Targeting α7 nicotinic acetylcholine receptors for chronic pain

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Despite rapid advances in the field of chronic pain, it remains extremely challenging in the clinic. Pain treatment strategies have not improved for decades as opioids remain the main prescribed drugs for chronic pain management. However, long-term use of opioids often leads to detrimental side effects. Therefore, uncovering the mechanisms underlying the development and maintenance of chronic pain may aid the discovery of novel therapeutics to benefit patients with chronic pain. Substantial evidence indicates downregulation of α7 nicotinic acetylcholine receptors (α7 nAChR) in the sciatic nerve, dorsal root ganglia, and spinal cord dorsal horn in rodent models of chronic pain. Moreover, our recent study and results from other laboratories demonstrate that potentiation of α7 nAChR attenuates pain behaviors in various murine models of chronic pain. This review summarized and discussed the preclinical evidence demonstrating the therapeutic potential of α7 nAChR agonists and allosteric modulators in chronic pain. This evidence indicates that potentiation of α7 nAChR is beneficial in chronic pain, mostly by alleviating neuroinflammation. Overall, α7 nAChR-based therapy for chronic pain is an area with great promise, but more research regarding its detailed mechanisms is warranted.

KEYWORDS
chronic pain, α7 nAChR, neuroinflammation, microglia, oxidative stress

Introduction

Despite rapid advances in the field of chronic pain, it remains extremely challenging in the clinic (Burma et al., 2017; Cornett et al., 2018). Pain treatment strategies have not improved for decades as opioids remain the main prescribed drugs for chronic pain management (Matthias et al., 2020; Goldstick et al., 2022). However, long-term use of opioids often leads to detrimental side effects, including respiratory depression, mental clouding, tolerance, addiction, constipation, nausea, and vomiting (Liu et al., 2019). Therefore, uncovering the mechanisms underlying the development and maintenance of chronic pain may aid the discovery of novel therapeutics to benefit patients with chronic pain.

Nicotinic acetylcholine receptors (nAChRs), a member of the cysteine-loop (Cys-loop) family of ligand-gated ion channels, are involved in various physiological
The role of α7 nAChR in chronic pain

It has been reported that α7 nAChR is expressed on neuronal cells in the dorsal root ganglia (DRG), spinal cord, and brain, as well as non-neuronal cells in the pain transmission pathway (Cordero-Erausquin et al., 2004). The protein level of α7 nAChR was significantly downregulated in the sciatic nerve, DRG, and spinal cord in oxaliplatin-induced neuropathic pain rats (Di Cesare Mannelli et al., 2014). Similarly, the protein level of α7 nAChR was considerably decreased after single prolonged stress (SPS) exposure in the spinal cord (Sun et al., 2019). Our recent study also revealed that the expression of spinal α7 nAChR was significantly downregulated in cancer-induced bone pain (CIBP) rats, and most of the α7 nAChR was localized in neurons (Yang et al., 2021). The ventrolateral periaqueductal gray (vPAG) and rostral ventromedial medulla (RVM) are the major components of the descending pain modulatory pathway, which are important targets of analgesic drugs (Bagley and Ingram, 2020). Umana et al. (2017) showed that 63% of PAG-RVM projection neurons expressed functional nAChR, which were exclusively the α7-subtype. These results indicate that decreased expression of α7 nAChR in the pain transmission pathway might be involved in the development of chronic pain.

Compelling evidence from our laboratory and others has demonstrated that activation of α7 nAChR could attenuate chronic pain, including neuropathic pain, inflammatory pain, and CIBP (Medhurst et al., 2008; Papke et al., 2015; Arias et al., 2020; Yang et al., 2021). Alsharari et al. evaluated pain behaviors in the α7 mutant mice (KO) and α7 hypersensitive mice (KI) expressing the L250T α7 nAChR and their respective WT mice in chronic pain models (Alsharari et al., 2013). While no significant change was observed between α7 KO mice and WT mice regarding thermal and mechanical allodynia after chronic nerve injury, α7 KI mice showed a significant reduction in these pain behaviors. Moreover, α7 KO mice displayed a marked increase in edema, hyperalgesia, and allodynia after intraplantar injection of complete Freund adjuvant (CFA).

Importantly, systemic administration of nicotine reversed established mechanical allodynia after CFA in WT mice, which was lost in the α7 KO mice. These results indicate that endogenous α7 nAChR plays a vital role in chronic pain. Recently, Khasabov et al. (2021) investigated whether loss of neuronal-specific TMEM35a, a novel chaperone for functional expression of the homomeric α7, in the spinal cord modulates pain in mice. They found that mice with tmem35a deletion exhibited thermal hyperalgesia and mechanical allodynia. Furthermore, they found that the spinal cord of tmem35a KO mice exhibited 72 differentially expressed genes compared with WT control, which was associated with neuroinflammation. Overall, these results indicate a promising therapeutic potential of α7 nAChR in chronic pain.

The therapeutic potential of α7 nAChR agonists and allosteric modulators in chronic pain

Nicotine modifies the activity of nAChRs, including α7 nAChR. α7 nAChR can also be activated by selective agonists (e.g., GTS-21 and TC-7020) and allosteric modulators (e.g., PNU-120596 and GAT107). The remainder of this review will provide
detailed insight into the therapeutic potential of α7 nAChR agonists and allosteric modulators in chronic pain.

**Nicotine**

It has been frequently reported that nicotine displays potent antinociceptive effects both in human and rodent studies (Richardson et al., 2012; Di Cesare Mannelli et al., 2013; Brunori et al., 2018; Costa et al., 2012) showed that both acute and chronic oral treatments with nicotine remarkably inhibited established mechanical allodynia in dextran sulfate sodium (DSS)-induced visceral pain in mice. Moreover, the antinociceptive effect of nicotine was completely abolished by co-treatment with methyllycaconitine (MLA), a selective α7 nAChR antagonist. Nevertheless, nicotine did not affect DSS-induced colonic damage and inflammation. In another study, Teng et al. (2019) demonstrated that intraperitoneal injection of nicotine considerably attenuated mechanical allodynia, cartilage degradation, and upregulation of matrix metalloproteinase-9 in monosodium iodoacetate (MIA)-induced osteoarthritis pain mice, which were entirely abolished by MLA. Their further study validated that nicotine alleviated MIA-induced pain behaviors and cartilage degradation via stimulating the α7 nAChR/mammalian target of the rapamycin signal pathway (Liu et al., 2021). These results indicate that nicotine attenuates chronic pain in an α7 nAChR-dependent manner. Nevertheless, it is worth noting that long-term nicotine administration led to mechanical allodynia, which could be reversed by the selective α7 nAChR agonist CDP-Choline (Zhang et al., 2021c).

**Selective α7 nAChR agonists**

The role of α7 nAChR in chronic pain was not fully elucidated until the discovery of selective agonists. Medhurst et al. revealed that intraperitoneal injection of (R)-N-(1-azabicyclo[2.2.2]oct-3-yl)-5-(2-pyridyl) thiophene-2-carboxamide) (compound B), a selective α7 nAChR agonist, completely reversed CFA-induced inflammatory pain in mice and rats in a dose-related manner (Medhurst et al., 2008). Importantly, subcutaneous treatment with selective low-brain penetrant α7 antagonist MLA did not affect compound B activity, while intrathecal injection of MLA completely inhibited the agonist effect. These results indicate that compound B alleviated inflammatory pain via activation of α7 nAChR in the central nervous system. In another study, Loram et al. (2010) showed that intrathecal injection of choline, a precursor for acetylcholine and a selective α7 nAChR agonist, considerably suppressed and reversed gp120-induced mechanical allodynia and upregulation of proinflammatory cytokines, including TNFα, IL-1β, and IL6 in the spinal cord. Moreover, they found that GTS-21, another selective α7 nAChR agonist, exhibited similar properties. Furthermore, both choline and GTS-21 treatment inhibited the activation of microglia in the spinal cord, as indicated by decreased expression of cd11b. These results indicate that α7 nAChR agonists might attenuate neuropathic pain via suppressing neuroinflammation. Consistently, Zhang et al. (2021b) demonstrated that microinjection of α7 nAChR siRNA into ipsilateral L4/5 DRG aggravated CFA-induced inflammatory pain, while intrathecal injection of GTS-21 attenuated the development of CFA-induced inflammatory pain. Their in vitro study showed that knockdown of α7 nAChR initiated the activation of TNF receptor-associated factor 6 (TRAF6) and nuclear factor kappa B (NF-kB) under CFA-induced inflammatory conditions, while α7 nAChR activation inhibited the upregulation of TRAF6 and NF-kB. These results indicate that GTS-21 might attenuate inflammatory pain by inhibiting the TRAF6/NF-kB signaling pathway. In a sciatic chronic constriction injury (CCI)-induced neuropathic pain model, Loram et al. (2012) demonstrated that subcutaneous injection of TC-7020, a selective α7 nAChR agonist, significantly attenuated CCI-induced mechanical allodynia. Moreover, TC-7020 downregulated the integrated density of activation transcription factor 3 and phosphorylated extracellular signal kinase as well as satellite cell activation in DRG in CCI rats. These results indicate that systemic administration of TC-7020 may attenuate neuropathic pain by reducing neuronal injury and immune cell activation in the DRG.

In a mouse model of visceral pain, Costa et al. (2012) showed that intraperitoneal injection of selective α7 nAChR agonist PNU-282987 significantly reduced DSS-induced mechanical allodynia. Similarly, Di Cesare Mannelli et al. (2014) demonstrated that PNU-282987 significantly reduced mechanical allodynia in oxalipatin-treated rats. Importantly, PNU-282987 treatment prevented the downregulation of α7 nAChR in the sciatic nerve, DRG, and spinal cord in oxalipatin-induced neuropathic pain rats. In our recent study, we further investigated the analgesic effect of PNU-282987 in a rat model of CIBP (Yang et al., 2021). We found that both acute and chronic treatment with PNU-282987 significantly attenuated mechanical allodynia in CIBP rats, which was completely blocked by pretreatment with MLA. Moreover, repeated administration of PNU-282987 reversed the downregulation of α7 nAChR and inhibited the upregulation of NF-kB in the spinal cord. These findings indicate that PNU-282987 might attenuate chronic pain by restoring the expression of α7 nAChR. In another study, Sun et al. (2017) demonstrated that intrathecal injection of PHA-543613, a selective α7 nAChR agonist, dose-dependently attenuated SPS-evoked mechanical allodynia, which was remarkably blocked by pretreatment with MLA.

Moreover, SPS-induced glial activation and upregulation of proinflammatory cytokines were considerably suppressed by PHA-543613, which was abolished by MLA. Their further study confirmed that repeated intrathecal injection of PHA-543613...
## TABLE 1  Summary of the therapeutic potential of α7 nAChR agonists and allosteric modulators in chronic pain.

| Compound   | Chemical structure | Model                          | Treatment strategy                                                                                     | Effects | Mechanisms                                      | References     |
|------------|--------------------|--------------------------------|------------------------------------------------------------------------------------------------------|---------|------------------------------------------------|---------------|
| Nicotine   | ![Nicotine](image) | MIA-induced osteoarthritis pain mice | Nicotine (0.5 and 1.0 mg/kg, i.p.) was administered once daily for seven consecutive days before MIA and 21 straight days after MIA. | PWT↑    | Activation of α7 nAChR | Teng et al., 2019 |
| Choline    | ![Choline](image)  | DSS-induced visceral pain mice   | Nicotine (0.1, 0.3, and 1.0 mg/kg, p.o.) was administered two times daily at 12-h intervals from day 3 to 6 of 4% DSS intake. | PNT↑    | Activation of α7 nAChR | Costa et al., 2012 |
|            |                    | gp120-induced neuropathic pain rats | Choline (0.1, and 1 μM, i.t.) was administered intrathecally either with or 30 min after intrathecal gp120. | PWT↑    | Activation of α7 nAChR Microglial activation↓ TNFα, IL-1β and IL6↓ | Loram et al., 2010 |
| Compound B | ![Compound B](image) | CFA-induced inflammatory pain in rats and mice | Compound B (1, 3, and 10 mg/kg, i.p.) was administered 23.5 h after CFA injection in rats. Compound B (5, 10, and 20 mg/kg, i.p.) was administered 23.5 h after CFA injection in mice. | PWT↑    | Activation of α7 nAChR | Medhurst et al., 2008 |
| GTS-21     | ![GTS-21](image)   | CFA-induced inflammatory pain mice | GTS-21 (5,000, 10,000, and 20,000 nM/mice, i.t.) was injected on day 3 after the CFA injection. | PWL↑    | Activation of α7 nAChR TRAF6↓ NF-κB↓ | Zhang et al., 2021b |
| TC-7020    |                    | gp120-induced neuropathic pain rats | GTS-21 (1, and 10 μg/mL, i.t.) was administered intrathecally either with or 30 min after intrathecal gp120. | PWT↑    | Activation of α7 nAChR Microglial activation↓ TNFα, IL-1β and IL6↓ | Loram et al., 2010 |
| PNU-282987 | ![PNU-282987](image) | CCI-induced neuropathic pain rats | PNU-282987 (30 mg/kg, p.o.) was administered acutely on day 21 or daily starting from the first day of oxaliplatin administration up to day 20. | PWT↑    | Activation of α7 nAChR | Di Cesare Mannelli et al., 2014 |
|            |                    | Oxaliplatin-induced neuropathic pain rats | PNU-282987 (0.1, 0.25, and 0.5 mg/kg, i.t.) was given on day 14 after surgery. PNU-282987 (0.5 mg/kg, i.t.) was given once daily from day 14 to 18 after surgery. | PWT↑    | Activation of α7 nAChR NF-κB↓ | Yang et al., 2021 |
|            |                    | Cancer-induced bone pain rats | PNU-282987 (0.1, 0.25, and 0.5 mg/kg, i.t.) was given on day 14 after surgery. PNU-282987 (0.5 mg/kg, i.t.) was given once daily from day 14 to 18 after surgery. | PWT↑    | Activation of α7 nAChR NF-κB↓ | Costa et al., 2012 |
|            |                    | DSS-induced visceral pain mice   | PNU-282987 (0.1, 0.3, and 1.0 mg/kg, i.p.) was administered two times a day at 12-h intervals from day 3 to 6 of 4% DSS intake. | PNT↑    | Activation of α7 nAChR | Costa et al., 2012 |
| PHA-543613 | ![PHA-543613](image) | Formalin-induced inflammatory pain rats | PHA-543613 (0.3 and 3 nmol, Intra-vlPAG) was administered 5 min before formalin injection. PHA-543613 (0.2–10 mg/kg, s.c.) was administered 15 min before formalin injection. | PWL↑    | Activation of α7 nAChR | Umana et al., 2017 |
|            |                    | SPS-induced chronic pain rats   | PHA-543613 (6 and 12 μg, i.t.) was administered for 8 consecutive days starting from the day of SPS. PHA-543613 (12 μg, i.t.) was administered on day 7 after SPS. | PWT↑    | Activation of α7 nAChR Microglial activation↓ Astrocytic activation↓ TNFα and IL-1β↓ | Sun et al., 2017 |

(Continued)
| Compound          | Chemical structure | Model                      | Treatment strategy                                                                 | Effects |
|-------------------|--------------------|----------------------------|------------------------------------------------------------------------------------|---------|
| SNL-induced neuropathic pain rats | PHA-543613 (12 µg, i.t.) was administered 7 or 21 days after SNL. | | | PWT↑, Activation of α7 nAChR, TNFα, IL-1β↑, dynorphin A↓ | Ji et al., 2019 |
| Preoperative stress-induced prolongation of postsurgical pain rats | PHA-543613 (12 µg, i.t.) was administered 30 min before SPS or on the first day after incisional surgery. PHA-543613 (12 µg, i.t.) was administered once daily for 5 consecutive days starting from 30 min before SPS. | | | PWT↑, Activation of α7 nAChR Microglial activation↓, TNFs and IL-1β↓, NF-κB↓ | Sun et al., 2019 |
| DDD-028           | Paclitaxel-induced neuropathic pain rats | DDD-028 (1, 5, 10, and 25 mg/kg, p.o.) was administered on day 10 after the initial injection of paclitaxel. DDD-028 (10 mg/kg, p.o.) was administered twice daily for 18 consecutive days starting from the beginning of the paditaxel injection. | | PWT↑, Activation of α7 nAChR Oxidative stress, Microglial activation↓, Astrocytic activation↓ | Micheli et al., 2021 |
| PNU-120596        | Carrageenan-induced inflammatory pain mice | PNU-120596 (1 and 4 mg/kg, s.c.) was administered 15 min before intraplantar injection of carrageenan. PNU-120596 (8 mg/kg, i.p.) was administered 3 h after carrageenan. | | PWT↑, Activation of α7 nAChR | Freitas et al., 2013b |
|                  | CCI-induced neuropathic pain mice | PNU-120596 (1, 2, and 4 mg/kg, i.p.) was administered 10 days after CCI. | | PWT↑, Activation of α7 nAChR | Freitas et al., 2013b |
|                  | Preoperative stress-induced prolongation of postsurgical pain rats | PNU-120596 (15 µg, i.t.) was administered once daily for 5 consecutive days starting from 30 min before SPS. | | PWT↑, Activation of α7 nAChR Microglial activation↓, TNFs and IL-1β↓, NF-κB↓ | Sun et al., 2019 |
| GAT107 /          | Formalin-induced inflammatory pain mice | GAT107 (0.1, 1, and 10 mg/kg, i.p.) was injected 15 min before formalin injection. Mice were administered with GAT107 (1 and 10 mg/kg, i.p.) for 6 days twice daily with 8 h apart and were challenged with GAT107 (1 or 10 mg/kg, i.p.) on day 7 and tested in the formalin test. | | PWT↑, Activation of α7 nAChR, Astrocytic activation↓, p38 MAPK↓ | Bagdas et al., 2016 |
|                  | CFA-induced inflammatory pain mice | GAT107 (1, 3, and 10 mg/kg, i.p.) was injected on day 3 after the CFA injection. GAT107 (0.3 and 3 µg/5 µL/mouse, i.t.) was injected on day 3 after CFA injection. GAT107 (3 and 9 µg/20 µL/mouse, i.pl.) was injected on day 3 after CFA injection. | | PWT↑, Activation of α7 nAChR Microglial activation↓, NF-κB↓, TNFα↓ | Bagdas et al., 2016 |
|                  | CCI-induced neuropathic pain mice | GAT107 (1, 3, and 10 mg/kg, i.p.) was injected 2 weeks after CCI surgery. | | PWT↑, Activation of α7 nAChR Microglial activation↓, NF-κB↓, TNFα↓ | Bagdas et al., 2016 |
| TQS /             | LPS-induced inflammatory pain | TQS (0.25, 1, and 4 mg/kg, i.p.) was given 30 min before LPS administration. | | PWT↑, Activation of α7 nAChR, Microglial activation↓, NF-κB↓ | Abbas et al., 2019 |

(Continued)


**α7 nAChR positive allosteric modulators**

Despite the encouraging therapeutic efficacy of α7 nAChR agonists in chronic pain, concerns have been raised regarding the long-term administration of α7 nAChR agonists due to rapid desensitization of α7 nAChR, receptor selectivity issues, and the narrow window of antinociceptive effect *in vivo* (Freitas et al., 2013a; Toma et al., 2020). These limitations hinder the application of α7 nAChRs as analgesics in the clinic. However, they led to the development of α7 nAChR positive allosteric modulators (PAMs), which potentiate α7 currents in the presence of an endogenous agonist such as acetylcholine and choline. Type I PAMs facilitate agonist response with little effect on desensitization of α7 nAChRs, while type II PAMs facilitate agonist response and retard the obvious desensitization profile of the agonist response (Toma et al., 2020). Freitas et al. (2013b) investigated two types of PAMs in chronic pain models. They found that both NS1738 (type I) and PNU-120596 (type II) considerably attenuated thermal hyperalgesia, while only PNU-120596 considerably reduced edema in carrageenan-induced inflammatory pain mice. Moreover, PNU-120596 reversed established thermal
hyperalgesia and edema induced by carrageenan. Additionally, a single dose of PNU-120596 significantly attenuated mechanical allodynia and thermal hyperalgesia in CCI mice, while NS1738 was ineffective. Importantly, the analgesic effect of PNU-120596 was completely blocked by systemic administration of the α7 nAChR antagonist MLA. Furthermore, an ineffective dose of selective α7 nAChR agonist PHA-543613 produced anti-allodynic effects in CCI mice by co-administration of PNU-120596. Their further study demonstrated that systemic administration of PNU-120596 dose-dependently attenuated nociceptive behaviors in the formalin test (Freitas et al., 2013c). Moreover, PNU-120596 enhanced the effects of nicotine and PHA-543613 in the formalin test. Our recent study also demonstrated that PNU-120596 enhanced the analgesic effect of PNU-282987 in a rat model of CIBP (Yang et al., 2021). Consistently, Sun et al. demonstrated that repeated intrathecal injection of PNU-120596 during the perioperative period shortened the duration of post-surgical pain after SPS and suppressed SPS-potentiated microglia activation, which was markedly abolished by pretreatment with MLA (Sun et al., 2019). These results indicate that type II α7 nAChR PAM PNU-120596 shows a potent analgesic effect in chronic pain. In another study, Bagdas et al. (2016) investigated the antinociceptive role of GAT107, a potent α7 nAChR type II PAM with intrinsic allosteric agonist activities. They found that GAT107 dose-dependently attenuated CFA-induced inflammatory pain and CCI-induced neuropathic pain. Moreover, intrathecal, but not intraplantar, injection of GAT107 reversed established pain behaviors in CFA mice. Furthermore, intrathecal GAT107 treatment inhibited the activation of astrocytes and upregulated p38 mitogen-activated protein kinase in the spinal cord. In a mouse model of lipopolysaccharide (LPS)-induced inflammatory pain, Abbas et al. (2019) investigated the analgesic effect of 3a,4,5,9b-tetrahydro-4-(1-naphthalenyl)-3H-cyclopentan[c]quinoline-8-sulfonamide (TQS), an α7 nAChR type II PAM. They found that systemic administration of TQS prevented LPS-induced mechanical allodynia and thermal hyperalgesia by decreasing the expression of Iba-1, p-NF-κB, and TNF-α in CA1 and dentate gyrus regions of the hippocampus. Their further study demonstrated that TQS attenuated LPS-induced inflammatory pain via downregulation of hippocampal brain-derived neurotrophic factor and Na⁺/K⁺/Cl⁻ and upregulation of K⁺/Cl⁻ (Abbas et al., 2021). Recently, Bagdas et al. (2021) demonstrated that acute systemic administration of PAM-4, a highly selective α7-nAChR PAM, dose dependently reversed formalin-induced inflammatory pain and CCI-induced neuropathic pain without the development of any motor impairment, which was entirely blocked by MLA. Moreover, PAM-4 reversed CCI-induced depression-like behavior and anxiogenic-like effects. These results indicate that PAM-4 reduces both sensorial and affective behaviors in chronic pain via α7 nAChR potentiation. Interestingly, secreted mammalian Ly6/urokinase plasminogen activator receptor-related protein-1 (SLURP-1) has been identified as an endogenous ligand of α7 nAChR (Chimenti et al., 2003). It was reported that SLURP-1 is expressed in the dorsal horn of the spinal cord and a subset of primary peptidergic sensory neurons in the dorsal root ganglia (Moriwaki et al., 2009). Therefore, it is plausible that SLURP-1 might play a role in chronic pain via activation of α7 nAChR. Collectively, these results indicate a promising future for the management of chronic pain via α7 nAChR positive allosteric modulators.

α7 nAChR silent agonists

Silent agonists are an additional class of α7 nAChR ligands (Papke et al., 2014). Toma et al. (2019) demonstrated that R-47, an α7 nAChR silent agonist, prevented and reversed paclitaxel-induced mechanical allodynia in mice in an α7 nAChR-dependent manner. Moreover, R-47 prevented paclitaxel-induced loss of intraepidermal nerve fibers and activation of microglia in the spinal cord. Importantly, R-47 did not affect tumor cell viability or interfere with the antitumor activity of paclitaxel in tumor-bearing mice. These results indicate that R-47 might be a promising strategy for preventing and treating paclitaxel-induced neuropathic pain.

Concluding remarks and future perspective

Data from our laboratory and others have shown decreased expression of α7 nAChR in the pain transmission pathway. Notably, the potentiation of α7 nAChR exerts a potent analgesic effect against chronic pain in preclinical studies (Gong et al., 2015; Criado et al., 2016; Quadri et al., 2018; Yang et al., 2018; Apyrani et al., 2020). This review summarized and discussed the therapeutic potential of α7 nAChR agonists and allosteric modulators in chronic pain (Table 1). This evidence showed that potentiation of α7 nAChR attenuates chronic pain mainly through modulating neuroinflammation (Figure 1). However, these findings raise further questions.

First of all, most of the studies mentioned above focused on behavioral tests after treatment rather than the underlying mechanisms. It is well-established that neuroinflammation plays a pivotal role in the development of chronic pain (Zhou et al., 2019; Jiang et al., 2020; Chen et al., 2021). The studies mentioned above demonstrated that α7 nAChR potentiation significantly inhibited the activation of microglia and astrocytes, as well as suppressed the release of proinflammatory cytokines in the spinal cord of rodent models of chronic pain. Nevertheless, how activation of α7 nAChR in neuronal and non-neuronal cells may reduce neuroinflammation in chronic pain remains elusive. Therefore, detailed mechanisms underlying the analgesic effects of α7 nAChR agonists and allosteric modulators must be
In preclinical studies, the mechanisms underlying the analgesic effect of $\alpha_7$ nAChR agonists and allosteric modulators in chronic pain. IL-1$\beta$, interleukin 1$\beta$; IL-6, interleukin 6; nAChR, nicotinic acetylcholine receptor; and TNF-$\alpha$, tumor necrosis factor-$\alpha$.

clarified. For instance, activation of adenosine monophosphate-activated protein kinase (AMPK) signaling might be an interesting hypothesis accounting for the analgesic effects of $\alpha_7$ nAChR agonists and allosteric modulators. It has been frequently reported that potentiation of $\alpha_7$ nAChR elicits activation of AMPK signaling in various diseases (Hasan et al., 2018; Lin et al., 2019; Shao et al., 2019; Xu et al., 2021). Previous studies showed that AMPK activation significantly suppressed neuroinflammation in the central nervous system in chronic pain (Song et al., 2015; Zhang et al., 2021a; Wan et al., 2022). Recently, our laboratory demonstrated that induction of AMPK-mediated mitochondrial biogenesis plays a pivotal role in alleviating chronic pain (Sun et al., 2022). Therefore, it is worth finding whether activation of AMPK signaling contributes to the analgesic effects of $\alpha_7$ nAChR agonists and allosteric modulators.

Second, the conclusions of the studies mentioned above were based on male rodents only, which is a weakness of the work. Emerging evidence indicates a sex difference in susceptibility to chronic pain (Won et al., 2020). For instance, microglia are required for mechanical pain hypersensitivity in male mice, whereas female mice achieve similar levels of pain hypersensitivity using adaptive immune cells, likely T lymphocytes (Sorge et al., 2015). Recently, it was reported that $\alpha_7$
nAChR regulated hippocampal adult-neurogenesis in a sexually dimorphic fashion (Otto and Yakel, 2019). Therefore, future studies should determine the role of α7 nAChR in chronic pain in females.

Besides, although compelling evidence supports the pivotal role of α7 nAChR potentiation in chronic pain, what contributes to the decreased expression level of α7 nAChR in the pain transmission pathway remains unclear. More importantly, the mechanism behind agonist-induced upregulation of α7 nAChR is unknown. Therefore, the mechanisms underlying the decreased expression level of α7 nAChR in chronic pain and agonist-induced upregulation of α7 nAChR await further investigation.

Finally, despite the encouraging therapeutic efficacy of α7 nAChR agonists and allosteric modulators in chronic pain in preclinical studies, no relevant clinical trials are available. Notably, α7 nAChR agonists and allosteric modulators have shown good safety, tolerability, and efficacy profiles in other diseases such as schizophrenia (Zhang et al., 2012; Xia et al., 2020). However, concerns have been raised regarding the long-term administration of α7 nAChR agonists due to rapid desensitization of α7 nAChR, receptor selectivity issues, and the narrow window of antinociceptive effect in vivo. These limitations must be addressed before applying α7 nAChR as an analgesic in the clinic. Overall, these studies suggest that α7 nAChR is a promising therapeutic target for chronic pain, but more research regarding its detailed mechanisms is warranted.

Author contributions

X-BT conceived the idea. Y-QZ and D-QL wrote and edited the manuscript. CL, A-JX, Y-KT, and WM reviewed and edited the manuscript. All authors read and approved the final manuscript.

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