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REVIEW

The nicotinic modulation of pain

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Abstract: Pain is a physiological unpleasant sensation that associated with actual or potential tissue damage and affects the major part of human population. Numerous modulatory system control pain through a complex process. The drugs that regulate the modulators involving in this process are currently available; however, the studies to understand the process and develop new agents are still going on. In this review, it is aimed to relay information about how nicotinic receptors contribute the pain modulation. It is obvious that a wide variety of nicotinic receptors is located in both peripheral and central areas. Among these receptors α7, α4β2 and α9α10 receptor subtypes draw attention in terms of pain modulation. The fact that different receptor subtypes involve in different processes of different pain conditions leads to provide beneficial results from the agonism of α7, α4β2 and antagonism of α9α10. The major restraint of the usage of nAChR agonists is their adverse effects. However, nowadays, the side effects are reduced by the clinical developments. Additionally, positive allosteric modulators that amplify the effectiveness of nAChR ligands are in demand.

Keywords: Analgesia; nicotinic receptors; positive allosteric modulators.

INTRODUCTION

International Association for the Study of Pain (IASP) describes pain as an unpleasant sensory and emotional experience arising from any part of the body and associated with actual or potential tissue damage, or described in terms of such damage. The pain is an ‘experience" and in this respect it differs from ‘nociception'. Nociception is called a neural process provides transduction and transmission of a noxious stimulus to the brain by using pain pathways (Merskey and Bogduk, 1994; Steeds, 2009). The pain may demonstrate acute or chronic characteristic depend on the underlying pathogenesis. Acute pain begins suddenly and probably has limited duration (Macintyre *et al*., 2010) while the chronic or persistent pain can be described as continuous or repeating pain, lasting the outside limits of the typical progress of acute disease, more than 3 to 6 months. Chronic pain pathophysiology comprises two categories as nociceptive or neuropathic (Feinberg *et al*., 2013). Since different mechanisms role in the pathophysiology of acute and chronic pain and even nociceptive and neuropathic pain, it is possible that all the drug classes may not relieve all pain types. Modulation of pain is a highly complex process involving various mediators and receptors over an extensive network from the periphery to the central nervous system (CNS). The rate of participation of the chemicals and receptor types in the modulation depend on the pain types and noxious stimulus. It is clear that the nicotinic receptors (nAChR) which discussed in this review, participate in many of these pain modulation pathways, hence it may explain why nicotinic receptor ligands acting as analgesic are effective in both acute and chronic pain conditions. nAChRs are transmembrane channels with numerous subtypes (Molas and Dierssen, 2014). Because different nAChR subtypes assume different roles in pain modulation, sometimes agonism is beneficial while sometimes antagonism is advantageous. More recently allosteric modulators other than orthosteric ligands are more in demand. Allosteric modulators bind to the receptor at a site other than the orthosteric ligands bind and lead a conformational change (Neubig *et al*., 2003). Positive allosteric modulators of nAChRs contribute the analgesia that induced by nAChR activation via maintaining the cholinergic tone (Uteshev, 2014).

In this review, the nicotinic side of the pain modulation is discussed. The experimental studies are examined to answer how nAChRs involve in this modulation.

Nicotinic receptors in pain modulation

Cholinergic receptors that are located in the autonomic ganglion and striated muscle cells are called as nAChR. nAChRs are divided into two groups as ‘neuron type’ and ‘muscle type’ because they are blocked by different antagonists. Neuronal nAChRs are blocked by hexamethonium and its analogs, muscle type nAChRs are blocked by decamethonium and skeletal muscle relaxants like it (Taylor, 1996). nAChRs are transmembrane channels that belong to the "Cys-loop” superfamily (Molas and Dierssen, 2014). The nAChRs involving in pain modulation are neuronal type nAChRs, and all the

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mentioned nAChRs in this review are neuronal type nAChRs. Presently, it is known that nine α (α2- α10) and three β (β2–β4) subunits exist (Kalamida *et al*., 2007). The α5 and β3 subunits are considered to be 'modulatory' subunits because they do not form functional receptors when expressed alone or when expressed as the sole α or β subunit in heteromeric channels (Vincler, 2005). Additionally, nAChRs could be examined under two classes: (a) the high-affinity agonist binding class, which do not bind α- bungarotoxin (α-bgtx), later investigated to be the heteromeric nAChRs formed by α2-α6 and β2-β4 subunits; and (b) a second class that binds agonists with μm affinities and binds α-bgtx with nm affinities, later shown to be generally homomeric molecules formed by α7–α9 subunits (Kalamida *et al*., 2007).

The presence of nAChRs seems to be nearly omnipresent in pain pathways. They are found in primary afferents, spinal cord excitatory and inhibitory interneurons and projecting neurons, the medial habenula and in brain nuclei with descending spinal projections. However, their expression is not homogeneous between these populations of neurons, nor is the expression uniform within each of the aforementioned groups. nAChRs, whose roles in nicotine addiction and autonomic physiology are well known, also have significant analgesic effects on animal models and humans (Vincler, 2005). The nicotine that stimulates neurons on the descending inhibitory pain pathways by activating the neurons in the subcortical area of the brain and revealing ACh secretion possesses weak antinociceptive character (Arneric, 2000). The effect of nicotine is completely antagonized by injection of antagonists' combinations, not by a single antagonist. Thereby, it is thought that multiple descending inhibitory pathways are involved in antinociception induced by nicotine (Decker and Meyer, 1999). According to another view, the stimulation of spinal cord by activation of nAChRs may also lead antinociception via increasing intracellular Ca^{++} (Arneric, 2000).

nAChRs are found in the periphery including macrophages, immune cells and in the CNS including many areas contributing to pain such as ascending and descending nociceptive pathways, spinal cord dorsal horn (DH) and in sensory nerve afferents (AlSharari *et al*. 2013; Umana *et al*. 2013; Tracey, 2002). While there is a great deal of studies about the roles of nAChRs in these areas, it is continued to elucidate the contribution of them in different areas and pain pathologies. For instance, there is evidence that ascending nociceptive control, a new spinostriatal pain modulation pathway, is mediated by a nicotinic mechanism in the rostral ventral medulla (Gear and Levine, 2009). More recently, it has also been shown that ascending nociceptive control mediates the analgesic effect of acupuncture and acupuncture-induce analgesia depends on nAChR mechanisms on rostral ventral medulla, like ascending nociceptive control-induced analgesia (Tobaldini, 2014). In Ghasemzadeh and Rezayof (2015) study, the role of ventral hippocampal nAChRs in stress-induced analgesia in mice were assessed by intra- ventral hippocampal microinjection of nicotine (enhancement of stress-induced analgesia) and mecamylamine (prevention of stress-induced analgesia) and. When the results were evaluated in the tail flick test, it is deduced that exposure to acute stress induces stress-induced analgesia time-dependently and the ventral hippocampal cholinergic system may be involved in stress-induced analgesia via nAChRs.

The investigations direct the α7 and α4β2 as nAChRs subtypes because of their distribution in neuronal pathways and their involvement in pain modulation. These subtypes are primarily present in CNS pain pathways as well as the periphery. There is evidence for nAChR expression on both DRG and trigeminal ganglion neurons. DRG neurons predominantly express α7 nAChRs (Genzen, *et al*., 2001). α7 nAChRs are also expressed on macrophages and immune cells and contribute to peripheral and central antinociception (De Jonge and Ulloa, 2007). α4β2 subunits are located extensively in the central and peripheral nervous system. The anatomical distribution of α4β2 nAChRs in the brain and the spinal cord strengthen the role of this receptor in nociceptive transmission (Nirogi *et al*., 2013). There are also α3- and β4-containing subtypes that are principally highly expressed in the autonomic ganglia, and are also expressed in subsets of neurons in the brain (Picciotto *et al*., 2001).

It has been observed that the residual nicotine-induced analgesia remained in α4-subunit knockout (KO) mice. This data reveals a role for non-α4-containing receptors such as α3- and β4-subunits (Marubio *et al*., 1999). However, a recent study demonstrated that α3β4 nAChRs do not significantly take part in mediating antinociception after acute nicotine and AuIB, a selective α3β4 antagonist, administration (Jackson *et al*., 2013). The α5 subunits that are incorporated with α4β2, α3β4, or α3β2 nAChR subtypes, do not shape functional receptors alone, they could only significantly alter the properties of the channel (Umana *et al*., 2013). It was reported that α5 nAChRs are involved in nicotine-mediated antinociception because α5 KO mice remain sensitive, even a little, to nicotine induce antinociception (Jackson *et al*., 2010).

α7 subtype

The proofs about α7 nAChR subtype that contributes to the chronic inflammatory and neuropathic pain are increasing day by day. The homomeric α7 is a subtype that is expressed abundantly in the CNS and the periphery (Girod *et al*., 1999). It is known that the acute antinociceptive effects of the nicotine and nAChR agonists are thought to occur via the activation of nAChRs localized on neurons, chronic administration of nicotine reduces immune and inflammatory responses

(Kalra *et al*., 2004). nAChRs are at the periphery at sites of injury where inflammation is initiated (Genzen *et al*., 2001). Preclinical investigations have indicated the therapeutic potential of targeting α7 nAChRs-mediated anti-inflammation via modulating of pro-inflammatory cytokines. This "cholinergic anti-inflammatory pathway" harmonizes the immune system through convergent events of ACh and α7 nAChRs, expressed on macrophages and immune cells (Tracey, 2002; De Jonge and Ulloa, 2007). Recent studies have identified the role of pro-inflammatory cytokines in the development and maintenance of chronic pain conditions (Watkins *et al*., 2001). In the periphery, the pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α (TNF-α), which are released by macrophages, promote to behavioral hypersensitivity following peripheral nerve injury. The α7 nAChR on macrophages is an essential mediator of inflammation. Stimulation of the α7 nAChR on macrophages inhibits the release of TNF-α (Vincler, 2005).

The contribution of α7 nAChRs in nicotinic analgesia has been determined following choline administration both spinally (i.t.) and supraspinally (i.c.v.) to mice. The antinociception induced by the α7 agonist choline was observed in a dose-dependent manner in the tail-flick test as an acute pain model. Moreover, methyllycaconitine and α-bgtx, the α7 antagonists, significantly antagonized the choline-induced antinociception, not dihydro-β-erythroidine and mecamylamine, the nicotinic antagonists that are non-significant affinity for α7 receptor subtypes. DMXB (3-(2, 4)-dimethobenzylidine anabaseine) and 4-OH-DMXB, α7 partial agonists, did not produce any significant antinociceptive effect although they dose-dependently antagonized the choline-induced antinociception following spinal injection. This antagonism has been thought that it is associated with their partial agonistic properties of the α7 receptors (Damaj *et al*., 2000). To determine the effects CDP-choline (cytidine-5'-diphosphate-choline; citicoline) and its metabolites when administered i.c.v. in rat models of inflammatory and neuropathic pain and to explain mechanism of CDP-choline, Hemicholinium-3 (HC-3), neuronal high-affinity choline uptake inhibitor; mecamylamine, the α7-selective nAChR antagonist α-bgtx and the $GABA_B$ receptor antagonist CGP-35348 (3-aminopropyl-diethoxy-methyl-phosphinic acid), atropine, and non-selective opioid receptor antagonist naloxone were used by Bagdas *et al*. (Bagdas *et al*., 2011). CDP-choline reversed the mechanical hyperalgesia dose and time-dependently as choline, in both carrageenan-evoked inflammatory and chronic constriction injury (CCI)-induced neuropathic pain models. This antihyperalgesic effect was blocked by central administration of HC-3, mecamylamine, α-bgtx and CGP-35348. In contrast, i.c.v. pretreatment with naloxone only prevented the CDP-choline-induced antihyperalgesic effect in the neuropathic pain model while atropine did not change the antihyperalgesic effect in these models. Recently, the contribution of the endogenous α7 nAChRs in pain and inflammation has been investigated in the α7 KO mice and its complementary α7 hypersensitive mice knock-in (KI) expressing the L250T. α7 nAChRs and their respective wild-type (WT) mice α7 KO and KI mice demonstrated no remarkable alterations in pain thresholds assessed by acute noxious thermal and mechanical stimulus when compared to WT mice. Whereas α7 KO mice demonstrated no changes in thermal and mechanical allodynia compared to WT mice in neuropathic pain model; α7 KI mice display a significant attenuation in the pain responses. However, the apparent enhancement in edema, hyperalgesia, and allodynia induced by intraplantar Complete Freund's adjuvant (CFA) injection, inflammation model, was seen in the α7 KO mice compared to WT mice. On the contrary, α7 KI mice demonstrated a lower degree of hyperalgesia and allodynia in inflammation model. The reversing effect of systemic nicotine on mechanical allodynia that was induced following intraplantar CFA injection observed in WT mice was disappeared in the α7 KO animals (AlSharari *et al*., 2013). These studies obviously prove that α7 nAChRs activation in the CNS has an important function in antinociception in neuropathic pain, but mostly in chronic inflammatory pain associated with nerve injury via reducing inflammation. In another study that is based on the role of systemic delivery of a α7 nAChR agonist in attenuation of neuropathic pain and relation to immune-mediated pro-inflammation, osmotic mini-pumps containing TC-7020 (5-methyl-N-[2-(pyridin-3-ylmethyl)-1-azabicyclo[222]oct-3-yl]thiophene-2-carboxamide), α7 nAChR selective agonist, were implanted 10 to 14 days after sciatic CCI or sham surgery. The hind paw withdrawing thresholds after the application of mechanical stimuli in rats were evaluated TC-7020 (s.c.) significantly decreased CCI-induced allodynia. Spinal cords were gathered two weeks later and processed for microglial and astrocyte activation markers within the ipsilateral L4-L6 DH. Additionally, ipsilateral L4-5 DRG were processed for neuronal injury and satellite cell activation markers. According to the results of obtained from in vivo activity tests and the other analyses apart from in vivo activity tests, it has been indicated that systemic α7 nAChR agonist might be effective in the management of neuropathic pain by abolishing neuronal injury and immune cells activation observed in the periphery (Loram *et al*., 2012). Eventually, α7 nAChR agonists such as AR-R 17779 ((3S)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidine]-2'-one hydrochloride), GTS21 (3-[(2,4-dimethoxy)benzylidene]-anabaseine), 4OHGTS (3-(4-hydroxy,2-methoxybenzylidene) anabaseine), ARR17779 ((-)-spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one]), CAP55, Exo2 (exo-2-(2-pyridyl)-7-azabicyclo[2.2.1] heptane) and PNU-282987 ([N-[(3R)-1-Azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide

hydrochloride]) are focused as possible agents for management of pain and inflammation in preclinical studies (Damaj *et al*., 2000; De Jonge and Ulloa, 2007). For instance, Di Cesare Mannelli *et al*. (2014a), have been reported that α7 nAChR is involved in oxaliplatin-dependent neuropathology, as neuropathic pain model, and the agonists (R)-ICH3 ((R)-(−)-3-methoxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene sesquifumarate), the most active enantiomer of the novel and selective α7 nAChR agonist, and PNU-282987 prevented the alterations of the pain threshold evoked by mechanical and thermal stimuli in paw-pressure, Von Frey and cold-plate tests and protected nervous tissue with concomitant glial activation. Since glial cells play a role both in pain and in neuroprotection, a α7 AChR-dependent modulation of glial functions is suggested to distinguish rescue signals from the pathological pain-mediating pathway.

α4β2 subtypes

The α4 and β2 subunits have been described as two of the subunits that handle the effect of systemic nicotinic agonists primarily via the activation of descending noradrenergic and serotonergic systems. These subtypes are seemed to be more responsible for acute antinociception than other subtypes (Vincler, 2005). The α4β2 subtype is located extensively in the central and peripheral nervous system. The anatomical distribution of α4β2 nAChRs in the brain and the spinal cord strengthen the role of this receptor in nociceptive transmission (Nirogi *et al*., 2013). Pre-synaptic α4β2 nAChRs is crucial in the modulation of pain which is regulated by some neurotransmitters like ACh, dopamine, gamma-aminobutyric acid (GABA) and norepinephrine. Both spinal and supraspinal pathways contribute to the neuropathic and inflammatory pain mechanisms which are mediated by α4β2 nAChR ligands. Selective α4β2 nAChR ligands are being developed for neuropathic and inflammatory pain treatment because of their extensive efficacy (Tracey, 2002). In a study in which the mice lacking the α4β2 subunit of the nAChR were used, it has been shown that the mutant mice exhibit a decreased antinociception induced by nicotine in the hot-plate test and reduced sensitivity to nicotine in the tail-flick test. Additionally, patch-clamp recordings further reveal that raphe magnus and thalamic neurons no longer respond to nicotine (Marubio *et al*., 1999). Epibatidine, an alkaloid found in the skin of *Dendrobatidae* frogs, has structural similarities to nicotine (Fisher *et al*., 1994). Epibatidine is a α4β2 nAChR agonist and also interact with nAChR like α7 and α1β1δγ lesser (Sullivan *et al*., 1994). It is claimed that the analgesic effect of epibatidine is developed via α4β2 nAChR. The anti-nociceptive effect of epibatidine were tested, and epibatidine dose-dependently reversed inflammatory and neuropathic hyperalgesia in all tests (Kesingland *et al*., 2000). These results also showed that the critical role of α4β2 nAChR subunit for modulating antinociception. In another study performed with epibatidine, it was found minimum 100 times more potent when injected into the dorsal raphe nucleus, a site of action for epibatidine, compared to systemically administration. Moreover, administration of epibatidine only to dorsal raphe displays analgesic action, not into the PAG area, outside the dorsal raphe (Cucchiaro *et al*., 2005). Therefore, it can be thought that α4β2 nAChRs located in particularly dorsal raphe are more involved in nicotinic antinociception due to the analgesic effect of epibatidine is regionally selective. The cardiovascular system and CNS toxicity developed due to epibatidine's nonselective activity at peripheral neuromuscular and ganglionic nAChRs, it is unsuitable for further development (Hurst *et al*., 2013). Thus, different α4β2 nAChR ligands such as ABT-594 (5-((2*R*)-azetidinylmethoxy)-2-chloropyridine; Tebanicline), ABT-894 (3-(5,6-dichloro-pyridin-3-yl)-1(S),5(S)-3,6-diazabicyclo[320]heptane; Sofinicline), A-85380 (3-(2(S)-azetidinylmethoxy) pyridine), A-366833 (5-[(1R,5S)-3,6-diazabicyclo[320]heptan-6-yl]nicotinonitrile) and TC-6499 (is not disclosed) are being improved (Nirogi *et al*., 2013). Each study that is performed with these agents support the role of this receptor subtype in antinociception and provide new information about the roles of nAChRs in pain modulation. The nAChR agonist ABT-594, which has a similar high efficacy with epibatidine, also acts rather the α4β2 heteromeric than the α7 homomeric receptor subtype (Hurst *et al*., 2013), and provides broad-spectrum antinociceptive efficacy in acute and chronic pain models (Kesingland *et al*., 2000; Bannon *et al*., 1998). ABT-594 was basically demonstrated to provide a better side-effect profile when compared to the epibatidine, thanks to its low affinity for neuromuscular α_1-containing nAChR and reduced intrinsic activity at α_3-containing receptors (Munro *et al*., 2000). However, the proofs demonstrate that the acute safety profile of ABT-594 is not significantly improved over other nicotinic analgesics (Boyce *et al*., 2000; Rowbotham *et al*., 2009). ABT-594 has been gotten as far as Phase II trials in humans. However, further research in this area is ongoing. ABT-894 called drug is a α4β2 nAChR full agonist which is promising hope, safety and good tolerance (Hurst *et al*., 2013). Compatible with α4β2 nAChR selectivity, ABT-894 has shown improved pharmacological effects with fewer adverse effects rather than ABT-594 in several preclinical neuropathic pain models (Rowbotham *et al*., 2012). A-366833 and A-85380 are other ligands of nAChR and exhibits more selectivity to α4β2 nAChRs. When the analgesic effects of them were evaluated, they were found effective in too many pain models such as acute, persistent, inflammatory and neuropathic pain models (Curzon *et al*., 1998; Rueter *et al*., 2003; Rueter *et al*., 2006; Ji *et al*., 2007; Nirogi *et al*., 2011). Therefore, they exhibit a wide spectrum of analgesic efficiency without side effects. These favorable results support the importance of α4β2 nAChR in pain modulation and clinical improvement of new agents directed toward α4β2 nAChR agonists.

Despite the studies shows that these agonists show high efficacy in inflammatory animal models, there are also some other studies indicated that some agonists show insufficient efficacy. The series of nAChR agonists, non-selective nAChR agonists, and α4β2 or α7 selective agonists were evaluated in pain models induced by formalin and CFA. All of these agonists showed different effectiveness, some were positive, some others negative. Moreover, electrophysiological recordings obtained from spinal cord slice revealed a potent nicotine-induced enhancement in inhibitory synaptic transmission that was regulated partially by α4β2 and only minimally by α7 subtypes. It has been concluded after the evaluating the results obtained from this study and previous studies, that α4β2 agonism is required but not adequate to induce analgesia, and that the spinal cord has a main role since spinal cord is the site of the molecular action of nAChRs to produce analgesia (Gao *et al*., 2010). Recently, analgesic efficacy and safety of ABT-894 has been assessed in two separate randomized, double-blind, multicenter, placebo-controlled clinical trials in patients suffered from pain induced by diabetic peripheral neuropathy. In contrast to previous studies, although ABT-894 were well tolerated in all doses, and showed well safety profile, none of the ABT-894 dose groups showed efficacy compared to placebo in both trials (Rowbotham *et al*., 2012). Even though these results indicate that nAChRs may not be a feasible idea for the neuropathic pain therapy, α4β2 receptors-mediated analgesia is still promising in pain management with other involving mechanisms as well as however it is not satisfactory to focus on just α4β2 receptors.

Allosteric modulators for α7 and α4β2 subtypes

Allosteric modulation is defined as; proteins could exist in multiple conformational states and binding of allosteric ligands to proteins changes the energy barriers or "isomerization coefficients" between various states (Arias, 2010). Allosteric modulators bind to the receptor at a site other than the orthosteric ligands bind and the conformational change occurs by this binding process (imidazoline). Allosteric modulation is obtained by negative and positive allosteric modulators (PAMs) (Arias, 2010). Positive allosteric modulation (also known as allosteric activation) occurs when the binding of one ligand enhances the attraction between substrate molecules and other binding sites. Negative allosteric modulation (also known as allosteric inhibition) occurs when the binding of one ligand decreases the affinity for substrate at other active sites (Neubig *et al*., 2003). PAMs for nAChRs are responsible for maintaining the cholinergic tone. It is known that age, disease and trauma-related neuropathology do not make functional nicotinic receptors disappeared but decrease their expression and/or activation levels in a subunit- and brain region-specific manner. Therefore, nicotinic-PAM-based treatments are expected to augment the endogenous cholinergic tone in a spatially and temporally restricted manner creating the potential for differential efficacy and improved safety as compared to exogenous orthosteric nicotinic agonists that activate nicotinic receptors indiscriminately (Uteshev, 2014). Since α7 and α4β2 nAChRs subtypes play roles in the modulation of pain, the studies on PAMs of these receptors' subtypes are accelerated. Ivermectin, 5-hydroxyindole, genistein, NS-1738 (N-[5-chloro-2-hydroxyphenyl]-N9-(2-chloro-5-[trifluoromethyl] phenyl)), compound 6, galantamine, LY-2087101 ([2-(4-fluoro-phenylamino)-4-methyl-thiazol-5-yl]-thio-phen-3-yl-methanone), PNU-120596 (1-[5-chloro-2,4-dimethoxyphenyl]-3-[5-methylisoxazol-3-yl]-urea) and TQS (3a,4,5,9b-Tetrahydro-4-(1-naphthalenyl)-3*H*-cyclopentan[*c*]quinoline-8-sulfonamide) are PAMs effective at the α7 nAChRs and 17 β-estradiol, Zinc, NS9283 (3-[3-(pyridine-3-yl)-1,2,4-oxadiazol-5-yl]benzonitrile), LY-2087101 ([2-[(4-Fluorophenyl)amino]-4-methyl-5-thiazolyl]-3-thienyl-methanone) are at α4β2 nAChR (Lee *et al*., 2011; Freitas *et al*., 2013a). It may be identified that there are two types of modulators as Type I and II. Type I predominately affects the apparent peak current, and Type II enhances both the apparent peak current and alters the desensitization profile of the agonist response. Some studies showed that type II PAMs are more effective than Type I PAMs (Bertrand and Gopalakrishnan, 2007; Lee *et al*., 2011). Although, some studies indicated that both alone usage of PAMs, such as α7 PAM PNU-120596, and combined usage with nAChR agonist could be an effective treatment for inflammatory and neuropathic pain (Freitas *et al*., 2013b), some studies investigated that the usage of PAMs alone, such as α4β2 NS-9283, is not effective (Lee *et al*., 2011). Because PAMs are valuable for pain relieving, discovering of new agents is in demand. Most recently, two novel α7 PAMs, PMP-311 ((S)-2-(2-((Pyridin-3-yloxy) methyl) piperazin-1-yl)oxazolo[4,5- b]pyridine) and PMP-072 ((R)-N-(4-methoxyphenyl)-2-((pyridin-3-yloxy) methyl) piperazine-1-carboxamide), have been investigated in modulating collagen-induced arthritis in mice and found effective (van Maanen, 2015). All the data considered together, it is possible to say that a PAM-plus agonist approach could be therapeutically effective for multiple nAChR subtypes, including in α7 and α4β2 nAChRs.

α9α10 subtypes

The activation of nAChRs is intensely involved in pain modulation while new findings point the blockage of various nAChR. α-conotoxins are small peptides produced by carnivorous marine snails known as *Conus.* RgIA and Vc1.1, which are members of a subclass of these peptides provide acute and long-lasting analgesia. Pharmacological studies suggest that RgIA and Vc1.1 molecules are selective antagonists of α9α10 nAChRs (McIntosh *et al*., 2009). Expression of α9α10 subtypes have been shown in DRG neurons and a range of immune

cells (Holtman *et al*., 2011). Peripherally administered α9α10 selective α-conotoxin antagonists have provided antinociception in acute, neuropathic and tonic inflammatory pain models without motor toxicity (Vincler and McIntosh, 2007). In the study performed by Vincler *et al*. (Vincler *et al*., 2006) the involvement of α9α10 nAChRs in the pathophysiology of peripheral nerve injury was evaluated by using nerve injury pain model. Consistent with previous studies on this topic (Hone *et al*., 2009), they have been demonstrated that the antagonism of this nAChR subtype decreases the number of choline acetyltransferase-positive cells, macrophages, and lymphocytes at the site of injury. More recently, it has been claimed that α9α10 nAChRs antagonists may be served as a new class of curative for neuropathic pain that protects peripheral nervous tissues as well as prevents central maladaptive plasticity by inhibiting the activation of glial cell (Di Cesare Mannelli *et al*., 2014b). Moreover, the nAChR blockage may relieve pain related to small-fiber neuropathies via antagonizing the nicotine-evoked expansion in the axonal excitability of unmyelinated C-fiber axons in isolated segments of peripheral human nerves (Lang *et al*., 2005). After the reveal that the antinociception of RgIA and Vc1.1 is related to α9α10 nAChR antagonism, a few new compound act on these receptors has been recently synthesized. nAChR antagonist ZZ-204G ([5,5′,5″,5‴-(1,2,4,5-benzenetetrail) tetrakis-[1-(3-fenylpyridinium)-4-penten] tetrabromit), has been studied in order to characterize a new molecule structure and it was observed that ZZ-204G inhibited α9α10 nAChRs. Its in-vivo efficacy was tried on rats with formalin test, neuropathic pain (chronic pinched nerve injury) model and thermal nociception (tail-flick) model. The results of this study have been indicated that ZZ-204G is analgesic with low doses but it doesn't change motor function (Holtman *et al*., 2011). Similarly, the effect of a novel nAChR antagonist, bis-analog ZZ1-61c was evaluated on chemotherapy-induced pain and this compound showed both preventive and restorative effects via antagonistic action at the α9α10 nAChR as mentioned for ZZ-204G, without motor dysfunction or muscular weakness (Wala *et al*., 2012). Shortly the blockage of α9α10 nAChRs plays an effective role in nicotinic pain modulation, and the antagonists can be used as new analgesic agents for pain treatment.

Nicotinic pain modulation via non-cholinergic systems

nAChRs are broadly found in postsynaptic neurons and also on presynaptic terminals in the nervous system. nAChRs regulate neurotransmitter release as some other receptor types do, such as mAChRs. The releasing of neurotransmitter is crucial in pain nicotinic pain modulatory system which in turn might modulate the release of several neurotransmitters in the brain, including GABA, glutamate as well as endogenous opioid peptides (Hamurtekin *et al*., 2007). GABA is the main inhibitory neurotransmitter in the CNS. The interaction between the cholinergic system and GABAergic transmission in CNS has been studied for a long time. Cholinergic and GABAergic neurons may be located in identical regions in the CNS (Hamurtekin *et al*., 2007). GABA neurons and receptors, distributed in supraspinal sites, organize the perception and response to painful impetus and in the spinal cord this neurotransmitter system regulates sensory information proceeding (Enna and McCarson, 2006). Different nAChRs located near transmitter release sites on GABAergic nerve terminals are responsible for modulating GABA release (De Filippi *et al*., 2005). The rat hippocampal synaptosomes have both α7 and α4β2 nAChR subtypes, which could regulate GABA release (Zappettini *et al*., 2011).

The different nAChR subtypes also modulate the release of glutamate (De Filippi *et al*., 2005). As mentioned before, different nAChRs mediate both nociception and antinociception. It has been shown that pronociceptive effects of nAChR agonists are reliant on glutamate release and stimulation of spinal N-methyl-D-aspartate (NMDA) receptors, ionotropic glutamate (iGlu) receptor channels (Young *et al*., 2008). Glutamate is a major excitatory neurotransmitter in the spinal cord. It is crucial in the handling of sensual information in the spinal cord DH and is known to provide improved excitability of DH neurons in chronic pain conditions (Chen and Pan, 2003). The central sensitization is also crucial in the creation of hypersensitivity and causes hyperalgesia and allodynia. One of the mechanisms implicated in central sensitization is the stimulation of the NMDA receptors, by glutamate (Cury *et al*., 2011). The antagonism of the pronociceptive α9α10 nAChRs may provide analgesia via decreasing the release of glutamate. On the other hand, Young *et al*. (Young *et al*., 2008) study shows inhibitory cholinergic tonus that is induced by peripheral injury at spinal α3β2 nAChRs without receptor loss may lead to mechanical hypersensitivity after spinal nerve ligation. It is also revealed that glutamate releasing from primary afferent C-fibers may be indirectly inhibited by α3β2 nAChRs in the lower lumbar rat spinal cord DH, and responsiveness to painful mechanical stimuli is diminished. Additionally, it has been reported by Stella and Piomelli (2001) that the anandamide formation is modulated by cholinergic contribution. This inhibitory endocannabinoid is produced by brain neurons and provides analgesia by activating cannabinoid receptors. The formation of anadamide is mediated by coactivation of α7 nAChRs and NMDA receptor (Stella and Piomelli, 2001).

In terms of nAChRs mediated neurotransmitter release it is possible to say that nicotine acting on presynaptic nAChRs is able to increase cytosolic Ca^{++} concentration and affect certain presynaptic neuronal actions in the nerve endings such as the release of neurotransmitter, both directly by permeation via α7 nAChR channel and indirectly by operating voltage-gated calcium channels

(VGCC) triggered by the depolarization evoked by Na^+ flow predominantly via non-α7 receptor subtypes (Marchi and Grilli, 2010). N-type VGCC transduces electrical action into different cellular activities, modulate Ca^{++} homeostasis and play an important role in transforming pain signals. They are mainly expressed in nerve terminals, where they monitor the release of neurotransmitter. α-conotoxins Vc1.1 and RgIA, very potent antagonists of α9 subunit-containing nAChRs, also robustly block N-type VGCC flow in α9 nAChR KO mice and the sensory DRG neurons of rodents (Callaghan, 2008-2010). However, Vc1.1 and RgIA do not interact directly with N-type VGCCs (Ca_v22), but via a novel mechanism mediated by G protein-coupled $GABA_B$ receptor (Adams and Berecki, 2013). Aforementioned nAChRs enhance GABA release at different synapses. Increased GABA-induced by nAChRs may also enhance the inhibition of N-type VGCC currents via stimulating $GABA_B$ receptors, the metabotropic receptor of GABA.

Lastly, it is possible to discuss cholinergic-opioidergic interaction on pain. The antinociceptive effects of nicotine and alternative nAChRs agonists, such as epibatidines, α4β2 nAChR agonist, derivatives are mediated by the stimulation of endogenous opioid peptide (EOP) systems. EOP system includes enkephalin, endorphin, and dynorphin, all of them play an important role in analgesia by acting on μ-, δ- and κ- opioid receptors. The reports indicate that nicotine-evoked antinociception was prevented by naloxone. Thereby, enkephalin-mediated μ-opioid receptor signaling is crucial for nicotine-evoked antinociception since nicotine activated opioidergic neuron in spinal cord induces the releasing of EOPs by way of the stimulation of spinal nAChRs and generates an antinociceptive action (Kiguchi *et al*., 2008). Consistent with this results, CDP-choline, α_7 nAChRs agonist, i.c.v. induced antinociception also reversed by opioid receptor antagonist naloxone (Hamurtekin *et al*., 2007).

Summary

It is obvious that the cholinergic system is involved in pain process mediating by neuronal nAChRs as discussed in this review. Electrophysiological and neurochemical studies indicate that these receptors are located in both peripheral and central areas and play a role in a variety of acute or chronic pain conditions depend on the receptor subtypes and located area. In the periphery, among the nAChRs, α7 subtypes seems to handle nicotinic antinociception. Neuronal and non-neuronal ACh released from peripheral sources such as sensory neurons or different cell types of the skin such as keratinocytes and fibroblasts, respectively, following cutaneous injury can activate sensory afferents through nAChRs as well as mAChRs (Bernardini *et al*., 2001). nAChRs are at sites of injury where inflammation is initiated. Inflammation is a complex process in which immune cells such as macrophages and cytokines such as TNF-α involve. The stimulation of the α7 nAChR on macrophages inhibits the release of TNF-α, both a pro-inflammatory and a pain mediator (Vincler, 2005; Freitas *et al*., 2013a). This "cholinergic anti-inflammatory pathway" harmonizes the immune system through convergent events of ACh and α7 nAChRs. Since their peripheral effects associated with this harmonization, α7 nAChR agonists may be a potential option to ameliorate pain-associated behavioral hypersensitivity, the pro-inflammatory cytokines-mediated process following peripheral nerve injury in chronic pain conditions.

Yet another branch of nicotinic antinociception is central pain modulation. The studies show clearly the role of α7, α4β2 and α9α10 receptor subtypes. The α4 and β2 subunits, most highly expressed nAChRs in the CNS have been identified as two of the subunits that primarily activate descending noradrenergic and serotonergic systems for this analgesic effect (Vincler, 2005). In acute antinociception, α4β2 subtypes seem to be more responsible for nicotinic antinociception compared to other subtypes. Although the acute antinociceptive effects of the nAChR agonists are thought to occur via the activation of nAChRs localized on neurons, chronic administration of nicotine reduces immune and inflammatory responses (Kalra *et al*., 2004). In this point, a7 subtypes that also involve in peripheral antinociception, become a part of the activity in nAChR-mediated central antinociception. Although most of the studies have focused on the cholinergic anti-inflammatory reflex mediated by the vagus nerve regulating peripheral macrophage functions, centrally mediated cholinergic components are the matter of regulating the activation of microglia. (Vincler, 2005). Similar to periphery, also in the CNS, microglia, the resident macrophages, release pro-inflammatory cytokines such as TNF-α that contribute to behavioral hypersensitivity following sciatic nerve injury and spinal nerve transection (Stuesse *et al*., 2000; Raghavendra *et al*., 2003). Another substantial advancement in the pathophysiology of neuropathic pain is the confidence of nerve injury-induced behavioral hypersensitivity on central neural immune interactions as well as peripheral interactions. Finally, selective α7 nAChR agonists may be helpful in reducing inflammation and, consequently, the chronic inflammatory pain that is associated with nerve injury since recent studies have identified the role of pro-inflammatory cytokines in the development and maintenance of chronic pain conditions as neuropathic pain.

Another relatively recent approach is applying the nicotinic-PAM-based treatments. They are expected to augment the endogenous cholinergic tone in a spatially and temporally restrained approach generating the potential for differential efficacy and enhanced safety as compared to exogenous orthosteric nAChR agonists that stimulate nAChR indiscriminately (Uteshev, 2014). Since

α7 and α4β2 nAChRs subtypes rather play roles in the modulation of pain, the studies on PAMs of these receptors' subtypes are accelerated. More exciting is that the combination of agonists with selective PAMs will provide an efficacious strategy for targeting specific receptor populations and optimizing their physiological impact. Because nAChRs agonists are hopeful in the management of neuropathic and inflammatory pain, the using nAChR-PAMs, especially as an adjuvant, is, thereby, rational. Another nicotinic antinociception mechanism that is drawn attention and is thought to be very effective in the treatment of chronic pain types such as neuropathic pain is antagonism of a9a10. Selective antagonists of α9α10 nAChR such as RgIA and Vc1.1 produce acute analgesia as well as long standing analgesia. These peptides speed the healing of nerve function after injury, likely through immune/ inflammatory-mediated mechanisms (Del Bufalo *et al*., 2014). Involvement of α9α10 nAChRs in the pathophysiology of peripheral nerve injury as mentioned for α7 by decrease the number of macrophages and T cells near the nerve injury site has also been pointed out in the text (Vincler *et al*., 2006). Therefore, effective analgesic treatment is thought to be provided by these agents and the antagonists which act on this subtype are currently in clinical development for neuropathic pain treatment.

CONCLUSION

Neuronal nAChR subtypes have been showed to modulate the acute and chronic pain by contributing to peripheral and central antinociception. In this regard, nAChR mediated agents are promising. The agonists and some antagonists, depend on receptor subtypes, are being developed and the favorable results are being obtained from the studies. Moreover, to amplify the effectiveness of nAChR ligands, the PAMs are developed. The main restraint of the clinical usage of nAChR agonists is their adverse effects common to broad cholinergic stimulation such as serious convulsions. Thus, it is so important to identify the best subtype to reduce or remove the cholinergic side effects. Nowadays, these drug's adverse effects are reduced thanks to the clinical developments. Although there are lots of unanswered questions, there are still valid reasons to discover new analgesics effective on nAChR because nicotinic ligands seem to be efficient against numerous stimulus approaches and possess a wide efficacy against a series of clinically important pain conditions.

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