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Original Article

Activation of spinal alpha-7 nicotinic acetylcholine receptor attenuates remifentanil-induced postoperative hyperalgesia

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Abstract: The activation of alpha-7 nicotinic acetylcholine receptors (α7-nAchRs) are currently being considered as novel therapeutic approaches for managing hyperalgesia in inflammation and chronic neuropathic pain, but the role of a7-nAChRs on opioids induced hyperalgesia remain unknown. The present study investigated the effects of α7-nAChRs selective agonists PHA-543613 and type II positive allosteric modulators (PAMs) PNU-120596 in remifentanil induced postoperative hyperalgesia. As the results shown, intrathecal treatment with both α7-nAChRs agonists and type II PAMs could attenuate remifentanil induced hyperalgesia by increasing paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL). Furthermore, we also investigated the protein level of proinflammatory cytokines and phosphorylation N-methyl-d-aspartate receptor 2B subunit (p-NR2B) in the spinal cord. Our data indicated that activation of α7-nAChRs decreased the proinflammatory cytokines (TNF-α, IL-6) and p-NR2B protein level in the spinal cord. The depression of the increased levels of proinflammatory cytokines and p-NR2B after remifentanil treatment may contribute to the anti-hyperalgesia effects of PHA-543613and PNU-120596 via α7-nAChRs. Therefore, our findings demonstrated that α7-nAChRs may be potential candidates for treating opioids induced hyperalgesia.

Keywords: Nicotinic receptors, remifentanil, hyperalgesia

Introduction

Opioids are the most widely used analgesic in the treatment of severe pain, but their prolonged use could induce serious hyperalgesia (OIH). Both animal experiments [1] and clinical studies [2] have shown that exposure of opioid could enhance the pain sensitivity [3], manifested as increased sensitivity to noxious stimuli [4]. As a μ-receptor agonist, remifentanil was widely used during the operation. It is an ultra-short-acting opioid, which acts with rapid onset and short duration of action. Intraoperative remifentanil could cause postoperative hyperalgesia and increase the use of analgesic treatments [5]. However, the underlying mechanisms remain unclear.

Sensitization of nociceptive pathways has been considered as neuroplasticity mechanism, and it has become increasingly recognized that spinal glial cells might be dynamic regulators of this network [6]. Spinal glial cells might function in central sensitization [7] and pathological pain [8], which has been shown in experimental animal models of peripheral inflammation, spinal injury, and nerve injury [9]. In our recent study, the spinal glial cells were active in remifentanil induced hyperalgesia [10]. This study also shown the activation of glia is associated with an increase of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL)-6 [11]. The activation of spinal glia and the release of proinflammatory cytokines should be important in the development of remifentanil induced hyperalgesia.

Many studies demonstrated that the mechanisms of OIH might be related to spinal N-methyl-d-aspartate receptor (NMDAR)-dependent central sensitization [12]. NMDA receptor antagonists, such as ketamine, could inhibit central sensitization and prevent remifentanil induced hyperalgesia [5, 13]. The NR2B is functional subunit of NMDAR and p-NR2B plays a critical role in the development and maintenance of central sensitization [14]. Our previous studies found that remifentanil-induced hyperalgesia was associated with an enhancement of p-NR2B in the spinal dorsal horn [15]. These results indicate that activation of NMDA receptors contribute to the hyperalgesia in OIH.

Multiple subtypes of nAChRs are expressed in pain transmission pathways [16] and α7-nAChRs have been shown expressed in the spinal cord dorsal horn [17]. Agonists of α7-nAChRs are considered as therapeutic approaches for managing hyperalgesia in inflammation and chronic neuropathic pain [18]. The α7-nAChRs agonists elicited significant anti-inflammatory and anti-nociceptive effects in these models [19]. Therefore, the α7-nAChR represents a promising target for analgesic agents. Previous studies also demonstrated that type II PAMs could facilitate endogenous neurotransmission and enhance the efficacy and potency of an agonist without directly stimulating the agonist-binding sites [20, 21].

Therefore, the aim of the present study was to evaluate whether α7-nAChRs selective agonists (PHA-543613) and type II PAMs (PNU-120596) could produce anti-hyperalgesia effects in remifentanil-induced hyperalgesia. We also evaluated whether PNU-120596 could enhance the PHA-543613 anti-nociceptive effects in this model. In addition, we investigated the effects of PHA-543613 and PNU-120596 on proinflammatory cytokines and p-NR2B in spinal cord.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing 220-250 g were used for this study. Animals were obtained from the Laboratory Animal Center of Drum Tower Hospital, housed in cages with a 12 h light/dark schedule at room temperature of 22±2°C, and allowed free access to food and water. All experiments were approved by the Animal Care and Use Committee [22].

Drugs

Remifentanil (batch number: 120801, Ren Fu Co, China) was dissolved NaCl 0.9% and infused subcutaneously over a period of 30 min using an apparatus pump rate was 0.8 ml/h. PHA-543613 and PNU-120596 (Sigma Chemical Co., St. Louis, MI) were dissolved in 5% DMSO. Methyllycaconitine (MLA) (Sigma Chemical Co., St. Louis, MI) was dissolved in NaCl 0.9%. The intrathecal injection of PNU-120596 (2 μg, 4 μg, 8 μg) and PHA-543613 (3 μg, 6 μg, 12 μg) were performed 24 h after remifentanil subcutaneously infused. The MLA was intrathecal injected 30 min before PNU-120596 and PHA-543613.

Surgical procedure

The rat model of postoperative pain was performed as previously described by Brennan et al [23]. Briefly, rats were anesthetized with sevoflurane (induction 3%; surgery 1%) via a nose mask. A 1 cm longitudinal incision was made through the skin and fascia, starting at 0.5 cm from the edge of the heel and extending toward the toes of the right hind paw. The plantaris muscle was elevated using forceps and incised longitudinally, leaving the muscle origin and insertion intact. After hemostasis with gentle pressure, the skin was closed with two mattress sutures of 5-0 nylon. The wound site was covered with aureomycin ointment.

Experimental design

Experiment 1: remifentanil-induced postoperative hyperalgesia behaviors: We used 3 groups of rats (n=8) in this study. PWMT and PWTL were examined respectively at 24 h before incision (baseline) and 2 h, 6 h, 24 h and 48 h after surgery. Group C: rats underwent a sham procedure; Group I: rats underwent a surgical incision without remifentanil; Group R: rats underwent a surgical incision and remifentanil was infused subcutaneously.

Experiment 2: effects of intrathecal administration of PHA-543613 and PNU-120596 on pain behaviors induced by remifentanil: We investigated the effects of PHA-543613 and PNU-120596 respectively. The PWMT and PWTL were examined at 1 h before injection (baseline) and 0.5, 1, 2, 4 and 6 h after administration. The dosage of PHA-543613 and PNU-120596 were selected based on a previous study [24].

For PHA-543613, 6 groups of rats (n=8) were involved in. Group R; Group DMSO: rats received intrathecal injection 20 μl 5% DMSO; Group PHA (3 μg, i.t.), Group PHA (6 μg, i.t.), Group PHA (12 μg, i.t.): rats received intrathecal injection 3 μg, 6 μg, 12 μg PHA-543613 respectively; Group MLA (10 μg, i.t.): rats received intrathecal injection 10 μg MLA 30 min before PHA (12 μg, i.t.).

For PNU-120596, 6 groups of rats (n=8) were involved in. Group R; Group DMSO; Group PNU (2 μg, i.t.), Group PNU (4 μg, i.t.), Group PNU (8 μg, i.t.): rats received intrathecal injection 2 μg, 4 μg, 8 μg PNU-120596 respectively; Group MLA (10 μg, i.t.): rats received intrathecal injection 10 μg MLA 30 min before PNU (8 μg, i.t.).

For the effects of PHA-543613 with PNU-120596, 3 groups of rats (n=8) were involved in. Group R; Group DMSO; Group PHA+PNU: rats received intrathecal injection 6 μg PHA-543613 and 4 μg PNU-120596.

Experiment 3: effects of intrathecal injection of PHA-543613 and PNU-120596 on proinflammatory cytokines and p-NR2B in the spinal cord: To determine whether PHA-543613 and PNU-120596 disturbed proinflammatory cytokines and p-NR2B in the spinal cord, 5 groups of rats (n=4) were used, including Group C, Group I, Group R, Group PHA (12 μg, i.t.), Group PNU (8 μg, i.t.).

Mechanical allodynia

Mechanical allodynia was assessed using von Frey filaments (Stoelting, Wood Dale, IL), which were applied to the right hindpaw according to our previous study [25]. As described by Chaplan et al [26], PWMT was measured using a set of von Frey filaments (2 g-15 g). Filaments were pressed vertically against the plantar surface with sufficient force to cause a slight bending against the paw and were held for 6 to 8 s with a 5min interval between stimulations. Brisk withdrawal or paw flinching were considered positive responses. Each rat was tested 5 times per stimulus strength. The lowest von Frey filament, which had 3 or more positive responses, was regarded as the PWMT.

Thermal hyperalgesia

Thermal hyperalgesia to radiant heat was determined according to a method described by Hargreaves et al [27]. In brief, rats were placed in clear plastic cages on an elevated glass plate and the radiant thermal stimulator (BME410A, Institute of Biological Medicine, Academy of Medical Science, China) was focused onto the plantar surface of the hindpaw through the glass plate. The nociceptive end-points in the radiant heat test were the characteristic lifting or licking of the hindpaw, and the time to the end-point was considered PWTL. The cutoff time of 25 s was used to avoid tissue damage. There were five trials per rat and 5 min intervals between trials. The mean PWTL was obtained from the latter three stimuli.

Western blotting

Rats were deeply anesthetized with 5% sevoflurane and the right dorsal horn of the spinal cord L4-L5 segments were removed rapidly and stored in liquid nitrogen. Tissue samples were homogenized in lysis buffer. The homogenate was centrifuged at 13,000 rpm for 10 min at 4°C and supernatant was removed. The protein concentration was determined by the BCA Protein Assay Kit, following the manufacturer's instructions. Samples (70 μg) were separated on SDS-PAGE (6%) and transferred onto a nitrocellulose membrane. The filter membranes were blocked with 5% nonfat milk for 1h at room temperature and incubated with the primary antibody IL-6 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, 1:400 dilution), TNF-α (Abcam, 1:600 dilution), p-NR2B (phosphor-Tyr 1472 NR2B, CST, Biotechnology, 1:500 dilution). The membrane was washed with TBST buffer and incubated for 1 h with the secondary antibody conjugated with horseradish peroxidase (1:5000, Jackson Immuno Research, USA) for 1 h at room temperature. Next, the immune complexes were detected using the ECL system (Santa Cruz Biotechnology, CA, USA). β-Actin was used as a loading control for total protein. The images of Western blot products were collected and analyzed by Quantity One V4.31 (Bio-Rad, USA).

Statistical analysis

Data are expressed as the mean ± SD (standard deviation). Changes in PWMT and PWTL after inoculation and administration were com-

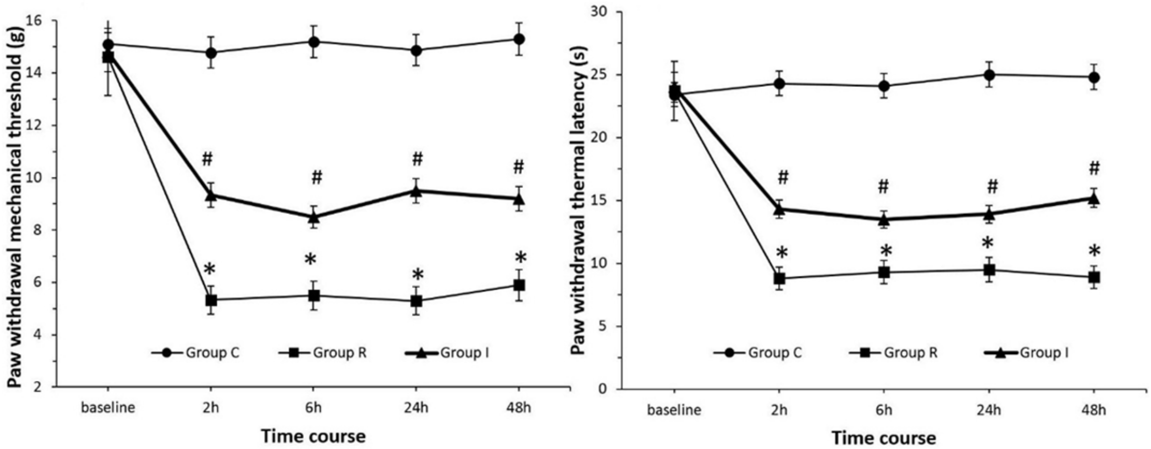

Figure 1. Remifentanil-induced postoperative hyperalgesia. PWMT and PWTL were evaluated at 24 h before incision (baseline) and 2 h, 6 h, 24 h and 48 h after surgery. All data represent as mean ± SD (n=8). #$P < 0.05$ vs. group C, *$P < 0.05$ vs. group I.

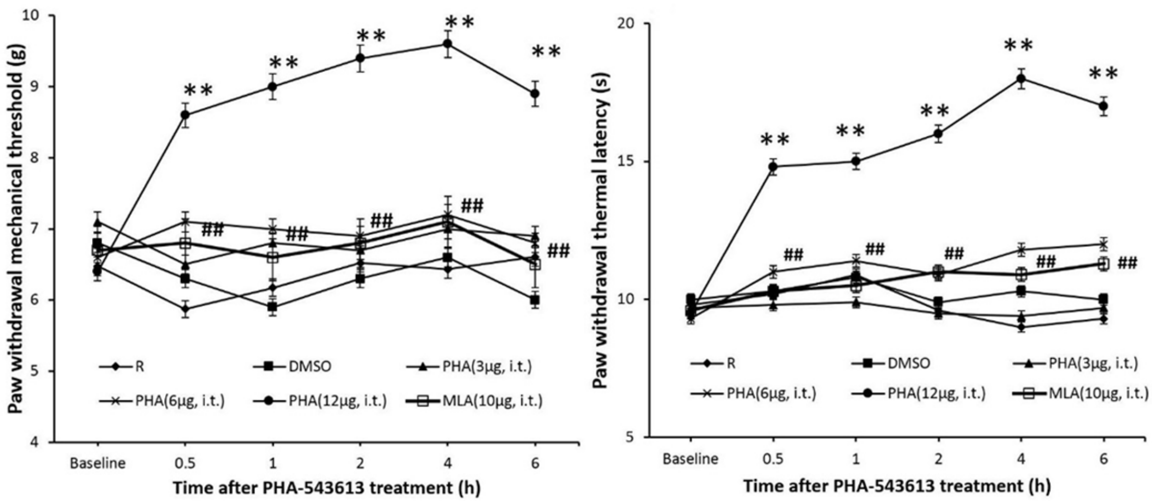

Figure 2. Intrathecal injection of PHA-543613 attenuated remifentanil-induced hyperalgesia dose-dependently. PWMT and PWTL increased significantly at 0.5, 1, 2, 4 and 6 h after intrathecal administration of PHA-543613 12 μg (**$P < 0.01$ compared with the group R and group DMSO). Pre-injection of MLA 10 μg inversed the analgesic effect of PHA (12 μg, i.t.) (##$P < 0.01$ compared with the group PHA 12 μg i.t.). All data represent as mean ± SD (n=8).

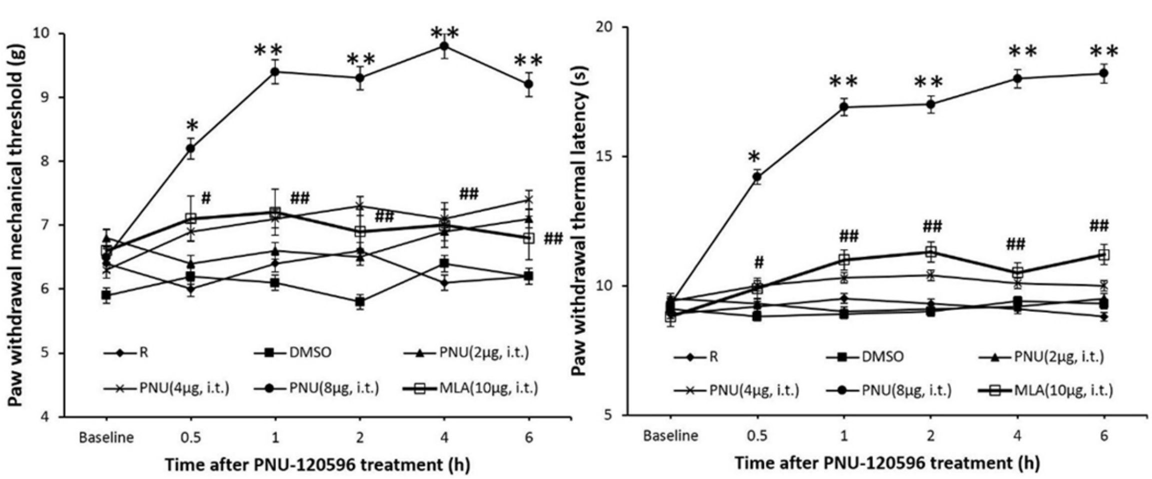

Figure 3. Effect of PNU-120596 on PWMT and PWTL in remifentanil induced hyperalgesia. Intrathecal administration of PNU-120596 increased both PWMT and PWTL dose-dependently. PWMT and PWTL increased significantly at 0.5, 1, 2, and 4 h after intrathecal administration of PNU-120596 8 μg ($^{*}P < 0.05$, $^{**}P < 0.01$ compared with group R and group DMSO). Similar with the PHA-543613, pre-injection of MLA 10 μg almost abolished this analgesic effect ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the group PNU 8 μg i.t.). All data represent as mean ± SD (n=8).

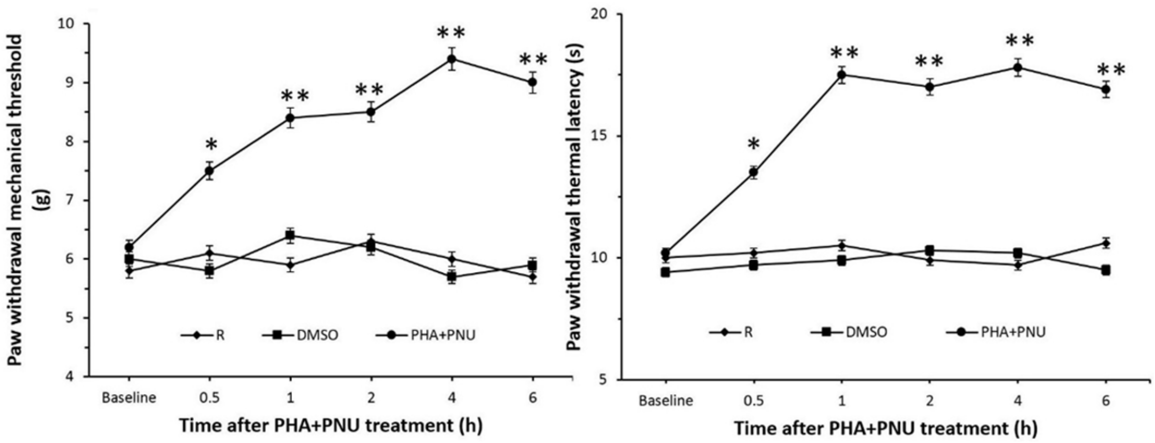

Figure 4. Effects of the non-analgesic dose combination of PNU-120596 (6 μg, i.t.) and PHA-543613 (4 μg, i.t.) in remifentanil induced hyperalgesia. All data represent as mean ± SD. Both PWMT and PWTL increased significantly at 0.5, 1, 2, and 4 hours after intrathecal administration of this combination ($^{*}P < 0.05$, $^{**}P < 0.01$ compared with group R and group DMSO).

pared with basal values, respectively, using a 2-way analysis of variance for repeated measures, followed by Bonferroni correction for between-group comparisons. Western blotting data were analyzed using a 1-way analysis of variance for overall differences among groups followed by Bonferroni correction for between-group comparisons. Statistical analysis was performed using SPSS 16.0 software. The *P* value < 0.05 was considered statistically significant.

Results

Remifentanil-induced postoperative hyperalgesia

There was no significant difference of PWMT and PWTL in rats of group C, group R and group I before operation. Compared with baseline and group C, the plantar incision induced a decrease of PWMT ($P < 0.05$) and PWTL) ($P <$ 0.05 in the operated paw during the postoperative period. Intraoperative infusion of remifentanil significantly enhanced hyperalgesia induced by the plantar incision. This was manifested by a decrease in PWMT ($P < 0.05$) and PWTL ($P < 0.05$) compared with group I (**Figure 1**).

Effects of intrathecal administration of PHA-543613 and PNU-120596 on pain behaviors induced by remifentanil

The intrathecal administration of PHA-543613 attenuated remifentanil-induced hyperalgesia dose-dependently. There was no significant difference of PWMT and PWTL in PHA (3 μg, i.t.) and PHA (6 μg, i.t.) group compared with DMSO group. PHA (12 μg, i.t.) significantly increased PWMT ($P < 0.01$) and PWTL ($P < 0.01$) compared with DMSO groups at 0.5, 1, 2, 4 and 6 h after administration. MLA (10 μg, i.t.) almost abolished the analgesic effect of PHA (12 μg, i.t.) ($P < 0.01$) (**Figure 2**).

The effects of PNU-120596 were similar with PHA-543613. There was no significant difference of PWMT and PWTL in PNU (2 μg, i.t.) group and PNU (4 μg, i.t.) group compared with the DMSO group. The PNU (8 μg, i.t.) group significantly increased PWMT ($P < 0.01$) and PWTL ($P < 0.01$) compared with DMSO groups at 0.5, 1, 2, 4 and 6 hours after administration. Intrathecal injection MLA 10 μg greatly decreased the analgesic effect of PNU (8 μg, i.t.) ($P < 0.05$) (**Figure 3**).

Furthermore, we investigated the non-analgesic effect dose of PHA-543613 (6 μg, i.t.) and

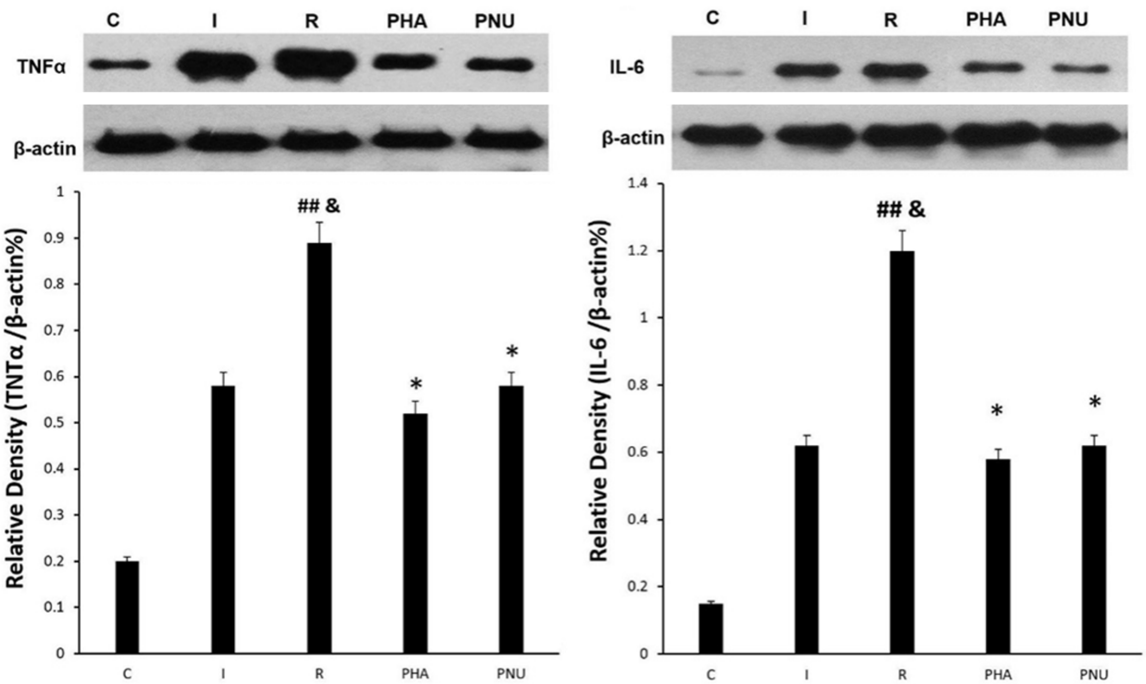

Figure 5. Effects of i.t. 12 μg PHA-543613 and 8 μg PNU-120596 on TNF-α, IL-6 expression. Remifentanil increased expression of TNF-α and IL-6 in the spinal cord (##$P < 0.01$ compared with the group C, &$P < 0.05$ compared with the group I). Intrathecal administration of 12 μg PHA-543613 and 8 μg PNU-120596 decreased the TNF-α and IL-6 protein levels at 4 h after the injection (*$P < 0.05$ compared with the group R) (n=4).

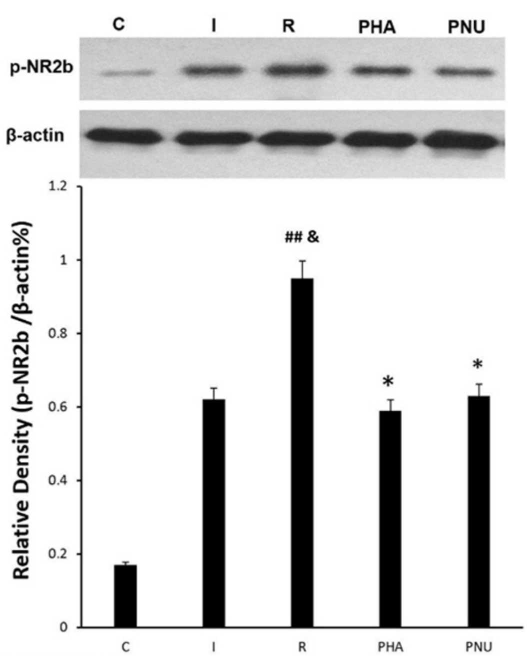

Figure 6. Effects of i.t. 12 μg PHA-543613 and 8 μg PNU-120596 on p-NR2B protein. The expression of p-NR2B protein increased greatly in remifentanil group (##$P < 0.01$ compared with the group C, &$P < 0.05$ compared with the group I). Intrathecal administration of 12 μg PHA-543613 and 8 μg PNU-120596 decreased the p-NR2B protein levels at 4 h after the injection (*$P < 0.05$ compared with the group R) (n = 4).

PNU-12059610 (4 μg, i.t.). The data showed that the combination of this non-analgesic effect dose could attenuate the remifentanil-induced hyperalgesia (**Figure 4**).

Intrathecal administration of PHA-543613 and PNU-120596 decreased the expression of proinflammatory cytokines and p-NR2B in remifentanil induced hyperalgesia

To investigate the effect of PHA-543613 and PNU-120596 on proinflammatory cytokines and p-NR2B expression, the lumbar enlargement was quickly dissected under deep anesthesia at 4h after i.t. administration of 12 μg PHA-543613 and 8 μg PNU-120596 on 24 h after the operation. Administration of 12 μg PHA-543613 and 8 μg PNU-120596 significantly decreased the TNF-α, IL-6 (**Figure 5**) and p-NR2B (**Figure 6**) protein level in the spinal cord.

Discussion

Intraoperative infusion of remifentanil is associated with postoperative hyperalgesia [28] and increases postoperative analgesic requirements in both animal models [29] and human clinical trials [30]. Through the underlying mechanisms remain unclear, many studied indicated proinflammatory cytokines and p-NR2B may contribute to remifentanil induced hyperalgesia.

Previous studies have demonstrated that the injuries in peripheral tissue or nerve could produce proinflammatory cytokines [31], such as IL-6 and TNF-α. These proinflammatory cytokines play important roles in mediating exaggerated pain states [32]. The spinal cord TNF-α may promote central sensitization by increasing glutamate release from presynaptic terminals [33]. Anti-inflammatory cytokines could block the induction of proinflammatory cytokines and suppressed inflammation-induced NMDAR phosphorylation [34]. Therefore, central sensitization could be enhanced and maintained by proinflammatory cytokines [35].

The NMDARs play an important role in synaptic transmission and central sensitization [36]. Our previous study indicated that noncompetitive NMDAR antagonist (ketamine) could inhibit the development of remifentanil induced postoperative hyperalgesia [15]. In the present study, our results showed that the infusion of remifentanil increased the expression of p-NR2B in spinal cord at 24 hours after surgery. These findings suggest that NMDARs contributed to remifentanil induced hyperalgesia.

Activation of α7-nAChRs by agonists and type II PAMs could inhibit proinflammatory cytokines [37]. Munro et al. showed that PNU-20596 produces anti-hyperalgesic effects in the formalin, carrageenan or complete Freund's adjuvant (CFA) tests rats through a decrease in TNF-α and IL-6 levels [38]. And Kelen et al. also showed that the effect of α7-nAChRs selective agonist was similar with type II PAMs in neuropathic pain [27]. In our study, the data demonstrated that both selective α7-nAChRs agonists PHA-543613 and type II PAMs PNU-120596 could attenuate remifentanil-induced hyperalgesia dose-dependently. Our results also showed the combination of PHA-543613 and PNU-120596 could produce enhanced anti-hyperalgesia effects compared to each drug given alone. Furthermore, we investigated the protein levels of TNF-α, IL-6 and p-NR2B in spinal cord. The result showed that activation of α7-nAChRs inhibited the production of proinflammatory cytokines and p-NR2B in the spinal dorsal horn. By this mechanism, α7-nAChRs may improve remifentanil induced postoperative hyperalgesia.

In summary, our present study demonstrated that both α7-nAChRs selective agonists and type II PAMs have anti-hyperalgesia effects in remifentanil induced postoperative hyperalgesia. The depression of the increased levels of proinflammatory cytokines (IL-6, TNF-α) and p-NR2B after remifentanil treatment in spinal cord may contribute to the anti-hyperalgesia effects of PNU-120596 and PHA-543613. Though α7-nAChRs are currently being developed for the treatment of cognitive deficits in schizophrenia or Alzheimer's patients, we demonstrated in our study that α7-nAChRs may be potential candidates for treating opioids induced hyperalgesia.

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Disclosure of conflict of interest

None.

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