Original Article

Differential involvement of GABA_A and GABA_B receptors in propofol self-administration in rats

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Aim: Propofol has shown abuse potential. The aim of the present study is to investigate the effects of GABA_A antagonist and GABA_B agonist on propofol reinforcement.

Methods: Sprague-Dawley rats were trained to self-administer propofol at a dose of 1.7 mg/kg per infusion under a fixed ratio (FR1) schedule of reinforcement for 14 d. In a separate set of experiments, food-maintained self-administration under a fixed ratio (FR5) schedule and locomotor activities of Sprague-Dawley rats were examined.

Results: GABA_A receptor antagonist bicuculline (0.25 mg/kg, ip) significantly increased the number of injections and active responses. Pretreatment with GABA_B receptor agonist baclofen (3 mg/kg, ip) significantly decreased the number of active responses and total infusions of propofol during the training session. Moreover, microinjection of baclofen (50 and 100 ng/side) into the ventral tegmental area (VTA) significantly decreased the number of active responses and total infusions of propofol. Neither baclofen (1–3 mg/kg, ip) nor bicuculline (0.25–1 mg/kg, ip) affected food-maintained responses or motor activities.

Conclusion: Propofol maintains its reward properties partially through GABA_A receptor activation. Stimulation of GABA_B receptors in VTA may counteract the reinforcing properties of propofol.

Keywords: addiction; propofol; drug abuse; GABA receptors; baclofen; bicuculline; ventral tegmental area (VTA); locomotor activity

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Introduction

Propofol (2, 6-disopropylphenol) is an intravenous, short-acting anesthetic that has been widely used for sedation and anesthesia. Clinical surveys reveal that propofol may have abuse potential and dependency^[1]. The abuse potential of propofol has also been demonstrated in preclinical studies using conditioned place preference (CPP)^[2, 3] and self-administration^[4], which are the classic methods for the evaluation of the psychodependence of drugs.

There are two types of neurons within the ventral tegmental area (VTA), primary dopaminergic projection neurons and secondary γ -aminobutyric acid (GABA) inhibitory interneurons. The mesolimbic dopamine system, which originates in the VTA and connects to the nucleus accumbens (NAc), is a critical component of reward and addiction. All drugs of abuse enhance the activity of the mesolimbic dopaminergic circuit of reward, which leads to the release of dopamine (DA)

in the NAc^[5, 6]. The release of DA in the NAc indicates that the reinforcement potential of propofol may account for the regulation of the activity of the reward pathway. Both GABA_A and GABA_B receptor subtypes have been identified within the VTA. The activation of GABA_A on GABAergic interneurons increases the activity of dopamine neurons in the VTA through disinhibition^[7, 8]. In contrast, GABAergic neurons dampen DA neurons via inhibitory GABA_B receptors on dopaminergic VTA neurons^[9]. The microinjection of baclofen, a GABA_B receptor agonist, into the VTA reduces heroin-induced DA release in the NAc and inhibits heroin and morphine self-administration in a dose-dependent manner^[10]. However, the role of each GABA receptor subtype in propofol reinforcement is not clear. Therefore, the present study examined the effects of baclofen and bicuculline on propofol reinforcement using intravenous self-administration.

Materials and methods

Animals

Experiments were performed in male Sprague-Dawley rats (280–300 g) that were purchased from the Experimental Animal Center of Zhejiang Province (Hangzhou, China). Rats

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were housed individually in home cages in a temperature-controlled ventilated colony room with a reversed 12–12 h light/dark cycle (lights on at 7:00 pm). *Ad libitum* food and water were provided in the home cage. The Institutional Animal Care and Use Committee of Zhejiang Province approved all procedures. Animal care was provided by trained vivarium staff at the Laboratory of Ningbo Addiction Research and Treatment Center (Ningbo, China). All animals were euthanized by carbon dioxide inhalation after pentobarbital anesthesia at the termination of experiments.

Drugs

Propofol (10 mg/mL; Diprivan, Astrazeneca, Italy) was prepared immediately before use and was injected intravenously. The propofol dose (1.7 mg/kg per infusion) that was used for the self-administration experiments was based on a previous study^[4]. Baclofen and bicuculline were purchased from Sigma Chemical Co (St Louis, USA). Both drugs were dissolved in sterile saline.

Apparatus

Thirty-two custom-made operant Plexiglas boxes (Ningbo Addiction Research and Treatment Center, China) were used for propofol training as described previously^[10]. Briefly, each box was equipped with two nose-poke apparatuses that were located 5 cm above the floor. There was a green LED light inside of each nose-poke hole. A house light (28 V, 0.1 mA) was situated on the wall above the nose-poke holes. Drug solution was delivered through Tygon tubing that was protected by a leash assembly and suspended through the ceiling of the chamber from a plastic fluid swivel. The leash assembly was modified to fit a custom-made fluid connector that was fixed on the animal's jacket. The Tygon tubing was attached to a syringe pump that delivered fluid at a speed of 1.2 mL/min using a 5-mL syringe. Experimental events were controlled by an IBM-compatible PC using an MED Associates interface and running self-programmed software written in Borland Delphi 6.0.

Surgery

The rats were surgically implanted with chronic indwelling intravenous catheters under sodium pentobarbital anesthesia using a previously described method^[10]. The catheters were flushed daily with a 0.2 mL saline-heparin solution (25 U/mL heparin) to maintain catheter patency. The rats were treated post-surgically with penicillin B for 5 d to prevent infection. All of the animals were allowed to recover for at least 7 d^[10].

In some experiments, bilateral guide cannulae (20-gauge Small Parts Inc, Roanoke, VA, USA) were implanted in the VTA (6.0 mm posterior to bregma, 1.0 mm lateral to midline, and 7.8 mm ventral to the surface of the cortex using a 10° angle) according to a previously described method^[11]. Guide cannulae were lowered into place and attached to the skull via dental acrylic. Obturators were extended 0.5 mm beyond the tip of each cannula to prevent obstruction by debris.

Microinjection procedure

Animals that had been trained for 14 d received microinjections beginning on d 15. Obturators were removed, and bilateral infusion cannulae were inserted to extend 0.5 mm beyond the tip of the guide cannulae. All injections into the VTA were delivered using a microinjection pump (MD-1001, Bioanalytical System Inc, West Lafayette, IN, USA) in a volume of 0.5 μ L/side over 5 min.

Propofol self-administration training

The rats were trained to self-administer drugs as described previously^[10]. For each daily 3 h training session, the rats were moved from their home cages to the operant chambers, and their connectors were attached to the infusion lines. Each session started with the illumination of the green light inside of the active nose-poke hole. The rats received a single propofol infusion (1.7 mg/kg per infusion) following the completion of the ratio requirement (FR1) in the active nose-poke hole. Each infusion was paired with a 5 s illumination of the house light and the noise of the infusion pump. A time-out period was imposed for 30 s, during which further responses produced no programmed consequences. However, these responses were recorded. Illumination of the green light in the active nose-poke hole signaled the end of the 30 s timeout period. Responses in the inactive nose-poke hole produced no programmed consequences. The sessions ended after 3 h or 50 propofol infusions, whichever event occurred first.

Sucrose self-administration training

The rats were trained to nose poke for sucrose pellets under a fixed ratio (FR5) schedule of reinforcement daily 1-h session for 7 d. The paradigm for sucrose self-administration was similar to the paradigm for heroin self-administration except that rats received a 45 mg sucrose pellet (Dustless precision pellets, Bio-Serv, NJ) that was delivered via a sucrose cup. During the session, each nose-poke in the active hole resulted in the delivery of a food pellet only. Nose pokes in the inactive hole had no programmed consequence. Active nose-poke responses, inactive nose-poke responses, and the number of sucrose pellets that were earned during each training session were recorded by a computer. Twenty minutes prior to the session on d 8, the trained rats received saline, baclofen (1.0, 2.0 or 3.0 mg/kg, ip), or bicuculline (0.25, 0.5, or 1.0 mg/kg, ip).

Locomotor test after withdrawal

We examined the effects of the intraperitoneal administration of either baclofen or bicuculline on locomotion in a novel context to further assess the possible nonspecific effects of baclofen or bicuculline on general activity. The locomotor activity of naïve rats ($n=8$ in each groups) was monitored for 3 h. The total distance traveled was recorded and analyzed as the measure of locomotion using MED Associates SOF-811 Open-field Activity Software.

Histology

The rats were anesthetized and transcardially perfused with

phosphate-buffered saline followed by a 4% polyformaldehyde solution. The brains were sectioned in the coronal plane to a thickness of 50 μm using a Cryostat Microtome (Leica CM1850; Leica, Wetzlar, Germany). The placement of the cannula tip for infusions was located using light microscopy and mapped onto a schematic diagram of the rat brain using a previously published method^[11]. Only animals with a proper functioning cannula and a correct location of the probe were included in the study.

Specific experiments

Experiment 1

Rats were trained for propofol self-administration (1.7 mg/kg per infusion) for 14 d. The rats ($n=24$) were randomly assigned to one of 4 groups and were injected with vehicle, 1.0 mg/kg, 2.0 mg/kg, or 3.0 mg/kg (ip) baclofen 20 min prior to testing.

Experiment 2

The rats ($n=24$) underwent the 14-d training and were then injected with vehicle or 0.25 mg/kg, 0.5 mg/kg, or 1.0 mg/kg (ip) bicuculline 20 min prior to testing.

Experiment 3

Bilateral microinjections were performed into VTA as described above. The rats ($n=6$ in each group) were placed into the self-administration chambers 10 min after intra-VTA baclofen (50 and 100 ng/side) or vehicle injection. Self-administration was examined as described above.

Statistical analysis

The number of infusions or responses in active or inactive holes during self-administration was analyzed using one-way ANOVA. A Newman-Keuls multiple comparison with an alpha level of 0.05 was used for *post hoc* comparisons between group means.

Results

Systemic pretreatment with baclofen decreased propofol self-administration

The animals exhibited reliable propofol self-administration, which was indicated by the increase in active responses and infusions during the acquisition, and maintained stable self-administration during the late training sessions. ANOVA revealed a significant main effect of baclofen treatment on the number of active responses [$F_{(3, 20)}=3.32$, $P<0.05$] (Figure 1A) and the number of infusions [$F_{(3, 20)}=8.1$, $P<0.05$] (Figure 1B). There was no significant difference in the number of inactive nose-poke responses between the groups [$F_{(3, 20)}=0.99$, $P>0.05$] (Figure 1A). Multiple comparisons showed that 3.0 mg/kg baclofen decreased the number of active responses compared to the control group ($P<0.01$) (Figure 1A). Baclofen at doses of 2.0 or 3.0 mg/kg decreased the number of infusions compared to the control group ($P<0.05$, $P<0.01$) (Figure 1B).

Figure 1. Effect of baclofen on intravenous propofol self-administration under an FR1 schedule. Baclofen (vehicle, 1.0, 2.0, or 3.0 mg/kg, $n=6$ in each group) was injected ip 20 min before training. Results represent the mean $\pm$ SEM of the number of total active and inactive responses (A) and infusions (B) in rats. ^a $P<0.05$, ^c $P<0.01$ compared to the saline group.

Systemic pretreatment with bicuculline enhanced propofol self-administration

ANOVA revealed a main effect of bicuculline treatment in the number of active responses [$F_{(3, 20)}=3.66$, $P<0.05$] (Figure 2A) and the number of infusions [$F_{(3, 20)}=3.18$, $P<0.05$] (Figure 2B). There was no difference of the number of inactive nose-poke responses between the groups [$F_{(3, 20)}=1.43$, $P>0.05$] (Figure 2A). Multiple comparisons demonstrated that 0.25 mg/kg bicuculline increased the number of active responses compared to the control group ($P<0.01$) (Figure 2A). Bicuculline at doses of 0.25 or 0.5 mg/kg increased the number of infusions compared to the control group ($P<0.01$, $P<0.05$) (Figure 2B).

Microinjection of baclofen into the VTA inhibited propofol self-administration

ANOVA revealed a main effect of baclofen infusion into the VTA on the number of active responses [$F_{(2, 15)}=21.23$, $P<0.05$] (Figure 3A) and the number of infusions [$F_{(2, 15)}=63.51$, $P<0.05$] (Figure 3B). There was no difference in the number of inactive nose-poke responses among the groups [$F_{(2, 15)}=0.081$, $P>0.05$] (Figure 3A). Multiple comparisons demonstrated that both doses of baclofen significantly decreased the number of active responses ($P<0.01$) (Figure 3A) and the number of infusions ($P<0.01$) (Figure 3B).

Figure 2. Effect of bicuculline on intravenous propofol self-administration under an FR1 schedule. Bicuculline (vehicle, 0.25, 0.50, or 1.0 mg/kg, $n=6$ in each group) was injected ip 20 min before training. Results represent the mean $\pm$ SEM of the number of total active and inactive responses (A) and infusions (B) in rats. ^b $P<0.05$, ^c $P<0.01$ compared to the saline group.

Neither baclofen nor bicuculline affected food-maintained behavior or locomotor activity

One-way ANOVA revealed no apparent variance of baclofen treatment on the number of active [$F_{(3, 24)}=1.27$, $P>0.05$] or inactive nose-poke responses [$F_{(3, 24)}=1.36$, $P>0.05$] or the number of sucrose pellets in food-maintained testing [$F_{(3, 24)}=0.32$, $P>0.05$] (Figure 4A). Pretreatment with bicuculline did not alter the number of active [$F_{(3, 24)}=0.94$, $P>0.05$] or inactive nose-poke responses [$F_{(3, 24)}=1.9$, $P>0.05$] or the number of sucrose pellets

Figure 4. Effects of baclofen on food-maintained self-administration and locomotor activities (A). Baclofen (vehicle, 1.0, 2.0, or 3.0 mg/kg, $n=7$ in each group) was administered systemically 20 min prior to training. Results represent the mean $\pm$ SEM number of the total active and inactive responses and sucrose pellets in rats. (B) Baclofen (vehicle, 1.0, 2.0, or 3.0 mg/kg, $n=8$ in each group) was administered systemically 20 min prior to testing. Each bar represents the mean $\pm$ SEM distance of rats.

in food-maintained testing [$F_{(3, 24)}=0.32$, $P>0.05$] (Figure 5A). Pretreatment with either baclofen [$F_{(3, 28)}=1.53$, $P>0.05$] (Figure 4B) or bicuculline [$F_{(3, 28)}=0.60$, $P>0.05$] (Figure 5B) did not affect the locomotor activity.

Figure 3. Effect of the microinjection of baclofen into the VTA on intravenous propofol self-administration under an FR1 schedule. Baclofen (vehicle, 50, or 100 ng/side, $n=6$ in each group) was injected intra-VTA before the beginning of training. Results represent the mean $\pm$ SEM number of active and inactive responses (A) and infusions (B) in rats. (C) Location of cannula tips in the VTA. Symbols (black spots) indicate where the cannula tips were placed. ^c $P<0.01$ compared to the saline group.

Figure 5. Effects of bicuculline on food-maintained self-administration and locomotor activities (A). Bicuculline (vehicle, 0.25, 0.50, or 1.0 mg/kg, $n=7$ in each group) was administered systemically 20 min prior to training. Results represent the mean $\pm$ SEM number of the total active and inactive responses and sucrose pellets in rats. (B) Bicuculline (saline, 0.25, 0.50, or 1.0 mg/kg, $n=8$ in each group) was administered systemically 20 min prior to testing. Each bar represents the mean $\pm$ SEM distance of rats.

Discussion

The main findings of this study are that bicuculline pretreatment at a lower dose increased the active responses and infusions of propofol self-administration. However, baclofen pretreatment using a systemic or intra-VTA infusion reduced the active responses and infusions of propofol self-administration in a dose-dependent manner in rats. Neither bicuculline nor baclofen affected food-maintained responses or locomotor activities. These results suggest that the action of bicuculline or baclofen on propofol reinforcement cannot account for the impairment of coordinated motor ability.

Drugs of abuse enhance DA release in the NAc via a disinhibition of GABAergic neurons in the VTA^[12]. Propofol is a positive allosteric modulator of the GABA_A receptor. Benzodiazepines have recently been shown to increase the firing of DA neurons in the VTA via the positive modulation of GABA_A receptors on nearby interneurons^[7]. In the present study, a low dose of bicuculline, an antagonist of GABA_A receptors, significantly increased propofol self-administration, which suggests a compensatory enhanced behavioral response as a result of the partial reversal of GABA_A activation by propofol. Both pre-synaptic and post-synaptic GABA_A receptors exist in the VTA. The activation of pre-synaptic GABA_A receptors leads to a decrease in GABA release from GABAergic interneurons and a subsequent increase in dopaminergic neu-

rons. However, the activation of post-synaptic GABA_A receptors may reduce the activity of dopaminergic neurons^[13]. A large dose of bicuculline did not affect propofol reinforcement, which may be explained by the blockade of post-synaptic GABA_A receptors. The present results are consistent with previous reports that the disinhibition of GABA_A receptors triggers drug-evoked synaptic plasticity in excitatory afferents that synapse on dopaminergic neurons, which underlies drug reinforcement^[7, 14].

The capacity of GABA_B receptors to modulate the DA projection from the VTA to the NAc has been the subject of much research. GABA_B receptor agonists reduce the activity of the mesolimbic DA system via direct actions on dopaminergic VTA neurons^[15, 16]. The present results showed that the systemic administration of the GABA_B receptor agonist baclofen inhibited propofol self-administration in a dose-dependent manner. This result suggests that GABA_B receptors regulate the reinforcement of propofol. The results of this study are consistent with those of previous studies of other addictive drugs^[17–20]. For example, baclofen inhibits morphine and heroin self-administration in rats^[10, 21]. Additionally, baclofen pretreatment antagonizes the morphine-induced increase in DA levels in the NAc, which is mediated by GABA_B receptors^[22]. The majority of VTA GABA_B receptors are localized on dopaminergic neurons^[23]. The present results also showed that an intra-VTA injection of baclofen reduced propofol self-administration, which is consistent with a previous report that the microinjection of baclofen into the VTA, but not into the NAc, decreases opiate reinforcement^[21, 24]. The inhibitory action of baclofen was blocked by an intra-VTA infusion of a GABA_B receptor antagonist^[21]. Moreover, the inhibitory action of systemically administered baclofen suggests that baclofen acted on GABA_B receptors on dopaminergic neurons within the VTA to inhibit propofol reinforcement.

In conclusion, the present data provided evidence that propofol maintains its reward properties partially through the activation of GABA_A receptors, but stimulation of the GABA_B receptors in the VTA may counteract the reinforcing of properties of propofol.

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Author contribution

Bo YANG, Wen-hua ZHOU, and Qing-quan LIAN designed the studies; Bo YANG, Ben-fu WANG, Miao-jun LAI, Fu-qiang ZHANG, and Xiao-wei YANG performed the studies; Bo YANG, and Wen-hua ZHOU analyzed the data; Bo YANG, Wen-hua ZHOU, and Qing-quan LIAN wrote the manuscript.

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