Published in final edited form as:

FEBS Lett. 2018 April ; 592(7): 1045–1062. doi:10.1002/1873-3468.12884.

Nicotinic Acetylcholine Receptors in Neuropathic and Inflammatory Pain

Arik J. Hone[1] and **J. Michael McIntosh**[1,2,3]

[1]Department of Biology, University of Utah, Salt Lake City, Utah, USA

[2]Department of Psychiatry, University of Utah, Salt Lake City, Utah, USA

[3]George E. Whalen Veterans Affairs Medical Center, Salt Lake City, Utah, USA

Abstract

Nicotinic acetylcholine receptors (nAChRs) are actively being investigated as therapeutic targets for the treatment of pain and inflammation, but despite more than 30 years of research, there are currently no FDA approved analgesics that target these receptors. Much of the initial research effort focused on the α4β2 subtype, but more recently, additional subtypes have been identified as promising new therapeutic targets and include α6β4, α7 and α9-containing subtypes. This Review will focus on the distribution of these nAChRs in the cell types involved in neuropathic pain and inflammation as well as current pharmacological compounds that target them.

Keywords

Neuropathic pain; inflammatory pain; cancer pain; nicotinic acetylcholine receptors; α-conotoxin RgIA

Introduction

nAChRs are numerous, diverse, and composed of different subunits forming multiple subtypes

nAChRs belong to the Cys-loop superfamily of receptors that include $GABA_A$, glycine, and serotonin $5HT_3$. Each receptor is composed of five individual subunits that assemble together to form pentameric ligand-gated ion channels. In the human genome, there are sixteen genes that encode the various subunits and include α1-α7, α9, α10, β1-β4, δ, ε, and γ. The α1, β1, δ, ε, and γ subunits form the nAChR subtype found at the neuromuscular junction. The remaining α and β subunits assemble in various combinations to form numerous and distinct receptor subtypes, and almost all of these subtypes are expressed by neurons. A limited subset has been reported to be expressed by non-neuronal cells and will be an important topic of this review. In the central nervous system (CNS), the most abundant

Corresponding Author: Arik J. Hone PhD, Department of Biology, University of Utah, Salt Lake City, Utah 84112, uuneurotox@yahoo.com.

Conflict of interest statement: Conotoxins including some of those referenced in this paper and quaternary ammonium salts have been patented by the University of Utah, Hainan University, or the University of Kentucky with JMM listed as an inventor.

nAChRs contain α4 and β2 subunits and form the α4β2* subtype; the asterisk indicates that additional subunits are potentially present in native nAChRs. Other abundant subtypes expressed by CNS neurons include α6β2* and α7*. In the peripheral nervous system (PNS), the most abundant subtypes are α3β2* and α3β4* and are prominently expressed by ganglionic neurons [1–5]. nAChRs are also expressed by a variety of non-neuronal cells including cochlear hair cells [6], various immune cells, keratinocytes [7] and chromaffin cells of the adrenal gland [8], among others. For an in depth review of the structure, function, and distribution of nAChRs in general see [9–11].

nAChR subtypes display different sensitivities to ligands

The diversity of nAChR subunits and their ability to assemble in multiple combinations gives rise to myriad subtypes with different sensitivities to ligands. The canonical ligand-binding site of heteromeric nAChRs composed of α and β subunits is located at the interface between each α and β subunit. Thus, a receptor composed of $(\alpha4)_2$ and $(\beta2)_3$ subunits would have two canonical ligand-binding sites. Similarly, a receptor with a stoichiometry of $(\alpha4)_3(\beta2)_2$ would also have two similar ligand-binding sites. However, although these sites may be identical at the amino acid level, recent studies suggest that they may not be equivalent in terms of ligand affinity and mechanism of action. One of the first pieces of evidence in support of this hypothesis comes from studies using the analgesic compound sazetidine-A [12]. Initially, it was reported that this compound desensitized rat α4β2 nAChRs heterologously expressed in HEK293 cells without inducing functional responses. Subsequently, it was discovered that sazetidine-A was, in fact, capable of evoking ionic currents in *Xenopus laevis* oocytes expressing human α4β2 nAChRs [13]. The authors demonstrated that sazetidine-A showed differential effects on α4β2 nAChRs depending on the stoichiometry of the receptors present. Receptors with a stoichiometry of $(\alpha4)_2(\beta2)_3$ were activated by sazetidine-A with a 99% efficacy, relative to the maximal response to acetylcholine, whereas those with a stoichiometry of $(\alpha4)_3(\beta2)_2$ responded with an efficacy level of only 6%. Additional studies using concatamers of α4 and β2 subunits [14] confirmed and extended the observations of Zwart *et al.*, [13] to include additional agonist compounds. These studies concluded that $(\alpha4)_2(\beta2)_3$ nAChRs showed high sensitivity (HS) to agonists ligands whereas those with an $(\alpha4)_3(\beta2)_2$ stoichiometry showed low sensitivity (LS). It has also been shown that a 3rd non-canonical ligand-binding site is present between the α4-α4 interface of the $(\alpha4)_3(\beta2)_2$ LS stoichiometry, the α5-α4 interface of the $(\alpha4)_2(\beta2)_2\alpha5$ subtype, and the β3-α4 interface in the $(\alpha4)_2(\beta2)_2\beta3$ subtype [15–18]. Interestingly, some agonist ligands are excluded from these non-canonical sites. Sazetidine-A, for example, is unable to bind to the α4-α4 interface of the $(\alpha4)_3(\beta2)_2$ LS stoichiometry. This differential agonist sensitivity of nAChRs with alternate stoichiometries is not limited to receptors composed of α4 and β2 subunits. Receptors containing α3 and β4 subunits also show the same pattern of differential sensitivity to agonists [19, 20]. Additionally, it has been shown that the potency and efficacy of a given ligand could be altered if the 5th subunit in the receptor complex was an α5 subunit i.e. receptors with an $(\alpha3)_2(\beta4)_2\alpha5$ stoichiometry.

Lastly, a new class of ligands has been discovered that, rather than acting as agonists, act as positive allosteric modulators (PAMs) of receptor function [21, 22]. Several types of PAMs

have been identified for the α7 subtype. Type I PAMs increase peak amplitudes of agonist-induced currents without large effects on response kinetics, and type II PAMs increase receptor function by limiting desensitization and prolonging responses [23–25]. A third type of PAM, called ago-PAMs, show intrinsic allosteric agonist activity as well as Type II effects [26, 27]. PAMs and other types of ligands that increase receptor activity have also been reported for α4β2 nAChRs [28–30]. For example, the agonist NS9283 selectively binds to the orthosteric agonist site at the α4-α4 interface of the $(\alpha4)_3(\beta2)_2$ receptor and increases receptor sensitivity to agonists but cannot, by itself, gate the receptor due to the lack of a second binding site [31, 32]. PAMs and other ligands that increase receptor activity without activating them have been proposed as promising therapeutics with reduced side effects. Rather than chronically stimulating the receptor targets with an agonist that may ultimately result in desensitized receptors, it might be more desirable to selectively increase receptor-response to endogenous cholinergic tone.

Subtype-selective ligands are critical for avoiding off-target effects

The enormous diversity of nAChR subtypes not surprisingly makes designing and developing subtype-selective ligands a challenging endeavor and the successful development of nicotinic agonists as therapeutics for the treatment of pain has been hampered because of it. Candidate therapeutics that that have off-target activity on α3-containing subtypes are particularly problematic as they provoke a variety of autonomic nervous system side effects including cardiovascular and gastrointestinal [33]. The α4β2 agonist ABT-594 [34], for example, has been shown to be analgesic in a several models of neuropathic pain in rodents [35–37] as well as in humans with diabetic neuropathy [38, 39]. However, serious side effects limit its therapeutic use as an analgesic [38, 40, 41]. Nevertheless, low doses of ABT-594 that do not activate α3-containing nAChRs may be useful as an adjunct therapy or in combination with ligands that increase receptor activity, but do not activate the receptor, to treat pain [42–44].

α6β4 nAChRs in pain

Subunit composition and tissue distribution

The α6β4* subtype has a very limited distribution in the central and peripheral nervous systems and very little is known about its functional roles. Expression of α6β4* nAChRs in the PNS was demonstrated in functional studies of rat DRG neurons where they are co-expressed with several other nicotinic subtypes including α3β4*, α7, and a β2-containing subtype [1]. Immunohistochemical studies of mouse DRG indicate that α6 subunits are expressed by small to medium diameter neurons [45]. Subsets of these α6-positive neurons express markers for nociceptive neurons; 66% were A-β positive, 26% isolectin B4 positive (IB4+), and 8% were calcitonin gene-related peptide positive (CGRP+) (8%). These studies indicate that neurons that express α6-containing nAChRs may be putative nociceptors.

Potential roles in the sensory system

The functional role of α6β4* nAChRs in DRG neurons is mostly unknown, but the expression of α6 subunits in nociceptors suggests they may be involved in the sensory processing of information regarding pain. Functional studies in *Xenopus* oocytes expressing

α6β4 nAChRs indicate that stimulation with a nicotinic agonist results in cross-inhibition of co-expressed purine P2X receptors through distinct mechanisms depending on the P2X receptor subtype expressed. Simulation of oocytes co-expressing α6β4 and P2X2 receptors resulted in prolonged desensitization of P2X2 receptors whereas co-expressing P2X3 receptors with α6β4 nAChRs resulted in reduced sensitivity to ATP. Reduced P2X3 sensitivity to ATP occurred even in the absence of α6β4 stimulation suggesting that there may be direct physical interaction between the two receptor types [46]. Both mechanisms, desensitization and decreased sensitivity to ATP, might be expected to have inhibitory effects on P2X receptor signaling of pain. Indeed, drugs that inhibit P2X receptors are being investigated for their use in treating chronic pain conditions [47, 48].

Targeting α6β4 for treating neuropathic pain

Recent studies of neuropathic pain by Mogil *el al.* [45] have shown that *CHRNA6* expression levels are inversely correlated with the manifestation of neuropathic pain symptoms in mice as well as in humans. This coupled with the studies by Lester *et al.* [46] suggest that stimulation of α6β4* nAChRs may produce analgesia through P2X receptor inhibition or some other as yet unidentified mechanism. Ligands that selectively target α6β4 over other nicotinic subtypes may be critical to avoid undesirable secondary side effects. As discussed above, nAChR subunits assemble in various combinations to form different receptor subtypes, and each of these subtypes displays distinct, yet overlapping, sensitivities to ligands. This is especially true for the more closely related subtypes such as α3β2 and α3β4 but is also true for α3β4 and α6β4 subtypes.

Stimulation of α3-containing nAChRs in the PNS may produce a number of side-effects, most notably cardiovascular and gastrointestinal [49]. The α3β4* subtype is prominently expressed in a variety of PNS neurons including those of DRG [1, 50, 51], superior cervical ganglia [2, 5], cardiac ganglia [52], and ganglia innervating the viscera [2, 4]. α3β2* nAChRs are also present in cardiac [52] and superior cervical [2] ganglia. Additionally, α3β4* nAChRs are the predominant nAChR subtype expressed by human adrenal chromaffin cells [53], the primary source of circulating catecholamines. Exposure of adrenal chromaffin cells to nicotinic agonists increases cell excitability and may result in increased release of catecholamines, potentially producing cardiovascular effects [54]. Lastly, stimulation of α3β4* receptors may, in fact, be pro-nociceptive. In human sural nerve, activation of α3β4* nAChRs by nicotine has an excitatory effect by lowering the threshold for the firing of action potentials [55]. Thus, in neuropathic pain stimulation of both α3β4* and α6β4* may produce counteracting effects hence the critical need for agonists that selectively target α6β4 over α3β4 nAChRs.

α7 nAChRs in pain and inflammation

Subunit composition and tissue distribution

nAChRs containing the α7 subunit are widely distributed throughout the nervous system and is one of the few mammalian nicotinic subunits shown to form homopentameric receptors. It has been suggested that most α7 receptors expressed in rat brain are homopentamers [56, 57], however recently it has been shown that subpopulations of basal forebrain,

hippocampal, and cortical neurons express a heteromeric α7β2 subtype [58–61]. Peripherally, α7* nAChRs are expressed by numerous ganglionic neurons including those found in ciliary [62], superior cervical [63], and dorsal root [50, 51] ganglia. An α7α10 subtype has also been reported in sympathetic neurons [64], but additional studies corroborating the presence of such a subunit combination are needed. Adrenal chromaffin cells are also widely reported to express α7 nAChRs and presumably are involved in the secretory function of these cells, although their exact functional role is currently unknown [65–67].

Primary DRG afferents have been reported to express α7 nAChRs and are involved in the modulation of glutamate release onto dorsal horn neurons, enhancing excitatory transmission at this synapse [68]. Modulating nAChR activity in DRG neurons has been proposed to be one mechanism of producing nAChR-mediated spinal analgesia [69]. Direct excitation by nicotinic agonists of inhibitory neurons in the dorsal horn enhances the activity of GABAergic/glycinergic interneurons, although most of this effect has been attributed to non-α7 subtypes [70–75]. DRG-expressed α7 nAChRs have also been shown to modulate the production of nitric oxide [76], an effect that may confer neuroprotection to injured or axotomized DRG neurons [77]. Importantly, α7* nAChRs have received significant attention in the area of pain and inflammation research due to their expression by numerous types of non-neuronal cells of the immune system including lymphocytes [78–80], monocytes/macrophages [81–84], and microglial cells [85, 86].

Potential roles of α7 in the immune system

It is not surprising given the expression of α7* nAChRs by immune cells that they have been implicated in various immunological processes including the modulation of the inflammatory response in conditions such as sepsis [87] and various other inflammatory diseases [88]. Early elucidation of the role of α7 nAChRs in inflammation comes from the work of Tracey *et al.* [81, 89–91] who proposed the cholinergic anti-inflammatory pathway, a potential communication link between the immune and nervous systems. This model proposes that the immune and nervous systems interact via the vagus nerve. Electrical or pharmacological stimulation of the vagus nerve reduces circulating levels of tumor necrosis factor-α (TNF-α) in models of endotoxemia [92, 93] and macrophages expressing α7* nAChRs have been suggested to play a pivotal role [81, 91, 94]. Functionally, α7* nAChRs have been shown to be coupled to the JAK-STAT pathway of macrophage inactivation [95]. Treatment of primary mouse peritoneal macrophages with lipopolysaccharide (LPS) results in the recruitment of the tyrosine kinase janus kinase-2 (JAK2). JAK2 phosphorylates signal transducer and activator of transcription 3 (STAT3), and once phosphorylated, dimerizes and translocates to the nucleus to inhibit the transcription, and ultimately the production, of pro-inflammatory cytokines. Stimulation of the vagus nerve *in vivo* correlates with the activation of STAT3. Additionally, stimulation of α7* nAChRs expressed by monocytes/macrophages has been shown to inactivate nuclear factor-κB (NF- κB)-mediated production of pro-inflammatory cytokines [82, 96]. NF-κB is a nuclear transcription factor that initiates the production of pro-inflammatory mediators including TNF-α, prostaglandin E2 (PGE_2), and macrophage inflammatory protein-1α (MIP-1α), among others. In LPS-challenged human peripheral monocytes, activation of α7* nAChRs by nicotine inhibited the synthesis of all

three of these pro-inflammatory mediators. Thus, in this model, the release of acetylcholine by the vagus nerve stimulates α7* nAChRs expressed by monocytes/macrophages to activate the JAK-STAT anti-inflammatory signaling pathway while simultaneously inactivating NF-κB pro-inflammatory pathways. However, the apparent lack of direct innervation of the spleen by the vagus nerve has called this model into question.

Alternative hypotheses postulate that a non-neural cholinergic pathway is important in the anti-inflammatory actions of nicotinic agonists in models of inflammatory disease [97, 98]. Various immune cells, including T cells, are known to be capable of synthesizing and releasing acetylcholine [99]. Subsets of splenic T cells have been shown to be a source of acetylcholine that activates this non-neural cholinergic anti-inflammatory pathway via α7* nAChRs [100]. These T cells are activated by norepinephrine released by α7*-expressing celiac neurons that directly innervate the spleen [101] providing a critical communication link between the nervous and immune systems. As discussed below, pharmacological stimulation of α7* nAChRs in this pathway has been shown to be anti-inflammatory in a number of inflammatory disease models. A summary of the cells types involved in pain and inflammation as well as the effects of α7 nAChR stimulation is presented in Table 1.

Targeting α7 for treating neuropathic and inflammatory pain

Nicotinic receptors containing the α7 subunit have been investigated for some time as potential targets for the modulation of pain and inflammation. Initially, research efforts were focused on drugs with agonist mechanisms of action. The prototypical agonist and endogenous neurotransmitter choline has been shown to be analgesic in several models of pain and inflammation in rodents [102–105], although positive results have not always been observed in humans [106]. In a comparative study of choline and the α7 nAChR partial agonist GTS-21, both compounds were capable of reducing HIV glycoprotein 120 (gp120)-induced increases in IL-1β protein as well as mRNA for various inflammatory mediators [107]. Furthermore, both compounds reduced gp120-induced mechanical allodynia. GTS-21 has also been shown to be analgesic in an incisional model of post-operative pain [108]. Unfortunately, despite positive results with choline and GTS-21, long term treatment with α7 agonists may have undesirable consequences. The expression and upregulation of α7 nAChRs has been associated with pro-oncogenic activity in several types of cancer [109, 110]. Of critical importance in the context of neuropathic pain and inflammation, is the fact that some α7-selective agonists may not be truly selective, but instead may also activate α9-containing nAChRs which, as will be discussed below, may produce counteracting effects [111]. Choline is one such example of an α7 agonist that also activates α9-containing nAChRs [6, 112, 113]. These and other concerns have led to the consideration of α7-targeting compounds with alternative mechanisms of action.

PAMs are ligands that lack intrinsic agonist activity but function by modulating the activity of the target receptor in the presence of an agonist. As discussed above, Type I PAMs increase responses without affecting desensitization whereas Type II PAMs increase receptor response by reducing desensitization. In addition, novel ligands called ago-PAMs that display both intrinsic agonist activity as well as PAM activity have been discovered [27]. Numerous PAM ligands have been tested in animal models of pain and inflammation.

GAT107 is an example of an ago-PAM that was shown to be effective in a battery of neuropathic and inflammatory pain models [114]. In a comparative study of NS1738 (Type I) [21] and PNU120596 (Type II) [22], the latter was shown to be effective in reducing inflammatory pain in the formalin test [115] as well as neuropathic pain in the chronic constriction injury (CCI) model [116]. Thus, one strategy for producing analgesia via α7 nAChRs without chronically activating the receptors is to use a PAM to selectively enhance receptor function in response to endogenous cholinergic tone [117]. PNU120596 has also been shown to enhance the anti-nociceptive effects of α7 agonists in the formalin test [118].

Lastly, an additional class of α7 ligands under consideration for the treatment of pain and inflammation belong to the so-called silent agonist class. These compounds are reported to have very little agonist activity by themselves but have substantially increased activity in the presence of a PAM. Two such compounds, NS6740 [119, 120] and PMP-072 [121], have been shown to be analgesic in CCI and anti-inflammatory in the collagen-induced arthritis (CIA) model, respectively. Interestingly, the therapeutic properties of both compounds are reported to be mediated through receptors in the non-conducting state suggesting that a mechanism other than classical ion channel function is present in immune cells. A summary is presented in Table 2 of the ligands discussed above and the effects of α7 nAChR stimulation on the target cell population. For further review of the involvement of α7 nAChRs in pain and inflammation and therapeutic strategies for targeting them see Bagdas *et al.*, [122].

α9 nAChRs in neuropathic pain and inflammation

Subunit composition and tissue distribution

The sequence encoding the α9 subunit was first identified by PCR screening of a rat genomic cDNA library [6]. *In situ* hybridization studies of rat embryos identified mRNA transcripts for α9 in the pituitary, tongue, olfactory epithelium, and hair cells of the cochlea. Injection of *Xenopus laevis* oocytes with cRNA for α9 resulted in the formation of functional homopentamers as determined by electrophysiology. A few years later, a related nicotinic subunit was discovered that is now known as α10 [113, 123, 124]. However, in contrast to α9, oocytes injected with cRNA or cDNA constructs for α10 failed to show functional responses. Furthermore, injection of α10 with α2-α6 or β2-β4 subunit cRNA also failed to produce functional responses, but instead co-injection with α9 yielded currents with distinct biophysical properties that were distinguishable from those of oocytes expressing α9 homopentamers [123]. Currents in oocytes injected with α7 and α10 were indistinguishable from oocytes expressing α7 homopentamers. These experiments demonstrated that α9 and α10 subunits were capable of assembling together to form functional heteropentamers and displayed properties similar to the nAChRs present in cochlear hair cells [123, 125, 126].

With respect to pain and inflammation, two cell types may be of particular relevance: DRG neurons and immune cells. mRNA transcripts for α10 subunits have consistently been reported in DRG neurons [1, 127, 128], but transcripts for α9 subunits are found inconsistently or in low abundance [1, 128, 129]. Furthermore, protein for α9 subunits has not been reported [129], nor have currents that could be attributed to α9-containing nAChRs

[1, 50]. Therefore, the expression of α9-containing nAChRs in rodent DRG neurons remains unclear. In contrast, transcripts for both α9 and α10 subunits have consistently been reported in a variety of native immune cells, and cell lines derived from immune cells including monocytes and macrophages [83, 130, 131], mast cells [132], B cells [124, 133], and T lymphocytes [124, 134]. Functional responses in human primary mononuclear leukocytes and the monocyte U937 cell line have been demonstrated and are functionally coupled to purine P2X receptors [83, 135–138]. A summary of the cell types involved in pain and inflammation as well as the effects of α9 nAChR stimulation is presented in Table 3.

Potential roles of α9-containing nAChRs in neuropathic pain and inflammation

While the exact role of α9* nAChRs is still under investigation, their very presence in immune cells suggests that they may be involved in immunological processes. Functionally, α9* nAChRs have been shown to modulate the release of IL-1β from human monocyte-derived U937 cells as well as from mouse peripheral blood mononuclear leukocytes [83, 135, 136]. It is important to point out that in these studies nicotine functioned as an agonist in contrast to the antagonist action observed for oocyte- and hair cell-expressed α9* nAChRs [6, 112, 123]. Furthermore, unlike oocyte- and hair cell-expressed α9* nAChRs, those found in U937 cells and leukocytes do not function as canonical ligand-gated ion channels; application of agonists that activate oocyte- or hair cell expressed α9-containing nAChRs do not evoke ion currents in tested immune cells. Nevertheless, exposure of U937 cells to nicotine, choline, or acetylcholine was shown to inhibit the release of IL-1β, an effect that could be prevented by pre-exposing the cells to nAChR antagonists. Such an effect *in vivo* might be expected to reduce inflammation. However, several antagonists of α9-containing receptors have been shown to be analgesic and anti-inflammatory in animal models of neuropathic pain and inflammation, as will be discussed below. Clearly, more work is needed to investigate these unusual properties of immune cell expressed α9* nAChRs and their involvement in inflammatory processes *in vivo*. Separately, nicotine has also been shown to reduce the proliferation of pro-inflammatory monocytes in bone marrow of LPS challenged mice, an effect attributed to α9* nAChRs [133]. Similarly, mice in the experimental autoimmune encephalomyelitis (EAE) model show a reduction in the number of pro-inflammatory monocytes and neutrophils infiltrating the CNS with nicotine treatment [139].

Several models of pain and inflammation have demonstrated that α9* nAChRs play a role in modulating the pathophysiology associated with each respective nerve injury model and include neuropathic pain resulting from traumatic injury to nerves [140] and chemotherapeutic-induced neuropathy [141]. Studies using mice with germline deletion of the α9 gene (*CHRNA9*) also point to role in immunomodulation. *CHRNA9* knockouts (α9 KOs) subjected to traumatic nerve injury (CCI) or inflammatory pain (paw injections of complete Freund's adjuvant; CFA) developed mechanical hyperalgesia to a lesser degree than wild type (WT) mice and recovered from CCI more quickly [142]. In the oxaliplatin model of peripheral neuropathy, α9 KOs were resistant to the development of long-lasting cold allodynia, a side effect characteristic of treatment with this chemotherapeutic drug in humans [143]. Lastly, in the EAE model of multiple sclerosis, α9 KOs showed a delayed onset and an overall reduction in severity of symptoms relative to WTs [144]. These disease

modifying effects observed in CCI and CFA models of pain and in EAE are consistent with immunomodulation and suggest that α9* nAChRs are involved *per se* in the pro-inflammatory aspects of some inflammatory conditions.

Targeting α9-containing nAChRs for treating neuropathic and chemotherapeutic-induced pain

Rats subjected to traumatic nerve injury show a reduction of immune cell infiltration into the site of injury when treated with the selective α9-containing nAChR antagonist α-conotoxin (α-Ctx) RgIA [145, 146]. Antagonists of α9-containing nAChRs have also been shown to prevent or reduce the pathophysiological changes in DRG neurons observed in traumatic and chemical nerve injury models [140, 141]. Furthermore, α9 KO mice that developed temporary cold allodynia were resistant to treatment with an analog of RgIA (RgIA4) in contrast to their WT cohorts that showed significantly reduced responses to cold allodynia. These studies suggest that for certain conditions, an α9-containing nAChR antagonist may be a useful therapeutic for treating patients that have experienced nerve injury or have undergone treatment with chemotherapeutics. They also suggest that therapeutics that target α9-containing subtypes may be useful as prophylactics for preventing the onset and severity of certain inflammatory diseases, or may be given pre-operatively to prevent neuropathies associated with trauma to nerves during surgery.

The observations that α-Ctx antagonists of α9-containing subtypes were analgesic and anti-inflammatory provided the initial impetus for the development of a drug that targets these receptors [145, 147]. Current research efforts have focused on both peptides and small molecules. Several conopeptides, each targeting a particular receptor, ion channel, or transporter, have been investigated as potential therapeutics for diverse neurological conditions [148–150]. One such peptide, ω-Ctx MVIIA, that targets voltage-gated calcium channels, was approved by the FDA in 2004 as ziconotide and is used in the treatment of intractable or opiod-resistant types of pain [151, 152]. Another ω-Ctx, CVID, has also been investigated as a treatment of neuropathic, inflammatory, and cancer pain and is reported to have substantially reduced side effects compared to MVIIA [153–155]. Vc1.1, also known as ACV1, was the first nAChR-targeting α-Ctx considered for the treatment of neuropathic pain [156, 157]. Vc1.1 was shown to alleviate neuropathic pain symptoms and accelerate the functional recovery of sensory neurons in rats subjected to CCI or partial sciatic nerve ligation (PSNL) [147]. Unfortunately, therapeutic benefit was not observed in humans during Phase II clinical trials and, consequently, further investigation was discontinued [158, 159]. Subsequently, it was determined that Vc1.1 was several orders of magnitude less potent on human α9α10 nAChRs compared to the rodent receptor [160] which may, in part, account for the lack of therapeutic effects in humans.

At the time of RgIA's discovery, other conopeptides had been shown to inhibit α9-containing nAChRs but none of them were sufficiently α9-selective. Thus, RgIA provided a promising platform with which to develop novel therapeutic ligands that selectively target α9-containing nAChRs. However, RgIA, like Vc1.1, was also several orders of magnitude less potent on human vs rat α9α10 nAChRs [160]. α-Ctxs are relatively small peptides usually 13–17 amino acids in length that are easily produced by standard chemical synthesis

techniques. Furthermore, derivatives of the native peptides can be generated to improve target specificity, potency, stability, and bioavailability. In an effort to improve the potential of RgIA as a human therapeutic, a series of analogs was synthesized by replacing key residues of the native peptide sequence with select amino acids or non-standard amino acids to improve the potency for human α9-containing nAChRs [143]. One of these analogs, RgIA4, showed a ~300-fold increase in potency and, critically, was >1,000-fold more potent on α9α10 nAChRs than α7 nAChRs. As previously discussed, RgIA4 has been shown to be an effective analgesic and prophylactic in the oxaliplatin model of neuropathic pain [112, 143]. Additional conopeptides have also been recently discovered that selectively target α9-containing nAChRs [161, 162]. These peptides are structurally unrelated to RgIA and Vc1.1 yet analgesic, further validating α9-containing nAChRs as therapeutic targets for the treatment of neuropathic pain. However, some studies have postulated that the mechanism of action of Vc1.1 and several other α-Ctxs is mediated through G-protein coupled $GABA_B$ receptors [163]; for recent reviews see [164, 165].

A separate class of non-peptide small molecule antagonists of α9-containing nAChRs has recently been discovered and shown to have analgesic properties. A series of highly potent azaaromatic quaternary ammonium analogs was synthesized and shown to be effective analgesics in both the CCI model of neuropathic pain as well as the formalin test model of inflammatory pain [166]. Two of these compounds, ZZ-204G and ZZ1-61c, were selected for further study to determine their ability to produce analgesia in different pain models. ZZ-204G reduced mechanical hyperalgesia in rats subjected to CCI and also reduced inflammatory pain in the formalin test model [167]. In the vincristine model of chemotherapeutic-induced neuropathy, ZZ1-61c was demonstrated to effectively reduce mechanical hyperalgesia and allodynia in established neuropathy [168]. A summary of the ligands targeting α9-containing nAChRs and the effects observed in models of neuropathic pain and inflammation is presented in Table 4.

Treating neuropathic pain and the avoidance of drug abuse, dependence, and addiction

Nicotinic agonists that target CNS α4β2* nAChRs may have the potential for abuse and addiction. The α4β2 subtype has been widely implicated in the reinforcing properties of nicotine [169]. Partial agonists of α4β2 nAChRs including varnicline, dianicline, and cytisine reduce the actions of nicotine and are effective treatments for nicotine addiction [170, 171]. However, non-nicotine tobacco components may also contribute to tobacco use and therefore the addiction potential of α4β2 agonists may well be lower than that of tobacco [172]. As previously discussed, the α6β4* subtype has a very limited distribution pattern and functional responses attributable to this subtype have only been demonstrated in the hippocampus of adolescent mice (but not adults) and in rat DRG neurons. Their apparent absence in reward centers of the brain makes it unlikely that α6β4 agonists would have reinforcing properties. The α7 subtype, while highly expressed by a number of neuronal cell types in reward centers, appears to be important for modulating the activity of the β2-containing nAChRs involved in nicotine addiction, but does not appear to facilitate the reinforcing properties of nicotine [173]. In fact, stimulation of α7 nAChRs in the nucleus accumbens with selective agonists in models of smoking cessation has been shown to reduce nicotine consumption whereas inhibition using an antagonist has the opposite effect [174].

Interestingly, the smoking cessation drug varenicline is a full agonist of α7 nAChRs [175, 176], a property that may mediate some of the observed therapeutic effects of this drug. Lastly, α9-containing nAChRs appear to be rare or absent from neurons in the brain [6, 113, 123, 177] and ligands of α9-containing nAChRs that have been shown to be effective in neuropathic pain and chemotherapeutic-induced neuropathies have not shown agonist activity on other nAChRs subtypes including those involved in nicotine addiction.

Concluding comments

nAChRs represent promising targets for the development of new non-opioid therapeutics that treat neuropathic pain and a variety of inflammatory conditions. While previous research efforts have focused on drugs that target CNS α4β2* nAChRs, many α4β2-targeting drugs have suffered from narrow therapeutic-indices resulting in substantial adverse side effects [38, 41]. Furthermore, drugs that target α4β2 nAChRs may have reinforcing properties and consequently abuse potential. There are several potential advantages for targeting α6β4, α7, or α9-containing subtypes for analgesia and anti-inflammatory effects. Firstly, drugs that target these receptors are unlikely to have reinforcing properties; the α6β4 subtype has not been shown to be expressed in rewards pathways, stimulation of α7 nAChRs does not enhance nicotine consumption, and α9 nAChRs appear to be restricted to the periphery. Secondly, since the target cell populations are peripheral, candidate therapeutics would not need to cross the blood-brain barrier, avoiding possible interactions with CNS nAChRs. However, the successful development of α6β4-, α7-, or α9-targeting therapeutics is not without potential complications. One issue that may rise from targeting the α6β4 subtype is its overlapping pharmacological profile with the closely related α3-containing nAChRs. Agonists of α6β4 nAChRs would likely need to be devoid of activity on α3-containing subtypes to avoid the autonomic side effects that have plagued other nAChR-targeting drugs.

Agonists, PAMs, and other ligands that target α7 nAChRs have shown considerable promise as therapeutic candidates for a number of inflammatory conditions (Table 2). These compounds likely mediate their anti-inflammatory and analgesic effects through the modulation of immune cell activity specifically, the inhibition of pro-inflammatory cytokine release and the proliferation of pro-inflammatory types of immune cells (Table 3). Similarly, it appears likely that modulation of immune cell activity also occurs with ligands that target α9-containing nAChRs. In support of a peripheral site of action, conopeptides that selectively target α9-containing nAChRs are unlikely to cross the blood-brain barrier. Importantly, α9-targeting conopeptides discriminate well between α9 and α7 subtypes. Ligands that inhibit α9 and α7 nAChRs may have counteracting effects; stimulation of α7 is analgesic and anti-inflammatory whereas inhibition of α9-containing nAChRs is needed. It is worth pointing out that in some immune cells (human monocyte cell line U937), nicotinic agonists have been shown to inhibit the release of the pro-inflammatory cytokine IL-1β while selective antagonists of either α7 or α9-containing nAChRs blocks this effect. Clearly, more research is needed to further elucidate the potential roles of α7 and α9-containing nAChRs in native systems.

Acknowledgments

Portions of the work summarized in the review were supported by NIH grants P01 GM48677, R01 GM103801, DOD grant W81XWH-15-2-0057, and a sponsored research agreement from Kineta Inc. to J.M.M.

Abbreviations

nAChR	nicotinic acetylcholine receptor
α-Ctx	α-conotoxin
PAM	positive allosteric modulator
DRG	dorsal root ganglia
CCI	chronic constriction injury
CNS	central nervous system
PNS	peripheral nervous system
EAE	experimental autoimmune encephalomyelitis
TNF-α	tumor necrosis factor-α
IL-1β	interleukin-1β

References

1. Hone AJ, Meyer EL, McIntyre M, McIntosh JM. Nicotinic acetylcholine receptors in dorsal root ganglion neurons include the alpha6beta4* subtype. FASEB J. 2012; 26:917–26. [PubMed: 22024738]
2. Mao D, Yasuda RP, Fan H, Wolfe BB, Kellar KJ. Heterogeneity of nicotinic cholinergic receptors in rat superior cervical and nodose Ganglia. Mol Pharmacol. 2006; 70:1693–9. [PubMed: 16882879]
3. Poth K, Nutter TJ, Cuevas J, Parker MJ, Adams DJ, Luetje CW. Heterogeneity of nicotinic receptor class and subunit mRNA expression among individual parasympathetic neurons from rat intracardiac ganglia. J Neurosci. 1997; 17:586–96. [PubMed: 8987781]
4. Zhou X, Ren J, Brown E, Schneider D, Caraballo-Lopez Y, Galligan JJ. Pharmacological properties of nicotinic acetylcholine receptors expressed by guinea pig small intestinal myenteric neurons. J Pharmacol Exp Ther. 2002; 302:889–97. [PubMed: 12183644]
5. David R, Ciuraszkiewicz A, Simeone X, Orr-Urtreger A, Papke RL, McIntosh JM, Huck S, Scholze P. Biochemical and functional properties of distinct nicotinic acetylcholine receptors in the superior cervical ganglion of mice with targeted deletions of nAChR subunit genes. Eur J Neurosci. 2010; 31:978–93. [PubMed: 20377613]
6. Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S. Alpha 9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. Cell. 1994; 79:705–15. [PubMed: 7954834]
7. Nguyen VT, Ndoye A, Grando SA. Novel human alpha9 acetylcholine receptor regulating keratinocyte adhesion is targeted by Pemphigus vulgaris autoimmunity. Am J Pathol. 2000; 157:1377–91. [PubMed: 11021840]
8. Criado M. Acetylcholine nicotinic receptor subtypes in chromaffin cells. Pflugers Arch. 2017
9. Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev. 2009; 89:73–120. [PubMed: 19126755]
10. Dani JA. Neuronal Nicotinic Acetylcholine Receptor Structure and Function and Response to Nicotine. Int Rev Neurobiol. 2015; 124:3–19. [PubMed: 26472524]

11. Zoli M, Pistillo F, Gotti C. Diversity of native nicotinic receptor subtypes in mammalian brain. Neuropharmacology. 2015; 96:302–11. [PubMed: 25460185]
12. Xiao Y, Fan H, Musachio JL, Wei ZL, Chellappan SK, Kozikowski AP, Kellar KJ. Sazetidine-A, a novel ligand that desensitizes alpha4beta2 nicotinic acetylcholine receptors without activating them. Mol Pharmacol. 2006; 70:1454–60. [PubMed: 16857741]
13. Zwart R, Carbone AL, Moroni M, Bermudez I, Mogg AJ, Folly EA, Broad LM, Williams AC, Zhang D, Ding C, Heinz BA, Sher E. Sazetidine-A is a potent and selective agonist at native and recombinant alpha 4 beta 2 nicotinic acetylcholine receptors. Mol Pharmacol. 2008; 73:1838–43. [PubMed: 18367540]
14. Carbone AL, Moroni M, Groot-Kormelink PJ, Bermudez I. Pentameric concatenated (alpha4)(2)(beta2)(3) and (alpha4)(3)(beta2)(2) nicotinic acetylcholine receptors: subunit arrangement determines functional expression. Br J Pharmacol. 2009; 156:970–81. [PubMed: 19366353]
15. Mazzaferro S, Benallegue N, Carbone A, Gasparri F, Vijayan R, Biggin PC, Moroni M, Bermudez I. Additional acetylcholine (ACh) binding site at alpha4/alpha4 interface of (alpha4beta2)2alpha4 nicotinic receptor influences agonist sensitivity. J Biol Chem. 2011; 286:31043–54. [PubMed: 21757735]
16. Harpsoe K, Ahring PK, Christensen JK, Jensen ML, Peters D, Balle T. Unraveling the high- and low-sensitivity agonist responses of nicotinic acetylcholine receptors. J Neurosci. 2011; 31:10759–66. [PubMed: 21795528]
17. Eaton JB, Lucero LM, Stratton H, Chang Y, Cooper JF, Lindstrom JM, Lukas RJ, Whiteaker P. The unique alpha4+/−alpha4 agonist binding site in (alpha4)3(beta2)2 subtype nicotinic acetylcholine receptors permits differential agonist desensitization pharmacology versus the (alpha4)2(beta2)3 subtype. J Pharmacol Exp Ther. 2014; 348:46–58. [PubMed: 24190916]
18. Jain A, Kuryatov A, Wang J, Kamenecka TM, Lindstrom J. Unorthodox Acetylcholine Binding Sites Formed by alpha5 and beta3 Accessory Subunits in alpha4beta2* Nicotinic Acetylcholine Receptors. J Biol Chem. 2016; 291:23452–23463. [PubMed: 27645992]
19. Stokes C, Papke RL. Use of an alpha3beta4 nicotinic acetylcholine receptor subunit concatamer to characterize ganglionic receptor subtypes with specific subunit composition reveals species-specific pharmacologic properties. Neuropharmacology. 2012; 63:538–46. [PubMed: 22580377]
20. George AA, Lucero LM, Damaj MI, Lukas RJ, Chen X, Whiteaker P. Function of Human α3β4α5 Nicotinic Acetylcholine Receptors Is Reduced by the α5(D398N) Variant. Journal of Biological Chemistry. 2012; 287:25151–25162. [PubMed: 22665477]
21. Timmermann DB, Gronlien JH, Kohlhaas KL, Nielsen EO, Dam E, Jorgensen TD, Ahring PK, Peters D, Holst D, Christensen JK, Malysz J, Briggs CA, Gopalakrishnan M, Olsen GM. An allosteric modulator of the alpha7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo. J Pharmacol Exp Ther. 2007; 323:294–307. [PubMed: 17625074]
22. Hurst RS, Hajos M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, Groppi VE, Allaman G, Ogier R, Bertrand S, Bertrand D, Arneric SP. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci. 2005; 25:4396–405. [PubMed: 15858066]
23. Gronlien JH, Hakerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, Malysz J. Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. Mol Pharmacol. 2007; 72:715–24. [PubMed: 17565004]
24. Williams DK, Wang J, Papke RL. Investigation of the molecular mechanism of the alpha7 nicotinic acetylcholine receptor positive allosteric modulator PNU-120596 provides evidence for two distinct desensitized states. Mol Pharmacol. 2011; 80:1013–32. [PubMed: 21885620]
25. Andersen ND, Nielsen BE, Corradi J, Tolosa MF, Feuerbach D, Arias HR, Bouzat C. Exploring the positive allosteric modulation of human alpha7 nicotinic receptors from a single-channel perspective. Neuropharmacology. 2016; 107:189–200. [PubMed: 26926428]
26. Thakur GA, Kulkarni AR, Deschamps JR, Papke RL. Expeditious synthesis, enantiomeric resolution, and enantiomer functional characterization of (4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): an allosteric agonist-positive allosteric modulator of alpha7 nicotinic acetylcholine receptors. Journal of medicinal chemistry. 2013; 56:8943–7. [PubMed: 24090443]

27. Papke RL, Horenstein NA, Kulkarni AR, Stokes C, Corrie LW, Maeng CY, Thakur GA. The activity of GAT107, an allosteric activator and positive modulator of alpha7 nicotinic acetylcholine receptors (nAChR), is regulated by aromatic amino acids that span the subunit interface. J Biol Chem. 2014; 289:4515–31. [PubMed: 24362025]

28. Wang J, Kuryatov A, Jin Z, Norleans J, Kamenecka TM, Kenny PJ, Lindstrom J. A Novel alpha2/alpha4 Subtype-selective Positive Allosteric Modulator of Nicotinic Acetylcholine Receptors Acting from the C-tail of an alpha Subunit. J Biol Chem. 2015; 290:28834–46. [PubMed: 26432642]

29. Olsen JA, Kastrup JS, Peters D, Gajhede M, Balle T, Ahring PK. Two distinct allosteric binding sites at alpha4beta2 nicotinic acetylcholine receptors revealed by NS206 and NS9283 give unique insights to binding activity-associated linkage at Cys-loop receptors. J Biol Chem. 2013; 288:35997–6006. [PubMed: 24169695]

30. Sala F, Mulet J, Reddy KP, Bernal JA, Wikman P, Valor LM, Peters L, Konig GM, Criado M, Sala S. Potentiation of human alpha4beta2 neuronal nicotinic receptors by a Flustra foliacea metabolite. Neurosci Lett. 2005; 373:144–9. [PubMed: 15567570]

31. Wang J, Kuryatov A, Sriram A, Jin Z, Kamenecka TM, Kenny PJ, Lindstrom J. An Accessory Agonist Binding Site Promotes Activation of alpha4beta2* Nicotinic Acetylcholine Receptors. J Biol Chem. 2015; 290:13907–18. [PubMed: 25869137]

32. Olsen JA, Ahring PK, Kastrup JS, Gajhede M, Balle T. Structural and functional studies of the modulator NS9283 reveal agonist-like mechanism of action at alpha4beta2 nicotinic acetylcholine receptors. J Biol Chem. 2014; 289:24911–21. [PubMed: 24982426]

33. Jain KK. Modulators of nicotinic acetylcholine receptors as analgesics. Curr Opin Investig Drugs. 2004; 5:76–81.

34. Holladay MW, Wasicak JT, Lin NH, He Y, Ryther KB, Bannon AW, Buckley MJ, Kim DJ, Decker MW, Anderson DJ, Campbell JE, Kuntzweiler TA, Donnelly-Roberts DL, Piattoni-Kaplan M, Briggs CA, Williams M, Arneric SP. Identification and initial structure-activity relationships of (R)-5-(2-azetidinylmethoxy)-2-chloropyridine (ABT-594), a potent, orally active, non-opiate analgesic agent acting via neuronal nicotinic acetylcholine receptors. Journal of medicinal chemistry. 1998; 41:407–12. [PubMed: 9484491]

35. Bannon AW, Decker MW, Curzon P, Buckley MJ, Kim DJ, Radek RJ, Lynch JK, Wasicak JT, Lin NH, Arnold WH, Holladay MW, Williams M, Arneric SP. ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine]: a novel, orally effective antinociceptive agent acting via neuronal nicotinic acetylcholine receptors: II. In vivo characterization. J Pharmacol Exp Ther. 1998; 285:787–94. [PubMed: 9580627]

36. Lynch JJ 3rd, Wade CL, Mikusa JP, Decker MW, Honore P. ABT-594 (a nicotinic acetylcholine agonist): anti-allodynia in a rat chemotherapy-induced pain model. Eur J Pharmacol. 2005; 509:43–8. [PubMed: 15713428]

37. Gao B, Hierl M, Clarkin K, Juan T, Nguyen H, Valk M, Deng H, Guo W, Lehto SG, Matson D, McDermott JS, Knop J, Gaida K, Cao L, Waldon D, Albrecht BK, Boezio AA, Copeland KW, Harmange JC, Springer SK, Malmberg AB, McDonough SI. Pharmacological effects of nonselective and subtype-selective nicotinic acetylcholine receptor agonists in animal models of persistent pain. Pain. 2010; 149:33–49. [PubMed: 20167427]

38. Rowbotham MC, Duan WR, Thomas J, Nothaft W, Backonja MM. A randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of ABT-594 in patients with diabetic peripheral neuropathic pain. Pain. 2009; 146:245–52. [PubMed: 19632048]

39. Rowbotham MC, Arslanian A, Nothaft W, Duan WR, Best AE, Pritchett Y, Zhou Q, Stacey BR. Efficacy and safety of the alpha4beta2 neuronal nicotinic receptor agonist ABT-894 in patients with diabetic peripheral neuropathic pain. Pain. 2012; 153:862–8. [PubMed: 22386472]

40. Boyce S, Webb JK, Shepheard SL, Russell MG, Hill RG, Rupniak NM. Analgesic and toxic effects of ABT-594 resemble epibatidine and nicotine in rats. Pain. 2000; 85:443–50. [PubMed: 10781917]

41. Dutta S, Hosmane BS, Awni WM. Population analyses of efficacy and safety of ABT-594 in subjects with diabetic peripheral neuropathic pain. AAPS J. 2012; 14:168–75. [PubMed: 22328206]

42. Lee CH, Zhu C, Malysz J, Campbell T, Shaughnessy T, Honore P, Polakowski J, Gopalakrishnan M. alpha4beta2 neuronal nicotinic receptor positive allosteric modulation: an approach for improving the therapeutic index of alpha4beta2 nAChR agonists in pain. Biochem Pharmacol. 2011; 82:959–66. [PubMed: 21763685]
43. Munro G, Dyhr H, Grunnet M. Selective potentiation of gabapentin-mediated antinociception in the rat formalin test by the nicotinic acetylcholine receptor agonist ABT-594. Neuropharmacology. 2010; 59:208–17. [PubMed: 20562022]
44. Zhu CZ, Chin CL, Rustay NR, Zhong C, Mikusa J, Chandran P, Salyers A, Gomez E, Simler G, Lewis LG, Gauvin D, Baker S, Pai M, Tovcimak A, Brown J, Komater V, Fox GB, Decker MW, Jacobson PB, Gopalakrishnan M, Lee CH, Honore P. Potentiation of analgesic efficacy but not side effects: co-administration of an alpha4beta2 neuronal nicotinic acetylcholine receptor agonist and its positive allosteric modulator in experimental models of pain in rats. Biochem Pharmacol. 2011; 82:967–76. [PubMed: 21620806]
45. Wieskopf JS, Mathur J, Limapichat W, Post MR, Al-Qazzaz M, Sorge RE, Martin LJ, Zaykin DV, Smith SB, Freitas K, Austin JS, Dai F, Zhang J, Marcovitz J, Tuttle AH, Slepian PM, Clarke S, Drenan RM, Janes J, Al Sharari S, Segall SK, Aasvang EK, Lai W, Bittner R, Richards CI, Slade GD, Kehlet H, Walker J, Maskos U, Changeux JP, Devor M, Maixner W, Diatchenko L, Belfer I, Dougherty DA, Su AI, Lummis SC, Imad Damaj M, Lester HA, Patapoutian A, Mogil JS. The nicotinic alpha6 subunit gene determines variability in chronic pain sensitivity via cross-inhibition of P2X2/3 receptors. Sci Transl Med. 2015; 7:287ra72.
46. Limapichat W, Dougherty DA, Lester HA. Subtype-specific mechanisms for functional interaction between alpha6beta4* nicotinic acetylcholine receptors and P2X receptors. Mol Pharmacol. 2014; 86:263–74. [PubMed: 24966348]
47. Gum RJ, Wakefield B, Jarvis MF. P2X receptor antagonists for pain management: examination of binding and physicochemical properties. Purinergic Signal. 2012; 8:41–56. [PubMed: 22086553]
48. North RA, Jarvis MF. P2X receptors as drug targets. Mol Pharmacol. 2013; 83:759–69. [PubMed: 23253448]
49. Haass M, Kubler W. Nicotine and sympathetic neurotransmission. Cardiovasc Drugs Ther. 1997; 10:657–65. [PubMed: 9110108]
50. Rau KK, Johnson RD, Cooper BY. Nicotinic AChR in subclassified capsaicin-sensitive and -insensitive nociceptors of the rat DRG. J Neurophysiol. 2005; 93:1358–71. [PubMed: 15483069]
51. Genzen JR, Van Cleve W, McGehee DS. Dorsal root ganglion neurons express multiple nicotinic acetylcholine receptor subtypes. J Neurophysiol. 2001; 86:1773–82. [PubMed: 11600638]
52. Bibevski S, Zhou Y, McIntosh JM, Zigmond RE, Dunlap ME. Functional nicotinic acetylcholine receptors that mediate ganglionic transmission in cardiac parasympathetic neurons. J Neurosci. 2000; 20:5076–82. [PubMed: 10864965]
53. Hone AJ, McIntosh JM, Azam L, Lindstrom J, Lucero L, Whiteaker P, Passas J, Blazquez J, Albillos A. alpha-Conotoxins Identify the alpha3beta4* Subtype as the Predominant Nicotinic Acetylcholine Receptor Expressed in Human Adrenal Chromaffin Cells. Mol Pharmacol. 2015; 88:881–93. [PubMed: 26330550]
54. Hone AJ, Michael McIntosh J, Rueda-Ruzafa L, Passas J, de Castro-Guerín C, Blázquez J, González-Enguita C, Albillos A. Therapeutic concentrations of varenicline in the presence of nicotine increase action potential firing in human adrenal chromaffin cells. Journal of Neurochemistry. 2017; 140:37–52. [PubMed: 27805736]
55. Lang PM, Burgstahler R, Sippel W, Irnich D, Schlotter-Weigel B, Grafe P. Characterization of neuronal nicotinic acetylcholine receptors in the membrane of unmyelinated human C-fiber axons by in vitro studies. J Neurophysiol. 2003; 90:3295–303. [PubMed: 12878715]
56. Chen D, Patrick JW. The alpha-bungarotoxin-binding nicotinic acetylcholine receptor from rat brain contains only the alpha7 subunit. J Biol Chem. 1997; 272:24024–9. [PubMed: 9295355]
57. Drisdel RC, Green WN. Neuronal alpha-bungarotoxin receptors are alpha7 subunit homomers. J Neurosci. 2000; 20:133–9. [PubMed: 10627589]
58. Liu Q, Huang Y, Xue F, Simard A, DeChon J, Li G, Zhang J, Lucero L, Wang M, Sierks M, Hu G, Chang Y, Lukas RJ, Wu J. A novel nicotinic acetylcholine receptor subtype in basal forebrain

cholinergic neurons with high sensitivity to amyloid peptides. J Neurosci. 2009; 29:918–29. [PubMed: 19176801]

59. Azam L, Winzer-Serhan U, Leslie FM. Co-expression of alpha7 and beta2 nicotinic acetylcholine receptor subunit mRNAs within rat brain cholinergic neurons. Neuroscience. 2003; 119:965–77. [PubMed: 12831856]
60. Liu Q, Huang Y, Shen J, Steffensen S, Wu J. Functional alpha7beta2 nicotinic acetylcholine receptors expressed in hippocampal interneurons exhibit high sensitivity to pathological level of amyloid beta peptides. BMC Neurosci. 2012; 13:155. [PubMed: 23272676]
61. Thomsen MS, Zwart R, Ursu D, Jensen MM, Pinborg LH, Gilmour G, Wu J, Sher E, Mikkelsen JD. alpha7 and beta2 Nicotinic Acetylcholine Receptor Subunits Form Heteromeric Receptor Complexes that Are Expressed in the Human Cortex and Display Distinct Pharmacological Properties. PLoS One. 2015; 10:e0130572. [PubMed: 26086615]
62. Shoop RD, Martone ME, Yamada N, Ellisman MH, Berg DK. Neuronal acetylcholine receptors with alpha7 subunits are concentrated on somatic spines for synaptic signaling in embryonic chick ciliary ganglia. J Neurosci. 1999; 19:692–704. [PubMed: 9880590]
63. Voitenko LP, Voitenko SV, Skok MV, Purnyn HE, Skok VI. Nicotinic acetylcholine receptor subtypes in rat superior cervical ganglion neurons as studied by sequential application of two alpha-subunit-specific antibodies. Neurosci Lett. 2001; 303:37–40. [PubMed: 11297818]
64. Lips KS, Konig P, Schatzle K, Pfeil U, Krasteva G, Spies M, Haberberger RV, Grando SA, Kummer W. Coexpression and spatial association of nicotinic acetylcholine receptor subunits alpha7 and alpha10 in rat sympathetic neurons. J Mol Neurosci. 2006; 30:15–6. [PubMed: 17192608]
65. Perez-Alvarez A, Hernandez-Vivanco A, Gregorio SA, Tabernero A, McIntosh JM, Albillos A. Pharmacological characterization of native alpha7 nAChRs and their contribution to depolarization-elicited exocytosis in human chromaffin cells. Br J Pharmacol. 2012; 165:908–21. [PubMed: 21790533]
66. Criado M, Dominguez del Toro E, Carrasco-Serrano C, Smillie FI, Juiz JM, Viniegra S, Ballesta JJ. Differential expression of alpha-bungarotoxin-sensitive neuronal nicotinic receptors in adrenergic chromaffin cells: a role for transcription factor Egr-1. J Neurosci. 1997; 17:6554–64. [PubMed: 9254668]
67. Gahring LC, Myers E, Palumbos S, Rogers SW. Nicotinic receptor Alpha7 expression during mouse adrenal gland development. PLoS One. 2014; 9:e103861. [PubMed: 25093893]
68. Genzen JR, McGehee DS. Short- and long-term enhancement of excitatory transmission in the spinal cord dorsal horn by nicotinic acetylcholine receptors. Proc Natl Acad Sci U S A. 2003; 100:6807–12. [PubMed: 12748382]
69. Matsumoto M, Xie W, Inoue M, Ueda H. Evidence for the tonic inhibition of spinal pain by nicotinic cholinergic transmission through primary afferents. Mol Pain. 2007; 3:41. [PubMed: 18088441]
70. Cordero-Erausquin M, Pons S, Faure P, Changeux JP. Nicotine differentially activates inhibitory and excitatory neurons in the dorsal spinal cord. Pain. 2004; 109:308–18. [PubMed: 15157692]
71. Takeda D, Nakatsuka T, Gu JG, Yoshida M. The activation of nicotinic acetylcholine receptors enhances the inhibitory synaptic transmission in the deep dorsal horn neurons of the adult rat spinal cord. Mol Pain. 2007; 3:26. [PubMed: 17894865]
72. Takeda D, Nakatsuka T, Papke R, Gu JG. Modulation of inhibitory synaptic activity by a non-alpha4beta2, non-alpha7 subtype of nicotinic receptors in the substantia gelatinosa of adult rat spinal cord. Pain. 2003; 101:13–23. [PubMed: 12507696]
73. Genzen JR, McGehee DS. Nicotinic modulation of GABAergic synaptic transmission in the spinal cord dorsal horn. Brain Res. 2005; 1031:229–37. [PubMed: 15649448]
74. Rashid MH, Furue H, Yoshimura M, Ueda H. Tonic inhibitory role of alpha4beta2 subtype of nicotinic acetylcholine receptors on nociceptive transmission in the spinal cord in mice. Pain. 2006; 125:125–35. [PubMed: 16781069]
75. Seddik R, Schlichter R, Trouslard J. Modulation of GABAergic synaptic transmission by terminal nicotinic acetylcholine receptors in the central autonomic nucleus of the neonatal rat spinal cord. Neuropharmacology. 2006; 51:77–89. [PubMed: 16678861]

76. Papadopolou S, Hartmann P, Lips KS, Kummer W, Haberberger RV. Nicotinic receptor mediated stimulation of NO-generation in neurons of rat thoracic dorsal root ganglia. Neurosci Lett. 2004; 361:32–5. [PubMed: 15135886]
77. Thippeswamy T, Jain R, Mumtaz N, Morris R. Inhibition of neuronal nitric oxide synthase results in neurodegenerative changes in the axotomised dorsal root ganglion neurons: Evidence for a neuroprotective role of nitric oxide in vivo. Neurosci Res. 2001; 40:37–44. [PubMed: 11311403]
78. Toyabe S, Iiai T, Fukuda M, Kawamura T, Suzuki S, Uchiyama M, Abo T. Identification of nicotinic acetylcholine receptors on lymphocytes in the periphery as well as thymus in mice. Immunology. 1997; 92:201–5. [PubMed: 9415027]
79. Zanetti SR, Ziblat A, Torres NI, Zwirner NW, Bouzat C. Expression and Functional Role of alpha7 Nicotinic Receptor in Human Cytokine-stimulated Natural Killer (NK) Cells. J Biol Chem. 2016; 291:16541–52. [PubMed: 27284006]
80. De Rosa MJ, Dionisio L, Agriello E, Bouzat C, Esandi Mdel C. Alpha 7 nicotinic acetylcholine receptor modulates lymphocyte activation. Life Sci. 2009; 85:444–9. [PubMed: 19632243]
81. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature. 2003; 421:384–8. [PubMed: 12508119]
82. Yoshikawa H, Kurokawa M, Ozaki N, Nara K, Atou K, Takada E, Kamochi H, Suzuki N. Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-kappaB phosphorylation and nuclear factor-kappaB transcriptional activity through nicotinic acetylcholine receptor alpha7. Clin Exp Immunol. 2006; 146:116–23. [PubMed: 16968406]
83. Hecker A, Kullmar M, Wilker S, Richter K, Zakrzewicz A, Atanasova S, Mathes V, Timm T, Lerner S, Klein J, Kaufmann A, Bauer S, Padberg W, Kummer W, Janciauskiene S, Fronius M, Schweda EK, Lochnit G, Grau V. Phosphocholine-Modified Macromolecules and Canonical Nicotinic Agonists Inhibit ATP-Induced IL-1beta Release. J Immunol. 2015; 195:2325–34. [PubMed: 26202987]
84. Rosas-Ballina M, Goldstein RS, Gallowitsch-Puerta M, Yang L, Valdes-Ferrer SI, Patel NB, Chavan S, Al-Abed Y, Yang H, Tracey KJ. The selective alpha7 agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. Mol Med. 2009; 15:195–202. [PubMed: 19593403]
85. Shytle RD, Mori T, Townsend K, Vendrame M, Sun N, Zeng J, Ehrhart J, Silver AA, Sanberg PR, Tan J. Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. J Neurochem. 2004; 89:337–43. [PubMed: 15056277]
86. De Simone R, Ajmone-Cat MA, Carnevale D, Minghetti L. Activation of alpha7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. J Neuroinflammation. 2005; 2:4. [PubMed: 15670336]
87. Cedillo JL, Arnalich F, Martin-Sanchez C, Quesada A, Rios JJ, Maldifassi MC, Atienza G, Renart J, Fernandez-Capitan C, Garcia-Rio F, Lopez-Collazo E, Montiel C. Usefulness of alpha7 nicotinic receptor messenger RNA levels in peripheral blood mononuclear cells as a marker for cholinergic antiinflammatory pathway activity in septic patients: results of a pilot study. J Infect Dis. 2015; 211:146–55. [PubMed: 25092899]
88. Bencherif M, Lippiello PM, Lucas R, Marrero MB. Alpha7 nicotinic receptors as novel therapeutic targets for inflammation-based diseases. Cell Mol Life Sci. 2011; 68:931–49. [PubMed: 20953658]
89. Saeed RW, Varma S, Peng-Nemeroff T, Sherry B, Balakhaneh D, Huston J, Tracey KJ, Al-Abed Y, Metz CN. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. J Exp Med. 2005; 201:1113–23. [PubMed: 15809354]
90. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med. 2003; 9:125–34. [PubMed: 14571320]
91. Gallowitsch-Puerta M, Tracey KJ. Immunologic role of the cholinergic anti-inflammatory pathway and the nicotinic acetylcholine alpha 7 receptor. Ann N Y Acad Sci. 2005; 1062:209–19. [PubMed: 16461803]

92. Bernik TR, Friedman SG, Ochani M, DiRaimo R, Ulloa L, Yang H, Sudan S, Czura CJ, Ivanova SM, Tracey KJ. Pharmacological stimulation of the cholinergic antiinflammatory pathway. J Exp Med. 2002; 195:781–8. [PubMed: 11901203]

93. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000; 405:458–62. [PubMed: 10839541]

94. Ulloa L. The vagus nerve and the nicotinic anti-inflammatory pathway. Nat Rev Drug Discov. 2005; 4:673–84. [PubMed: 16056392]

95. de Jonge WJ, van der Zanden EP, The FO, Bijlsma MF, van Westerloo DJ, Bennink RJ, Berthoud HR, Uematsu S, Akira S, van den Wijngaard RM, Boeckxstaens GE. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. Nat Immunol. 2005; 6:844–51. [PubMed: 16025117]

96. Wang H, Liao H, Ochani M, Justiniani M, Lin X, Yang L, Al-Abed Y, Wang H, Metz C, Miller EJ, Tracey KJ, Ulloa L. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med. 2004; 10:1216–21. [PubMed: 15502843]

97. McAllen RM, Cook AD, Khiew HW, Martelli D, Hamilton JA. The interface between cholinergic pathways and the immune system and its relevance to arthritis. Arthritis Res Ther. 2015; 17:87. [PubMed: 25889979]

98. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: a critical review. Auton Neurosci. 2014; 182:65–9. [PubMed: 24411268]

99. Fujii T, Mashimo M, Moriwaki Y, Misawa H, Ono S, Horiguchi K, Kawashima K. Physiological functions of the cholinergic system in immune cells. J Pharmacol Sci. 2017; 134:1–21. [PubMed: 28552584]

100. Rosas-Ballina M, Olofsson PS, Ochani M, Valdes-Ferrer SI, Levine YA, Reardon C, Tusche MW, Pavlov VA, Andersson U, Chavan S, Mak TW, Tracey KJ. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science. 2011; 334:98–101. [PubMed: 21921156]

101. Vida G, Pena G, Deitch EA, Ulloa L. alpha7-cholinergic receptor mediates vagal induction of splenic norepinephrine. J Immunol. 2011; 186:4340–6. [PubMed: 21339364]

102. Bagdas D, Sonat FA, Hamurtekin E, Sonal S, Gurun MS. The antihyperalgesic effect of cytidine-5'-diphosphate-choline in neuropathic and inflammatory pain models. Behav Pharmacol. 2011; 22:589–98. [PubMed: 21836465]

103. Gurun MS, Parker R, Eisenach JC, Vincler M. The effect of peripherally administered CDP-choline in an acute inflammatory pain model: the role of alpha7 nicotinic acetylcholine receptor. Anesth Analg. 2009; 108:1680–7. [PubMed: 19372354]

104. Rowley TJ, McKinstry A, Greenidge E, Smith W, Flood P. Antinociceptive and anti-inflammatory effects of choline in a mouse model of postoperative pain. Br J Anaesth. 2010; 105:201–7. [PubMed: 20511332]

105. Damaj MI, Meyer EM, Martin BR. The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. Neuropharmacology. 2000; 39:2785–91. [PubMed: 11044748]

106. Sidhu N, Davies S, Nadarajah A, Rivera J, Whittington R, Mercier RJ, Virag L, Wang S, Flood P. Oral choline supplementation for postoperative pain. Br J Anaesth. 2013; 111:249–55. [PubMed: 23568851]

107. Loram LC, Harrison JA, Chao L, Taylor FR, Reddy A, Travis CL, Giffard R, Al-Abed Y, Tracey K, Maier SF, Watkins LR. Intrathecal injection of an alpha seven nicotinic acetylcholine receptor agonist attenuates gp120-induced mechanical allodynia and spinal pro-inflammatory cytokine profiles in rats. Brain Behav Immun. 2010; 24:959–67. [PubMed: 20353818]

108. Rowley TJ, Payappilly J, Lu J, Flood P. The antinociceptive response to nicotinic agonists in a mouse model of postoperative pain. Anesth Analg. 2008; 107:1052–7. [PubMed: 18713928]

109. Mucchietto V, Fasoli F, Pucci S, Moretti M, Benfante R, Maroli A, Di Lascio S, Bolchi C, Pallavicini M, Dowell C, McIntosh M, Clementi F, Gotti C. alpha9- and alpha7-containing receptors mediate the pro-proliferative effects of nicotine in the A549 adenocarcinoma cell line. Br J Pharmacol. 2017 [Epub ahead of print].

110. Bordas A, Cedillo JL, Arnalich F, Esteban-Rodriguez I, Guerra-Pastrian L, de Castro J, Martin-Sanchez C, Atienza G, Fernandez-Capitan C, Rios JJ, Montiel C. Expression patterns for

nicotinic acetylcholine receptor subunit genes in smoking-related lung cancers. Oncotarget. 2017; 8:67878–67890. [PubMed: 28978081]

111. Vincler M, Vetter DE, McIntosh M. α9α10 and α7 nicotinic acetylcholine receptors have opposite roles in the immune response to peripheral nerve injury. Biochem Pharmacol. 2007; 74:SMA49.
112. Christensen SB, Hone AJ, Roux I, Kniazeff J, Pin JP, Upert G, Servent D, Glowatzki E, McIntosh JM. RgIA4 Potently Blocks Mouse alpha9alpha10 nAChRs and Provides Long Lasting Protection against Oxaliplatin-Induced Cold Allodynia. Front Cell Neurosci. 2017; 11:219. [PubMed: 28785206]
113. Sgard F, Charpantier E, Bertrand S, Walker N, Caput D, Graham D, Bertrand D, Besnard F. A novel human nicotinic receptor subunit, alpha10, that confers functionality to the alpha9-subunit. Mol Pharmacol. 2002; 61:150–9. [PubMed: 11752216]
114. Bagdas D, Wilkerson JL, Kulkarni A, Toma W, AlSharari S, Gul Z, Lichtman AH, Papke RL, Thakur GA, Damaj MI. The alpha7 nicotinic receptor dual allosteric agonist and positive allosteric modulator GAT107 reverses nociception in mouse models of inflammatory and neuropathic pain. Br J Pharmacol. 2016; 173:2506–20. [PubMed: 27243753]
115. Freitas K, Carroll FI, Damaj MI. The antinociceptive effects of nicotinic receptors alpha7-positive allosteric modulators in murine acute and tonic pain models. J Pharmacol Exp Ther. 2013; 344:264–75. [PubMed: 23115222]
116. Freitas K, Ghosh S, Ivy Carroll F, Lichtman AH, Imad Damaj M. Effects of alpha7 positive allosteric modulators in murine inflammatory and chronic neuropathic pain models. Neuropharmacology. 2013; 65:156–64. [PubMed: 23079470]
117. Uteshev VV. The therapeutic promise of positive allosteric modulation of nicotinic receptors. Eur J Pharmacol. 2014; 727:181–5. [PubMed: 24530419]
118. Freitas K, Negus SS, Carroll FI, Damaj MI. In vivo pharmacological interactions between a type II positive allosteric modulator of alpha7 nicotinic ACh receptors and nicotinic agonists in a murine tonic pain model. Br J Pharmacol. 2013; 169:567–79. [PubMed: 23004024]
119. Briggs CA, Gronlien JH, Curzon P, Timmermann DB, Ween H, Thorin-Hagene K, Kerr P, Anderson DJ, Malysz J, Dyhring T, Olsen GM, Peters D, Bunnelle WH, Gopalakrishnan M. Role of channel activation in cognitive enhancement mediated by alpha7 nicotinic acetylcholine receptors. Br J Pharmacol. 2009; 158:1486–94. [PubMed: 19845675]
120. Papke RL, Bagdas D, Kulkarni AR, Gould T, AlSharari SD, Thakur GA, Damaj MI. The analgesic-like properties of the alpha7 nAChR silent agonist NS6740 is associated with non-conducting conformations of the receptor. Neuropharmacology. 2015; 91:34–42. [PubMed: 25497451]
121. van Maanen MA, Papke RL, Koopman FA, Koepke J, Bevaart L, Clark R, Lamppu D, Elbaum D, LaRosa GJ, Tak PP, Vervoordeldonk MJ. Two novel alpha7 nicotinic acetylcholine receptor ligands: in vitro properties and their efficacy in collagen-induced arthritis in mice. PLoS One. 2015; 10:e0116227. [PubMed: 25617631]
122. Bagdas D, Gurun MS, Flood P, Papke RL, Damaj MI. New Insights on Neuronal Nicotinic Acetylcholine Receptors as Targets for Pain and Inflammation: A Focus on alpha7 nAChRs. Curr Neuropharmacol. 2017 [Epub ahead of print].
123. Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J. alpha10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. Proc Natl Acad Sci U S A. 2001; 98:3501–6. [PubMed: 11248107]
124. Lustig LR, Peng H, Hiel H, Yamamoto T, Fuchs PA. Molecular cloning and mapping of the human nicotinic acetylcholine receptor alpha10 (CHRNA10). Genomics. 2001; 73:272–83. [PubMed: 11350119]
125. Glowatzki E, Fuchs PA. Cholinergic synaptic inhibition of inner hair cells in the neonatal mammalian cochlea. Science. 2000; 288:2366–8. [PubMed: 10875922]
126. McIntosh JM, Plazas PV, Watkins M, Gomez-Casati ME, Olivera BM, Elgoyhen AB. A novel alpha-conotoxin, PeIA, cloned from Conus pergrandis, discriminates between rat alpha9alpha10 and alpha7 nicotinic cholinergic receptors. J Biol Chem. 2005; 280:30107–12. [PubMed: 15983035]

127. Lips KS, Pfeil U, Kummer W. Coexpression of alpha 9 and alpha 10 nicotinic acetylcholine receptors in rat dorsal root ganglion neurons. NeuroScience. 2002; 115:1–5. [PubMed: 12401316]

128. Haberberger RV, Bernardini N, Kress M, Hartmann P, Lips KS, Kummer W. Nicotinic acetylcholine receptor subtypes in nociceptive dorsal root ganglion neurons of the adult rat. Auton Neurosci. 2004; 113:32–42. [PubMed: 15296793]

129. Callaghan B, Adams DJ. Analgesic alpha-conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in sensory neurons of alpha9 nicotinic receptor knockout mice. Channels (Austin). 2010; 4:51–4. [PubMed: 20368690]

130. Biallas S, Wilker S, Lips KS, Kummer W, Grando SA, Padberg W, Grau V. Immunohistochemical detection of nicotinic acetylcholine receptor subunits alpha9 and alpha10 in rat lung isografts and allografts. Life Sci. 2007; 80:2286–9. [PubMed: 17331545]

131. Mikulski Z, Hartmann P, Jositsch G, Zaslona Z, Lips KS, Pfeil U, Kurzen H, Lohmeyer J, Clauss WG, Grau V, Fronius M, Kummer W. Nicotinic receptors on rat alveolar macrophages dampen ATP-induced increase in cytosolic calcium concentration. Respir Res. 2010; 11:133. [PubMed: 20920278]

132. Kindt F, Wiegand S, Niemeier V, Kupfer J, Loser C, Nilles M, Kurzen H, Kummer W, Gieler U, Haberberger RV. Reduced expression of nicotinic alpha subunits 3, 7, 9 and 10 in lesional and nonlesional atopic dermatitis skin but enhanced expression of alpha subunits 3 and 5 in mast cells. Br J Dermatol. 2008; 159:847–57. [PubMed: 18671783]

133. St-Pierre S, Jiang W, Roy P, Champigny C, LeBlanc E, Morley BJ, Hao J, Simard AR. Nicotinic Acetylcholine Receptors Modulate Bone Marrow-Derived Pro-Inflammatory Monocyte Production and Survival. PLoS One. 2016; 11:e0150230. [PubMed: 26925951]

134. Peng H, Ferris RL, Matthews T, Hiel H, Lopez-Albaitero A, Lustig LR. Characterization of the human nicotinic acetylcholine receptor subunit alpha (alpha) 9 (CHRNA9) and alpha (alpha) 10 (CHRNA10) in lymphocytes. Life Sci. 2004; 76:263–80. [PubMed: 15531379]

135. Richter K, Mathes V, Fronius M, Althaus M, Hecker A, Krasteva-Christ G, Padberg W, Hone AJ, McIntosh JM, Zakrzewicz A, Grau V. Phosphocholine - an agonist of metabotropic but not of ionotropic functions of alpha9-containing nicotinic acetylcholine receptors. Sci Rep. 2016; 6:28660. [PubMed: 27349288]

136. Zakrzewicz A, Richter K, Agne A, Wilker S, Siebers K, Fink B, Krasteva-Christ G, Althaus M, Padberg W, Hone AJ, McIntosh JM, Grau V. Canonical and Novel Non-Canonical Cholinergic Agonists Inhibit ATP-Induced Release of Monocytic Interleukin-1beta via Different Combinations of Nicotinic Acetylcholine Receptor Subunits alpha7, alpha9 and alpha10. Front Cell Neurosci. 2017; 11:189. [PubMed: 28725182]

137. Backhaus S, Zakrzewicz A, Richter K, Damm J, Wilker S, Fuchs-Moll G, Kullmar M, Hecker A, Manzini I, Ruppert C, McIntosh JM, Padberg W, Grau V. Surfactant inhibits ATP-induced release of interleukin-1beta via nicotinic acetylcholine receptors. J Lipid Res. 2017; 58:1055–1066. [PubMed: 28404637]

138. Amati AL, Zakrzewicz A, Siebers K, Wilker S, Heldmann S, Zakrzewicz D, Hecker A, McIntosh JM, Padberg W, Grau V. Chemokines (CCL3, CCL4, and CCL5) Inhibit ATP-Induced Release of IL-1beta by Monocytic Cells. Mediators Inflamm. 2017; 2017:1434872. [PubMed: 28757683]

139. Jiang W, St-Pierre S, Roy P, Morley BJ, Hao J, Simard AR. Infiltration of CCR2+Ly6Chigh Proinflammatory Monocytes and Neutrophils into the Central Nervous System Is Modulated by Nicotinic Acetylcholine Receptors in a Model of Multiple Sclerosis. J Immunol. 2016; 196:2095–108. [PubMed: 26810225]

140. Di Cesare Mannelli L, Cinci L, Micheli L, Zanardelli M, Pacini A, McIntosh JM, Ghelardini C. alpha-conotoxin RgIA protects against the development of nerve injury-induced chronic pain and prevents both neuronal and glial derangement. Pain. 2014; 155:1986–95. [PubMed: 25008370]

141. Pacini A, Micheli L, Maresca M, Branca JJ, McIntosh JM, Ghelardini C, Di Cesare Mannelli L. The alpha9alpha10 nicotinic receptor antagonist alpha-conotoxin RgIA prevents neuropathic pain induced by oxaliplatin treatment. Exp Neurol. 2016; 282:37–48. [PubMed: 27132993]

142. Mohammadi S, Christie MJ. alpha9-nicotinic acetylcholine receptors contribute to the maintenance of chronic mechanical hyperalgesia, but not thermal or mechanical allodynia. Mol Pain. 2014; 10:64. [PubMed: 25274008]

143. Romero HK, Christensen S, Di Cesare Mannelli L, Gajewiak J, Ramachandra R, Elmslie KS, Vetter DE, Ghelardini C, Ladonato SP, Mercado JL, Olivera BM, McIntosh JM. Inhibition of α9α10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain. Proc Natl Acad Sci U S A. 2017; 114:1825–1832.

144. Simard AR, Gan Y, St-Pierre S, Kousari A, Patel V, Whiteaker P, Morley BJ, Lukas RJ, Shi FD. Differential modulation of EAE by alpha9*- and beta2*-nicotinic acetylcholine receptors. Immunol Cell Biol. 2013; 91:195–200. [PubMed: 23399696]

145. Vincler M, Wittenauer S, Parker R, Ellison M, Olivera BM, McIntosh JM. Molecular mechanism for analgesia involving specific antagonism of alpha9alpha10 nicotinic acetylcholine receptors. Proc Natl Acad Sci U S A. 2006; 103:17880–4. [PubMed: 17101979]

146. Ellison M, Haberlandt C, Gomez-Casati ME, Watkins M, Elgoyhen AB, McIntosh JM, Olivera BM. Alpha-RgIA: a novel conotoxin that specifically and potently blocks the alpha9alpha10 nAChR. Biochemistry. 2006; 45:1511–7. [PubMed: 16445293]

147. Satkunanathan N, Livett B, Gayler K, Sandall D, Down J, Khalil Z. Alpha-conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurones. Brain Res. 2005; 1059:149–58. [PubMed: 16182258]

148. Livett, BG.Khalil, Z.Down, J.Sandall, DW., Keays, D., inventors. α-Contoxoin peptides with analgesic properties. US Patent. 7,348,400 B2. 2008.

149. Armishaw CJ. Synthetic alpha-conotoxin mutants as probes for studying nicotinic acetylcholine receptors and in the development of novel drug leads. Toxins (Basel). 2010; 2:1471–99. [PubMed: 22069647]

150. Shen GS, Layer RT, McCabe RT. Conopeptides: From deadly venoms to novel therapeutics. Drug Discov Today. 2000; 5:98–106. [PubMed: 10675883]

151. Atanassoff PG, Hartmannsgruber MW, Thrasher J, Wermeling D, Longton W, Gaeta R, Singh T, Mayo M, McGuire D, Luther RR. Ziconotide, a new N-type calcium channel blocker, administered intrathecally for acute postoperative pain. Reg Anesth Pain Med. 2000; 25:274–8. [PubMed: 10834782]

152. Olivera BM, Cruz LJ, de Santos V, LeCheminant GW, Griffin D, Zeikus R, McIntosh JM, Galyean R, Varga J, Gray WR, et al. Neuronal calcium channel antagonists. Discrimination between calcium channel subtypes using omega-conotoxin from Conus magus venom. Biochemistry. 1987; 26:2086–90. [PubMed: 2441741]

153. Kolosov A, Aurini L, Williams ED, Cooke I, Goodchild CS. Intravenous injection of leconotide, an omega conotoxin: synergistic antihyperalgesic effects with morphine in a rat model of bone cancer pain. Pain Med. 2011; 12:923–41. [PubMed: 21539704]

154. Kolosov A, Goodchild CS, Cooke I. CNSB004 (Leconotide) causes antihyperalgesia without side effects when given intravenously: a comparison with ziconotide in a rat model of diabetic neuropathic pain. Pain Med. 2010; 11:262–73. [PubMed: 20002322]

155. Sadeghi M, Murali SS, Lewis RJ, Alewood PF, Mohammadi S, Christie MJ. Novel omega-conotoxins from C. catus reverse signs of mouse inflammatory pain after systemic administration. Mol Pain. 2013; 9:51. [PubMed: 24139484]

156. Metabolic, PL. [Accessed: March 1st 2007] ACVI: A Novel Therapeutic For Neuropathic Pain. Technical Summary of Preclinical and Phase I Data. 2006. http://www.metaboliccomau/_files/QTO7TA4EWA/ACV_NonConfidentialPackage_February2007.pdf

157. Sandall DW, Satkunanathan N, Keays DA, Polidano MA, Liping X, Pham V, Down JG, Khalil Z, Livett BG, Gayler KR. A novel alpha-conotoxin identified by gene sequencing is active in suppressing the vascular response to selective stimulation of sensory nerves in vivo. Biochemistry. 2003; 42:6904–11. [PubMed: 12779345]

158. AdisInsight. [Accessed: April 17th, 2017] ACV1. 2016. http://adisinsight.springer.com/drugs/800020787

159. Metabolic, PL. [Accessed: August 9th, 2017] Metabolic discontinues clinical drug programme for neuropathic pain drug. ACV1. 2007. http://www.asx.com.au/asxpdf/20070814/pdf/313yjgpf7jl4lg.pdf

160. Azam L, McIntosh JM. Molecular basis for the differential sensitivity of rat and human alpha9alpha10 nAChRs to alpha-conotoxin RgIA. J Neurochem. 2012; 122:1137–44. [PubMed: 22774872]

161. Luo S, Zhangsun D, Harvey PJ, Kaas Q, Wu Y, Zhu X, Hu Y, Li X, Tsetlin VI, Christensen S, Romero HK, McIntyre M, Dowell C, Baxter JC, Elmslie KS, Craik DJ, McIntosh JM. Cloning, synthesis, and characterization of alphaO-conotoxin GeXIVA, a potent alpha9alpha10 nicotinic acetylcholine receptor antagonist. Proc Natl Acad Sci U S A. 2015; 112:E4026–35. [PubMed: 26170295]

162. Zhangsun D, Zhu X, Kaas Q, Wu Y, Craik DJ, McIntosh JM, Luo S. alphaO-Conotoxin GeXIVA disulfide bond isomers exhibit differential sensitivity for various nicotinic acetylcholine receptors but retain potency and selectivity for the human alpha9alpha10 subtype. Neuropharmacology. 2017 [Epub ahead of print].

163. Callaghan B, Haythornthwaite A, Berecki G, Clark RJ, Craik DJ, Adams DJ. Analgesic alpha-conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABAB receptor activation. J Neurosci. 2008; 28:10943–51. [PubMed: 18945902]

164. Hone AJ, Servent D, McIntosh JM. alpha9-containing nicotinic acetylcholine receptors and the modulation of pain. Br J Pharmacol. 2017 [Epub ahead of print].

165. Sadeghi M, McArthur JR, Finol-Urdaneta RK, Adams DJ. Analgesic conopeptides targeting G protein-coupled receptors reduce excitability of sensory neurons. Neuropharmacology. 2017 [Epub ahead of print].

166. Zheng G, Zhang Z, Dowell C, Wala E, Dwoskin LP, Holtman JR, McIntosh JM, Crooks PA. Discovery of non-peptide, small molecule antagonists of alpha9alpha10 nicotinic acetylcholine receptors as novel analgesics for the treatment of neuropathic and tonic inflammatory pain. Bioorg Med Chem Lett. 2011; 21:2476–9. [PubMed: 21397497]

167. Holtman JR, Dwoskin LP, Dowell C, Wala EP, Zhang Z, Crooks PA, McIntosh JM. The novel small molecule alpha9alpha10 nicotinic acetylcholine receptor antagonist ZZ-204G is analgesic. Eur J Pharmacol. 2011; 670:500–8. [PubMed: 21944926]

168. Wala EP, Crooks PA, McIntosh JM, Holtman JR Jr. Novel small molecule alpha9alpha10 nicotinic receptor antagonist prevents and reverses chemotherapy-evoked neuropathic pain in rats. Anesth Analg. 2012; 115:713–20. [PubMed: 22610850]

169. McGranahan TM, Patzlaff NE, Grady SR, Heinemann SF, Booker TK. alpha4beta2 nicotinic acetylcholine receptors on dopaminergic neurons mediate nicotine reward and anxiety relief. J Neurosci. 2011; 31:10891–902. [PubMed: 21795541]

170. Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, Lancaster T. Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev. 2016:CD006103. [PubMed: 27158893]

171. Tonstad S, Holme I, Tonnesen P. Dianicline, a novel alpha4beta2 nicotinic acetylcholine receptor partial agonist, for smoking cessation: a randomized placebo-controlled clinical trial. Nicotine Tob Res. 2011; 13:1–6. [PubMed: 21041839]

172. Costello MR, Reynaga DD, Mojica CY, Zaveri NT, Belluzzi JD, Leslie FM. Comparison of the reinforcing properties of nicotine and cigarette smoke extract in rats. Neuropsychopharmacology. 2014; 39:1843–51. [PubMed: 24513971]

173. Brunzell DH, McIntosh JM, Papke RL. Diverse strategies targeting alpha7 homomeric and alpha6beta2* heteromeric nicotinic acetylcholine receptors for smoking cessation. Ann N Y Acad Sci. 2014; 1327:27–45. [PubMed: 24730978]

174. Brunzell DH, McIntosh JM. Alpha7 nicotinic acetylcholine receptors modulate motivation to self-administer nicotine: implications for smoking and schizophrenia. Neuropsychopharmacology. 2012; 37:1134–43. [PubMed: 22169946]

175. Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. Mol Pharmacol. 2006; 70:801–5. [PubMed: 16766716]

176. Campling BG, Kuryatov A, Lindstrom J. Acute activation, desensitization and smoldering activation of human acetylcholine receptors. PLoS One. 2013; 8:e79653. [PubMed: 24244538]

177. Information N. C. f. B. [Accessed: August 30th 2017] Gene National Center for Biotechnology Information. 1999. https://www.ncbi.nlm.nih.gov/gene/231252

178. Haberberger RV, Henrich M, Lips KS, Kummer W. Nicotinic receptor alpha 7-subunits are coupled to the stimulation of nitric oxide synthase in rat dorsal root ganglion neurons. Histochem Cell Biol. 2003; 120:173–81. [PubMed: 12898272]

179. Takahashi HK, Iwagaki H, Hamano R, Yoshino T, Tanaka N, Nishibori M. alpha7 Nicotinic acetylcholine receptor stimulation inhibits lipopolysaccharide-induced interleukin-18 and −12 production in monocytes. J Pharmacol Sci. 2006; 102:143–6. [PubMed: 16960420]

180. Maldifassi MC, Atienza G, Arnalich F, Lopez-Collazo E, Cedillo JL, Martin-Sanchez C, Bordas A, Renart J, Montiel C. A new IRAK-M-mediated mechanism implicated in the anti-inflammatory effect of nicotine via alpha7 nicotinic receptors in human macrophages. PLoS One. 2014; 9:e108397. [PubMed: 25259522]

181. Li J, Mathieu SL, Harris R, Ji J, Anderson DJ, Malysz J, Bunnelle WH, Waring JF, Marsh KC, Murtaza A, Olson LM, Gopalakrishnan M. Role of alpha7 nicotinic acetylcholine receptors in regulating tumor necrosis factor-alpha (TNF-alpha) as revealed by subtype selective agonists. J Neuroimmunol. 2011; 239:37–43. [PubMed: 21911260]

182. Liu Z, Han B, Li P, Wang Z, Fan Q. Activation of alpha7nAChR by nicotine reduced the Th17 response in CD4(+)T lymphocytes. Immunol Invest. 2014; 43:667–74. [PubMed: 24949556]

183. Wu S, Zhao H, Luo H, Xiao X, Zhang H, Li T, Zuo X. GTS-21, an alpha7-nicotinic acetylcholine receptor agonist, modulates Th1 differentiation in CD4+ T cells from patients with rheumatoid arthritis. Exp Ther Med. 2014; 8:557–562. [PubMed: 25009619]

184. Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. J Immunol. 2009; 183:6681–8. [PubMed: 19846875]

185. Hu Y, Liu R, Li J, Yue Y, Cheng W, Zhang P. Attenuation of Collagen-Induced Arthritis in rat by nicotinic alpha7 receptor partial agonist GTS-21. Biomed Res Int. 2014; 2014:325875. [PubMed: 24719855]

186. Li X, Hu Y, Wu Y, Huang Y, Yu S, Ding Q, Zhangsun D, Luo S. Anti-hypersensitive effect of intramuscular administration of alphaO-conotoxin GeXIVA[1,2] and GeXIVA[1,4] in rats of neuropathic pain. Prog Neuropsychopharmacol Biol Psychiatry. 2016; 66:112–9. [PubMed: 26706456]

Table 1

Effect of α7 nAChR activation on cell types involved in pain and inflammation

Cell type	Agonist	Effects of α7 activation	Ref.
Rat DRG neurons	Nicotine	Neuroprotective effects from enhanced NO production	[76, 178]
Human monocytes	Nicotine	Reduced NF-κB, MIP-1α, PGE_2 production	[82]
	GTS-21	Reduced TNF-α production	[84]
	Nicotine	Reduced IL-18 and IL-12 production	[179]
	Nicotine	Reduced TNF-α, IL-1β, and IL-12 production; reduced proliferation of pro-inflammatory monocytes; increased secretion of IL-10	[133]
Human macrophages	Nicotine	Induction of IRAK-M expression	[180]
Mouse macrophages	A-833834	Reduced TNF-α production	[181]
Rodent microglia	Nicotine	Reduced TNF-α production	[85, 86]
Human T lymphocytes	Nicotine	Modulation of T cell activation state; reduced Th17 response	[182]
	GTS-21	Reduced Th1 cell differentiation; reduced production of IFN-γ	[183]
Mouse T lymphocytes	Nicotine	Reduced TNF-α, IFN-γ, NF-κB, and IL-17 production; reduced clinical symptoms of EAE	[184]
Human NK cells	PNU282987	Reduced NF-κB and IFN-γ production	[79]

Table 2

Compounds that target α7 nAChRs, mechanism of action, and therapeutic effects in models of pain and inflammation

Compound	Mechanism of Action	Pain/inflammation model	Therapeutic effects	Ref.
Choline	Agonist	Post-operative incisional pain	Reduced mechanical allodynia	[104]
		Formalin test	Reduced nociceptive behaviors Reduced	[118]
CDP-Choline	Agonist	CCI	mechanical hyperalgesia	[102]
		Carrageenan-induced inflammatory pain	Reduced mechanical hyperalgesia and edema	[103]
PHA-54361	Agonist	Formalin test	Reduced nociceptive behaviors	[118]
GTS-21	Partial agonist	Post-operative incisional pain	Reduced sensitivity to heat stimulus	[108]
		CIA	Reduced clinical symptoms of arthritis	[185]
PNU-120596	Type II PAM	Formalin test	Reduced nociceptive behaviors	[115, 118]
		CCI	Reduced mechanical allodynia and thermal hyperalgesia	[116]
		Carrageenan-induced inflammatory pain	Reduced thermal hyperalgesia and edema	[116]
GAT-107	ago-PAM	Formalin test	Reduced nociceptive behaviors	[114]
		CCI	Reduced mechanical allodynia	[114]
		LPS	Reduced mechanical allodynia	[114]
		CFA	Reduced mechanical allodynia and thermal hyperalgesia	[114]
NS6740	Silent agonist	Formalin test	Reduced nociceptive behaviors and edema	[120]
		CCI	Reduced mechanical allodynia	[120]
PMP-072	Silent agonist	CIA	Reduced clinical symptoms of arthritis	[121]

Table 3

Distribution of α9-containg nAChRs in cell types involved in pain and inflammation

Cell type	Ligand	Effects of α9-targeting ligands	Ref.
Human monocytes (U937 cell line)	Choline, nicotine	Reduced IL-1β release	[135, 136]
Mouse monocytes	Nicotine	Inhibition of monocyte infiltration into the CNS	[139]
Mouse bone marrow-derived cells	Nicotine	Reduced production of IFN-γ and proliferation of pro-inflammatory monocytes	[133]

Table 4

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

Compound	Mechanism of Action	Pain or inflammation model	Therapeutic effects	Ref.
RgIA	antagonist	CCI	Reduced mechanical allodynia and mechanical hyperalgesia, infiltration of immune cells	[145]
		Oxaliplatin-induced neuropathy	Reduced mechanical hyperalgesia, cold allodynia; disease modifying effects	[141]
RgIA4	antagonist	Oxaliplatin-induced neuropathy	Reduced mechanical hyperalgesia and cold allodynia; disease modifying effects	[143]
Vc1.1	antagonist	CCI	Reduced mechanical allodynia, mechanical hyperalgesia, infiltration of immune cells; disease modifying effects	[145, 147]
GeXIVA	antagonist	CCI	Reduced mechanical hyperalgesia	[161, 186]
ZZ-204G	antagonist	CCI	Reduced mechanical hyperalgesia	[167]
		Formalin test	Reduced inflammatory pain	[167]
ZZ1-61c	antagonist	Vincristine-induced neuropathy	Reduced mechanical allodynia and mechanical hyperalgesia	[168]