$GABA_A$ receptor partially mediated propofol-induced hyperalgesia at superspinal level and analgesia at spinal cord level in rats[1]

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ABSTRACT

AIM: To observe effects of propofol on nociceptive response at superspinal and spinal level in rats. **METHODS:** Two hundreds and fifty-eight Sprague-Dawley male rats were randomized into thirty-two groups. Propofol and bicuculline were microinjected into lateral ventricle (icv), ventrolateral periaqueductal gray (vlPAG), intrathecal (ith), and intraperitoneal (ip). The noxious responses were evaluated by hot plate and formalin test. **RESULTS:** In hot-plate test, systemic and superspinal administration of propofol (40 mg·kg^{-1} ip, 100 μg in 10 μL, icv, and 4 μg in 0.4 μL vlPAG microinjection) produced hyperalgesia (P<0.01). Hyperalgesia induced by vlPAG microinjection of propofol was significantly antagonized by 69.8 %, 71.2 %, 98.8 % at 10, 20, and 30 min by microinjection of bicuculline (10 ng in 0.4 μL, vlPAG) (P<0.01). Analgesia induced by ith propofol (100 μg·10 μL^{-1}) was antagonized about 81.3 %, 54.8 %, 80.8 %, and 97.4 % at 10, 20, 30 and 40 min by ith bicuculline (P<0.05). In formalin test, systemic and superspinal administration of propofol (40 mg·kg^{-1} ip, 4 μg in 0.4 μL, vlPAG) also produced hyperalgesia (P<0.01). The increased formalin pain scores were antagonized about 57.1 % by bicuculline (10 ng, vlPAG) (P<0.05) at 60 min after formalin injection. The decreased formalin pain scores induced by ith propofol (100 μg in 10 μL) were antagonized about 66.7 % at 30 min by ith bicuculline (P<0.05) after formalin injection. Hyperalgesia produced by ip propofol in both hot plate and formalin test could not be antagonized by vlPAG administration of bicuculline. **CONCLUSION:** $GABA_A$ receptor partly mediated propofol-induced hyperalgesia at superspinal and analgesia at spinal cord in rats.

INTRODUCTION

Propofol (Pro), an intravenous anesthetic agent, the active component is 2,6-diisopropyl phenol[1], has powerful hypnotic effects. There is controversy about its analgesic properties. Propofol can depress nociceptive transmission in the rat spinal cord *in vitro*[2,3] or *in vivo*[4,5], however, there are other reports that subhypnotic dosage of propofol can increase sensitivity to nociceptive stimulation[6-8]. The underlying mechanisms of this difference are unclear. Propofol, whose main site of action is gamma-aminobutyric acid ($GABA_A$) receptor[9,10], could directly activate $GABA_A$ receptors

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and enhance GABA-activated whole-cell currents and single-channel currents[11-13].

The periaqueductal gray matter (PAG), the region of the midbrain that surrounds the cerebral aqueduct, is involved in functions related to pain, fear, vocalization, and cardiovascular control[14]. Fifty percent of the total population of neurons is GABAergic in the midbrain[15]. GABA-mediated neuronal elements play a prominent role in the intrinsic neuronal circuitry of the PAG[16] and regulate the descending antinociceptive systems that arise from the PAG[17]. Most GABAergic neurons in the PAG are tonic active interneurons[16]. Autoradiographic study showed that $GABA_A$ receptors were dense, but $GABA_B$ receptors were scarce in the PAG[18]. The ventrolateral portion of the PAG (vlPAG) has preferential projections toward the nucleus raphe magnus, rostroventrolateral reticular nucleus, lateral paragigantocellular nucleus, and spinal cord[19]. All of those nucleuses are related with pain modulation. Activation of the vlPAG, by electrical stimulation or using excitatory amino acid or GABAergic antagonist, induces antinociception[20,21].

Spinal sensory processing and its ascending transmission are under tonic local inhibitory control that mediated partly by the inhibitory amino acids, γ-aminobutyric acid (GABA) and glycine. Intrathecal administration of GABA and $GABA_A$ receptor agonists increases the nociceptive threshold in a variety of animals[22,23] and inhibits the responses of nociceptive transmission cells to both pinch and iontophoretically applied glutamate[24].

Now that propofol can activate $GABA_A$ receptor directly and indirectly, and $GABA_A$ receptor plays a contrary role in pain modulation at spinal and superspinal level. We speculated that propofol could produce hyperalgesia at the superspinal level by inhibition of the endogenous pain descending inhibition (DI) system and direct analgesic effects at the spinal level. This hypothesis is helpful to understand the controversy about analgesic properties of propofol[2-7]. The effects of pain modulation induced by injection of propofol in the vlPAG, intracerebroventricular, or intrathecal in conscious rat had never been investigated. The present study aimed to determine 1) whether system administration of subhypnotic dosage of propofol has analgesic effects, 2) whether propofol produce the hyperalgesic and analgesic effects respectively at superspinal, particularly vlPAG, and spinal level, 3) whether $GABA_A$ receptor participated in the above effects.

MATERIALS AND METHODS

Animals This research was carried out according to guidelines of the Jiangsu Council on Animal Care. Two hundred and eighty-eight male Sprague-Dawley rats (clean grade) weighing 250-300 g were provided by Experiment Animal Center of Xuzhou Medical College. After cannula implantation, rats were housed individually in clear plastic cages in a temperature-controlled room (23 ºC) with a 12 h light/12 h dark cycle. Food and water were available *ad libitum*.

Drugs Propofol (Fresenius kabi AB. Lot BF478) 10 g/L was dissolved in intralipid (SIGMA); (+)-bicuculline (SIGMA, Lot. 61K1473) was dissolved in artificial cerebral spinal fluid (ACSF, pH 7.35-7.45) and kept at 4 ºC in a doses of 10, 25, and 100 μg/L in a light excluding vial. Fresh propofol and bicuculline solution was prepared every testing day. ACSF contained (in mmol/L) NaCl 117, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and dextrose 11.4.

Intracerebroventricular and vlPAG cannulation Rats were implanted, under pentobarbital anesthesia (50 mg/kg, ip), with a 12 mm stainless steel guide cannula (OD 0.4 mm, ID 0.3 mm) aimed stereotaxically to lateral ventricle or vlPAG. The coordinate of lateral ventricle was as follows referring to the atlas of Paxinos and Watson[25], with flat-skull position and bregma as the reference: Bregma -1.0 mm, Lateral 1.0 mm, Ventral 4.0 mm. The coordinate of vlPAG cannulation was Bregma -7.6 mm, Lateral 0.5 mm, Ventral 4.0 mm. The cannula was secured in place by dental base acrylic resin powder. A stainless steel stylet of the same length was left in place to ensure the patency of the guide cannula. Rats were recuperation at least 5 d after surgery before experiment was performed.

Intracerebral injections were made in the conscious animal. The microinjections were performed with a stainless steel injection cannula (OD 0.28 mm, and 2 mm longer than the guide cannula) introduced through the guide cannula until its tip was 2 mm below the cannula end. A volume of 0.4 μL or 10 μL was injected into vlPAG or lateral ventricle over a period of 30 s using a 0.5 μL or 25 μL microsyringe. The movement of an air bubble inside the PE-10 polyethylene tubing connecting the microsyringe to the needle confirmed drug flow. The rate of drug delivery was 0.4 μL/min (vlPAG) and 5 μL/min (icv). The injection cannula was left in place for a further 30 s to avoid reflux. At the end of the experiment, the site of cannulation was con-

firmed by injecting pontamine blue dye.

Intrathecal injection Intrathecal injection was made in the conscious animal as described by Hylden[26] and Mestre[27]. After local anesthetized with 2 % lidocaine 0.2 mL subcutaneous injection at lumbar area, the rat was handled gently and braced over a 500 mL centrifuge tube to splay the intervertebral spaces of the lumbar (L) spine. While firmly holding the rat's vertebral column, a 27G needle attached to a 25-μL microsyringe was inserted into the intervertebral space between L4 and L5. A sudden lateral movement of the tail indicated entry into the subarachnoid space. A volume of 10 μL was injected over a 30 s period and the injection cannula was left in place for a further 30 s. Kaneko and Hammond[28] reported that the dosage of 0.1 μg bicuculline ith, which did not exhibit its own behavioral effect. In our preliminary experiments, we found that intrathecal administration of a high dosage of bicuculline (0.3 μg) could result in signs of toxicity, for example, prominent repetitive tail flexing, convulsion, and twitch. So the dosage of 0.1 μg bicuculline was used in present study.

Section one: hot plate test The heat nociceptive response of rats was assessed using hot plate test in five experiments. Baseline nociceptive threshold of each rat was obtained before any drug administration. The metal plate surface maintained at (52.0±0.1) ºC. Licking the hind-paw (hot-plate latency, HPL) was nociceptive endpoint and cut-off time was 30 s. The HPL were obtained at 10, 20, 30, 40, 50, and 60 min after second injection.

Subhypnotic dosage of propofol (40 mg/kg) was injected intraperitoneally to study its system action on response of heat noxious stimulation in rats. To investigate the effects of propofol on response of heat noxious stimulation at superspinal, particularly vlPAG, propofol was administrated by icv (100 μg in 10 μL) and vlPAG (4 μg in 0.4 μL) microinjection. Intrathecal injection (100 μg in 10 μL, it) was used to assess the antinociceptive effect of propofol at spinal level. Bicuculline was injected (10 ng in 0.4 μL vlPAG, 0.1 μg in 10 μL icv, 0.1 μg in 10 μL ith) to determine the action of $GABA_A$ receptor in the above effects. The animal received firstly either bicuculline or ACSF by icv, ith, or vlPAG microinjection as injection A. Five minutes later, they received either propofol or intralipid at same or another site as injection B. Hot-plate latency (HPL) was measured at 5, 10, 20, 30, 40, 50, and 60 min after injection B.

Section two: formalin test The inflammation noxious responses were assessed using formalin test in three experiments. Formalin test was carried out in clear Plexiglas cubicles. Formalin 2.0 % 100 μL was injected into the plantar surface of one hindpaw. Pain-induced behaviors were rated as follows: 0 corresponds to normal weight bear on the injected paw, 1 to favoring, 2 to lifting with toes touching the foot at most, and 3 to licking or biting the affected paw[29,30].

To investigate the effects of propofol on response of inflammation noxious stimulation at vlPAG or spinal level, the animal received firstly injection of bicuculline (10 ng in 0.4 μL^{-1} vlPAG, 0.1 μg in 10 μL^{-1} ith) as injection A. Five minutes later, they received propofol (4 μg in 0.4 μL^{-1} vlPAG, 100 μg in 10 μL^{-1} ith) at same site as injection B. The effects of propofol system administration were investigated by 40 mg/kg ip. Bicuculline was used to determine the action of $GABA_A$ receptor in the above effects. ACSF and intralipid were used as control. Formalin was injected 5 min after injection B. Pain score were evaluated at 1, 5, 10, 20, 30, 40, 50, and 60 min after formalin injection.

Statistical analysis All data were expressed as mean±SD. The difference between baseline and postdrug was analyzed by paired *t*-test. The difference between groups was analyzed by one-way analysis of variance (ANOVA) or unpaired *t*-test as appropriate. $P<0.05$ was considered statistically significant.

RESULTS

Hyperalgesia produced by propofol microinjection into vlPAG and icv Compared to baseline, HPL was decreased by 18.0 %, 25.1 %, and 24.0 % respectively at 10, 20, and 30 min after microinjection of propofol (100 μg, icv) ($P<0.01$), but could not be antagonized by either icv or vlPAG injection of bicuculline (Fig 1A, 1B).

HPL was decreased by 23.5 %, 29.1 %, and 25.4 % respectively at 10, 20, and 30 min after microinjection of propofol (4 μg, vlPAG) compared to baseline ($P<0.01$). The above effects were antagonized by 69.8 %, 71.2 %, and 98.8 %, respectively at 10, 20, and 30 min timepoint after microinjection of bicuculline (10 ng, vlPAG) ($P<0.01$). The single injection of bicuculline (10 ng, vlPAG) did not affect the rat HPL (Fig 2A).

A biphasic behavioral response was produced after intraplantar injections of formalin. The first phase occurs about 0-10 min after the injection, and then a

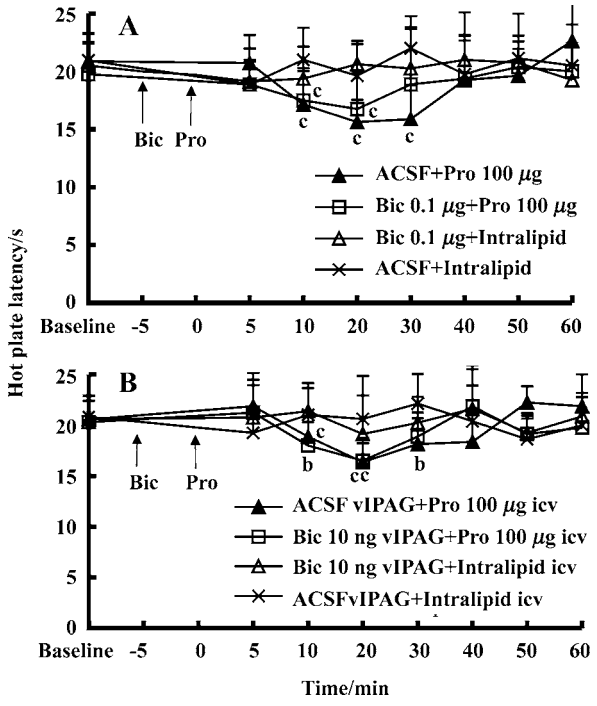

Fig 1. Time course of propofol icv and bicuculline icv (A) or vlPAG (B) microinjection in hot plate test in rats. *n*=8. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* baseline.

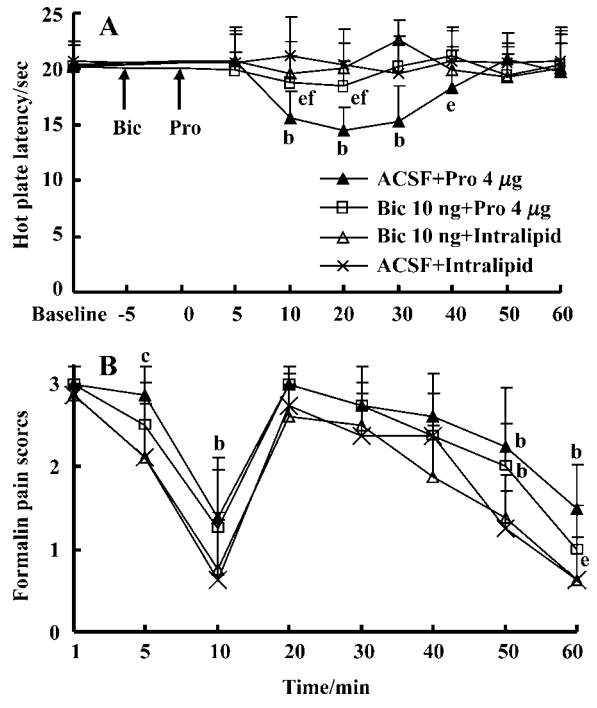

Fig 2. Effects of propofol and bicuculline vlPAG microinjection in hot plate (A) and formalin test (B) in rats. *n*=8. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* control. [e]*P*<0.05, [f]*P*<0.01 *vs* ACSF+Pro 4 μg.

second phase appeared from 20 to 60 min after a quiescent period. Formalin pain scores were increased by 35.3 %, 120.0 %, 80.0 %, and 140.0 % at 5, 10, 50, and 60 min after microinjection of propofol (4 μg, vlPAG) (*P*<0.01 *vs* control). The increased formalin pain score were antagonized by 57.1 % by bicuculline (10 ng, vlPAG) (*P*<0.05) at 60 min after formalin injection. The single injection of bicuculline (10 ng, vlPAG) did not affect the formalin pain score, which suggested that the hyperalgesia produced by propofol microinjection into vlPAG in formalin test was mainly mediated by $GABA_A$ receptor at vlPAG (Fig 2B).

Analgesia produced by propofol ith Propofol ith significantly increased the pain threshold in rats. Compared to baseline, HPL was increased by 19.6 %, 25.8 %, 31.9 %, and 23.3 % at 10, 20, 30, and 40 min after injection of propofol (100 μg, ith) (*P*<0.01). Analgesia induced by injection of propofol (100 μg, ith) was antagonized by 81.3 % (*P*<0.05), 54.8 % (*P*<0.05), 80.8 % (*P*<0.01), and 97.4 % (*P*<0.01) at 10, 20, 30, and 40 min after injection of bicuculline (0.1 μg, ith) (Fig 3A). Injection of bicuculline (0.1 μg, ith) did not affect the rat baseline HPL.

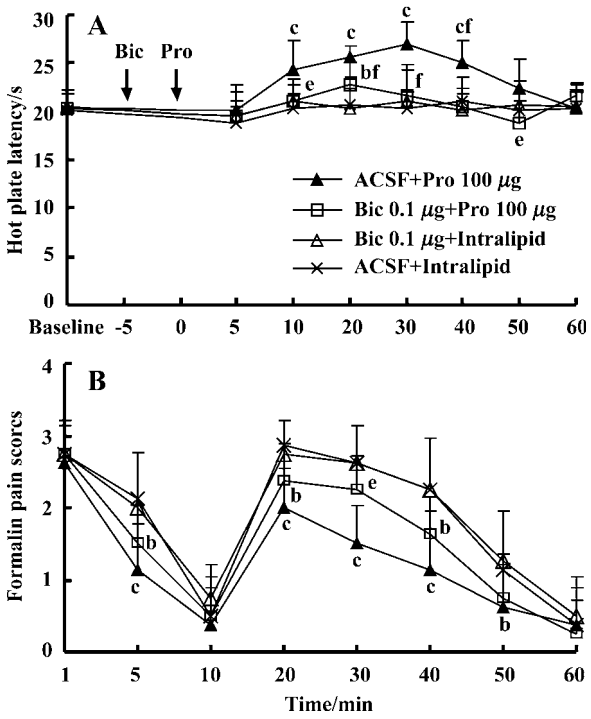

Fig 3. Effects of propofol and bicuculline ith in hot plate (A) and formalin test (B) in rats. *n*=8. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* baseline. [e]*P*<0.05, [f]*P*<0.01 *vs* ACSF+Pro 100 μg.

Formalin pain scores were decreased by 47.1 %, 30.4 %, 42.9 % and 50.0 % at 5, 20, 30 and 40 min

after microinjection of propofol (100 μg, ith) (P<0.01). The decreased formalin pain score were antagonized about 66.7 % by bicuculline (0.1 μg, ith) (P<0.01) at 30 min after formalin injection. The single injection of bicuculline (0.1 μg, ith) did not affect the formalin pain score. These results suggested that the analgesia produced by propofol ith in formalin test was mainly mediated by $GABA_A$ receptor at spinal cord. (Fig 3B).

Effects of systemic administration of propofol Systemic administration of propofol (40 mg·kg^{-1}, ip) induced hyperalgesia (P<0.01). Compared to baseline, HPL was decreased by 26.5 %, 40.1 %, 42.6 %, and 30.2 % at 10, 20, 30, and 40 min after propofol ip (P <0.01). Microinjection of bicuculline (10 ng, vlPAG) could not antagonize the hyperalgesia induced by systemic administration (ip) of propofol (Fig 4A). In our preliminary experiments, we found that hyperalgesia could not be detected in the high doses of propofol (>40 mg/kg)-induced sedative rats.

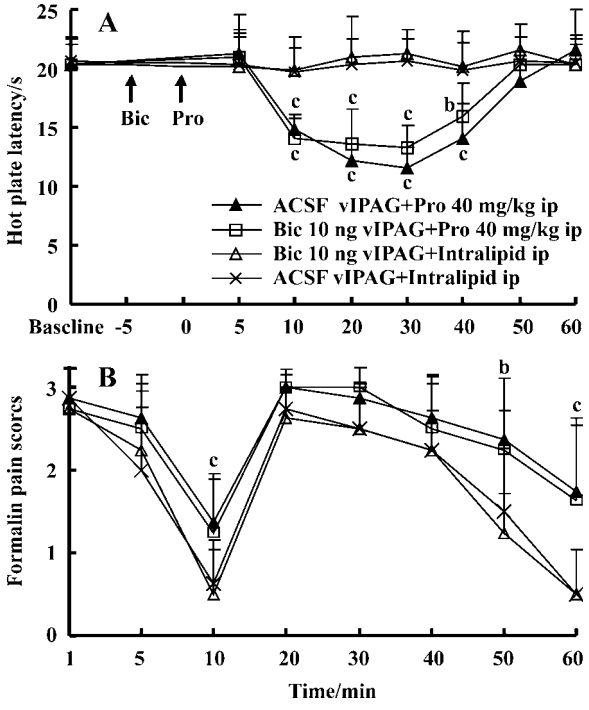

Fig 4. Effects of propofol ip and bicuculline vlPAG microinjection in hot plate (A) and formalin test (B) in rats. n=8. Mean±SD. ^bP<0.05, ^cP<0.01 *vs* baseline.

Systemic administration of propofol (40 mg/kg, ip) also induced hyperalgesia in formalin test. Compared to control group, formalin pain scores were increased by 31.3 % (P<0.05), 120.0 % (P<0.01), 58.3 % (P<0.05), and 250.0 % (P<0.01) at 5, 10, 50, and 60 min after intraperitoneal administration of propofol (40 mg/kg, vlPAG). The increased formalin pain scores cannot be antagonized by bicuculline (10 ng, vlPAG) at any time point after formalin injection. These results suggested that $GABA_A$ receptor at vlPAG did not mediate the hyperalgesia induced by systemic administration of propofol (40 mg/kg, ip) (Fig 4B).

DISCUSSION

In agreement with Ewen and Petersen-Felix's reports[6-8], we found that intraperitoneal administration of subhypnotic dosage propofol produced significantly hyperalgesia assessed by the hot plate test and formalin test in conscious rats. Further, we found that propofol icv and vlPAG microinjection also produced significantly hyperalgesia. The hyperalgesia produced by propofol vlPAG microinjection could be partly antagonized by microinjection of bicuculline at same site. But the hyperalgesia induced by injection of propofol at intracerebroventricular and intraperitoneal could not be antagonized by injection of bicuculline at neither intracerebroventricular nor vlPAG.

The cause of the hyperalgesia may be the inhibition by propofol on the endogenous pain descending inhibition (DI) system at superspinal level. Endogenous pain modulation system mainly consisted of hypothalamus, parabrachial nucleus (PBN), nucleus tractus solitarius (NTS), rostroventromedial medulla (RVM), dorsal reticular nucleus of the medulla, and periaqueductal gray (PAG). PAG and RVM play a more important role in pain modulation. A sheer abundance and ubiquity GABAergic neurons at the superspinal level contribute to pro- and anti-nociceptive effects. Endogenous descending inhibition system derived from or relay at PAG and RVM are under tonic inhibitory control of GABAergic neurons. Application of $GABA_A$ receptor blocker, bicuculline, into both the ventromedial medulla[21,31] and the PAG[32-34] can disinhibit, activate neurons and produce antinociception. In contrast, inhibition of neurons in the ventromedial medulla[33,35] or vlPAG[36,37] by microinjection of the $GABA_A$ agonist muscimol or THIP can produce hyperalgesia. The propofol-induced hyperalgesia may result from activation of $GABA_A$ receptors or increase of GABA-activated currents[11-13] on DI system at the superspinal level, particularly at vlPAG.

Bicuculline is a competitive antagonist of $GABA_A$ receptor, the behavioral effects of bicuculline administration can only be due to reversal of GABA-mediated

inhibition. In other word, the dimension of the observed behavioral effect of bicuculline should be proportional to the occupancy of $GABA_A$ receptors by GABA. Bicuculline 10 ng vlPAG microinjection had no significant effects on pain threshold baseline in rats, but can significantly antagonize the hyperalgesic effects of propofol vlPAG microinjection. It suggested that the hyperalgesia induced by injection of propofol at vlPAG was at largely mediated by $GABA_A$ receptor.

Why bicuculline cannot antagonize the hyperalgesia of propofol ip and icv? We supposed that administration of propofol by ip and icv also affected $GABA_A$ receptors in other regions (eg RVM) of the DI system except PAG. The difference of solubility and diffusivity between propofol[38] and bicuculline and the complicated interaction between nucleuses[39] could contribute to the failure of antagonism. It was also possible that other transmitter/receptor systems were involved in propofol-induced hyperalgesia.

Several studies reported that system administration of subhypnotic dosage of propofol produced hyperalgesia effects[6,7,37] and large dosage, which made loss of right reflex[4,5], produced analgesia effects. We considered that the low concentration of propofol (presumably subhypnotic dosage) preferential activated the $GABA_A$ receptor in the endogenous pain DI system at superspinal level, thus produced hyperalgesia. The large concentration of propofol (presumably anesthesia dosage) depressed ascending nociceptive signal from DH, thus produced analgesia.

$GABA_A$ receptor is enriched in the spinal dorsal horn (DH), especially in superficial laminae, wherein they are localized on the terminals of small and large diameter primary afferent fibres (PAFs)[40]. In addition, they occur on intrinsic DH neurons, including the projection neurons (PNs)[41]. GABAergic inhibitory interneurons (ININs) play a critical tonically inhibitory role in antinociceptive processes in the spinal cord[42]. Intrathecal administration of GABA or $GABA_A$ receptor agonists can produce analgesia effects at variety of animals[22-24]. Propofol could depress nociceptive transmission in the rat spinal cord *in vitro*[2,3] or *in vivo*[4,5] in previous study. In present study, our results demonstrate that intrathecal injection of propofol in conscious rats could produce the analgesic effects, which could be partially antagonized by bicuculline. It suggested that $GABA_A$ receptor mediated the analgesic effects of propofol at spinal cord level.

In conclusion, $GABA_A$ receptor partly mediated propofol-induced hyperalgesia at superspinal and analgesia at spinal cord in rats.

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