bi-track tape recorder. Data were input into a computer after the management by data analyzer and analyzed by Chart v5.1 software. The noxious stimulation evoked by electrostimulator on right sciatic nerve was a train of impulses (parameters: 5 mA in strength, 0.3 ms in wave width, 5 ms in interval, 5 pulses included in one

series impulse). Moving arthrosis and touching fur were set as non-traumatic stimulation in order to identify the pain reactive neuron. After the experiment, the position of the microelectrode tip was marked through electrifying the recording microelectrode filled with 2% of Pontamine Sky Blue with direct current (25 μ A at negative electrode for 20 s).

2.4 Statistical analysis Data was managed by SPSS10.0 software and expressed by mean $\pm$ SD. Paired *t* test and *t* test were introduced in the analysis. *P* < 0.05 was considered statistically significant.

3 Results

3.1 The effect of GABA injection into intracerebroventricle (ICV) on electric activities of PEN in the NAc Twenty-eight PEN were completely recorded in the NAc from 20 morphine-dependent rats, in which 15 were recorded from the GABA group and 13 were recorded from the control group. The variations in the frequency and latency of PEN electric activities in the two groups were observed and compared. Figure 1 indicated that GABA caused the decrease in the pain-evoked discharge frequency and the prolongation of latency of PEN in the NAc of the morphine-dependent rats.

The net increased value (NIV) of the discharge frequency of PEN was (4.33 $\pm$ 0.72) Hz and the mean latency was (0.16 $\pm$ 0.04) s before GABA injection. The NIV of the discharge frequency of PEN decreased and the latency started to prolong right after GABA injection. The NIV of discharge frequency of PEN decreased from (4.33 $\pm$ 0.72) Hz to (-0.49 $\pm$ 0.06) Hz and the mean latency prolonged from (0.16 $\pm$ 0.04) s to (0.31 $\pm$ 0.06) s at 2 min after the injection of GABA. Both the discharge frequency and the latency recovered at 18 min after GABA injection. The comparisons between the GABA group and the control group in the same time among every index during 0-18 min after GABA injection were significant

Fig. 1 Effects of GABA on the PEN electric activities in the NAc of morphine-dependent rats. $\uparrow$: stimulus artifact; $\blacktriangle$: the time point of GABA injection; X: the time course before GABA injection; 0, 4, 16 and 30: time points (min) examined after injection of GABA.

Fig. 2 Effects of the ICV injection of GABA on the evoked discharge of PEN in NAc of morphinism rats. X: the time course before injection of GABA or saline; ■: injection of GABA or saline. * $P < 0.05$, ** $P < 0.01$ vs saline group.

Table1 Antagonism of Bic to the effects of GABA on the evoked discharge of PEN in NAc

Time after injection (min)	Control group		Bic group	
	Frequency NIV (Hz)	Latency (s)	Frequency NIV (Hz)	Latency (s)
Before GABA injection	4.76±0.97	0.14±0.03	4.68±0.13	0.14±0.01
After GABA injection 0	0.32±0.03	0.23±0.09	0.71±0.05	0.22±0.03
After Bic injection 2 (0')	-0.53±0.16	0.29±0.05	3.41±0.09**	0.17±0.07**
4 (2')	1.14±0.56	0.27±0.04	3.97±0.08**	0.16±0.02**
8 (6')	2.52±0.77	0.22±0.01	4.23±0.10*	0.16±0.01*
12 (10')	3.56±0.34	0.18±0.05	4.56±0.19	0.15±0.02
22 (20')	4.38±0.28	0.16±0.01	4.60±0.08	0.15±0.03
32 (30')	4.75±0.31	0.15±0.03	4.70±0.07	0.14±0.01

* $P < 0.05$, ** $P < 0.01$ vs control group. 0, 2, 4...32: time points after GABA injection; 0', 2', 6',...30': time points after Bic injection.

different ($P < 0.01$; Fig. 2).

3.2 Bic injection interdict the inhibitory influence of GABA injection on the electric activities of PEN Twenty-eight discharges of PEN were completely recorded in the NAc from 24 morphine-dependent rats, in which 17 were recorded from GABA+Bic group, and 11 were recorded from GABA+saline group. The electric activities of PEN weakened after GABA injection. GABA_A-receptor antagonist, Bic, could block the effects induced by GABA.

The analysis of discharge frequency of twenty-eight PEN indicated that the NIV of the discharge frequency of PEN was (4.68±0.13) Hz and the mean latency was (0.14±0.01) s before GABA injection. The NIV decreased to (0.71±0.05) Hz and the mean latency prolonged to (0.22±0.03) s soon after GABA injection. The NIV increased to (3.41±0.09) Hz and the latency shortened to (0.17±0.07) s at 2 min after Bic injection. The reduced reaction of PEN electric activity started to increase soon after Bic injection. The discharge frequency of

PEN increased noticeably after Bic injection 2 min later and recovered from (3.41±0.09) Hz to (4.70±0.07) Hz. The mean latency shortened from (0.17±0.07) s to (0.14±0.01) s. The comparison of every index during the period of 0-6 min after Bic injection between GABA+Bic group and GABA+saline groups in the same time were significant different ($P < 0.01$, $P < 0.05$; Table 1).

3.3 The effect of GABA injection into NAc on the electric activities of PEN in NAc Discharge frequencies of 44 PEN were completely recorded in the NAc from 38 morphine-dependent rats, in which 10 were recorded from the control group, 18 from the GABA+saline group and 16 from the GABA+Bic group. GABA in NAc caused a decrease in the pain-evoked discharge frequency and a prolongation in latency of PEN. Bic counteracted the effects induced by GABA (Fig. 3).

The NIV of discharge frequency of PEN was (5.41±0.77) Hz, and the mean latency was (0.23±0.01) s before GABA injection.

Fig. 3 Effects of GABA on the electric activities of PEN in NAc of morphine-dependent rats. X: the time course before GABA injection; ■: GABA injection; □: Bic or saline injection. 0, 2, 4...32: time points after injection of GABA or 0.9%NaCl; 0', 2', 6'...30': time points after injection of Bic. * $P < 0.05$, ** $P < 0.01$ vs Saline group; # $P < 0.05$, ## $P < 0.01$ vs GABA+saline group.

The NIV of the discharge frequency of PEN decreased to (0.65 ± 0.03) Hz, and the mean latency prolonged to (0.38 ± 0.05) s after GABA injection 2 min later. The comparisons among every index during 0-8 min after GABA injection between the GABA group and the control group in the same time were significant different ($P < 0.01$).

For the GABA+Bic group, when injecting Bic 2 min after GABA injection, the PEN electric activities started to recover. The NIV of discharge frequency of PEN recovered to (4.57 ± 0.74) Hz and the mean latency recovered to (0.27 ± 0.05) s in the GABA group. The comparison among every index during 6-10 min after Bic injection between the GABA+Bic group and the GABA+saline group in the same time were significantly different ($P < 0.01$). There was no significant difference compared with control group ($P > 0.05$).

4 Discussion

Experimental evidence has proven that GABA participates in CNS analgesia modulation activity. The aim of this study is to figure out whether GABA takes part in morphine addiction and withdrawal based on the experiments by taking NAc as target area and taking PEN as target cells. The results indicated that the NIV of discharge frequency of PEN reduced after GABA was injected into ICV of morphine-dependent rats without changing the nociceptive stimulus intensity, which suggested GABA could cause the excited threshold value of PEN to increase. Moreover, the NIV of discharge frequency of PEN increased after Bic injection, which revealed that the excited threshold value of PEN decreased by blocking GABA_A receptors. Therefore, the pos-

sible reason for the reduced discharge frequency of PEN was that GABA changed the PEN excitability by binding with the GABA_A receptors. After injection of GABA, the latency of the electric activities of PEN in NAc prolonged, which suggested that GABA inhibited the electric activity of brain central nuclei before spinal cord pain signals transmitted to NAc. What's more, the latency of the electric activities of PEN was shortened after Bic injection, which proved that the inhibitory effect of neurons was counteracted by blocking GABA_A receptor with Bic. These results were consistent with the assumption that GABA played an inhibitory role in pain modulation in CNS by binding with GABA_A receptor. In the present study, we also found the changes of discharge frequency of PEN by injecting GABA into NAc or ICV showed the similar results. The similar electric activity of PEN suggested GABA regulated morphine-dependent pain process in the mechanism that GABA could activate GABA_A receptor and then increase Cl⁻ conductance, and the inflow of Cl⁻ caused PEN hyperpolarization and then PEN electric activity was inhibited.

It has been pointed out that DANergic system in the brain is excited after taking in morphine, in which morphine can inhibit GABAergic interneurons and release DANergic neurons from the tension inhibition exerted by GABAergic interneurons^[7]. Brain microdialysis revealed that morphine especially made an increase of DA released from NAc^[8,9]. Administration of exogenous GABA would make GABAergic neurons get rid of morphine inhibitory effect, so the excitability of DANergic neuron was weakened and pain threshold value was heightened. The GABA density in NAc gradually reduces and the DA density gradually elevates as time

goes by, and then the excitability of GABA neuron is suppressed, which causes the pain threshold value to be reduced. In the present study, the effect of GABA on PEN gradually approached to the level of non-administration of GABA as time went by. This phenomenon is totally consistent with what has been observed in microdialysis studies. The mechanism of algaesthesia regulation of morphine-dependent rats and its receptor should be further studied.

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γ-氨基丁酸对吗啡成瘾大鼠中枢神经系统痛觉的调制

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摘要: 目的 观察γ-氨基丁酸(γ-aminobutyric acid, GABA)对吗啡依赖大鼠中枢神经系统伏隔核(nucleus accumbens, NAc)痛兴奋神经元(pain-excited neurons, PEN)电活动的影响。方法 脑室或NAc中注射GABA或荷包牡丹碱(bicuculline, Bic),电脉冲刺激右侧坐骨神经作为伤害性疼痛刺激,用玻璃微电极在细胞外记录中枢神经系统伏隔核痛兴奋神经元电活动的变化。结果 脑室及NAc中注入GABA均能使吗啡依赖大鼠NAc中PEN潜伏期延长、痛诱发放电频率减少;GABA_A受体拮抗剂Bic能够阻断GABA的上述效应。结论 外源性GABA在中枢痛觉调制中起抑制作用,GABA及GABA_A受体参与介导中枢伤害性信息的传递过程。

关键词: γ-氨基丁酸; 痛兴奋神经元; 伏隔核; 荷包牡丹碱; 吗啡依赖大鼠