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Review Article

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PHARMACOLOGICAL COMPONENTS ASSOCIATED WITH PAIN

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ABSTRACT

Pain is an unpleasant experience comes along with any kind of damage and effects daily routine negatively. Although there are various drugs, many of them could not completely succeed in relieving pain due to pain modulation is a complex process involving numerous mediators and receptors. Therefore, it is a rational approach to identifying the components involved in this complex process and develop new agents act on these components. In this respect, the involvement of muscarinic receptors in pain modulation has drawn attention in recent years. The aim of the review is to exhibit the involvement of the muscarinic receptor subtypes that contribute to pain modulation. The search strategy was performed with MeSH terms and free text words, using the bibliographic databases Science Direct and PubMed. The articles have been collected from the experimental animal studies. It is obvious that muscarinic receptors that are located in both peripheral and central areas are extensively involved in the pain process, besides the regional effectiveness of these receptors and their subtypes may vary. Since the muscarinic receptors are various and involve in many physiologic processes, the possibility of adverse effects is a problem in their clinical use. Thus, determining the receptor specificity is an important issue to understand what types of muscarinic receptors involve in pain modulation and to develop new drugs. The agonists of muscarinic receptors are promising for relieving pain although there are lots of unanswered questions.

INTRODUCTION

Pain is experienced by all the people, and various pharmacological groups are used to relieve pain. Various problems such as drug interactions, adverse effects, and tolerability problems could be observed with current agents. Studies are devoted to gain a better understand of pain and discover new agents, however, a lot of questions remain unanswered. 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.[1,2] Noxious stimuli are detected by nociceptors that are found in skin, muscle, connective tissues, blood vessels, and viscera.[3] They are sensory neurons giving rise to a nerve fiber. They have two main fiber types: Aδ and C fibers.[2] The nociceptors travel through the spinal cord and make synaptic connections with second order neurons in the gray matter column of the dorsal horn (DH). A part of second-order neurons have ascending axons and project to the brainstem or the thalamocortical system.[4] The impulses originated from brain stem nuclei, "descend" to the spinal level and affect the transmission of pain signals at the DH.[3,4] The relative balance between descending inhibition and facilitation can be changed by the type and intensity of the stimulus and also by the time following the injury. Somatosensory system that detects destructive and potentially tissue injurious stimuli plays a critical role as an essential protective mechanism including numerous interacting peripheral and central mechanisms.[5] These mechanisms are the highly complex process involving various mediators and receptors as seen in Table 1. Pain control is provided by the interaction of these chemicals and receptors over an extensive network from the periphery to the 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 muscarinic acetylcholine receptors (mAChRs) that discussed in this review, apparently play a role both directly and indirectly in pain modulation. The activation of mAChRs provides pain control by contributing to releasing various modulators and changing the permeability of various ion channels.[9] Moreover, they also mediate the analgesic effect of other analgesic agents.[10] In this review, the experimental studies that prove the involvement of mAChRs in pain modulation are mentioned.

MUSCARINIC RECEPTORS IN PAIN

Acetylcholine and muscarinic receptor subtypes in pain modulation

Acetylcholine (ACh) is a neurotransmitter found in both the peripheral and central nervous system in many organisms as well as humans.[11] According to several reports, ACh plays a role in the inhibition and regulation of the pain transmission.[10,12] The physiological effects of ACh are mediated by mAChRs or nicotinic acetylcholine receptors (nAChRs). It is known that nAChRs are also involved in pain modulation as well as mAChRs; however, it will be touched on mAChRs in this review.

Molecular cloning studies has identified five different mAChRs termed as M_1-M_5. There is consensus on mAChRs in peripheral tissues and the nervous system.[13] The M_1, M_3 and M_5 subtypes are selectively bind to $G_{q/11}$ proteins to activate phospholipase C, whereas the M_2 and M_4 subtypes are selectively coupled to the pertussis toxin-sensitive $G_{i/o}$ proteins that mediate the adenylyl cyclase inhibition.[14] mAChRs activating drugs have been in use for a long time to treat both acute and chronic pain. It is well known that treatment of postoperative pain, labor pain and cancer pain with cholinesterase inhibitors lead to strong and stable analgesia via cholinergic stimulation and following spinal mAChRs activation.[13] Some research reports that cholinergic agonists also have analgesic effects on animal experiments, as well as cholinesterase inhibitors. Additionally, mAChRs agonists are better alternative than opioid analgesics in that they do not show physical dependence.[15] However, some cholinergic side effects such as bradycardia, hypotension, diarrhea, frequent urination and lacrimation prevent their clinical benefits.[16] That‟s why using drugs that are selective for the subtypes taking roles in the modulation of pain, are gaining importance and lots of research triggering to investigate the mAChRs subtypes that participate in the antinociception. Using the genetic knockout (KO) animals, contributes to examine the importance of receptor subtypes and their ligands and receptor subtypes that have a high affinity.[13,17]

The perception and control of pain are provided through an extensive network from the periphery to the CNS. mAChRs involve in pain modulation at all levels. In peripheral antinociception, the functional roles of peripheral mAChR have been showed with a series of electrophysiological and neurochemical studies. It is suggested that the transmission of pain impulses may be suppressed via activation of mAChRs that are located on peripheral nociceptors of the skin. Neuronal and nonneuronal ACh released from peripheral sources such as sensory neurons or separate cell types of the skin such as keratinocytes and fibroblasts, respectively, following cutaneous injury can activate sensory afferents through muscarinic receptors as well as nicotinic receptors. The activation of mAChR provides desensitization in sensory neuron.[18]

The investigations are more focused on the spinal and supraspinal muscarinic pain modulation since the mAChRs are also expressed in central pain processing regions such as the spinal cord, thalamus, periaqueductal gray (PAG), and rostral ventrolateral medulla (RVM). Although M_1, M_2, M_3, and M_4 subtypes existed in the central area, the intensity and the localization of these muscarinic subtypes are different.[19] A primary site of action for cholinomimetics in nociceptive processing is the spinal cord. M_2 is the major mAChR subtype expressed in the spinal cord, whereas M_3 and M_4 subtypes represent only a fraction of the total mAChRs in the spinal cord.[20] These subtypes, especially M_2 and M_4, are important ones for pain, located in the spinal cord DH and nociceptive pathways as shown in behavioral, pharmacological, neurochemical and electrophysiological studies. The contribution of these subtypes in spinal pain modulation is emphasized in the further parts of the review. Additionally, investigations emphasize the M_1, M_2, and M_4 subtypes are involved in supraspinal pain modulation. There are proofs about the regulation of pain perception by mAChRs via supraspinal mechanisms, as well as spinal mechanisms. The supraspinal administration of muscarinic ligands shows that they have analgesic effects at the levels of the hypothalamus, PAG, RVM and amygdala.[13] It has been reported that mAChRs activation may play an analgesic role by affecting the electric activities of pain excited neurons and pain inhibited neurons in the caudate putamen, a region is known that contribute to nociceptive modulation.[21] Moreover, stimulation of mAChRs in the thalamus can influence the emotional part of analgesia.[22] The studies about which subtype is involved in pain control and how they contribute this process have been accelerated since the role of muscarinic pain modulation was elucidated.

M_1 subtypes

Previously Ghelardini et al.[23] reported that M_1 receptors participate in the central antinociception, and Zhuo and Gebhart[24] supported that the role of M_1 receptor subtype in the spinal cholinergic modulation. Afterward, Sheardown et al.[16] studied rat models of acute pain and indicated that M_1 receptor subtype is not necessary for antinociception. More recently, it has been shown that i.t. application of putative M_1 agonist McNA-343 ([4-[[N-(3-chlorophenyl) carbamoyl]oxy]-2butynyl] trimethylammonium chloride) caused dosedependent antinociceptive effect in tail-flick test.[25] Although there is a little evidence about M_1 receptors involve in spinal cholinergic modulation, its role is predominantly distinct in supraspinal cholinergic antinociception. In one of the studies suggesting its role, knockdown of the alpha subunit of $G_{q/11}$ proteins, provided by intracerebroventricular (i.c.v.) administration of antisense oligodeoxyribonucleotide, and in another one knockdown of central M_1 prevented the antinociception induced by systemically injection of oxotremorine (OXO), non-selective mAChR agonist, and physostigmine, acetylcholine esterase inhibitor.[26,27]

Moreover, the contribution of supraspinal M_1 mAChRs in morphine analgesia was investigated as discussed below.[28] In another study, the antinociceptive mechanism of xanomeline, an M_1/M_4-preferring agonist, was determined by using nonselective (scopolamine and pirenzepine), and selective mAChR antagonists MT-7 (muscarinic toxin-7), for M_1 receptor, and MT-3 (muscarinic toxin-3) for M_4 receptor in several models of inflammatory and neuropathic pain. Tactile allodynia (pain is induced by a stimulus that does not evoke pain in normal conditions) and heat hyperalgesia (an enhanced response to a painful stimulus) was significantly reversed by Xanomeline in animal models of neuropathic and inflammatory pain. Scopolamine and pirenzepine entirely antagonized the analgesic effect of xanomeline, supporting that the analgesic effect is associated with the muscarinic system. In addition, MT-7, the highly selective M_1 receptor toxin, nearly suppress the whole analgesic effect of xanomeline when injected supraspinal although, MT-3, the highly selective M_4 receptor toxin, reversed the analgesia relatively MT-3 also had no effect when given spinally. These results have been indicated the supraspinal M_4 receptors‟ weak role, as well as the predominant role of supraspinal M_1 receptors in analgesia.[29]

M_2 subtypes

M_2 receptor subtypes seem to handle both peripheral and central muscarinic antinociception. Reduction of the sensitivity of peripheral nociceptors to different painful stimuli by muscarinic agonist via the activation of cutaneous M_2 mAChR has been shown in M_2 KO mice.[17] Thus, M_2 mAChR agonists have potential as a peripheral analgesic, particularly when administered topically owing to the possessing various side-effects when administered systemically.

The M_2 subtype is the most crucial mAChR that mediate analgesia produced by muscarinic agonists in the spinal cord. Gomeza et al.[30] used the M_2 KO mice to investigate the pharmacological role of M_2 mAChRs. Antinociceptive effect of the non-selective mAChR agonist OXO on thermal thresholds evaluated by using tail-flick and hot-plate tests. Even though the tail-flick method estimates pain sensitivity mainly at the spinal level; the hot-plate test assesses pain responses and analgesia at the supraspinal level. The antinociception evoked by OXO disappeared in M_2 KO mice. This study obviously proves the role of M_2 subtypes in central muscarinic pain modulation. In a study, the effects of systemic arecaidine, M_2 mAChR agonist, administration on nociceptive responses evaluated in a murine model of nerve growth factor-induced pain. Antinociception of arecaidine by activation of M_2 mAChRs exerted analgesic action on DRG sensory neurons by negatively modulating vanilloid receptor subtype 1 (VR1) activity. These evidence also informative to show that there is a cross interaction between M_2 mAChRs and VR1 activity.[31]

It has been predicted that M_2 subtypes are mostly involved in acute pain modulation. In a study that performed with WAY-132983 ((3R,4R)-3-(3-hexylsulfanyl-pyrazin-2-yloxy) -1-aza-bicyclo[221] heptane), M_1/M_4-preferring agonist, this agent could not found effective in acute pain model and it was claimed that this ineffectiveness occurred due to its low affinity and potency for M_2 receptors.[32] In general, M_2 mAChR subtypes are also expressed on presynaptic terminals, as well as postsynaptic neurons, to modulate the releasing of some neurotransmitters, such as GABA, glutamate and ACh itself, as touched on below. Jeong et al.[33] searched if the stimulation of mAChRs may regulate glutamate releasing from primary afferents onto medullary DH neurons which receive Aδ- and C-fibers from orofacial tissues and contribute orofacial pain process including migraine and trigeminal neuralgia and showed that the stimulation of presynaptic M_2 mAChRs reduce action potential-dependent glutamate releasing onto medullary DH neurons. Therefore, M_2 mAChRs may also be promising targets for the management of pain arising from orofacial tissues.

Additionally, M_2 mAChRs seem to be involved in the affective dimension of pain. Raisings in vocalization thresholds (Pain behaviors evoked by noxious tail shock) produced by intra-nucleus parafascicularis (nPf) carbachol were reversed dose-dependently by local administration of the non-specific mAChR antagonist atropine. Localization studies show moderate to high expression of M_2 receptors in nPf, a thalamic site that takes part in the creation of affective responses to painful stimulus. Thereby, the antinociceptive effects induced by intra-nPf carbachol are most presumably mediated by M_2 receptors.[22]

M_3 subtypes

A study performed to understand the role of M_3 mAChRs of the spinal cord in pain modulation showed that the release of ACh modulated by presynaptic M_3 mAChRs which are involved in the second phase of nociception evoked by formalin due to significant increase of the ACh level in the second phase was inhibited by it injection of M_3 antagonist 4-DAMP.[34] This study supports the results obtained from Dawson et al.[35] in which M_1/M_3 receptor agonists L-689,660 and AF102B were found effective in the tail-flick test. In contrast, in the study performed by Cai et al.[36], it was shown that M_3 subtypes did not contribute to antinociception at the spinal level. As mentioned above, some contrary results also obtained for M_1 mAChRs. The reasons for this controversy are not apparent but are attributed to differences in animal strains, agents and assessment methods. Further investigations are required to identify this controversy. In Matera et al. study[37], new bis(ammonio)alkane-types mAChR agonists that incorporate the orthosteric muscarinic agonist iperoxo into a molecular fragment of the M_2-selective allosteric modulators W84 or naphmethonium, was synthesized and their analgesic action was assayed in vivo in the

acetic acid writhing test. Among these synthesized compounds, the naphmethonium-related compound, named as 8b, which showed the most potent antinociception without muscarinic side effects such as cardiovascular unwanted effects and the lowest intrinsic activity at M_3 mAChRs when compared with those measured at M_1 and M_2 subtypes. This fact may be explained that M_3 mAChRs prevent the muscarinic side effects and also they are involved in pain modulation less than other receptor subtypes.

M_4 subtypes

The antinociceptive effects of various centrally active muscarinic agonists has been evaluated by using M_2 KO, M_4 KO, and M_2/M_4 double-KO mice in order understand the involvement of the M_2 and M_4 mAChRs in muscarinic agonist-induced analgesia in the tail-flick and hot-plate tests.[38] The analgesic activity induced by subcutaneous (s.c.) administration of the non-selective mAChR agonist OXO entirely disappeared in M_2/M_4 double-KO mice in both tests. Previously, Gomeza et al.[30] showed that the analgesic action of OXO was significantly decreased in M_2 KO mice. However, it was indicated that non-M_2 mAChRs can also mediate profound antinociception because maximum analgesia could still be elicited in M_2 KO mice by increasing doses of OXO. The wholly disappearing of antinociception in M_2/M_4 double-KO mice suggests that both M_2 and M_4 mAChRs participate in mediating muscarinic antinociception at both spinal and supraspinal levels, and non-M_2/M_4 mAChRs do not involve in this effect.[38] similarly, CMI-936 (2-exo{5-(3-methyl-1,2,4oxadiazolyl)}-[221]-7-azabicycloheptane) and CMI-1145 (2-exo{5-(3-amino-1,2,4-oxadiazolyl)}-[221]-7azabicycloheptane) (s.c.), M4 preferring agonists, showed potential antinociceptive efficacy in the tail-flick test and this efficacy was reduced by M_2/M_4 preferring antagonists like hymbacin (s.c.), pertussis toxin (i.t.) and M_4 selective peptide antagonist, MT-3 (i.t.).[39] In another study, the changes of muscarinic M_4 receptor levels have been investigated by using M_4 mAChR subtype selective ligands with receptor autoradiography, on rats with acute and chronic arthritis, the model of pain. The heat-killed Mycobacterium butyricum was applied intradermally to rats and then observed 12 days for acute, 30 days for the chronic group. An important reduction of M_4 mAChR level, the down-regulation of M_4 mAChRs spinal cord of rats that have acute and chronic arthritis, occurred as a result of prolonged ACh, released highly against to the pain stimulus, stimulation.[40] In addition, M_1/M_4 preferring agonist WAY-132983, the agent which was found ineffective in acute pain model as aforementioned, generated strong and efficient antihyperalgesic and antiallodynic effects in rodent models of chemical irritant-induced visceral pain, chronic inflammatory, neuropathic, and incisional pain.[32] These findings show that M_4 mAChRs are participating muscarinic mechanisms of analgesia at the level of the spinal cord and M_4 mAChR selective agonists promise hope for using as analgesics.

M_5 subtypes

There are limited studies on M_5 receptors induced antinociception, and the involvement of M_5 mAChRs in pain modulation has not been exactly proved yet. However, because the existence of mRNA‟s of this subtype in the DRG area was shown, development of novel drugs act on M_5 mAChRs are on the agenda.[13] In the one recent of these studies, a complex role of M_5 mAChRs was also revealed. It has been demonstrated that the activation of the M_5 subtype expressed at primary afferent terminals potentiates primary afferent input, whereas stimulation of the M_5 subtype can also indirectly inhibit nociceptive primary afferent input through increased glutamate release from spinal interneurons and subsequent activation of group II/III metabotropic glutamate receptors (mGluRs).[20]

mAChRs involved in the antinociception induced by other analgesic treatments mAChRs mediate the antinociceptive effect of not only own agonist but also the other analgesic treatments such as morphine, clonidine, and spinal cord stimulation (SCS), so they are also called as mAChR ligands. It is proved that SCS can be used for neuropathic pain treatment.[41] An increased release of spinal ACh acting on mAChRs has been reported to be one of the mechanisms involved in SCS.[42] It has been shown that sub-effective dose of OXO may have a synergistic effect with SCS against painful hypersensitivity in SCS nonresponding rats.[43] In another study analgesic effect of SCS were completely blocked by atropine but it was not susceptible to the nicotinic antagonist mecamylamine, and there was only a partial attenuation produced by M_1 and M_2 antagonists. Interestingly, M_4 selective antagonist MT-3, blocked the SCS induced analgesia selectively.[42] Therefore, M_4 subtype could be defined as a key subtype for SCS induced analgesia. Da Silva et al.[44] has recently shown that low-frequency electro acupuncture-induced analgesia utilizes muscarinic mechanism in the dorsal anterior pretectal nucleus, which is located in descending pathways from the dorsolateral funiculus to the spinal dorsal horn for mediating nociception.

It has been investigated that morphine and clonidine, analgesic agents, are capable of increasing ACh release at spinal level and this endogenous ACh has a significant role in mediating the analgesic effect of these drugs.[10,12] Spinal ACh assists to the analgesic effect of systemic morphine through cholinergic receptors.[10] It has been suggested by a study that demonstrates that the interaction between ACh and endogenous opiate peptides (EOP) as morphine. ACh and EOP increase the release of each other which antagonized by their receptor antagonists, opiate antagonist; naloxone, and mAChRs inhibitor: atropine, or nAChRs inhibitor; hexahydric gallamine.[11] In another study to explain the role of spinal mAChRs participating in morphine analgesia, antinociceptive effect of morphine (s.c.) was inhibited by the mAChRs antagonist atropine and pirenzepine (i.t.), potently M_1 and also M_4 antagonist. In contrast, an M_2

antagonist methoctramine and M_3 antagonist 4-DAMP did not antagonize the antinociceptive effect of morphine.[25] In another study aimed to investigate the role of mAChR subtypes in the nucleus reticularis gigantocellularis/nucleus reticularis gigantocellularis alpha of the rat RVM in morphine-induced antinociception showed that morphine-induced antinociceptive effects partly involve the M_1 and M_3 mAChR of the rat nucleus reticularis gigantocellularis/nucleus reticularis gigantocellularis alpha. The M_1 mAChR antagonists, MT-1 (muscarinic toxin-1), selective M_1 antagonist, and pirenzepine, nonselective mAChR antagonist, inhibited the antinociception that was induced by both systemic administration and microinjections of morphine into the nucleus reticularis gigantocellularis/nucleus reticularis gigantocellularis alpha. The analgesic effect of the morphine obtained by the systemically administration was not reversed by pretreatment with M_2 antagonist methoctramine in the hot-plate and tail-immersion tests and low-dose M_3 antagonist 4-DAMP (1,1-Dimethyl-4difenylacethoxypiperydinium iodide) in the hot-plate test.[28] The interactions between spinal α_2-adrenoceptors and cholinergic interneurons are well known. It is thought that the ACh release as a result of exciting of spinal cholinergic neurons by α_2-adrenoceptor agonists after injury is crucial for the analgesia of spinal $\alpha_2$adrenoceptor activation.[12] It has been shown that $\alpha_2$adrenoceptor agonists such as dexmedetomidine as well as clonidine facilitate KCl-evoked ACh release from lumbar DH synaptosomes in neuropathic pain model by used spinal nerve ligated rats.[45] It has been revealed that atropine and pirenzepine reversed the anti-allodynic effect of clonidine (i.t.) in diabetic mice, but the M_2 and also M_3 mAChR antagonist were not succeed in antagonism as in previous study.[46] These results suggest the contributing role of M_1 or M_4 mAChRs in both spinal pain modulation and morphine and clonidine analgesia. As morphine and clonidine, the antinociception mechanisms of the non-steroidal anti-inflammatory drugs (NSAIDs), the drug class whose primary mechanism is COX (cyclo-oxygenase) inhibition and which is often used for the control of acute pain, may associate with the ACh release in the spinal cord. The relation between pre- and postsynaptic mechanisms that facilitate cholinergic transmission and the antinociception of NSAIDs has been suggested by a study in which atropine or Hemicholinium-3 (HC-3), neuronal high-affinity choline uptake inhibitor, antagonized the antinociception developed by NSAIDs in acute thermal pain model.[47]

Muscarinic pain modulation via non-cholinergic systems

Muscarinic pain modulation also provided by noncholinergic pain modulatory systems and it will be discussed briefly in this part of the review. mAChRs are broadly found in postsynaptic neurons and also in presynaptic terminals in the nervous system. Presynaptic mAChRs modulate the release of several neurotransmitters such as inhibitory GABA and glycine, excitatory glutamate, and ACh itself onto spinal DH neurons.[9,33] GABA is the primary inhibitory neurotransmitter in the CNS. The interaction between the muscarinic system and GABAergic transmission in CNS has been studied for a long time. Moreover, GABA neurons and receptors are also distributed in supraspinal sites that organize the perception and response to painful impulse, and this neurotransmitter system regulates sensory information proceeding in the spinal cord.[48] It has two receptors called ionotropic $GABA_A$, primarily postsynaptic, and metabotropic $GABA_B$, mostly presynaptic. The interaction between cholinergic system and GABAergic transmission in CNS has been studied for a long time and the various reports indicate that spinal mAChR activation produce antinociception via activation of mAChRs on the GABAergic interneurons and terminals to excite GABA release and then the DH neurons are inhibited by $GABA_A$ receptor-mediated Cl^- channels provoked by this released GABA.[49] It is also clearly seen that globus pallidus and substantia gelatinosa are involved in pain modulation, considering the muscarinic modulation of GABA release.[50] The stimulation of somatodendritic M_2, M_3, and M_4 on GABA interneurons assists the GABAergic transmission and causes inhibition of postsynaptic DH neurons. Contrary to rats, presynaptic M_2, M_3, and M_4 mAChR subtypes regulate GABAergic transmission in mice DH. The inhibitory GABAergic input to DH neurons is mostly weakened through the stimulation of M_2 and M_4 mAChRs, whereas M_3 activation assists the releasing of GABA. Endogenously released GABA in the spinal cord can preferentially activate presynaptic $GABA_B$ receptors.[51] For instance in Chen and Pan study[49], antinociception induced by it mAChR activation in streptozocin-treated rats blocked by it $GABA_B$ receptor antagonist, CGP55845 ([(2S)-3-[[(1S)-1-(3,4-Dichlorophenyl)ethyl] amino]-2-hydroxypropyl] (phenylmethyl)phosphinic acid hydrochloride). The activated presynaptic $GABA_B$ receptors may attenuate the spinal release of glutamate indirectly and contribute to spinal analgesia.[51]

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.[49] The heteroreceptor function of $GABA_B$ receptors also controls synaptic glycine release as well as glutamate release to spinal DH neurons.[52] Glycine is the other inhibitory neurotransmitter, and blocking of its receptors in the spinal cord is known to cause oversensitiveness of DH neurons and allodynia. The findings suggest that any impairment of glycinergic inhibitory synaptic transmission in the spinal DH is associated with the progress of neuropathic pain.[13]

mAChRs also directly contribute to the regulation of glycinergic, glutamatergic inputs on DH neurons in mice and rats as well as GABAergic modulation. Presynaptic M_2 on primary peripheral sensory neurons inhibit

excitatory glutamatergic input to DH neurons through diminishing the Ca^{++} influx into primary afferent terminals in rats[53] and M_2/M_4 and M_3 mAChRs subtypes on a subset of interneurons also inhibit. The inhibition of postsynaptic DH neurons by increasing glycinergic transmission is also provided by stimulation of somatodendritic M_2 and M_3 mAChRs on glycine interneurons. The M_3 subtype is mainly responsible for the muscarinic potentiation of synaptic glycine release in the rat spinal cord.[13,52]

As understood, glutamate transmission takes an important place in muscarinic pain modulation. Interactions between the muscarinic and glutamatergic neurotransmitter systems may change the neuronal excitability and synaptic transmission by synergistic activation of M_1 mAChRs and metabotropic glutamate receptors (mGluRs), group I (mGlu1 and mGlu5).[54]

M_1, M_2, M_3, and M_4 subtypes may also alter the activation of some ion channels when modify the neurotransmitter release. Therefore, the interaction between the ion channels and the muscarinic system is also a valuable focus point. In mice and rats, the stimulation of presynaptic M_1 can help the release of neurotransmitter from sympathetic neurons by M-type K^+ current suppression or weaken release through closing the voltage-activated N- and L-type Ca^{++} channels. Stimulation of presynaptic M_2 and M_4 inhibit the release of neurotransmitter through fast inhibition of N- and P/Q-type Ca^{++} channels.[13] The N- and P/O-type voltage-gated calcium channels subtypes are associated with the release of glutamate from trigeminal primary afferents[53] and may be implicated in the mAChRmediated presynaptic inhibition of glutamate release onto medullary DH neurons.

There are some evidence that the activation of mAChRs may contribute the pain modulation by acting on the pain modulatory ion currents. It was informed that increasing intracellular calcium content is important for cholinergic antinociception. T-type Ca^{++} channels are important in modulating intracellular Ca^{++} ion concentration nearby the resting membrane potential and the regulation of Ttype currents in reaction to stimulation of an array of G protein-coupled receptors, as M_1 mAChRs.[55] In Zhang et al.[56] study, alpha-cobratoxin, a neurotoxic protein that has the analgesic effect, reversibly inhibited T-currents dose-dependently. Selective M_4 mAChR antagonist tropicamide blocked this inhibitory effect. As M_4 mAChRs, M_1 mAChRs activation by $G_{q/11}$ also inhibits T-type flow by way of an unclear mechanism pathway and stimulation of M3 mAChR blocks T-type currents via a new PKC isoform pathway in ice DRG neurons.[57] It is known that mAChRs activate K^+ channels and the agents that open K^+ channels such as neuronal Kv7 and K^+ATP channels have been demonstrated to generate an antinociceptive effect in models of acute and chronic pain.[58,59] In one of the studies that show various types of K^+ channels such as K^+ATP appeared to be related with the antinociception of mAChR agonists, i.c.v. glibenclamide (K^+ATP channel blocker) antagonized the antinociceptive effect of i.c.v. pilocarpine.[60] In the further study, it was reported that the antinociception provoked by i.t. bethanecol was potentiated by nicorandil (K^+ channel opener) and partially antagonized by glibenclamide and charybdotoxin (both K^+ATP channel and Ca^{++}-activated K^+ channel blockers).[59] These outcomes indicated that the antinociception evoked by mAChRs agonists, especially through the activation of M_2 mAChR subtypes, both at the supraspinal and the spinal levels is reliant on opening of K^+ATP channel.[61] Also, group-I mGluRs inhibit the mAChR-dependent K^+ flow that is important for the after hyperpolarization that happens following an action potential and a leakage of K^+ flow in neurons. Pharmacological and electrophysiological studies show that group-I mGluRs on peripheral sensory neurons are crucial in chronic pain models.[62] It is possible that prevention of weakening the muscarinic K^+ flow may underlying the antinociception evoked by mGluR antagonists. Additionally, it should be noted that muscarinic stimulation decrease glutamate release as well, which provides mGluRs activation.[62,63] The activity of these neuropeptides may be reinforced via VR1 activity. One of the interactions of muscarinic pain modulation is with transient receptor potential VR1 known as TRPV1, a nonselective cation channel. It is triggered via injurious heat, protons and vanilloid agonists.[31] De Angelis et al.[31] show that the activation of M_2 mAChR leads to desensitization to mechanical and heat stimulus via a down-regulation of VR1 expression.

In the last step of muscarinic antinociception, it is possible to discuss muscarinic and opioidergic interaction on emotional modulation and defensive responses to pain. In Leite-Panissi study[64] it is demonstrated that antinociception evoked by carbachol or morphine sulfate administered into the central nucleus of the amygdala (involved in diverse emotional and cognitive functions related to responses to fear and orientation, defensive behavior, pain) is prevented by pretreatment with naloxone in the same region. It is indicated that the action of the cholinergic system is opioid-dependent in descending inhibitory pathways as well. Moreover, it is known that mAChRs activation also raises EOP concentration in the spinal cord and assist to antinociception by improvement of EOP stimulation of μ receptors. 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.[11]

Even though, the antinociception gained in DH can be referred to stimulation of cholinergic–opioidergic systems, it cannot be rejected the attendance of other hippocampal neurotransmitters in antinociceptive response mediating.

CONCLUSION

It is obvious that the muscarinic system is involved in pain process mediating by mAChRs as discussed in this

review. Electrophysiological and neurochemical studies indicate that these receptors are located in both peripheral and central areas; however, the density of the receptors differs. Although muscarinic receptors are more involved in central pain control, at spinal and supraspinal levels, peripherally control cannot be negligible. In the periphery, among the mAChRs, M_2 subtypes seems to be responsible for cholinergic antinociception. It is suggested that the transmission of pain impulses may be suppressed via activation of mAChRs that are located on peripheral nociceptors of the skin. 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 mAChRs.[18] Because main task of M_2 mAChRs is on the heart and soft muscle physiology, they may cause several systemic sideeffects, for this reason, M_2 agonists can be administrated as topical analgesics in the acute and chronic pain conditions which is managed peripherally with minimal adverse systemic effects.

It has been discussed in this review that there are some areas that intensely take part in pain modulation in CNS. The investigations are more focused on the spinal and supraspinal cholinergic pain modulation since the cholinergic receptor density is more excessive in spinal and supraspinal pain pathways. The obtained results from these investigations are considerable. In the respect of muscarinic antinociception; M_1, M_2, and M_4 mAChRs are important subtypes, especially M_2 and M_4, located in the spinal cord DH and nociceptive pathways. According to the preclinical pharmacological information, activations of M_4 and even M_1 receptors can be necessary for ACh releasing in the spinal level. This secretion leads to antinociception by the activation of M_2 receptors. Thereby, M_2 receptors are privileged for the mAChR-mediated spinal antinociception as well as peripheral antinociception. Also, investigations emphasize the M_2, M_4, and especially M_1 subtypes are involved in supraspinal pain modulation. It has been reported that mAChRs activation may possess an analgesic action via affecting the electric activities of pain-excited neurons and pain-inhibited neurons in the caudate putamen, a region is known that contribute to nociceptive modulation.[21] When all the data is considered, it is remarkable that M_1 subtype predominantly involve in supraspinal muscarinic antinociception whereas M_2 and M_4 subtypes involve in spinal antinociception. mAChs utilize various mechanisms such as modulation of neurotransmitter release and ion channels permeability concurrently with muscarinic antinociception.

In conclusion, it is possible to say that mAChRs regulate analgesia peripherally and centrally at spinal and supraspinal levels, and muscarinic antinociception extensively takes part in pain control. Moreover, mAChRs are related to analgesia induced by different pain treatments. Because ACh-activated cholinergic receptors involve in many physiological processes, the drugs that act on this receptor system may cause undesirable side-effects. Thereby, it is so important to identify the best subtype to reduce or remove the cholinergic side effects that are seen with the nonselective agonists and to design new therapeutic strategies. The muscarinic receptor-mediated agents are promising. Muscarinic agonists seem to be efficient against numerous stimulus approaches and possess a wide efficacy against a series of clinically important acute and chronic pain conditions. The treatments targeting central pain pathways provide more effective results than peripheral-targeting treatments for both chronic pain types such as neuropathic and inflammatory pain and acute pain. Nevertheless, it is rational to use these agents for both peripheral and central management of these pain conditions. Thereby, the selective agonists targeting M_1, M_2 or M_4 mAChRs are valuable agents because of providing an acceptable analgesia in the management of acute and chronic pain conditions that are induced by several disorders. It is also possible to utilize these agonists in controlling post-operative pain, labor pain, and orofacial pain such as migraine.

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