Original Research

Effects of intrathecal NMDA and AMPA receptors agonists or antagonists on antinociception of propofol

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KEY WORDS *N*-methyl-*D*-aspartate receptors; AMPA receptors; spinal injections; antinociception; propofol

ABSTRACT

AIM: To study the effects of intrathecal (it) agonists and antagonists of *N*-methyl-*D*-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors and NMDAR1 antisense oligodeoxynucleotides (AS ODN) on the antinociception of propofol. **METHODS:** Hot-plate test (HPPT) and acetic acid-induced writhing test were used to measure the nociceptive thresholds in mice. The effects of intrathecal NMDA, AMPA, MK-801, NBQX, or NMDAR1 AS ODN on the antinociception of propofol were observed. **RESULTS:** Propofol (25, 50 mg/kg, ip) displayed an appreciable antinociceptive effect in hot-plate test and acetic acid-induced writhing test. NMDA (12.5, 25 ng, it) or AMPA (1.25, 2.5 ng, it) exhibited no effects on the behavior but decreased HPPT significantly compared with basal HPPT and aCSF group ($P<0.05$, $P<0.01$). No effects on behavior and HPPT were obtained in NMDA (6.25 ng, it) or AMPA (0.625 ng, it) groups. NMDA (6.25, 12.5, and 25 ng, it) dose-dependently decreased the HPPT in propofol-treated group. AMPA (1.25, 2.5 ng, it) also decreased HPPT significantly. MK-801 (0.25, 0.5 μg, it) or NBQX (0.25, 0.5 μg, it) groups had no behavioral changes, two antagonists 0.5 μg but not 0.25 μg increased HPPT in conscious or propofol-treated mice. AS ODN (5, 10, and 20 μg, it) groups exhibited dose-dependent increased in HPPT in propofol-treated groups compared with aCSF group ($P<0.05$, $P<0.01$). **CONCLUSION:** Both agonists NMDA and AMPA reversed the antinociception of propofol. MK-801, NBQX, and NMDAR1 AS ODN potentiated the antinociceptive effects of propofol. Propofol produced antinociception through an interaction with spinal NMDA and AMPA receptors in mice.

INTRODUCTION

Ionotropic glutamate receptors can be broadly subdivided into *N*-methyl-*D*-aspartate (NMDA) and non-NMDA receptors and both types were involved in the nociceptive modulation in spinal cord. Previous researches had demonstrated that ionotropic glutamate receptor agonists potentiated nociceptive responses of dorsal horn neurons and induced or enhanced nociceptive behavior[1-3]. On the other hand, intrathecal injection of ionotropic glutamate receptor antagonists produced antinociceptive effects and blocked excitatory synaptic input to dorsal horn neurons[4,5]. Activation of NMDA receptor is related to the nerve lesion-induced hyperalgesia and allodynia, which depend on not only the sensory sensitivity increase of primary input but also the alteration of synaptic excitation.

Propofol (2,6-diisopropylphenol, Diprivan) has been widely used as intravenous anesthetic. Studies *in vitro* suggested that propofol-induced anesthesia was

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related to inhibiting NMDA receptor[6,7]. Many studies have also shown that propofol has analgesic properties[8-10]. It produced a significant reduction of the receptive field areas of spinal dorsal horn neurons induced by noxious and non-noxious stimuli. These results suggested that propofol might produce antinociception directly through the spinal cord[11]. But, the relationship of the propofol antinociception and spinal NMDA and non-NMDA receptors is unclear.

In the present study, we used the NMDA and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors agonists (NMDA and AMPA) and antagonists (MK-801 and NBQX) and NMDAR1 antisense oligodeoxynucleotides (AS ODN) injected intrathecally to investigate the interactions of propofol with spinal NMDA and AMPA receptor, and to explore the antinociceptive mechanisms of propofol at the spinal level.

MATERIALS AND METHODS

Animals Kunming mice (22±3 g, Grade II, Certificate No SCXK-SU-2002-0022) were obtained from the Experimental Animal Center of Xuzhou Medical College. Female mice were used in the hot-plate test. Male and female mice were used in acetic acid-induced writhing test. Mice were housed in a 12-h light/dark cycle (lighting 8:00-20:00) at room temperature 22±2 ºC. Food and water were given *ad libitum*. All experiments were performed at the same time between 8:00 and 12:00 to avoid diurnal variation in behavioral tests.

Chemicals Propofol and vehicle (10 % intralipid) were supplied by AstraZeneca Company; NMDA, AMPA, MK-801, and NBQX were purchased from ALEXIS (USA) and dissolved by artificial cerebral spinal fluid (aCSF, pH 7.0)[12]. Ice acetic acid was purchased from Beijing Chemical Factory and NMDAR1 AS ODN (sense: 5'-ATG AGC ACC ATG CAC CTG-3', antisense: 5'-CAG CAG GTG CAT GGT GCT-3', 4-21) were obtained from Shanghai BIOASIA Company and dissolved by distilled H_2O.

Hot-plate nociceptive test (HPPT)[13] The mice were intrathecally injected 5 μL aCSF, NMDAR1 sense ODN (5,10, and 20 μg), AS ODN (5,10, and 20 μg) every other day for 5 d (three injections in all). The dosing scheme of the AS ODN was chosen according to the study[15] by Rydh-Rinder M. The hot-plate test was performed on d 6 and the effects of propofol (50 mg/kg, ip) on HPPT were observed. Homeothermic water-box was heated to 55±0.5 ºC and then mice were placed onto the hot-plate. The latency to lick the hindpaw was recorded as the pain threshold of mice. All mice were tested twice at 5-min intervals and the mean value was considered as the basal pain threshold. The latency between 5 s and 30 s was qualified and a maximum of 60 s was allowed as the cut off time to avoid tissue damage.

Aceticacid-induced writhing test[13] Mice were ip administered NS or intralipid or propofol 30 min before ip injection of 0.6 % ice acetic acid 0.2 mL. We observed the number, latency and times of writhing mice within 15 min after last injection.

Intrathecal (it) injections in conscious mice[14] The intrathecal location of the needle tip was affirmed 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 intrathecally injected in 10 mice and which exhibited hindlimb paralysis immediately and lasted about 10 min in our pilot experiment.

Statistical analysis The data were expressed as mean±SD, and analyzed by one-way repeated-measure ANOVA and Student's *t* test for comparisons between groups. $P<0.05$ was considered statistically significant.

RESULTS

Effect of propofol in hot-plate test and aceticacid-induced writhing test in mice There was no significant changes in HPPT before and after it injection of 10 % intralipid compared with normal saline (NS) group. Propofol (12.5 mg/kg, ip) had no effect on the HPPT. The HPPT was dose-dependently increased in 25 and 50 mg/kg propofol groups compared with NS group ($P<0.05$, $P<0.01$, Tab 1).

No significant differences existed in the number of writhing mice, writhing latency and writhing times in intralipid group compared with NS group ($P>0.05$). The number of writhing mice had no difference in different doses of propofol groups, but the writhing times were decreased significantly with the doses of propofol increasing ($P<0.01$). The results suggested that propofol could inhibit nociception induced by 0.6 % ice acetic acid. The writhing latency was decreased significantly in different doses of propofol groups compared with NS and intralipid groups ($P<0.01$, Tab 2).

Effects of NMDA and AMPA receptor agonists and antagonists on HPPT in conscious mice NMDA (37.5-100 ng, it) or AMPA (5-10 ng, it) produced caudally directed biting and scratching behavior in mice. NMDA (12.5, 25 ng, it) groups exhibited no effects on

Tab 1. Effect of propofol on the pain threshold in hot-plate test (HPPT) in mice. *n* =12. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* NS group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* basal HPPT.

Group	Dose/ mg·kg^{-1}	HPPT/s Before treated	After treated 5 min	10 min	20 min	30 min	40 min
NS	-	21±5	20±4	20±3	21±4	22±5	21±5
Intralipid	-	22±4	21±3	19.4±2.6	20.2±2.6	22±4	22±4
Propofol	12.5	21±4	22±5	22±4	22±4	21.7±2.2	21.2±2.8
	25	22±4	26±3^{ce}	26±3^{ce}	24±3^{b}	23±3	23±4
	50	21.4±2.7	36±8^{cf}	33±7^{cf}	28±7^{bf}	25±5^{b}	22±5

Tab 2. Effect of propofol on acetic acid-induced writhing in mice. *n*=12. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* NS group.

Group	Dose/ mg·kg^{-1}	Writhing latency/min	Writhing times	Number of writhing mice
NS	_	3.8±0.9	44±10	12/12
Intralipid	_	3.9±1.0	45±10	12/12
Propofol	12.5	0.6±0.3^{c}	33±11^{b}	12/12
	25	0.4±0.4^{c}	27±9^{c}	11/12
	50	0.7±0.4^{c}	16±9^{c}	10/12

the behavior but decreased HPPT significantly compared with itself baseline and aCSF groups ($P<0.05$, $P<0.01$). AMPA (1.25, 2.5 ng, it) caused no exciting behavior but remarkably decreased HPPT ($P<0.05$, $P<0.01$). No effects on behavior and HPPT were obtained in NMDA (6.25 ng, it) or AMPA (0.625 ng, it) groups compared with itself baseline and aCSF group ($P>0.05$).

MK-801 (1-4 μg) or NBQX (1-4 μg) exhibited sedation and the muscle strength of hindlimb was decreased, MK-801 (4 μg, it) even produced hindlimb paralysis; MK-801 or NBQX (0.25, 0.5 μg, it) groups had no behavioral changes, above two antagonists (0.5 μg but not 0.25 μg) increased HPPT compared with itself baseline and aCSF group ($P<0.01$, Tab 3).

Effects of NMDA and AMPA receptor agonists and antagonists on HPPT in propofol-treated mice The mice were injected it aCSF 5 μL, NMDA, AMPA, MK-801 or NBQX at different doses 5 min after propofol (50 mg/kg, ip). NMDA (6.25, 12.5 and 25 ng, it) dose-dependently decreased the HPPT in propofol-treated

Tab 3. Effects of NMDA, AMPA, MK-801 or NBQX at different doses on the pain threshold in hot-plate test (HPPT) in conscious mice. *n*=12. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* aCSF group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* basal HPPT.

Group	Dose	HPPT/s Before treated	After treated 1 min	6 min	11 min	20 min	40 min
aCSF	-	19.9±2.4	19.8±2.2	19±3	19±3	20±4	20±5
NMDA	6.25 ng	19.3±2.5	18±3	19±3	18.7±2.5	18±2	18±3
	12.5 ng	20.6±2.6	20±3	16±3^{bf}	15±4^{bf}	18±5	20±4
	25 ng	20±5	16±3^{be}	13±4^{cf}	14±4^{cf}	17±3^{b}	20±4
AMPA	0.625 ng	21±4	18±5	20±5	20±5	22±5	24±3
	1.25 ng	22±2	22±3	16±3^{bf}	17±3^{f}	17±3^{b}	21±3
	2.5 ng	22±4	17±5^{be}	14±4^{cf}	16±5^{cf}	17.7±2.5^{be}	21±4
MK-801	0.25 μg	19.4±2.6	22±3	22±3	21±5	22±3	20±3
	0.5 μg	21±4	32±8^{cf}	33±7^{cf}	29±10^{b}	24±7	20±5
NBQX	0.25 μg	21±4	26±10	23±3	22.5±2.6	22.3±2.6	21.8±2.7
	0.5 μg	21±5	30±6^{cf}	35±6^{cf}	29±6^{bf}	24±5	20±4

group compared with aCSF group ($P<0.01$). AMPA 0.625 ng only decreased the HPPT at 6 min after intrathecal injection ($P<0.01$). AMPA (1.25, 2.5 ng, it) decreased HPPT significantly ($P<0.01$). MK-801 (0.5 μg) and NBQX (0.5 μg) significantly increased HPPT in propofol-treated group compared with aCSF group ($P<0.01$). MK-801 (0.25 μg) only increased the HPPT at 1 min and NBQX (0.25 μg) increased the HPPT significantly at 1 min and 6 min after intrathecal injection ($P<0.05$, $P<0.01$, Tab 4).

Effects of NMDAR1 AS ODN on the HPPT by hot-plate tests in propofol-treated mice No changes in behavior and HPPT were observed before and after treatment with aCSF, sense ODN (5, 10, and 20 μg, it) and 5 μg AS ODN; AS ODN (10, 20 μg, it) groups exhibited the hindlimb paralysis (4/10 in 10 μg group; 7/10 in 20 μg group) immediately after intrathecal injection and lasted for at least 6 h followed by a gradual and full recovery. No signs of impaired motor behavior could be observed on the day of the hot-plate test. Three doses of AS ODN groups exhibited dose-dependent increase in HPPT after propofol treatment compared with aCSF group ($P<0.05$, $P<0.01$, Tab 5).

DISCUSSION

In our experiment, propofol (25, 50 mg/kg, ip) displayed an appreciable antinociceptive effect in hot-plate test and acetic acid-induced writhing test. The results indicated the antinociception of propofol in two animal models. Propofol (25, 50 mg/kg, ip) caused no noticeable sedation but increased the latency to licking the hindlimb in the hot-plate test in mice. The same results were shown in a previous study by Anwar[16]. The appreciable antinociceptive effect lasted for 30 min and then returned to nociceptive threshold baseline. Propofol (12.5, 25, and 50 mg/kg, ip) dose-dependently decreased the writhing times in the acetic-acid-induced writhing test. This effect suggested that propofol also inhibited the chemical stimulus. But the writhing latency was decreased significantly in different doses of propofol groups compared with NS and intralipid groups. This phenomenon maybe related to the local pain of propofol injection itself. We have observed that ip injection of propofol itself could induce licking or abdominal contraction behavior in some mice. This contrary phenomenon of propofol in chemical assays deserves further studies.

Ionotropic glutamate receptor agonists NMDA and AMPA caused appreciable decrease of HPPT, and antagonists MK-801 or NBQX increased the HPPT significantly in the hot-plate test in conscious mice. These results were consistent with Aanonsen and Wilcox's findings[2] that NMDA might have a nociceptive action in the mouse spinal cord. Lutfy and Weber[17] have reported that antagonist of NMDA receptor ACEA-1021 had dose-dependent protection against nociceptive behaviors induced by intrathecal administration of NMDA in mice. Lutfy also suggested that inhibition of spinal non-NMDA receptors was the primary and necessary

Tab 4. Effects of NMDA, AMPA, MK-801 or NBQX at different doses on the pain threshold in hot-plate test (HPPT) in propofol-treated mice. n=12. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* aCSF group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* basal HPPT.

Group	Dose	HPPT/s Before treated	After treated 1 min	6 min	11 min	20 min	40 min
aCSF	-	21±4	32±4^{f}	34±3^{f}	29.1±2.4^{f}	27±4^{e}	23±3
NMDA	6.25 ng	22±5	24±4^{e}	25±3^{e}	24.2±2.5^{e}	25±3	22±3
	12.5 ng	21±4	18±3^{cf}	16±3^{cf}	15.7±2.3^{cf}	16.8±2.9^{cf}	18±4^{f}
	25 ng	21±4	16±6^{ce}	14±5^{cf}	15±6^{ce}	17±4^{ce}	20±4
AMPA	0.625 ng	22±3	27±7	26±3^{c}	26±4	24±4	20.6±2.2
	1.25 ng	22±4	24±3^{c}	16±5^{cf}	19±3^{ce}	21±3^{c}	22±4
	2.5 ng	21±3	16±5^{ce}	13±5^{cf}	18.4±2.8^{c}	19±4^{c}	21±5
MK-801	0.25 μg	22±4	29±8^{e}	25±6	22±6	22±7	22±6
	0.5 μg	21±4	38±9^{cf}	42±5^{cf}	35±7^{bf}	28±6^{f}	21±4
NBQX	0.25 μg	22±4	29±3^{bf}	27±7^{ce}	24±9	20±4	21±5
	0.5 μg	21±4	42±9^{cf}	45±7^{cf}	38±6^{cf}	28±5^{f}	22±4

Tab 5. Effects of NMDAR1 AS ODN on the pain threshold in hot-plate test (HPPT) in propofol-treated mice. *n*=10. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* aCSF group. [e]*P*<0.05, [f]*P*<0.01 *vs* basal HPPT; [h]*P*<0.05. [i]*P*<0.01 *vs* the HPPT after intrathecal AS ODN.

Group	Dose/μg	HPPT/s					
		Before treated	After intrathecal AS ODN	After ip propofol			
				5 min	10 min	20 min	30 min
aCSF	-	21±3	20±4	32±11[fi]	33±12[fi]	35±11[fi]	27±9[fi]
Sense	5	22±4	21.1±2.4	30±9[ei]	32±9[fi]	27±9	27±6
	10	21.2±2.7	20±5	33±8[fi]	35±10[fi]	32±9[fi]	25±8
	20	21±4	20±6	31±8[fi]	32±9[fi]	30±9[fi]	25±12
Antisense	5	21±2	22±4	29±7[bi]	25±10	27±4[cei]	25±4[b]
	10	20.1±2.4	27±6[e]	41±8[bfi]	45±8[cfi]	43±9[fi]	32±11[f]
	20	22±4	30±9[cf]	52±10[cfi]	53±8[cfi]	50±8[cfi]	37±8[bf]

mechanism of antinociception in the tail flick test in mice[18]. These studies suggested that NMDA and non-NMDA receptors in spinal cord played the important role in the antinociception of animals. We found that intrathecal injections of NMDA and AMPA caused appreciable decrease of HPPT, MK-801 and NBQX increased the HPPT significantly in propofol-treated mice, suggesting that NMDA and AMPA could reverse the antinociception of propofol, MK-801 and NBQX produced synergetic antinociceptive effects with propofol. We only used agonist or antagonist alone in our experiment, so we could not observe the interaction between the two agonists or antagonists. A detailed interactive study on these chemicals is being contemplated.

Previous studies[15] demonstrated that intrathecal injection of antisense oligodeoxynucleotids targeting the NMDAR1 produced an antinociceptive effect in mice. Pain behavior was significantly reduced by 40 % after 25 μg of antisense probes were given. The results suggested that a significant correlation existed between the reduction in pain behavior and NMDAR1 receptor binding. Intrathecal injection of different doses of NMDAR1 AS ODN all increased the HPPT significantly in conscious as well as propofol-treated mice, indicating that antinociception properties of propofol were mediated through NMDA receptor in spinal cord.

Our studies showed that propofol caused antinociception through involvement with spinal NMDA and AMPA receptors. But it is unclear whether these receptors mediate the nociceptive system in spinal cord at the same time or there are interactions among them, and whether propofol binds directly with the spinal NMDA and non-NMDA receptors or exerts an indirect action. To elucidate these questions further, experiments using propofol intrathecally injected are required. So a suitable vehicle for intrathecal injection propofol should be applied in the near future.

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