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

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. The abuse potential of propofol has also been demonstrated in preclinical studies using conditioned place preference (CPP) and self-administration, 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. 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 receptors increases the activity of dopamine neurons in the VTA through disinhibition. In contrast, GABAergic neurons dampen DA neurons via inhibitory GABA_B receptors on dopaminergic VTA neurons. 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. 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...
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[10]. 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, Bionalytical System Inc, West Lafayette, IN, USA) in a volume of 0.5 µL/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, which ever 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 group) 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).

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).
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 and inactive responses $[F(3, 24)=1.27, P>0.05]$ 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 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.
In the present study, a marked increase in dopamine neuron activity was observed in the VTA. The activation of pre-synaptic GABA<sub>A</sub> receptors is involved in this process. Both pre-synaptic and post-synaptic GABA<sub>A</sub> receptors exist in the VTA. The activity of pre-synaptic GABA<sub>A</sub> receptors leads to a decrease in GABA release from GABAergic interneurons and a subsequent increase in dopaminergic neurons. However, the activation of post-synaptic GABA<sub>A</sub> receptors may reduce the activity of dopaminergic neurons. A large dose of bicuculline did not affect propofol reinforcement, which may be explained by the blockade of post-synaptic GABA<sub>A</sub> receptors. The present results are consistent with previous reports that the disinhibition of GABA<sub>A</sub> receptors triggers drug-evoked synaptic plasticity in excitatory afferents that synapse on dopaminergic neurons, which underlies drug reinforcement.

The capacity of GABA<sub>B</sub> receptors to modulate the DA projection from the VTA to the NAc has been the subject of much research. GABA<sub>B</sub> receptor agonists reduce the activity of the mesolimbic DA system via direct actions on dopaminergic VTA neurons. The present results showed that the systemic administration of the GABA<sub>B</sub> receptor agonist baclofen inhibited propofol self-administration in a dose-dependent manner. This result suggests that GABA<sub>B</sub> receptors regulate the reinforcement of propofol. The results of this study are consistent with those of previous studies of other addictive drugs. For example, baclofen inhibits morphine and heroin self-administration in rats. Additionally, baclofen pretreatment antagonizes the morphine-induced increase in DA levels in the NAc, which is mediated by GABA<sub>B</sub> receptors. The majority of VTA GABA<sub>B</sub> receptors are localized on dopaminergic neurons. 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. The inhibitory action of baclofen was blocked by an intra-VTA infusion of a GABA<sub>B</sub> receptor antagonist. Moreover, the inhibitory action of systemically administered baclofen suggests that baclofen acts on GABA<sub>B</sub> 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<sub>A</sub> receptors, but stimulation of the GABA<sub>B</sub> 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.

References
1. Roussin A, Montastruc JL, Lapeyre-Mestre M. Pharmacological and clinical evidences on the potential for abuse and dependence of propofol: a review of the literature. Fundam Clin Pharmacol 2007; 21:
2. Pain L, Oberling P, Sandner G, Di Scala G. Effect of propofol on affective state as assessed by place conditioning paradigm in rats. Anesthesiology 1996; 85: 121–8.

3. Pain L, Oberling P, Sandner G, Di Scala G. Effect of midazolam on propofol-induced positive affective state assessed by place conditioning in rats. Anesthesiology 1997; 87: 935–43.

4. LeSage MG, Stafford D, Glowa JR. Abuse liability of the anesthetic propofol: self-administration of propofol in rats under fixed-ratio schedules of drug delivery. Psychopharmacology (Berl) 2000; 153: 148–54.

5. Wise RA. Dopamine, learning and motivation. Nat Rev Neurosci 2004; 5: 483–94.

6. Pain L, Gobaille S, Schleef C, Aunis D, Oberling P. In vivo dopamine measurements in the nucleus accumbens after nonanesthetic and anesthetic doses of propofol in rats. Anesth Analg 2002; 95: 915–9.

7. Tan KR, Brown M, Labouèbe G, Yvon C, Creton C, Fritschy JM, et al. Neural bases for addictive properties of benzodiazepines. Nature 2010; 463: 769–74.

8. Xi ZX, Stein EA. Nucleus accumbens dopamine release modulation by mesolimbic GABA<sub>A</sub> receptors--an in vivo electrochemical study. Brain Research 1998;798: 156–65.

9. Cousins MS, Roberts DC, de Wit H. GABA<sub>B</sub> receptor agonists for the treatment of drug addiction: a review of recent findings. Drug Alcohol Depend 2002; 65: 209–20.

10. Yoon SS, Lee BH, Kim HS, Choi KH, Yun J, Jang EY, et al. Potential roles of GABA receptors in morphine self-administration in rats. Neurosci Lett 2007; 428: 33–7.

11. Zhou W, Liu H, Zhang F, Tang S, Zhu H, Lai M, et al. Role of acetylcholine transmission in nucleus accumbens and ventral tegmental area in heroin-seeking induced by conditioned cues. Neuroscience 2007; 144: 1209–18.

12. Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 1992; 12: 483–8.

13. McBride WJ, Murphy JM, Ikemoto S. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 1999; 101: 129–52.

14. Laviolette, SR, Gallegos RA, Henriksen SJ, van der Kooy D. Opiate state controls bi-directional reward signaling via GABA<sub>B</sub> receptors in the ventral tegmental area. Nature Neurosci 2004; 7: 160–9.

15. Panagis G, Kastellakis A. The effects of ventral tegmental administration of GABA<sub>A</sub>, GABA<sub>B</sub>, NMDA and AMPA receptor agonists on ventral pallidum self-stimulation. Behav Brain Res 2002; 131: 115–23.

16. Westerink BH, Enrico P, Feimann J, De Vries JB. The pharmacology of mesocortical dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and prefrontal cortex of the rat brain. J Pharmacol Exp Ther 1998; 285: 143–54.

17. Roberts DC, Andrews MM. Baclofen suppression of cocaine self-administration: demonstration using a discrete trials procedure. Psychopharmacology (Berl) 1997; 131: 271–7.

18. Shoabi M, Swanner LS, Beyer CE, Goldberg SR, Schindler CW. The GABA<sub>B</sub> agonist baclofen modifies cocaine self-administration in rats. Behav Pharmacol 1998; 9: 195–206.

19. Walker BM, Koob GF. The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. Alcohol Clin Exp Res 2007; 31: 11–8.

20. Brebner K, Phelan R, Roberts DC. Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. Pharmacol Biochem Behav 2000; 66: 857–62.

21. Xi ZX, Stein EA. Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. J Pharmacol Exp Ther 1999; 290: 1369–74.

22. Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse 2003; 50: 1–6.

23. Margreta-Mitrovic M, Mitrovic I, Riley RC, Jan LY, Basbaum AI. Immunohistochemical localization of GABA<sub>B</sub> receptors in the rat central nervous system. J Com Neurol 1999; 405: 299–321.

24. Sahraei H, Amiri YA, Haeri-Rohani A, Sepehri H, Salimi SH, Pourmortabbed A, et al. Different effects of GABAergic receptors located in the ventral tegmental area on the expression of morphine-induced conditioned place preference in rat. Eur J Pharmacol 2005; 524: 95–101.