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Nicotinic acetylcholine receptors: Therapeutic targets for novel ligands to treat pain and inflammation☆

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Abstract

Nicotinic acetylcholine receptors (nAChRs) have been historically defined as ligand-gated ion channels and function as such in the central and peripheral nervous systems. Recently, however, non-ionic signaling mechanisms via nAChRs have been demonstrated in immune cells. Furthermore, the signaling pathways where nAChRs are expressed can be activated by endogenous ligands other than the canonical agonists acetylcholine and choline. In this review, we discuss the involvement of a subset of nAChRs containing α7, α9, and/or α10 subunits in the modulation of pain and inflammation via the cholinergic anti-inflammatory pathway. Additionally, we review the most recent advances in the development of novel ligands and their potential as therapeutics.

Keywords

Nicotinic acetylcholine receptor subunits α7, α9, and α10; Neuropathic pain; Chronic pain; Inflammatory pain; Chemotherapy-induced neuropathic pain; α-conotoxin RgIA

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) isolated from the electric organ of the *Torpedo marmorata* ray were first visualized using electron microscopy by Changeux and colleagues in 1973 [1,2]. These studies revealed that the receptors present in the ray electric organ were pentameric structures with a central pore that presumably allowed the flux of ions.

☆The multifaceted activities of nervous and non-nervous neuronal nicotinic acetylcholine receptors in physiology and pathology. Eds: Dr Cecilia Gotti, Prof Francesco Clementi, Prof Michele ZOli.

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CRediT authorship contribution statement

The authors contributed equally to manuscript conceptualization, writing the original draft, and writing, reviewing and, editing the revised manuscript.

Declaration of Interest Statement

The University of Utah has filed patent applications on conopeptides including those described in the present manuscript and on which J.M. M. is listed as an inventor.

Later studies would demonstrate that upon ligand-binding the pentameric structure would, in fact, rotate to open the channel and allow ion flux across the cell membrane generating an electrical current. Nicotinic receptors were later classified as the first member of a superfamily of ligand-gated ion channels that would expand to include γ-aminobutyric acid ($GABA_A$), glycine, 5-hydroxytryptamine type 3, and zinc-activated channels [3,4]. Through molecular biology techniques, it was later discovered that nAChRs are composed of five individual subunits rather than one large polypeptide. In fact, the nAChR subtype found at the neuromuscular junction is composed of four different gene products [5,6]. A total of 16 nicotinic genes have been identified in the human genome and code for α1-α7, α9, α10, β1-β4, δ, ε, and γ subunits. Myriad nAChR subtypes can be formed by different combinations of these subunits. For example, α4 and β2 subunits assemble together and comprise the most abundant nAChR subtype, α4β2 * (the asterisk denotes the potential presence of other subunits), in the mammalian brain. In the peripheral nervous system, α3β4 * nAChRs are most abundant. Subtypes containing more than one gene product are classified as heteromeric while those formed from a single gene product are known as homomeric nAChRs. Human homomeric subtypes have historically only included α7 and α9, but very recently we demonstrated that α10 subunits can assemble as functional homopentamers when heterologously expressed in *Xenopus laevis* oocytes [7]. Regardless of subunit combination, long-standing scientific precedent has held that nAChRs function as ligand-gated ion channels, but recent work in immune cells has challenged this notion [8].

Immune cells, especially monocytes and lymphocytes, are known to express several nAChR subunits [9–12]. Importantly, distinct subtypes of these cells express α7, α9, and/or α10 subunits [13,14]. The potential nAChR subtypes expressed by immune cells are shown in Fig. 1. However, patch-clamp electrophysiology studies have failed to detect acetylcholine-mediated currents in these cells [15]. Ionic currents mediated by α7 nAChRs have been observed in differentiated macrophages from the human THP-1 monocyte cell line, however [16]. Several ligands of α7, α9, and α10 nAChRs have been shown to modulate the release of inflammatory cytokines from immune cells [17–19]. These ligands include the canonical neurotransmitters acetylcholine and choline, but recently conjugates of choline and phosphocholine with soluble proteins including albumin and C-reactive protein have been shown to act as ligands of α7 and α9α10 nAChRs [20–23]. It is notable that immune cells are not considered ‘excitable’ yet express ion channels that are generally associated with neurons and other excitable cells. Though the exact mechanisms of how signal transduction occurs in immune cells is not fully understood, it is likely that canonical ion-channel functions are not involved. In this work, we review the involvement of nAChRs containing α7, α9, and/or α10 subunits in the modulation of pain and inflammation with a focus on novel ligands and mechanisms.

1.1. α7 nAChRs are broadly implicated in modulating the inflammatory responses of immune cells

Seminal studies in the early 2000s identified α7 nAChRs as prominent players in the modulation of inflammatory cytokine release by immune cells. Stimulation of the vagus nerve releases acetylcholine into the blood stream and activates nAChRs expressed by circulating immune cells. In vitro studies using human macrophages showed that stimulation

with acetylcholine inhibited the release of the inflammatory cytokines interleukin-1β (IL-1β), IL-6, IL-18 and tumor necrosis factor-α (TNF-α) [24]. Initially the nAChR mediating this response was unknown, but later experiments identified α7 as the principal subtype [25]. The link between the nervous system and the immune system can therefore be attributed to immune cell expressed α7 nAChRs along with the vagus nerve and collectively comprise two critical components of the cholinergic anti-inflammatory pathway (CAP) [26,27]. Mechanistically, stimulation of α7 nAChRs expressed by macrophages activates a number of important biochemical pathways involved in the inflammatory response. One pathway inhibits nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and another activates the janus-kinase-2 (JAK)-signal transducer and activator of transcription-3 (STAT3) pathway (JAK2-STAT). Inhibition of NF-κB signaling reduces the expression of and ultimately the release of TNF-α by macrophages and monocytes. A third pathway has been proposed that involves interleukin-1 receptor-associated kinases (IRAK) [28]. Stimulation of monocytes and macrophages with lipopolysaccharide (LPS) induces IRAK expression and TNF-α release through toll-like receptors (TLRs). Increased IRAK expression functions as a negative regulator of TLR functions. Human peripheral blood mononuclear cells (PBMCs) stimulated with nicotine showed up-regulation of IRAK-M expression, and this effect was dependent on α7 nAChR-mediated activation of the JAK2-STAT3 pathway [19]. Macrophages are just one example where a non-ionic mechanism of action mediated by α7 nAChRs has been demonstrated [29]. Non-ionic mechanisms have also been shown in a number of primary monocytes and monocytic cell lines and lymphocytes. In human pathologies, the expression and function of α7 nAChRs by immune cells may attenuate excessive inflammation. Clinical outcomes in patients with sepsis have been shown to closely correlate with levels of α7 nAChR mRNA in PBMCs [30]. Patients with lower levels of α7 nAChR mRNA experienced worse clinical outcomes including increased mortality, whereas those with higher levels showed attenuated signs and symptoms of sepsis. The inflammatory response that occurs in sepsis is reminiscent of the ‘cytokine-storm’ that occurs in COVID-19 disease, and α7 nAChRs have been proposed as pharmacological targets for attenuating the associated inflammatory response [31, 32]. Not surprisingly, broad involvement of α7 nAChRs in a variety of inflammatory conditions has generated immense interest in developing drugs that targets these receptors [33]. A summary of the interaction between α7 nAChRs and the downstream biochemical pathways is presented in Fig. 2.

1.2. Novel compounds that target α7 nAChRs are analgesic and antiinflammatory

The development of drugs that target α7 nAChRs is a major focus of research at several academic institutions and in the pharmaceutical industry, and a number of candidate compounds have been developed. Extensive research indicates that activation of α7 nAChRs is antiinflammatory and analgesic. However, recently developed compounds show a more diverse range of mechanisms rather than simple receptor agonism. These mechanisms include partial agonism, silent agonism, and positive allosteric modulation [34,35]. Some ligands show functional properties of both orthosteric agonists and positive allosteric modulators (PAM) and are called ago-PAMs. Agonists of α7 nAChRs have demonstrated efficacy in numerous models of pain and inflammation (Table 1). Two such compounds, PNU-282987 [36] and PHA-543613 [37], have been tested in a wide

range of disease models including neuropathic and inflammatory pain, chemotherapeutic-induced neuropathic pain (CIN), acute lung injury, inflammatory bowel disease (IBD), and chronic pain related to posttraumatic stress disorder (PTSD). In the rat chronic constriction injury (CCI) model of neuropathic pain, PNU-282987 was demonstrated to produce acute analgesia in response to noxious mechanical stimuli as measured by an Analgesy-meter [38]. Daily administration of PNU-282987 reduced macrophage infiltrate and attenuated pathophysiological changes of the sciatic nerve that are associated with CCI injury. Certain chemotherapeutics such as oxaliplatin, paclitaxel, and vincristine produce a type of neuropathy in humans that is characterized by decreased pain tolerance and severe cold allodynia. In a model of CIN, cold allodynia (cold plate test) and hyperalgesia (paw pressure and von Frey tests) were reduced by treatment with PNU-282987 [39]. Rats administered the chemotherapeutic oxaliplatin showed lower levels of α7 nAChR protein in the sciatic nerve, DRG, and spinal cord and PNU-282987 prevented this downregulation. PNU-282987 and another α7 nAChR agonist (*R*)-ICH3 also produced modulatory effects on microglia and astrocyte populations in the oxaliplatin model. Microglia numbers increased in the dorsolateral-periaqueductal grey, thalamus, and somatosensory areas in the brain but not in the dorsal horn of the spinal cord. By contrast, glial-fibrillary acidic protein-positive astrocyte numbers were increased in both the dorsal horn and in pain-related areas of the brain. Stimulation of glial cell and astrocyte populations by α7 nAChR agonists was hypothesized to be neuroprotective by preventing the pathophysiological changes to neurons and nerves induced by oxaliplatin. Both PNU-282987 and PHA-543613 also produced analgesia in the formalin model of inflammatory pain in mice [40,41]. Analgesia was observed in both the acute and tonic phases of pain that are induced by formalin administration. The antinociceptive effects of PNU-282987 were not observed in α7 knockout mice. Both compounds were also effective in the dextran sodium sulfate (DSS) model of colitis in rodents, but there were differences in their clinical profiles. PNU-282987 reduced referred mechanical hyperalgesia (von Frey test) associated with DSS-induced colitis but failed to attenuate the associated pathophysiological changes in colonic histology [42]. By contrast, PHA-543613 showed disease modifying properties including preservation of colon length and prevention of mucosal ulceration [43]. Interestingly, the disease modifying effects of PHA-543613 were only observed in male but not female mice. Although PNU-282987 failed to reduce myeloperoxidase activity, a marker of polymorphonuclear leukocyte recruitment, and levels of keratinocyte-derived chemokine in DSS, it was effective in modifying immune cell activity in the rat model of postoperative ileus (POI) and acute lung injury. Macrophage, but not neutrophil, infiltration into the muscle layer of the intestines was reduced in POI by administration of PNU-282987 [44]. In a model of acute lung injury, neutrophil recruitment, levels of TNF-α, IL-1β, IL-6 levels, and NF- κB activity were all reduced with PNU-282987 [45]. Similarly, PHA-543613 suppressed reactive astrocyte numbers and decreased spinal IL-1β and TNF-α levels in the rat PTSD-related chronic pain model [46]. Lastly, in an inflammation-driven model of systemic skin fibrosis, PHA-543613 prevented or reversed fibrosis in mice [47].

One potential issue with stimulation of α7 nAChRs to treat disease is the fact that this receptor subtype is highly expressed in multiple systems in the body, and over stimulation may result in unwanted effects on systems not related to the disease target of interest. A

potential solution would be to use a ligand that lacks full efficacy or to increase endogenous cholinergic tone through allosteric modulation. Partial agonists are ligands that activate the receptor or increase endogenous tone but to a lesser extent than the natural neurotransmitter, and silent agonists are those that lack intrinsic agonist activity and function by working through non-conducting states of the receptor. Examples of such ligand are GTS-21 [48] and NS-6740 [49]. GTS-21, an analog of the plant alkaloid anabasine (*Nicotiana glauca*), is a partial agonist of α7 nAChRs and has been shown to be analgesic in models of inflammatory pain. In the complete Freund's adjuvant (CFA) model, GTS-21 attenuated mechanical hyperalgesia (von Frey test) and thermal hyperalgesia (Analgesia Meter and cold plate test) [50]. Likewise, in the formalin model NS-6740 reduced nocifensive behaviors (paw licking) and was analgesic in both the acute and tonic phases of the inflammatory pain response [41]. In a different study, NS-6740 reduced mechanical allodynia (von Frey test) in mice subjected to CCI [51]. Ligands, such as GAT-107, that bind to the orthosteric binding-site as well as an allosteric modulatory site are called ago-PAMs [52,53]. In a mouse model of experimental autoimmune encephalomyelitis (EAE), GAT-107 reduced signs and symptoms of neuroinflammation as well as meningeal infiltration of immune cells in the spinal cord [35]. Mechanical allodynia (von Frey test) was also attenuated in CCI and LPS-induced inflammatory pain by GAT-107 treatment [54]. Interestingly, ligands that have no intrinsic agonist activity, but show only positive allosteric modulation, have also been shown to be analgesic and anti-inflammatory. The PAM PNU-120596 was analgesic in phase II of the formalin model [40,41] and was remarkably effective at reducing the signs and symptoms of DSS-induced colitis in mice including reduced severity of pathophysiological changes in colon histology [43]. These studies suggest that increasing the endogenous cholinergic tone through positive allosteric modulation might be an effective therapeutic strategy for the treatment of pain and inflammation without the risk of inducing desensitization which could result in antagonism of the CAP. However, in some cells over stimulation of α7 nAChRs with PNU-120596 proved to be cytotoxic from elevated levels of intracellular calcium [55]. Nevertheless, these studies provide strong evidence that ligands of α7 nAChRs exert their analgesic and anti-inflammatory properties through modulation of the activities of immune cells and specifically through inhibition of pro-inflammatory cytokine and chemokine release by these cells. A number of other compounds not mentioned here have been developed and assessed for their potential as analgesic and anti-inflammatory drugs as well as treatments for disorders of cognition [56–62]. Lastly, natural products such as formulated curcumin have shown promising results in rodent models of inflammatory pain and CIN [63,64].

1.3. Nicotinic acetylcholine receptors containing α9/α10 subunits are novel targets for pharmacological intervention in pain and inflammatory conditions

CHRNA9 and *CHRNA10* are the most recently discovered genes in the nAChR family [15, 69–71]. Initially discovered through a rat cDNA library screen, in situ hybridization studies showed that *CHRNA9* expression was localized to a discrete set of tissues outside of the central nervous system including the cochlea, pars tuberalis, and olfactory bulb. In humans, α9 was discovered in epidermal and oral keratinocytes and functionally regulates keratinocyte adhesion [72]. Some years after the discovery of the rat α9 subunit, human *CHRNA9* and *CHRNA10* were discovered through the screening of libraries obtained from

whole embryo and tonsil [15,71]. Importantly, α9 and α10 mRNAs were found in tonsil tissue-derived B-, and T-cells, peripheral blood lymphocytes, and monocytes. Subsequent work refined the expression patterns of α9 and α10 to CD3 +, CD4 +, and CD8 + T-cells as well as CD19 + and CD80 + B-cells [13]. These studies have spurred investigations into the role of α9 and α10 subunits in immune-system function including pathophysiological conditions such as cancer [73,74]. A summary of the interaction between α9/α10 nAChRs and the downstream biochemical pathways is presented in Fig. 3.

1.4. α-Conotoxins are analgesic, anti-inflammatory, and reduce signs and symptoms of disease in models of neuropathic pain

The expression of α9 and α10 subunits by immune cells suggests that α9/α10 nAChRs are involved in immune cell functions. Some of the first studies that implicated α9/α10 nAChRs in neuropathic pain came from experiments with α-conotoxins that selectively target α9/α10 nAChRs. One such α-conotoxin, Vc1.1, was shown to accelerate functional recovery of damaged nerves and alleviate pain in the rat CCI model of neuropathic pain [75]. Vc1.1 later entered human clinical trials as ACV-1 but failed to show the same efficacy for reducing human neuropathic pain, consistent with its low affinity for the human α9α10 subtype [76–78]. A second α-conotoxin called [S4Dap]Mr1.1 that is similar in sequence to Vc1.1 has recently been shown to be analgesic in the rat CCI model [79]. α-Conotoxin RgIA has been used in a number of neuropathic and inflammatory pain models and shown to be effective in reducing pain and inflammation. In the rat CCI model, RgIA reduced the signs and symptoms of neuropathy [80]. Specifically, RgIA reduced the histological changes in nerve morphology including decreased axonal compactness and diameter, loss of myelin sheath, and decreased nerve-fiber numbers. The therapeutic effects produced by RgIA in this model may be attributed to modulation in the activity of immune cells since inflammatory infiltrate including lymphocytes and CD86+ macrophages was reduced in the affected nerve and dorsal root ganglion (DRG) [80,81]. These disease-modifying effects were not limited to peripheral nerves as RgIA prevented the activation of microglia and astrocytes in the dorsal horn of the spinal cord. Further evidence that RgIA modulates the activity of immune cells was found in the DSS model of inflammatory bowel diseases (IBD) in mice [82]. In this study, colonic levels of TNF-α were reduced and pathological changes in colon morphology were prevented by administration of RgIA. Therapeutic effects were also observed in models of CIN. Morphological changes in rat DRG induced by oxaliplatin administration were significantly attenuated and the number of glial fibrillary acidic-protein positive astrocytes in the dorsal horn reduced [83]. Analogs of RgIA with increased potency were also analgesic in CIN. RgIA4 prevented oxaliplatin-induced cold allodynia and neuropathic pain in mice [84], and the therapeutic effects were dependent on the presence of CD3+ T-cells [85]. Strikingly, RgIA4 provided sustained protection against oxaliplatin-induced nerve injury in mice long after the last dose of the peptide [86]. Additional analogs of RgIA with increased potency and biostability are being developed as potential therapeutics for treatment of human neuropathic pain conditions [87–90].

Other α-conotoxins and small molecules that target α9α10 nAChRs have also been shown to be therapeutic in various neuropathy models. GeXIVA and is an α-conotoxin that is structurally quite different than Vc1.1, [S4Dap]Mr1.1, and RgIA yet was also analgesic and

reduced mechanical hyperalgesia (von Frey test) in rats subjected to CCI [88,91]. Small non-peptidic molecules have been synthesized that are analgesic and anti-inflammatory in CCI-induced neuropathy, formalin-induced inflammatory pain, and CIN. The tetrakis-quaternary ammonium compound ZZ-204G was analgesic across a battery of pain models including CCI, formalin-induced inflammatory pain, and the tail-flick model of thermal nociception [92]. The related compound ZZ1–61c prevented the induction of neuropathy by the chemotherapeutic vincristine [93]. Structurally diverse ligands selective for α9α10 nAChRs that are analgesic provide strong evidence in support of targeting α9/α10 nAChRs for treating neuropathic and inflammatory pain [94,95].

The ligands discussed above are all antagonists of α9α10 nAChRs, but some studies suggest that agonist ligands may have anti-inflammatory and/or analgesic properties. Recently, a number of novel small molecule agonists and antagonists that target α9α10 nAChRs were reported [96]. One of these compounds, the agonist *p*CF3 diEPP, was effective at inhibiting LPS-induced release of IL-6 from primary mouse macrophages and IL-6, TNF-α, and IL-1β from whole human blood cultures [97]. Additionally, *p*CF3 diEPP inhibited ATP-induced release of IL-1β from human peripheral blood mononuclear leukocytes. Choline is an agonist of α9α10 nAChRs and has analgesic and anti-inflammatory properties, but most studies attribute the analgesic effects of choline to agonism of α7 nAChRs. It is noteworthy that choline concentrations in the mM range were required for inhibition of TNF-α release from cultured mouse macrophages [98]. In other cell types such as human U937 monocytes, 10 μM choline was sufficient to inhibit IL-1β release [21]. Naturally occurring derivatives of choline have been shown to inhibit the release of cytokines from several immune cell types. Cytidine-5′-diphosphate choline, or CDP-choline, has been shown to be analgesic, but again most of these studies attribute the effects to α7 nAChRs [99–102]. Phosphocholine is an agonist of human monocyte expressed nAChRs containing α7, α9, and α10 subunits [20,21]. In these studies, both choline and phosphocholine inhibited ATP-stimulated release of IL-1β from human and murine monocytes and from the human monocyte U937 cell line. The effects of choline and phosphocholine were significantly inhibited by the potent and selective antagonist RgIA4, suggesting that α9α10 nAChRs are involved in the in modulation of immune cell function by choline and its derivatives. There is a large disparity between the potency of choline for activation of α7 vs α9-containing nAChRs; choline activates α9 and α9α10 nAChRs [71] in the low μM range whereas mM concentrations are required for activation of the α7 subtype. Concentrations in the mM range are probably not achieved in human blood [103], and thus it remains an open question as to which nAChR subtype mediates the therapeutic effects of choline. (Table 2).

1.5. Alternative mechanisms for the therapeutic effects of α-conotoxins that target α9α10 nAChRs

Certain α-conotoxin ligands that target α9α10 nAChRs have been shown to act as agonists of $GABA_B$ receptors [104–110]. Structure-activity studies have correlated potency for $GABA_B$ receptors with analgesia suggesting that a portion of the therapeutic effects might be accounted for by this mechanism. $GABA_B$ receptors are present in DRG, spinal cord and brain. Baclofen is a clinically used $GABA_B$ agonist with analgesic activity in animal models. However, its modest therapeutic effects are thought to be mediated through action on central

nervous system (CNS) rather than DRG expressed $GABA_B$ receptors [111–113]. The size and charge of α-conopeptides would likely significantly limit CNS concentrations of the peptides precluding therapeutic effects at spinal cord or brain $GABA_B$ receptors. Separately, it is noteworthy that highly potent α-conopeptide antagonists of α9α10 nAChR, that show analgesic activity, lack effects on $GABA_B$ receptors [84,89, 90]. In addition, studies utilizing mice with germline deletions of the α9 nAChR subunit indicate that analgesic activity of the peptides in CIN are dependent on the α9 nAChR subunit [84,85,90]. Furthermore, as reviewed above, disease-modifying aspects of analgesic α-conopeptides are consistent with immune system modulation mediated by α9-containing nAChRs; similar properties have not been noted for baclofen or other $GABA_B$ receptor agonists (for further review see [94,114] and references therein).(Table. 3).

2. Conclusions

A growing body of evidence establishes nAChRs containing α7, α9, and/or α10 subunits as promising molecular targets for pharmacotherapy of disease that involves pain and inflammation. It appears increasingly likely that inhibition of pain and inflammatory states by nAChRs occurs through modulation of immune cell function. However, at the present time the exact composition and stoichiometry of the nAChRs involved in modulating immune cell function isn't precisely known. Long-standing convention holds that α7 subunits form homopentamers and that α9 and α10 subunits are expressed together as heteropentamers in human. Recently, however, it was demonstrated that α7 subunits can combine with β2 subunits to form α7β2 heteromers and are expressed in certain areas of mammalian basal forebrain [118–120]. Immune cells are also known to express β2 subunits, and it is unknown whether they combine with α7 subunits in these cells. The stoichiometry of immune cell expressed receptors containing α9 and α10 subunits is perhaps more complicated. Not only can α9α10 nAChRs vary with respect to the ratio of α9 to α10 subunits [78,121], which alters the pharmacology of the receptor, but the individual subunits themselves can also be expressed as homopentamers [7,69,71]. Nevertheless, studies using ligands selective for α7 and α9α10 nAChRs strongly suggest that a number of immune cell types express these two subtypes [122]. Prospective pharmacotherapeutics may benefit from being subtype selective to avoid off-target effects such as reinforcing behaviors that, for example, have hindered the development of an analgesic drug that targets α4β2* nAChRs [123]. As mentioned above, agonists and PAMs of α7 nAChRs may produce unwanted side effects from elevated intracellular calcium levels which can be cytotoxic. Furthermore, α7 nAChRs are highly expressed in numerous areas of the CNS and PNS. Human adrenal chromaffin cells, for example, have enriched expression of α7 nAChRs and are involved in the stimulus-secretion coupling response [124]. Ligands that activate α7 nAChRs have been shown to be excitatory and increase catecholamine release from human chromaffin cells [125,126]. Such actions could increase plasma catecholamine levels and potentially trigger cardiovascular side effects. Therefore, research investigating potential side effects that may occur from systemic administration of α7 nAChR ligands is essential. Pharmacotherapeutics targeting α9α10 nAChRs might produce fewer side effects because of their restricted expression patterns in comparison to α7 nAChRs. α9α10 nAChR are expressed by immune cells, skin, anterior pituitary, and a limited number of other tissues but not in the CNS [127],

and therefore agonist ligands would likely be devoid of reinforcing behaviors. Interestingly, mRNA for the α9 subunit has been found in human [128] and α10 in rat [129] DRG though responses attributable to α9α10 nAChRs have thus far not been identified. Additional research is needed to further understand the physiological as well as the pathological roles of α7 and α9α10 nAChRs in pain and inflammation.

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Data availability

No data was used for the research described in the article.

Abbreviations:

CCI	chronic constriction injury
CIN	chemotherapy-induced neuropathic pain
EAE	experimental autoimmune encephalomyelitis
PTSD	posttraumatic stress disorder
PAM	positive allosteric modulator
LPS	lipopolysaccharide

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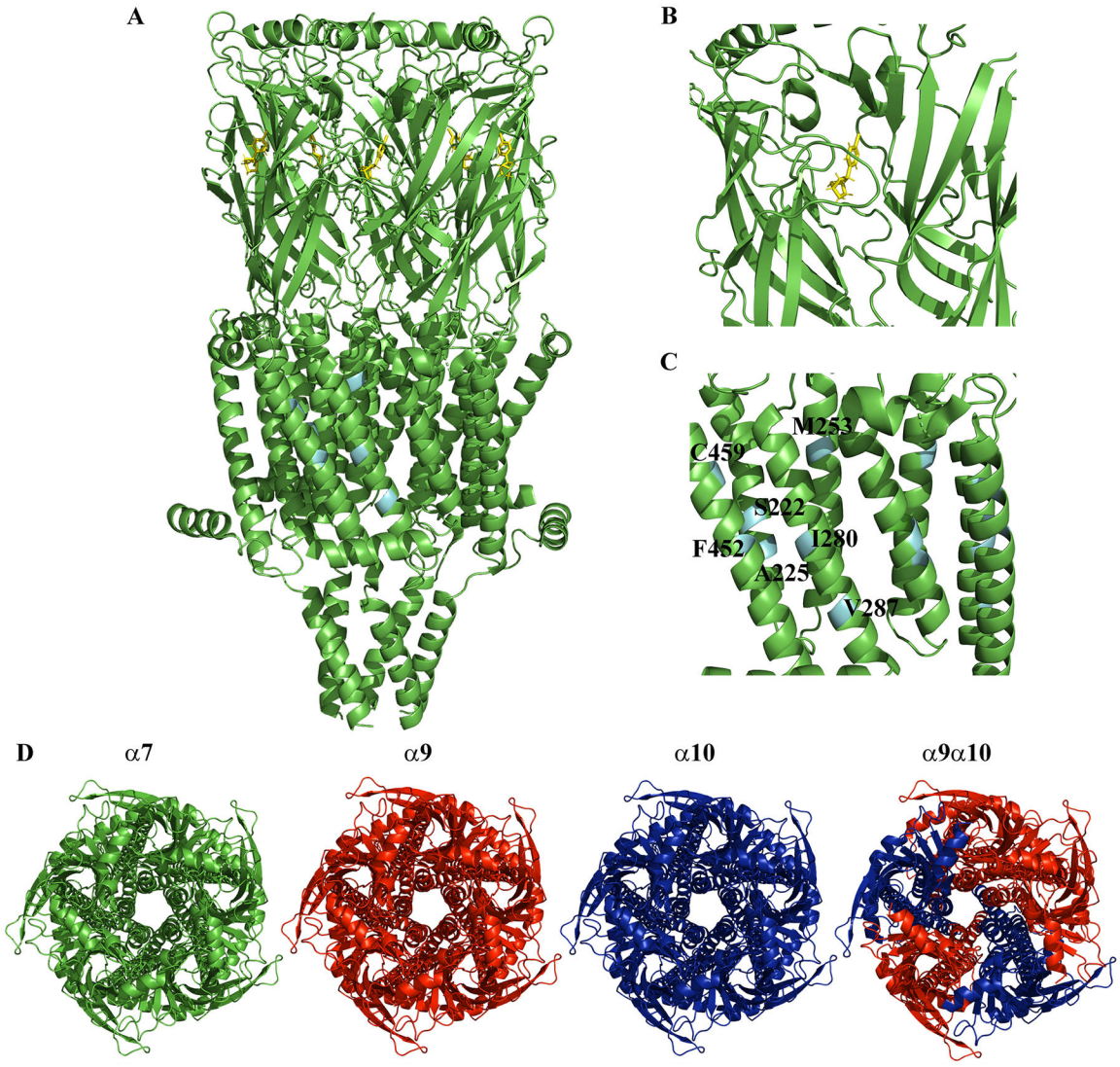

Fig. 1.

The nicotinic acetylcholine receptors (nAChRs) involved in pain and inflammation. (A) Cartoon rendition of the cryo-EM structure of the human homomeric α7 nAChR (PDB:7KOX) [130]. (B) A 20 Å spherical view of the orthosteric ligand-binding site shown with the frog neurotoxin epibatidine (yellow) from *Epipedobates tricolor*. (C) A 20 Å spherical view of the allosteric ligand-binding site. The labeled residues are involved in binding different ligands and are cyan colored. (D) Several immune cell types including lymphocytes, monocytes/macrophages, and granulocytes are known to express nAChRs containing α7, α9, and/or α10 nAChR subunits. These subtypes may include homomeric α7 (green), α9 (red) or α10 (blue) nAChRs. Heteromeric α9α10 (red and blue) are also expressed although the stoichiometry and ratio of α9 to α10 subunits has yet to be elucidated. The structures are color coded to depict graphically the different nAChR subtypes and generated using the α7 structure (PDB:7KOO) [130]. The receptors are in the closed position and oriented looking through the channel from extracellular space. All images were generated using PyMOL.

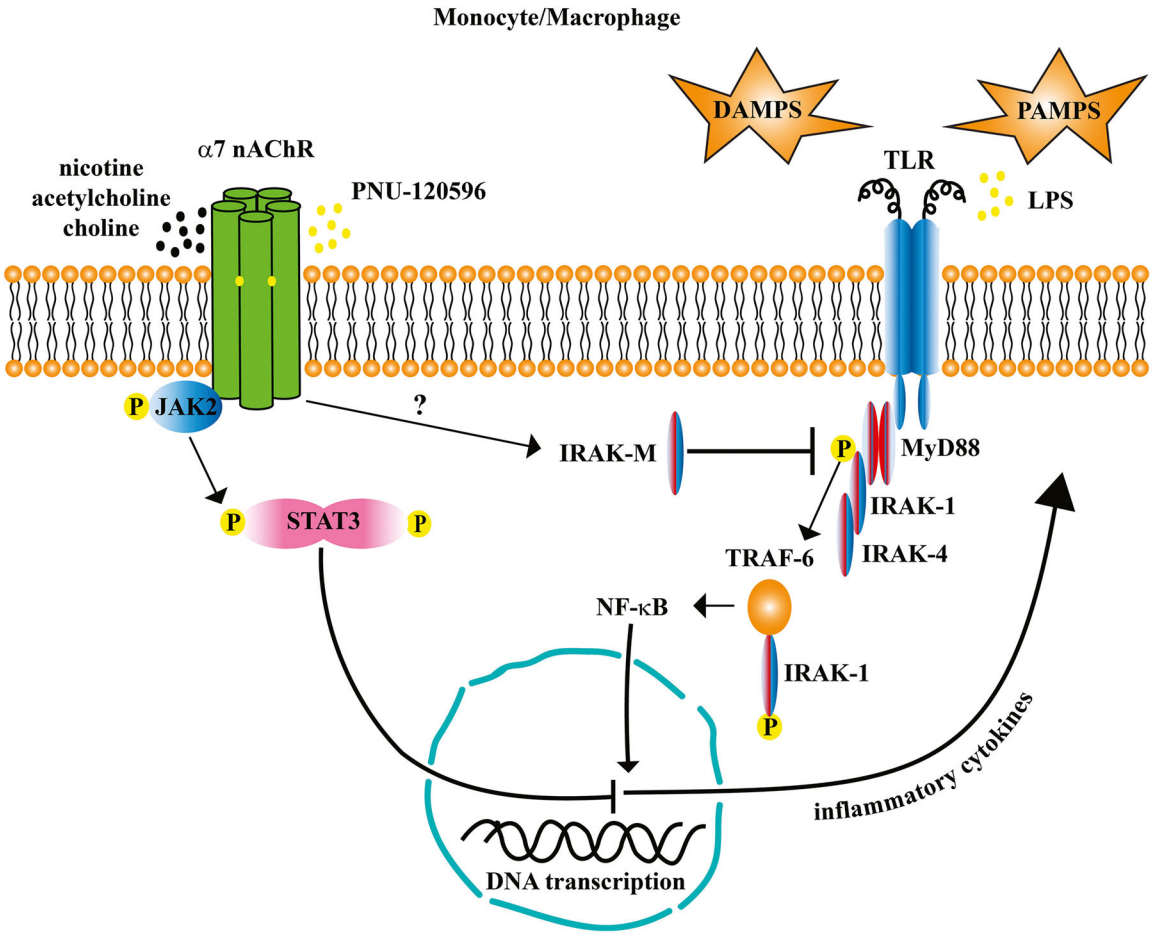

Fig. 2.

Nicotinic acetylcholine receptor (nAChR) α7 inhibits the production of inflammatory cytokines by suppressing downstream pathways triggered by stimulation of toll-like receptors (TLR) by damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS). In this model, stimulation of α7 nAChRs activates the Janus Kinase-2 (JAK2) signal-transducer and activator of transcription-3 (STAT3) signaling pathway. JAK2 phosphorylates STAT3 which dimerizes and translocates to the nucleus where it interferes with nuclear factor kappa-B (NF-κB) binding to DNA and prevents the transcription of genes for inflammatory cytokines. Interleukin-1 receptor associated kinase-M (IRAK-M), an IRAK specific to monocytes and macrophages, inhibits the phosphorylation of IRAK-1 by IRAK-4. The downstream effects are to inhibit the oligomerization of tumor necrosis-factor receptor-associated factor-6 (TRAF-6) and IRAK-1. The TRAF-6/TRAK-1 complex activates NF-κB which translocates to the nucleus and binds to DNA to initiate the production of inflammatory cytokines. How IRAK-M becomes activated by stimulation of α7 nAChRs is currently under investigation but may involve other intermediary molecules such as G-proteins. For brevity, not all intermediaries in the pathways are shown; for a thorough review of NF-κB signaling see Liu et al., [131].

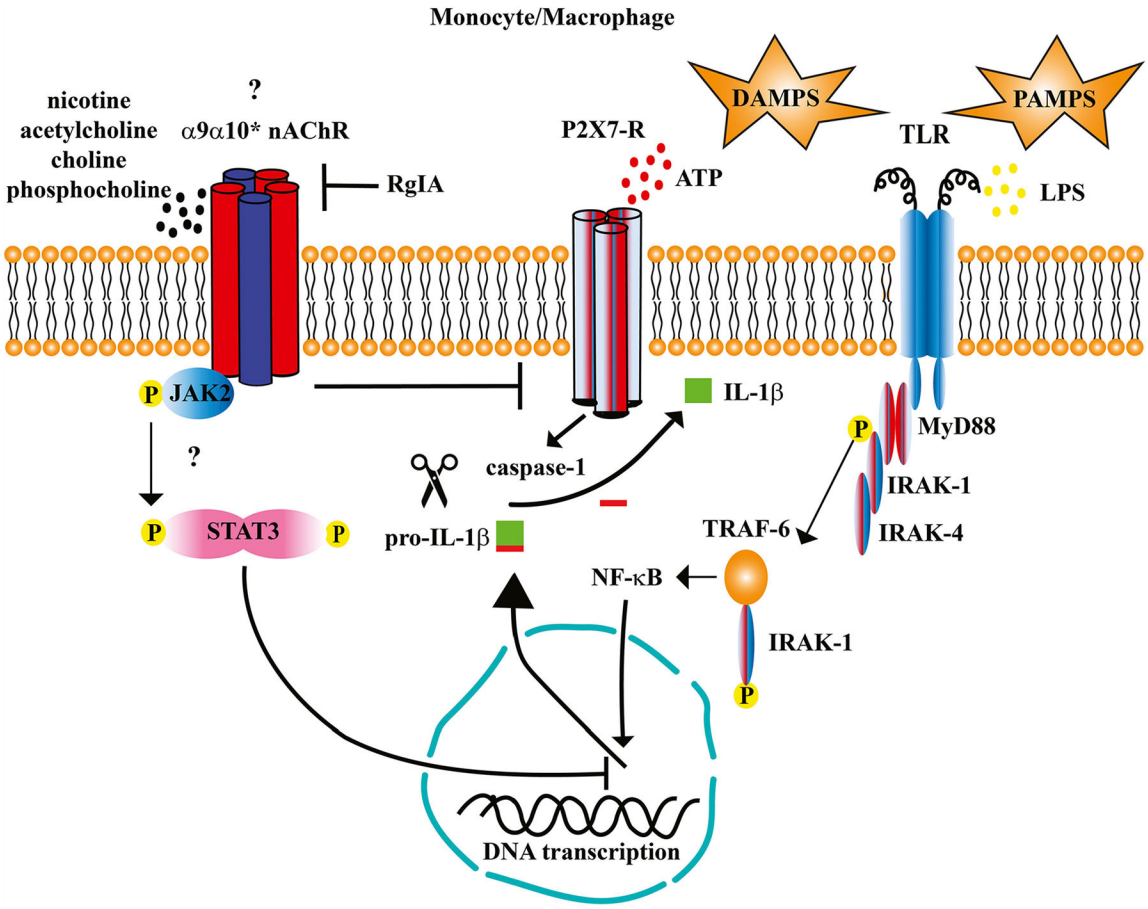

Fig. 3.

Stimulation of α9α10* (the asterisk indicates that the stoichiometry of the receptor expressed by immune cells is unknown and may also include other subunits) nicotinic acetylcholine receptors (nAChR) inhibits the production of inflammatory cytokines. Danger signals including stimulation of toll-like receptors (TLR) by damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS), and stimulation of purinergic P2×7-Rs by ATP increases the production of inflammatory cytokines including interleukin-1β (IL-1β). Stimulation of both TLRs and P2X-Rs is needed for the expression and assembly of the inflammasome complex (not shown) and subsequent cleavage of pro-IL-1β by activated caspase-1. In this model, agonists of monocytic α9α10* nAChRs such as nicotine, choline, and phosphocholine inhibit IL-1β release as demonstrated in human monocytes [20,21]. The mechanisms of how this occurs is currently under investigation, but one potential mechanism involves activation of the Janus Kinase-2 (JAK2) signal-transducer and activator of transcription-3 (STAT3) signaling pathway as demonstrated in breast cancer cells [132]. Stimulation of α9α10* nAChRs has also been shown to inhibit the release of tumor necrosis factor-α (TNF-α) and IL-6, but not the anti-inflammatory cytokine IL-10 in human whole blood cultures [97].

Table 1

Novel compounds that target α7 nAChRs, mechanism of action, and effects in models of pain and inflammation.

Ligand	Mechanism of Action	Disease Model	Pharmacological Effects	Ref.
PNU-282987	Agonist	CCI-induced neuropathic pain	Reduced hyperalgesia; reduced macrophage infiltration of the sciatic nerve; attenuated morphological changes of the sciatic nerve.	[38]
		CIN	Reduced cold allodynia and hyperalgesia; modulation of glial cell and astrocyte populations in the dorsal horn of the spinal column.	[39]
		Formalin-induced inflammatory pain	Reduced nocifensive behaviors; inhibition of phase I and II pain.	[41]
		DSS induced-colitis	Reduced referred mechanical hyperalgesia.	[42]
		POI	Reduced macrophage infiltration of inflamed intestinal tissue.	[44]
		Acute lung injury	Reduced neutrophil recruitment; reduced TNF-α, IL-1β, IL-6, IL-10 levels; reduced NF- κB activity.	[45]
PHA-543613	Agonist	Formalin-induced inflammatory pain	Reduced nocifensive behaviors; inhibition of phase I and II pain.	[40, 41]
		DSS-colitis	Reduced signs and symptoms of colitis; reduced severity of histological changes in colon morphology.	[43]
		PTSD-related chronic pain	Attenuated mechanical allodynia; suppressed reactive astrocyte numbers; decreased spinal TNF-α and IL-1β levels.	[46]
		Bleomycin-induced systemic sclerosis	Inhibition of profibrotic effects of bleomycin.	[47]
GTS-21	Partial agonist	CFA-induced chronic inflammatory pain	Attenuated mechanical hyperalgesia and thermal nociception.	[50]
		Morphine-exacerbated burn pain	Prevention of mechanical allodynia and thermal hyperalgesia.	[65]
NS6740	Silent agonist	Formalin-induced inflammatory pain	Reduced nocifensive behaviors; inhibition of phase I and II pain.	[41, 51]
		CCI-induced neuropathic pain	Reduced mechanical allodynia.	[51]
pCF_3 diEPP	Partial agonist	CFA-induced chronic inflammatory pain	Reduced mechanical allodynia and paw edema; reduced mechanical allodynia.	[66]
GAT-107	ago-PAM	Formalin-induced inflammatory pain	Reduced nocifensive behaviors; inhibition of phase II pain.	[41, 54]
		EAE	Reduced signs and symptoms of neuroinflammation; reduced immune cell meningial infiltrate in the spinal cord.	[35]
		CCI-induced neuropathic pain	Attenuation of mechanical allodynia and thermal hyperalgesia.	[54]
		LPS-induced inflammatory pain	Attenuation of mechanical allodynia.	[54]
PNU-120596	PAM	Formalin-induced inflammatory pain	Reduced nocifensive behaviors; inhibition of phase II pain.	[40, 41]
		DSS-induced colitis	Reduced signs and symptoms of colitis; reduced severity of histological changes in colon morphology.	[43]
TQS	PAM	LPS-induced inflammatory pain	Reduced mechanical and thermal hyperalgesia.	[67]
DDD-028	Unknown	CIN	Reduced mechanical hyperalgesia and mechanical allodynia.	[68]

CCI, chronic constriction injury; CIN, chemotherapeutic-induced neuropathic pain; DSS, dextran sodium sulfate; CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalomyelitis; LPS, lipopolysaccharide; PAM, positive allosteric modulator; POI, post operative ileus; PTSD, posttraumatic stress disorder.

Table 2

Antagonists that target α9-containing nAChRs, mechanism of action, and effects in models of pain and inflammation.

Ligand	Mechanism of Action	Disease Model	Pharmacological Effects	Ref.
Vc1.1	Antagonist	CCI-induced neuropathic pain	Reduced mechanical allodynia, mechanical hyperalgesia, infiltration of immune cells; disease modifying effects.	[75, 81]
Mr1.1	Antagonist	CCI-induced neuropathic pain	Reduced mechanical hyperalgesia.	[79]
RgIA	Antagonist	CCI-induced neuropathic pain CIN	Reduced mechanical allodynia and mechanical hyperalgesia, infiltration of immune cells. Reduced mechanical hyperalgesia, cold allodynia; disease modifying effects	[81] [83, 115]
RgIA4	Antagonist	CIN	Reduced mechanical hyperalgesia and cold allodynia; disease modifying effects.	[84, 85, 116]
RgIA-5474	Antagonist	CIN	Prevention of cold allodynia induction.	[89]
RgIA-5628	Antagonist	CIN	Prevention of cold allodynia induction.	[87]
RgIA-5524	Antagonist	CIN	Prevention of cold allodynia induction.	[90]
GeXIVA	Antagonist	CCI-induced neuropathic pain CIN	Reduced mechanical hyperalgesia. Long-lasting inhibition of mechanical and cold allodynia.	[88] [91, 117]
ZZ-204 G	Antagonist	CCI-induced neuropathic pain Formalin-induced inflammatory pain	Reduced mechanical hyperalgesia Reduced inflammatory pain.	[92] [92]
ZZ1–61c	Antagonist	CIN	Reduced mechanical allodynia and mechanical hyperalgesia.	[93]

CCI, chronic constriction injury; CIN, chemotherapeutic-induced neuropathic pain.

Table 3

Small molecules that target nAChRs containing α7, α9, or α10 subunits, mechanism of action, and pharmacological effects.

Ligand	Receptor Target	Mechanism of Action	Pharmacological Effect	Ref.
Choline	α7 α9α10	agonist partial agonist	Decreased TNF-α release from mouse macrophages; inhibition of IL-1β from primary human monocytes.	[21,98]
CDP-choline	α7	agonist	Reduced oxaliplatin-induced neuropathic pain in mice; reversal of CCI-induced mechanical hyperalgesia in mice; decreased mechanical hyperalgesia and paw edema in carrageenan-induced inflammatory pain.	[100–102]
Phosphocholine	α9α10	agonist	Inhibition of IL-1β from human U937 monocytes, monocytic THP-1, THP-1 derived M1-like macrophages, primary monocytes, and PBMCs.	[21,22]
pCF_3 diEPP	α9α10	partial agonist	Inhibition of TNF-α, IL-1β, and Il-6 release from whole human blood cultures; reduced IL-1β release from PBMCs, monocytic THP-1, THP-1 derived M1-like macrophages, and primary monocytes.	[97]

CDP-choline; cytidine-5′-diphosphate choline; PBMCs, peripheral blood mononuclear cells.