Relationship between gamma-hydroxybutyric acid receptors and the hypnotic and analgesic effects of emulsified inhalation anesthetics

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Abstract. – **OBJECTIVES:** This study was designed to investigate the role of gamma-hy-droxybutyric acid receptors (GHBR) in hypnosis and analgesia induced by emulsified inhalation anesthetics.

MATERIALS AND METHODS: After having established the mice model of hypnosis and analgesia by intraperitoneal injections of appropriate doses of enflurane, isoflurane, or sevoflurane, we intracerebroventricularly (ICV) or intrathecally injected different doses of NCS-382 (antagonist of GHBR) and, then, observed the effects on the sleeping time using awaken test and the pain threshold in hot-plate test (HPPT) using HPPT.

RESULTS: In the awaken test, 1, 5, and 25 µg of NCS-382 (ICV) significantly decreased the sleeping time of the mice treated with the three emulsified inhalation anesthetics mentioned above ($p < 0.05$ or 0.01). In the HPPT, 1, 5, and 25 µg of NCS-382 (intrathecally) did not affect the HPPT in conscious mice ($p > 0.05$); in contrast, 1, 5, and 25 µg of NCS-382 (intrathecally) significantly decreased the HPPT of the mice treated with emulsified inhalation anesthetics ($p < 0.05$ or 0.01).

CONCLUSIONS: The data presented in this study suggest that GHBR may be important targets for the hypnotic and analgesic effects induced by emulsified enflurane, isoflurane, and sevoflurane.

Key Words:
 Analgesia, Hypnosis, Inhalation anesthetics, Gamma-hydroxybutyric acid receptors.

Introduction

Because of their induction and wake up quickly, and anesthesia controllability, inhalation anesthetics are widely used in clinical trials, but their exact mechanisms of action still remain unknown[1,2]. In recent years, ligand-gated ion channels have emerged as the most promising molecular targets for inhalation anesthetics[3]. Various _in vitro_ studies have reported that enhancement of inhibitory neurotransmitter function or inhibition of excitatory neurotransmitter function or both are plausible mechanisms of anesthesia[4-7]. However, neither a specific nor a unified mechanism of action has been described. Gamma-hydroxybutyric acid receptors (GHBR) are founded in recent years and exist in central nervous system, With GHBR, specific combination can play a central inhibition effect. The study found that sodium oxybate has certain protective effect for rats' ischemia-reperfusion injury, which brain protection is related with GHBR[8]. Many studies have shown that inhalation anesthetics have hypnotic and analgesic properties and their analgesic properties are mediated by the spinal cord[9,10]. But, the relationship between GHBR and the hypnotic and analgesic effects of emulsified inhalation anesthetics is not fully understood.

In this study, we tested the hypothesis that GHBR may contribute to the hypnotic and analgesic effects of emulsified inhalation anesthetics. 6,7,8,9-tetrahydro-5-hydroxy-5H-benzocyclohept-6-ylideneacetic acid (NCS-382), which was the specificity antagonists of GHBR, was injected intracerebroventricularly (ICV) or intrathecally to examine the effect of GHBR on the hypnotic and analgesic effects of emulsified enflurane, isoflurane, and sevoflurane in a behavioral study.

Materials and Methods

Animals

This research was carried out according to the guidelines of the Jiangsu Council on Animal Care. Kunming mice (22 ± 3 g, 6-8 weeks, Certificate No. SCXK-SU-2007-0037) were obtained from the Experimental Animal Center of Xuzhou Medical College. Male and female mice

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were used in the awaken test. Female mice were used in the hot-plate test (HPPT). Mice were housed in a 12-h light: dark cycle at room temperature (22 ± 2°C). Food and water were given ad libitum. All experiments were performed at the same time between 8:00 and 12:00 AM to avoid diurnal variation in the behavioral tests.

Formulation of emulsified inhalation anesthetics

Emulsified inhalation anesthetics were made as described by Chiari et al[11].

Enflurane, isoflurane, and sevoflurane were dissolved in soy bean oil containing dispersed egg lecithin, which comprised the oil phase of the emulsion. Dissolution of egg lecithin in soy bean oil was facilitated with heat. After dissolution of lecithin, the oil phase was, then, cooled to approximately 10°C before addition of the inhalation anesthetic. The aqueous phase of the emulsion contained glycerin dissolved in water. The aqueous phase was cooled to a temperature similar to that of the oil phase (10°C). The oil phase was, then, added to the aqueous phase, while it was stirred vigorously to form the primary emulsion. The primary emulsion was homogenized at high pressure to form the final emulsion. After homogenization, the inhalation anesthetic emulsion was stored in glass vials, capped, and refrigerated (2-5°C) until use. Vials of the emulsion were warmed to 37°C for 2 h before administration. Our target concentration of anesthetics in emulsion was about 10%. After warming, the emulsion for 2 h at 37°C, the actual concentrations of enflurane, isoflurane, and sevoflurane in the emulsion were 9.7%, 10.1%, and 9.6%, respectively, and were determined by gas chromatography.

Intracerebroventricular injection

Intracerebroventricular injections were carried out into the left lateral ventricle of mice. Injections were performed using a Hamilton microsyringe fitted with a 26-gauge intracerebroventricular (ICV) needle, according to the method of Haley and McCormick[12]. The site of injection was 2-mm caudal and 2-mm lateral to the bregma, and 3 mm in depth from the skull surface. The solution was injected in a volume of 5 μL in 5 s.

Intrathecal injection

Intrathecal injections were performed free hand between spinal L5 and L6 segments according to the method of Hylden and Wilcox[13]. The intrathe-cal location of the needle tip was confirmed by a characteristic flick of the tail. The solution was injected in a volume of 5 μL in 5 s. Lidocaine (2%) 5 μL was injected intrathecally in 10 mice, which immediately exhibited hind limb paralysis that lasted about 10 min in our pilot experiments.

Awaken test

According to our pilot experiment, hypnotic doses of emulsified enflurane (22 mL/kg), isoflurane (12 mL/kg), sevoflurane (50 mL/kg) were injected intraperitoneally to establish the mouse model of hypnosis. One hundred and twenty Kunming mice (male or female) were divided randomly into 12 groups (n = 10): the emulsified intraperitoneal enflurane, isoflurane, sevoflurane + aCSF groups and the emulsified intraperitoneal enflurane, isoflurane, sevoflurane + NCS-382 (1, 5, and 25 μg) groups.
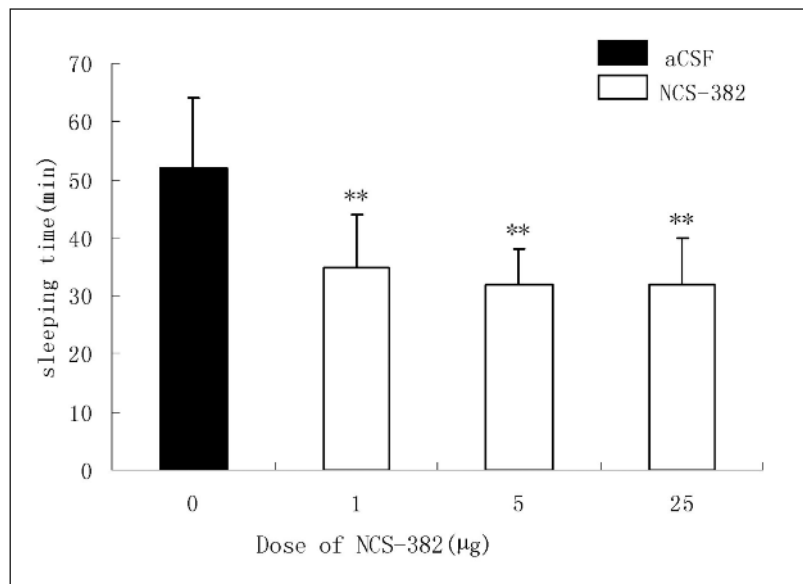
Every group was injected intraperitoneally with hypnotic doses of emulsified inhalation anesthetics and 1 min after righting reflex loss, each group was injected ICV with a CSF or different doses of NCS-382. Sleeping times (duration of the loss of righting reflex) were observed.

Hot-plate test

A homeothermic water box was heated to 55 ± 0.5°C, and then mice were placed onto the hot-plate. To avoid scalding of male reproductive organs and its possible influence on the experimental results, females were preferred. The latency to licking the hind paw was recorded as the pain threshold in HPPT of mice. All mice were tested twice at 5-min intervals and the mean value was considered as the basal pain threshold (basal HPPT) before any drug administration. The latency between 5 and 30 s was qualified and the cutoff time was 60s to avoid tissue damage. According to our pilot experiment, analgesic doses of emulsified enflurane (8 mL/kg), isoflurane (5 mL/kg), and sevoflurane (20 mL/kg) were injected intraperitoneally to establish the mouse model of analgesia. All three emulsified inhalation anesthetics exhibited a time-dependent anti-nociceptive effect that was evidenced by increased response latencies at about 10 min after intraperitoneal drug administration.

A total of 160 female Kunming mice were divided randomly into 16 groups (n = 10): the aCSF group; the NCS-382 (1, 5, and 25 μg) groups; the emulsified intraperitoneal enflurane, isoflurane, sevoflurane + aCSF groups and the emulsified intraperitoneal enflurane, isoflurane, sevoflurane +

Figure 1. Effects of NCS-382 on sleeping time in isoflurane-treated mice. NCS-382 (1, 5, and 25 μg) was injected ICV. Data are expressed as the mean ± SD, $n = 10$. Multiple group comparisons were performed by ANOVA, $^*p < 0.05$, $^{**}p < 0.01$ versus aCSF group.

NCS-382 (1, 5, and 25 μg) groups. The aCSF and NCS-382 groups were injected intrathecally with aCSF or different doses of NCS-382, respectively. The emulsified enflurane, isoflurane, and sevoflurane intraperitoneal groups were injected intraperitoneally with analgesic doses of emulsified inhalation anesthetics, and after 5 min, each group was injected intrathecally with aCSF or different doses of NCS-382. The basal HPPT and the HPPT at 5, 10, 15, 20, and 25 min after intrathecal injection of drugs were observed.

Statistical analysis

Results are expressed as the mean ± SD. The results obtained were statistically treated by applying the SPSS version 16.0 (SPSS, Chicago, IL, USA). Multiple group comparisons were performed by ANOVA followed by the Scheffe test. In HPPT, the difference between baseline and post-drug was analyzed using paired *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

Effects on sleep

Intracerebroventricular injection of NCS-382 (1, 5, and 25 μg) significantly decreased the sleeping time of the mice treated with emulsified enflurane, isoflurane, and sevoflurane ($p < 0.05$ and 0.01; Figures 1-3).

Figure 2. Effects of NCS-382 on sleeping time in enflurane-treated mice. NCS-382 (1, 5, and 25 μg) was injected ICV. Data are expressed as the mean ± SD, $n = 10$. Multiple group comparisons were performed by ANOVA, $^*p < 0.05$, $^{**}p < 0.01$ versus aCSF group.
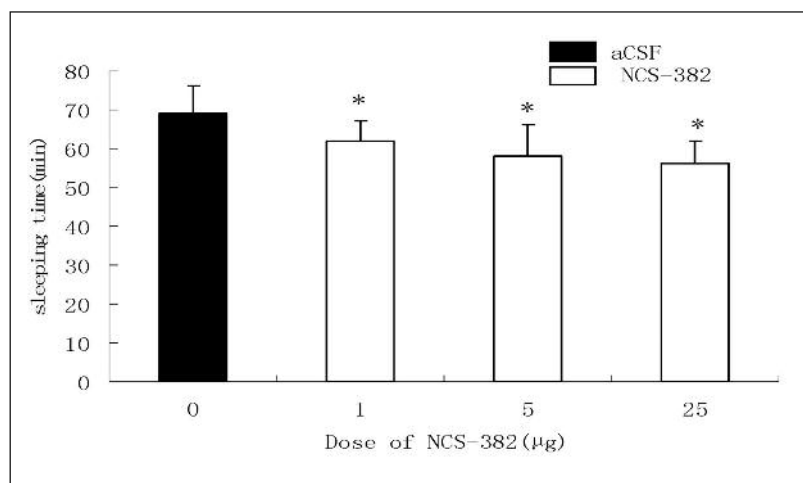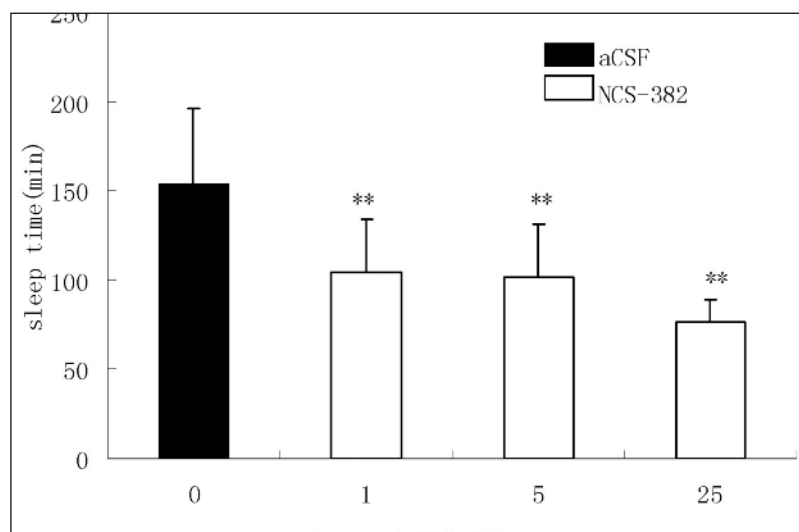
Figure 3. Effects of NCS-382 on sleeping time in sevoflurane-treated mice. NCS-382 (1, 5, and 25 µg) was injected ICV. Data are expressed as the mean ± SD, $n = 10$. Multiple group comparisons were performed by ANOVA, $^*p < 0.05$, $^{**}p < 0.01$ versus aCSF group.

Effects on hot-plate

The NCS-382 (1, 5, and 25 µg) groups exhibited no effects on HPPT compared with baseline values and aCSF groups ($p > 0.05$; Figure 4). All the three intraperitoneal emulsified inhalation anesthetics significantly increased the HPPT ($p < 0.01$) compared with baseline, and the increase of HPPT induced by three emulsified inhalation anesthetics can be abolished by intrathecally injected NCS-382 (Figures 5-7).

Discussion

Volatile anesthetics induce a wide spectrum of clinical effects such as unconsciousness, amnesia, analgesia, muscle relaxation, attenuation of protective reflex, and hemodynamic suppression. These diverse effects could reflect an integration of separate pharmacological actions of anesthetics[14]. Although intravenous (i.v.) injection of liquid volatile anesthetics is invariable fatal[15,16], studies in a variety of animals have shown that i.v. administration of lipid emulsions of isoflurane or halothane are safe and effective for induction of anesthesia[17-19]. A new formulation of volatile anesthetics has been developed that incorporates emulsification of these drugs into a lipid vehicle and, thus, facilitates their administration *in vivo*. Such a preparation may be clinically useful for anesthetic induction in the future[11].
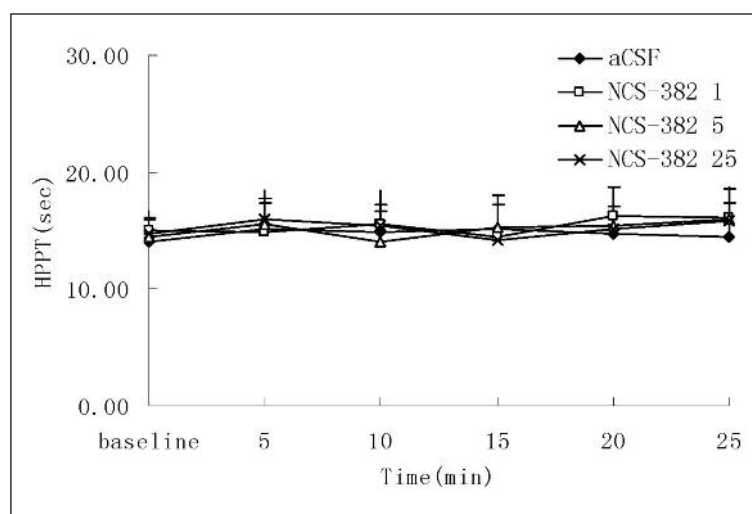

Figure 4. Effect of NCS-382 on the pain threshold in HPPT in conscious mice. NCS-382 (1, 5, and 25 µg) was injected intrathecally. Data are expressed as the mean ± SD, $n = 10$.

Figure 5. Effect of NCS-382 on the pain threshold in HPPT in isoflurane-treated mice. NCS-382 (1, 5, and 25 μg) was injected intrathecally. Data are expressed as the mean ± SD, $n = 10$. $^*p < 0.05$, $^{**}p < 0.01$ versus aCSF group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus basal HPPT.
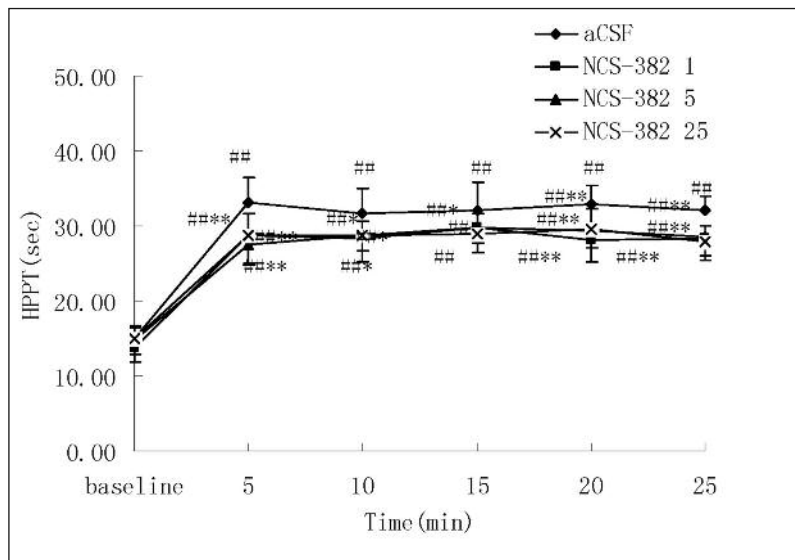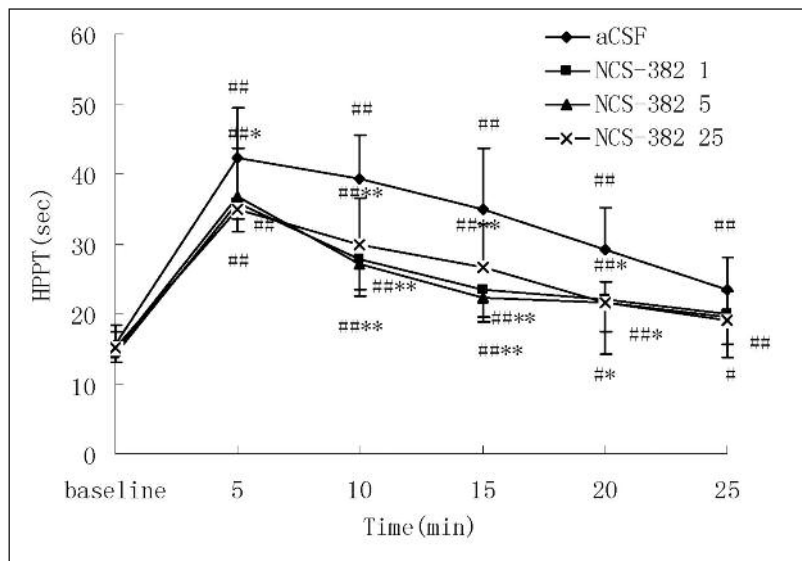
Figure 6. Effect of NCS-382 on the pain threshold in HPPT sevoflurane-treated mice. NCS-382 (1, 5, and 25 μg) was injected intrathecally. Data are expressed as the mean ± SD, $n = 10$. $^*p < 0.05$, $^{**}p < 0.01$ versus aCSF group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus basal HPPT.

Figure 7. Effect of NCS-382 on the pain threshold in HPPT enflurane-treated mice. NCS-382 (1, 5, and 25 μg) was injected intrathecally. Data are expressed as the mean ± SD, $n = 10$. $^*p < 0.05$, $^{**}p < 0.01$ versus aCSF group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus basal HPPT.
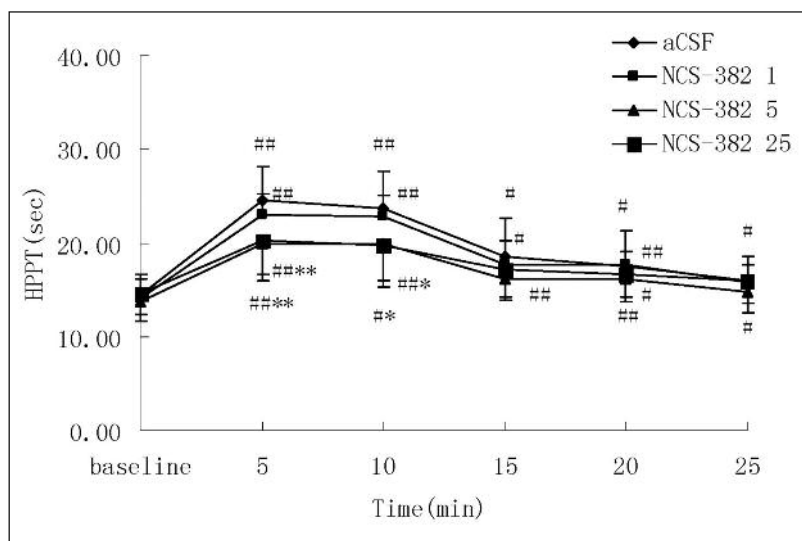
Inhalation anesthetics were often administrated through airway. It needs many specific types of equipment, and some experiments like HPPT cannot be done in case of inhalation. In awaken test, hypnotic doses of emulsified inhalation anesthetics can cause loss of righting reflex in mice, but hardly influence their breath and circulation function. In HPPT, we chose the doses of sub-anesthesia intraperitoneal injection to establish the analgesic model. The mice in such a model not only showed the effects of analgesia, but also had almost normal behavior and had no loss of the righting reflex. Therefore, the analgesia model can ultimately exclude the sedative effects. There are some differences between inhalation and intraperitoneal injection, for example, sevoflurane is known to exhibit rapid onset and recovery in case of inhalation, but the sleeping time and time course of analgesia of sevoflurane are much longer than those of other anesthetics. This is because the onset and recovery velocity are decided by absorption velocity of anesthetics in case of intraperitoneal injection and sevoflurane may be absorbed more slowly than other anesthetics, and this also can explain why the dose of sevoflurane applied to mice was four times that of isoflurane although the ratio of minimal alveolar concentration (MAC) between isoflurane (1.15% in humans) and sevoflurane (1.71%) is about 1.5.

In Awaken test, NCS-382 1, 5, and 25 μg (ICV) can significantly decrease the ST of the mice treated with isoflurane, enflurane, or sevoflurane ($p < 0.05$ or $p < 0.01$). This suggests that NCS-382 may be one of the important targets for the hypnotic effects of isoflurane, enflurane, and sevoflurane.

In HPPT, NCS-382 1, 5, and 25 μg (it) did not affect the HPPT in conscious mice ($p > 0.05$), in contrast, NCS-382 1, 5, and 25 μg (it) can significantly decrease the HPPT of the mice treated with isoflurane, enflurane, or sevoflurane ($p < 0.05$ or $p < 0.01$). This suggests that GHBR may be one of the important targets for the analgesic effects on thermal-induced nociception of isoflurane, enflurane, and sevoflurane.

Conclusions

We conclude that GHB may be an important target for the hypnotic and analgesic effects of emulsified inhalation anesthetics. Further research is needed for the elucidation of post-receptor mechanisms.

Conflict of interest

The Authors declare that they have no conflict of interests.

References

1) MASHOUR GA, FOMAN SA, CAMPAGNA JA. Mechanisms of general anaesthesia: from molecules to mind. Best Pract Res Clin Anaesthesiol 2005; 19: 349-364.

2) CAMPAGNA JA, MILLER KW, FOMAN SA. Mechanisms of actions of inhaled anesthetics. N Engl J Med 2003; 348: 2110-2124.

3) HEMMINGS HC JR, AKABAS MH, GOLDSTEIN PA, TRUDELL JR, ORSER BA, HARRISON NL. Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci 2005; 26: 503-510.

4) WESTPHALEN RI, HEMMINGS HC JR. Volatile anesthetic effects on glutamate versus GABA release from isolated rat cortical nerve terminals: basal release. J Pharmacol Exp Ther 2006; 316: 208-215.

5) WU XS, SUN JY, EVERS AS, CROWDER M, WU LG. Isoflurane inhibits transmitter release and the presynaptic action potential. Anesthesiology 2004; 100: 663-670.

6) HASENEDER R, KURZ J, DODT HU, KOCHS E, ZIEGLGANSBERGER W, SCHELLER M, RAMMES G, HAPFELMEIER G. Isoflurane reduces glutamatergic transmission in neurons in the spinal cord superficial dorsal horn: evidence for a presynaptic site of an analgesic action. Anesth Analg 2004; 98: 1718-1723.

7) MATUTE E, RIVERA-ARCONADA I, LOPEZ-GARCIA JA. Effects of propofol and sevoflurane on the excitability of rat spinal motoneurones and nociceptive reflexes in vitro. Br J Anaesth 2004; 93: 422-427.

8) GU SL, YOU WB, MA X, PENG B, DAI TJ. Effect of gamma-hydroxybutyric acid receptor on protection of hypoxia-reoxygenation injured hippocampus in rats by sodium oxybate. Chin J Pharmacol Toxicol 2006; 20: 91-95.

9) RAMPIL IJ. Anesthetic potency is not altered after hypothermic spinal cord transection in rats. Anesthesiology 1994; 80: 606-610.

10) SPADAVECCHIA C, LEVIONNOIS O, KRONEN PW, LEANDRI M, SPADAVECCHIA L, SCHATZMANN U. Evaluation of administration of isoflurane at approximately the minimum alveolar concentration on depression of a nociceptive withdrawal reflex evoked by transcutaneous electrical stimulation in ponies. Am J Vet Res 2006; 67: 762-769.

11) CHIARI PC, PAGEL PS, TANAKA K, KROLIKOWSKI JG, LUDWIG LM, TRILLO RA JR, PURI N, KERSTEN JR, WARLTIER DC. Intravenous emulsified halogenated anesthetics produce acute and delayed preconditioning against myocardial infarction in rabbits. Anesthesiology 2004; 101: 1160-1166.

12) HALEY TJ, McCORMICK WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br J Pharmacol 1957; 12: 12-15.

13) HYLDEN JL, WILCOX GL. Intrathecal morphine in mice: a new technique. Eur J Pharmacol 1980; 67: 313-316.

14) KISSIN I. General anesthetic action: an obsolete notion? Anesth Analg 1993; 76: 215-218.

15) STEMP LI. Intravenous injection of liquid halothane. Anesth Analg 1990; 70: 568.

16) KAWAMOTO M, SUZUKI N, TAKASAKI M. Acute pulmonary edema after intravenous liquid halothane in dogs. Anesth Analg 1992; 74: 747-752.

17) YANG XL, MA HX, YANG ZB, LIU AJ, LUO NF, ZHANG WS, WANG L, JIANG XH, LI J, LIU J. Comparison of minimum alveolar concentration between intravenous isoflurane lipid emulsion and inhaled isoflurane in dogs. Anesthesiology 2006; 104: 482-487.

18) ZHOU JX, LUO NF, LIANG XM, LIU J. The efficacy and safety of intravenous emulsified isoflurane in rats. Anesth Analg 2006; 102: 129-134.

19) MUSSER JB, FONTANA JL, MONGAN PD. The anesthetic and physiologic effects of intravenous administration of halothane lipid emulsion (5% vol/vol). Anesth Analg 1999; 88: 671-675.