Antinociception and prevention of hyperalgesia by intrathecal administration of Ro 25-6981, a highly selective antagonist of the 2B subunit of N-methyl-D-aspartate receptor

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Background: NR2B subunits (NMDA receptor 2B subunit) play an important role in generation of pain and forming central sensitization of pain. Ro 25-6981, a highly selective NR2B antagonist, gained much attention in recent years. In this study, we used a rat model of incisional pain to investigate effects of postoperative analgesia and changes of postoperative hyperalgesia induced by remifentanil through the pretreatment of intrathecal administration with Ro 25-6981.

Methods: The behavioral changes of rats have been evaluated by the paw withdrawal mechanical threshold and paw withdrawal thermal latency after intrathecal injection of Ro 25-6981. The expression of NR2B with tyrosine phosphorylation in the spinal dorsal horn was analyzed by Western blotting.

Results: Intrathecal injection of Ro 25-6981 significantly enhanced the paw withdrawal mechanical threshold and paw withdrawal thermal latency after the operation. Significant change has been observed after intrathecal injection of 800.0 μg of Ro 25-6981 and at 2 h after operation in the oblique pull test degree and BBB rating score. Pretreatment of Ro 25-6981 decreased the high level expression of NR2B with tyrosine phosphorylation in spinal dorsal horn of the rat model after the operation.

Conclusions: Intrathecal injection of Ro 25-6981 had significant analgesic effects on incision pain in rats and effectively attenuated postoperative hyperalgesia induced by remifentanil.

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1. Introduction

N-methyl-D-aspartate receptors (NMDARs) belong to a type of glutamate receptor, which is an excitatory neurotransmitter in the central nervous system. NMDARs are composed of the principal subunit NR1, the modulatory subunit NR2A-D and subunit NR3A-B (Mori and Mishina, 1995). NR2B subunit (NMDA receptor 2B subunit) plays an important role in generation of pain and central sensitization (Woolf and Thompson, 1991). The carboxyl terminus of the NR2B subunit has seventyrosine phosphorylation sites, and Tyr-1472 is the major site. Phosphorylation of Tyr-1472 has an influence for changes of synaptic plasticity and occurrence of long-term potentiation (LTP), while LTP and opioid-induced hyperalgesia (OIH) have the same signal transduction pathway and the common pharmacological pathway (Drdla et al., 2009; Nakazawa et al., 2001). The spinal cord dorsal horn of Tyr-1472 phosphorylation in NR2B has been reported in the development of inflammatory (Gu et al., 2002) and neuropathic pain model (Abe et al., 2005) of hyperalgesia induced works. We have observed the pain behavior of rats and the analgesic effects of Ro 25-6981 in rat incisional pain and hyperalgesia model. The electrophysiological results of the rat spinal cord showed that remifentanil can enhance the function of NMDA receptors and inward electric current (Guntz et al., 2005; Zhao and Joo, 2008). The strength of opioid-induced hyperalgesia is closely related to pharmacokinetics. Noxious stimulation of rapid onset and short-acting drugs such as remifentanil is more rapidly and prominently promoted after drug discontinuance compared with long-acting opioids (Derrode et al., 2003). Remifentanil has strong analgesic effect and eliminates rapidly as a new type of short-acting opioid receptor agonist and there are no accumulation effects after remifentanil intravenous infusion (Bürkle et al., 1996). In recent years, the reports about remifentanil induced hyperalgesia attracted more attention.
Although noncompetitive NMDA receptor antagonists are widely used in a variety of pain models for analgesic effect, they have serious side effects such as hallucinations, confusion, ataxia, movement disorders and tonic stupor psychosis (Parsons, 2001; Taniguchi et al., 1997). Selective antagonist of the NR2B subunit plays an effective analgesic effect with low toxic side effects. The low toxic side effects may be caused by the limited distribution of the NR2B subunit in the central nervous system, such as the spinal cord and other parts of forebrain (Boyce et al., 1999; Laurie et al., 1997). It is necessary to find the highly selective NR2B antagonist as the effective analgesic drug. Ro 25-6981, a highly selective NR2B antagonist, has been used in some pain models such as spinal nerve ligation (Boyce et al., 1999; Fischer et al., 1997; Mutel et al., 1998). However it has not been reported as the effective analgesic drug. It is necessary to determine the effect of postoperative analgesia and the changes of the postoperative hyperalgesia which has enhanced responses to normal stimulation (allodynia). In this study, we hypothesized that Ro 25-5981 could play an important role in the rat model of incision pain or the postoperative hyperalgesia. The effect of postoperative analgesia and the changes of the postoperative hyperalgesia induced by remifentanil through the pretreatment of intrathecal administration with Ro 25-6981 were investigated in the rat model of incisional pain.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 220–250 g, provided by the Laboratory Animal Center of Drum Tower Hospital, were used in the study. The rats were housed in standard laboratory conditions with free access to food and tap water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Nanjing University and followed the guidelines for the use of laboratory animals (Zimmermann, 1983).

2.2. Surgical process

Rats were anesthetized with sevoflurane by a nose mask. The incisional surgery of rats followed the procedures described by Brennan et al. (1996) under sterile conditions. A longitudinal 1 cm incision was made through the skin, starting at 0.5 cm from the edge of the heel and extending toward the toes of the right hindpaw. Using forceps elevated the plantaris muscle, leaving the muscle origin and insertion intact. After holding the wound with gentle pressure, the skin was closed and covered with Aureomycin ointment.

2.3. Drugs and chemicals

Ro 25-6981 (Tocris Co., England), remifentanil hydrochloride (batch number: 081101, Ren Fu Co., China), and sevoflurane (batch number: 08100931, Heng Rui Co., China), and sevoflurane (batch number: 08100931, Heng Rui Co., China) (0.04 mg/kg, 0.4 ml) was dissolved in saline (NaCl 0.9%) to a volume of 0.4 ml.

According to the different doses administered, 54 SD rats were randomly divided into 9 groups (n = 6) (Table 1). Within two weeks before the experiment, the rats were placed in the test room for 2 h every day to accustom various apparatuses. The drug Ro 25-6981 was injected intrathecally before surgical incision. The detailed information of the dose was shown in Table 1. Intrathecal injections (i.t.) were made through the intervertebral space in all rats between the L4 and L5 of the spinal cord, as described by Hylden and Wilcox (1980). Ro 25-6981 (dissolved in 5% DMSO) at the dose of 25 μl was administrated i.t. with a 28-gauge 1/2-inch stainless steel needle connected to a 50 μl Hamilton microsyringe, the animal being lightly restrained to maintain the position of the needle. Puncture of the dura was indicated behaviorally by a slight flick of the tail. Because intrathecal injection of 5% DMSO solvent had no effect on the rat behavior (Qu et al., 2009), in order to maintain consistency, all the rats received intrathecal injection with 5% DMSO solvent. Rat models of incisional pain in the right back paw were prepared before intrathecal injection in all groups except group C. In group M, (R + M)1, (R + M)2 and (R + M)3, remifentanil (0.04 mg/kg, 0.4 ml) was infused subcutaneously during surgical incision with a pump for 30 min, and in group C, I, R1, R2 and R3, 0.9% saline (0.4 ml) was infused subcutaneously in identical conditions for 30 min. For behavioral studies, paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) of the rats were tested. The changes of rat behavior were measured at 24 h before intrathecal injection and at 2 h, 6 h, 24 h, and 48 h after operation (n = 6). And the motor function indexes (inclined pull test and BASSO, BEATTIE and BRESNAHAN (BBB) rating) were also examined at the same time points. According to the changes in behavioral indicators of pain, the specimens of all groups were collected at 2 h, 6 h, and 48 h after operation (n = 4) for Western blot analysis.

In this study, the double-blind method was used. The administration of drug and the measurement of rat behavior (including PWMT and PWTL) and motor function were respectively conducted by different researchers.

2.5. Assessment of mechanical allodynia

To assess mechanical allodynia, PWMT was measured by using a set of von Frey filaments. The animals were placed in plastic boxes (20 × 20 × 15 cm) with a wire mesh bottom (1 × 1 cm) and allowed to acclimatize for 30 min. Each von Frey filament was applied vertically to the plantar surface adjacent to the wound of right hindpaw for 6–8 s with sufficient force. Positive responses were defined as paw flinching or brisk withdrawal. There was a five-minute interval between withdrawal responses. The PWMT in response to a series of von Frey filaments was determined by the “up-and-down” method and the data were analyzed through the nonparametric method of Dixon, as described by Chaplan et al. (1994). The measurement of PWMT was conducted three times at each time point.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Description</th>
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<tbody>
<tr>
<td>Group C</td>
<td>No intrathecal injection or surgical incision</td>
</tr>
<tr>
<td>Group I</td>
<td>Surgical incision without Ro 25-6981</td>
</tr>
<tr>
<td>Group R1</td>
<td>Intrathecal injection of Ro 25-6981 (200.0 μg)</td>
</tr>
<tr>
<td>Group R2</td>
<td>Intrathecal injection of Ro 25-6981 (400.0 μg)</td>
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<tr>
<td>Group R3</td>
<td>Intrathecal injection of Ro 25-6981 (800.0 μg)</td>
</tr>
<tr>
<td>Group M</td>
<td>Remifentanil infusion 0.4 ml (0.04 mg/kg)</td>
</tr>
<tr>
<td>Group (R + M)</td>
<td>Intrathecal injection of Ro 25-6981 (200.0 μg) +</td>
</tr>
<tr>
<td>Group (R + M)2</td>
<td>Remifentanil infusion 0.4 ml (0.04 mg/kg)</td>
</tr>
<tr>
<td>Group (R + M)3</td>
<td>Remifentanil infusion 0.4 ml (0.04 mg/kg)</td>
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2.6. Assessment of thermal hyperalgesia

PWTL was measured by using a radiant thermal (BME410A, Institute of Biological Medicine, Academy of Medical Science, China). Rats were placed in clear plastic cages (20 × 15 × 15 cm) with a glass floor (3 mm thick) and allowed to acclimatize for 30 min before test (Hargreaves et al., 1988). A radiant heat source was focused on the plantar surface adjacent to the wound of right hindpaw through the glass plate. Measurements of PWTL were recorded by a timer that was started by the activation of the heat source and stopped when withdrawal or licking of the hindpaw was detected with a photo-detector. The maximal cutoff time of 25 s was established to prevent tissue damage. There were five trials per rat with a 5-minute interval between consecutive tests. The mean PWTL was obtained from the last three stimuli. At each time point, the detection of PWTL was performed three times.

2.7. Assessment of motor function

The motor function was recorded by using inclined plate testing according to the method described by Rivlin and Tator (1977). The initial angle of the inclined plate was 25°. The rat was placed crosswise to the long axis of an inclined plate. If the rat maintained its body position for 5 s without falling from the inclined plate, the angle of the inclined plate was increased by 5° each time. Finally, the maximum angle of the inclined plate was recorded.

BBB rating is another method to estimate motor function. According to BBB rating, motor function of the rat hind limb was divided into 22 levels. A score of 21 indicates that the hind limb is completely normal and a score of 0 indicates paralysis (Basso et al., 1996). During observation of motor function, the rats should be placed in the center of the scope for about 4 min.

Assessment of motor function was conducted thrice at each time point.

2.8. Western blotting

The animals were anesthetized with sevoﬂurane (5%). The right dorsal horn of the spinal cord L4–L5 segments were removed in 2 min and stored in liquid nitrogen. Tissue samples were homogenized in lysis buffer. The homogenates were vortexed for 40 s, then centrifuged at 13,000 rpm for 10 min at 4 °C and supernatants were transferred to a new tube. The protein concentrations were measured by Bradford method and the protein samples were stored at −70 °C. The samples were separated on SDS-polyacrylamide gel electrophoresis (6%) and transferred onto a nitrocellulose membrane. The filter membranes were blocked with 5% nonfat milk for 1 h and incubated with the primary antibody phosphor-Tyr 1472 NR2B (1:1000; Milliore, Biotechnology, USA). The membrane was extensively washed with PBST buffer and incubated for 1 h with the secondary antibody (1:5000; Jackson ImmunoResearch, USA) at room temperature and visualized in ECl solution for 1 min followed by ﬁlm exposure for 1–10 min. The loading and blotting of equal amount of proteins were veriﬁed by the membrane with antibody against Na+/K+-ATPase α (Santa Cruz, Biotechnology, USA). A computer-assisted imaging analysis system (IPLab software, Scanalytics, Fairfax, VA) was used in measuring the density of speciﬁc bands.

2.9. Statistical analysis

All data were expressed as the mean ± standard deviation. Behavioral studies were analyzed using the Friedman test at each time point.

Fig. 1. Effect of Ro 25-6981 on the paw withdrawal mechanical threshold (n = 6) at 2 h, 6 h, 24 h and 48 h after operation. *P < 0.01 compared with C, #P < 0.01 compared with group I, and &P < 0.01 compared with group M.
point. In all experiment groups, the results of NR2B with tyrosine phosphorylation were analyzed by analysis of variance followed one-way ANOVA. In order to determine the differences among experimental groups, post hoc analysis was performed using the Bonferroni test for multiple comparisons. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of intrathecal Ro 25-6981 injection on PWMT and PWTL

Before the operation, no significant differences were observed in all groups on PWMT and PWTL ($P > 0.05$). However, nociceptive thresholds were decreased on PWMT ($P < 0.01$) and PWTL ($P < 0.01$) in groups I, R1, R2, M, (R + M)1, and (R + M)2 at every time point after the surgery compared with baseline. After 2 h of incision, nociceptive thresholds were increased on PWMT ($P < 0.01$) and PWTL ($P < 0.01$) in groups I, R1, R2, R3, M, (R + M)1, (R + M)2 and (R + M)3. Compared with group C, there were no significant changes of PWMT ($P > 0.05$) and PWTL ($P > 0.05$) in group R3 and group (R + M)3 after 2 h. Significant increases of PWMT ($P < 0.01$) and PWTL ($P < 0.01$) were observed in all groups compared with group M at any time points after the incision (Figs. 1 and 2).

3.2. Effect of intrathecal Ro 25-6981 injection on the motor function

In order to assess whether Ro 25-6981 had side effects, the effects of Ro 25-6981 on the motor function of the rats in each group were evaluated. Compared with other groups, the inclined plate testing degree in group R3 ($P < 0.01$) and group (R + M)3 ($P < 0.01$) significantly decreased after 2 h of the operation; compared with group C and other groups, the BBB rating score in group R3 ($P < 0.01$) and group (R + M)3 ($P < 0.01$) obvious decreased at the same time. Except for at the time point 2 h after the operation, no significant differences were observed in motor functions in each group before and after the operation ($P > 0.05$) (Figs. 3 and 4).

3.3. Effect of intrathecal Ro 25-6981 injection on Western blot

The expression levels of the NR2B with tyrosine phosphorylation at Tyr-1472 in the dorsal horn were evaluated by Western blot after intrathecal injection of Ro 25-6981. Compared with group C, the level of NR2B with tyrosine phosphorylation in the spinal dorsal cord was increased after the right hindpaw plantar incision in group I ($P < 0.01$). However, the level of NR2B with tyrosine phosphorylation was decreased in rats previously administrated by intrathecal injection with Ro 25-6981 after 2 h of the operation ($P < 0.01$). When compared with the sham group and the incisional pain group, the expression level of NR2B with tyrosine phosphorylation in spinal dorsal horn in rats receiving intraoperative remifentanil infusion was significantly up-regulated after the operation ($P < 0.01$). On the contrary, intrathecal injection of Ro 25-6981 could inhibit this increase at the high level of NR2B tyrosine phosphorylation caused by remifentanil ($P < 0.01$) (Figs. 5–7).

4. Discussion

Many studies have shown that in the rat lumbar spinal cord, the distribution of NMDA receptor NR2B is limited in the superficial dorsal horn (Boyce et al., 1999; Nagy et al., 2004). When noxious stimulus through the peripheral nerve receptors terminates in the central synaptic terminals of the superficial spinal dorsal horn, neurotransmitters

Fig. 2. Effect of Ro 25-6981 on the paw withdrawal thermal latency ($n = 6$) at 2 h, 6 h, 24 h and 48 h after operation. $^{*}P < 0.01$ compared with C, $^{*}P < 0.01$ compared with group I, and $^{*}P < 0.01$ compared with group M.
such as substance P, glutamic acid and aspartic acid can be released. These substances could activate the specific nociceptive neurons of the spinal dorsal horn and transmit the pain impulses (Zhuo, 2002). Wilson et al. (2005) found that the NR2B subunit expression in rat spinal dorsal horn was significantly increased after nerve injury through the NMDA receptor subunits. The ideal NR2B antagonist should have an effective analgesic effect with the least side-effects. There are some side-effects of the currently known NR2B antagonists such as the MK-801 for narrow therapeutic window (Boyce et al., 1999), marked antagonism of ifenprodil with 5-HT3 receptor (Barann et al., 1998; McCool and Lovinger, 1995), and adrenergic α1 receptor (Chenard et al., 1991). Ro 25-6981 has not only greater selectivity but also higher affinity to NR2B as a selective NR2B subunit antagonist (Fischer et al., 1997; Mutel et al., 1998).

Spinal dorsal horn is the primary integration hub of the peripheral afferent information. In this experiment, drug injection was administered by intrathecal injection. Ro 25-6981 exerted its analgesic effect mainly through antagonizing the spinal cord level of NR2B receptor. In this study, compared with the control group without surgical incision, thermal hyperalgesia and mechanical allodynia in surgical incision group after operation were lower, which indicated that the incision pain model was successfully established. The doses of Ro 25-6981 were selected according to our preliminary experiments. The results of our preliminary experiments showed that the analgesic effect of Ro 25-6981 was not significant when the dose was 50 μg or 100 μg. However, when the dose was 200 μg, the analgesic effect of Ro 25-6981 was significant. Thus, the analgesic effects of Ro 25-6981 at doses of 200 μg, 400 μg and 600 μg were investigated in this study. We observed that intrathecal injection of Ro 25-6981 can lead to obvious increase of thermal hyperalgesia and mechanical allodynia in rats after incision. In addition, analgesic effect of Ro 25-6981 increased with its dose enhancement, which indicated a dose-dependent analgesic effect. Intrathecal injection of Ro 25-6981 at 800.0 μg manifested no difference in analgesic effect when contrasted with the sham group at 2 h after the surgery. The analgesic effect of Ro 25-6981 at this dose is remarkable. However, this dose of Ro 25-6981 showed no analgesic effect in rats at other times after incision, which indicated that the drug metabolism of Ro 25-6981 in rat intrathecal was transient. Our data were consistent with the findings reported by Qu et al. in 2009. Ro 25-6981 had analgesic effect at 2 h after incision surgery. However, due to the fast pharmacokinetics of Ro 25-6981 in rats, the analgesic effect of Ro 25-6981 was not significant at 6 h after incision surgery. Collectively, we suppose that the effects of Ro 25-6981 in rat incision pain model were dependent on drug doses and drug metabolism.

To further identify Ro 25-6981 as the highly selective NR2B antagonist, the changes of the postoperative hyperalgesia induced by remifentanil through the pretreatment of intrathecal administration were observed with different doses of Ro 25-6981. In addition to the analgesic effect of opioid, the drugs also can activate the body’s mechanisms of promotion injury, which increase the body’s sensitivity to pain (Li et al., 2001). The establishment of central sensitization does not depend on the continued tissue damage through the peripheral nociceptors’ introduction and constitutes the hyperalgesia mechanism of pathophysiology (Coderre and Melzack, 1985). Intensity of OIH is
closely related to pharmacokinetics, rapid onset and short-lived drugs such as remifentanil. Compared with long-acting opioids, the developments of noxious stimulus are more rapidly and predominantly after the drug withdrawal (Derrode et al., 2003). However, it is difficult to distinguish from tissue injury or the combined effects of noxious stimulus and remifentanil regarding postoperative pain in clinic. The classical rat plantar incision pain model established by Brennan et al. (1996) is an effective way to explore hyperalgesia mechanism induced by remifentanil. The dose selection (0.04 mg/kg) of remifentanil in this experiment follows previous report. The rats showing administration of remifentanil showed a loss of righting reflex that was predictive of clinical anesthesia (Lozito et al., 1994). The thermal hyperalgesia and mechanical allodynia begin to increase when remifentanil continuous to infuse over 30 min after the incision of the model mice, and maintain for 7 d according to Célérier et al.’s research (2006). Therefore, the effects of Ro 25-6981 were studied at 2 h, 6 h, 24 h, and 48 h after incision. Compared with group of surgical incision group, nociceptive thresholds decreased in group of remifentanil infusion at every time point after the surgery. This dose of remifentanil could induce hyperalgesia of incisional surgery. Thermal hyperalgesia and mechanical in intrathecal injection of Ro 25-6981 were significantly increased at 2 h after surgery and showed a dose-dependent analgesic effect in hyperalgesia groups. The different doses of Ro 25-6981 showed no analgesic effect in rats at other times after incision, but were competent to alleviate hyperalgesia induced by remifentanil at every time point after surgery. Although the drug metabolism of Ro 25-6981 in rat intrathecal was transient, the pretreatment of intrathecal administration with a certain dose Ro 25-6981 could effectively attenuated the postoperative hyperalgesia induced by remifentanil.

The protein kinase C (PKC) activates protein phosphorylation and tyrosine kinase signaling cascade is an important factor in regulating NMDA receptor function (Chen and Roche, 2007). When remifentanil interacted with the μ-opioid receptor, postsynaptic membrane of NMDA receptor would be activated leading to increase Ca²⁺ ion permeability. Influx of Ca²⁺ inside the cell would further activate intracellular Ca²⁺-dependent PKC. After the activated PKC transferred from the cytoplasm to the membrane, the NMDA phosphorylation receptor would be activated and the influx of Ca²⁺ would be increased, thus forming a positive feedback. Remifentanil could excite NMDA receptor directly (Hahnenkamp et al., 2004). Recent studies also show that the μ-opioid receptor and the NMDA receptor are involved in pain control (Rodríguez-Muñoz et al., 2012). Protein phosphorylation is not only the primary mechanism for regulating NMDA receptor function, but also can change the peculiarity of the NMDA ion channel receptor. For example, subunit NR2 in its carboxy terminal can be tyrosine phosphorylated (Tezuka et al., 1999; Yu et al., 1997). Although both NR2A and NR2B can be phosphorylated by tyrosine in vitro, NR2B phosphorylation plays essential roles in activation of NMDA receptors, activation of nociceptors caused by spinal cord plasticity, and development of central sensitization (Guo et al., 2002). The structural and functional changes of tyrosine phosphorylated protein in the postsynaptic density can sustain excessive activation of the NMDA receptor. Ro 25-6981 can significantly reduce the opening frequency of the NMDA channel, and inhibits the ion inflow mediated by

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**Fig. 5.** Effect of Ro 25-6981 on the NR2B protein expression in the spinal cord at 2 h after the surgery (n = 4). Representative Western blot for tyrosine phosphorylation of NR2B (A); quantification of NR2B tyrosine phosphorylation in each group (B). *P < 0.05 compared with C, **P < 0.01 compared with group I, and ***P < 0.01 compared with group M.

**Fig. 6.** Effect of Ro 25-6981 on the NR2B protein expression in the spinal cord at 6 h after the surgery (n = 4). Representative Western blot for tyrosine phosphorylation of NR2B (A); quantification of NR2B tyrosine phosphorylation in each group (B). *P < 0.01 compared with C, **P < 0.01 compared with group I, and ***P < 0.01 compared with group M.
NMDA receptor. Therefore, we conclude that Ro 25-6981 can inhibit the NR2B receptor pathway through directly or indirectly inactivation of NMDA receptor which alleviates the hyperalgesia induced by remifentanil.

In this study, the motor function of rats through BBB rating and inclined plate testing in all groups was examined in order to estimate the side-effects of Ro 25-6981 had side-effects. The results showed that when intrathecal injection of Ro 25-6981 at 800.0 μg, the rat's motor function was limited after 2 h of the operation. We observed the analgesic effect of Ro 25-6981 increased with dose augment at the same time. Motor function in rats might be blocked due to larger dose intrathecal injection of Ro 25-6981. With the metabolism of drugs, rats gradually recovered motor function. Except for at the time point after 2 h of the operation, no differences were observed in motor functions in each group at other time points. The results of motor function in rats with the changes of drug metabolism and dose could exclude the possibility of nerve injury in rats because of intrathecal injection drugs.

NR2B subunit in the generation of pain and central sensitization plays an important role (LoGrasso and McKelvy, 2003). There are seven tyrosine phosphorylation sites in the cytoplasmic C-terminal of NR2B subunit and Tyr-1472 is the main site. Its phosphorylation has significant effect for changes of synaptic plasticity and occurrence of long-term potentiation (LTP). LTP and OIH have the common pharmacological and signal transduction pathway (Drdla et al., 2009; Nakazawa et al., 2001). Many studies have shown that tyrosine phosphorylation of the NR2B at Tyr-1472 in the spinal dorsal horn contributed to the development of hyperalgesia in neuropathic pain model (Abe et al., 2005) and inflammatory pain model (Guo et al., 2002). Therefore, we not only observed the behavioral changes in rats but also Ro 25-6981 induced antinociception in the rat model of incisional pain and hyperalgesia. Western blot analysis showed that pre-intrathecal injection of Ro 25-6981 decreased the higher level expression of tyrosine phosphorylation of NR2B in spinal dorsal horn and hyperalgesia. Ro 25-6981 may relieve incisional pain and remifentanil-induced hyperalgesia through the pathway of NR2B as the specific NR2B subunit antagonist.

In summary, behavioral test and Western blot analysis demonstrated pre-intrathecal injection of Ro 25-6981 had significant analgesic effects on incision pain in rats and effectively prevented postoperative hyperalgesia induced by remifentanil. These results might have some relation with inhibiting tyrosine phosphorylation of NR2B in superficial spinal cord of rats. The effects of Ro 25-6981 on locomotor functions of rats changed with the different doses and drug metabolism. Although Ro 25-6981 is currently studied for animal experiment, clinical application has not been carried out. The mechanism of these drugs may provide ideas to find out more value methods for the clinical treatment of incisional pain and hyperalgesia. Conclusively, our study investigated the analgesic effects through targeting NR2B in a rat incision pain model. And our data suggest that targeting NR2B in the spinal cord might be a new strategy for the treatment of clinical pain.

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