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The Cholinergic System as a Treatment Target for Opioid Use Disorder

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Abstract

Opioid overdoses recently became the leading cause of accidental death in the United States, marking an increase in the severity of the opioid use disorder (OUD) epidemic that is impacting global health. Current treatment protocols for OUD are limited to opioid medications, including methadone, buprenorphine and naltrexone. While these medications are effective in many cases, new treatments are required to more effectively address the rising societal and interpersonal costs associated with OUD. Here we review the opioid and cholinergic systems, and we examine the potential of acetylcholine (ACh) as a treatment target for OUD. The cholinergic system includes enzymes that synthesize and degrade ACh and receptors that mediate ACh's effects. ACh is involved in many central nervous system functions that are critical to the development and maintenance of OUD, such as reward and cognition. Medications that target the cholinergic system have been approved for the treatment of Alzheimer's disease, tobacco use disorder and nausea. Clinical and preclinical studies suggest that medications such as cholinesterase inhibitors and scopolamine, which target components of the cholinergic system, show promise for the treatment of OUD and further investigations are warranted.

1. Introduction

The United States is currently facing an opioid use disorder (OUD) epidemic, which started with large increases in opioid prescriptions in 1990 and expanded by the widespread availability of heroin and synthetic opioids [1, 2]. In addition to affecting the United States, OUD is problematic in several other countries and significantly contributes to global disease burden [3]. The current epidemic has resulted in an estimated 2.1 million individuals in the United States with OUD in 2016 [4, 5]. In 2016, over 42,000 people died from opioid overdose, making it the leading cause of accidental death in the United States [6]. Medication assisted treatment (MAT), including methadone, buprenorphine and naltrexone, is effective in reducing opioid use, rate of OUD-associated infections, and psychosocial consequences of OUD [7–9]. However, high rates of attrition limit the effectiveness of MAT, underscoring the need to develop novel primary or adjunct treatments for OUD [7, 8].

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Conflicts of Interest

Kevin P. Jensen, Elise E. DeVito, Sarah Yip, Kathleen M. Carroll and Mehmet Sofuoglu declare no conflicts of interest.

As will be discussed in this review, among potential treatment targets for OUD, the brain cholinergic system shows a particular promise. Acetylcholine (ACh) participates in a wide range of central nervous system (CNS) functions that are thought to be critical in development and maintenance of OUD including reward, motivation, attention, mood, nociception, stress response and neuroimmune functions [10–15]. Accumulating evidence from many studies support a close functional coupling between ACh and endogenous opioids. Further, preclinical and clinical studies suggest that medications targeting the cholinergic system may have utility for OUD treatment. This paper synthesizes studies that have examined the potential role of the cholinergic system as a treatment target for OUD. We first summarize clinical aspects of OUD, followed by current treatment approaches and clinical challenges. Next, we overview neurobiology, pharmacology and genetics of endogenous opioid and cholinergic system for CNS functions that are relevant for OUD. We then review preclinical and clinical studies that have examined the use of cholinergic medications for outcomes relevant for OUD. We conclude with a discussion of research gaps and future directions.

2. Overview of OUD

OUD is a chronic relapsing disorder characterized by compulsive and uncontrollable opioid use, most commonly heroin or prescription opioids. OUD increases the mortality rate of affected individuals 6 to 20 times over the general population, primarily due to overdose deaths [16]. Opioid overdose deaths are mainly due to respiratory depression; risk of overdose is accentuated by concurrent benzodiazepine use [17]. Typically, first exposure to opioids is through prescription opioids which is followed by non-prescription opioid and eventually heroin use [18]. Following initial exposure, individual vulnerability factors to develop OUD include depression, posttraumatic stress disorder (PTSD), presence of an additional substance use disorder and adolescence [19]. In addition, multiple genetic variations have been associated with the risk of developing OUD [20–22]. OUD is highly comorbid with many psychiatric and medical problems including depression, anxiety disorders, PTSD, chronic pain, and infections including the human immunodeficiency virus and Hepatitis C Virus [23–25].

3. Pharmacological treatment of OUD

The primary pharmacological approach for OUD is referred to as MAT, comprising methadone, buprenorphine and naltrexone. MAT reduces or eliminates opioid use, prevents overdose deaths, and reduces risk of contracting infections [26]. The principal limitation of MAT is high drop-out rates and subsequent relapse to opioid use. For buprenorphine and methadone maintenance treatments, retention rates at 1 year are typically less than 50% [7, 8]. Retention rates for injectable sustained-release naltrexone are even lower [27–30]. In addition, long-term treatment with opioid medication is associated with adverse effects including cognitive deficits, endocrine disturbances, decreased libido and increased pain sensitivity or hyperalgesia [31–34]. Thus, there is a great need to identify novel non-opioid medications for OUD treatment, including those that could be used alone or in combination with MAT.

4. Overview of the Opioid System

Endogenous opioids and their receptors are widely distributed in the CNS and peripheral tissues, reflecting their participation in multiple functions including reward, pain, emotion, cognition, and immune response. The endogenous opioid system includes four opioid receptors, mu (MOR), delta (DOR), kappa (KOR), and NOP, and 4 families of opioid peptides, β-endorphin, enkephalins, dynorphins and prepronociception [35]. Opioid receptors belong to the G-protein-coupled receptor superfamily and their activation leads to inhibition of cyclic adenosine monophosphate production and voltage-gated calcium channels, and activation of inwardly rectifying K+ channels and mitogen-activated protein kinase activity [36, 37]. These effects result in presynaptic inhibition of neurotransmitter release and inhibition of neuronal excitability. In the brain reward circuitry, MOR increases dopamine (DA) release by disinhibition; that is, inhibiting GABAergic interneurons which inhibit DA neurons [38].

4.1. Endogenous opioid peptides:

The endogenous opioid peptides are derived from 4 large precursor proteins (Table 1): 1) proopiomelanocortin (POMC), the precursor for β-endorphin; 2) preproenkephalin (PENK), the precursor for leucine (Leu)- and methionine (Met)-enkephalins; 3) preprodynorphin (PDYN), the precursor of dynorphins A and B, and neoendorphins [39–41] and 4) prepronociception (PNOC), the precursor of nociceptin or orphanin FQ (N/OFQ) [42]. The POMC synthesizing neurons are located in the arcuate nucleus of the hypothalamus and the nucleus tractus solitarius in the dorsal medulla. The arcuate nucleus POMC neurons project to cortical and limbic regions, including the amygdala, hypothalamus, nucleus accumbens, periaqueductal gray and ventral tegmental area. In contrast, the POMC neurons located in the nucleus tractus solitarius project mainly to the spinal cord and brainstem. PENK and PDYN are synthesized locally in neurons in multiple regions of the CNS including the cortex, hippocampus, basal ganglia, thalamus, hypothalamus, periaqueductal gray area, ventral tegmental area, rostral ventromedial medulla, and the dorsal horn of the spinal cord. PNOC is expressed in multiple areas in the CNS including amygdala, thalamus, subthalamic nuclei, hypothalamus and basal ganglia [42]. β-endorphin, N/OFQ, and enkephalins are released in response to a multitude of painful or stressful stimuli [43]. Analgesia induced by physical and mental stressors is cross-tolerant with morphine and blocked by naloxone [44]. Overall, endogenous opioids have anti-stress effects mediated by MOR, DOR and KOR activation.

4.2 Opioid receptors:

Opioid receptors are widely distributed in the central and peripheral neurons, neuroendocrine and immune cells [45]. In the CNS, opioid receptors are found in the ventral tegmental area, nucleus accumbens, hypothalamus, prefrontal cortex and amygdala. This distribution pattern is consistent with their participation in analgesia, reward, cognitive and emotional functions. The MOR, DOR, and KOR have differential sensitivity to endogenous opioid peptides. While β-endorphin has a high affinity to MOR and DOR, enkephalins and dynorphin have high affinity for DOR and KOR, respectively.

4.2.1 MOR: MOR is the main target for the rewarding, analgesic and addictive effects of opioids like morphine, heroin, fentanyl and oxycodone. For example, unlike wild-type mice, MOR gene knockout mice do not show preference for morphine in a conditioned place preference task, do not self-administer morphine or heroin, and do not show signs of opioid withdrawal following chronic opioid exposure [46, 47]. MOR also has a key role in mediating rewarding effects from non-opioid drugs of abuse including alcohol, cocaine, nicotine and tetrahydrocannabinol. MOR knockout mice are less sensitive than wild-type mice to rewarding effects of these drugs of abuse [48–50]. These effects are mediated mainly by MORs that are located on GABAergic inhibitory cells in the ventral tegmental area, which provides tonic inhibition to ventral tegmental area DA neurons. The inhibition of GABAergic cells by MOR (i.e., disinhibition), results in increased DA release in the nucleus accumbens [51]. In humans, naltrexone, an antagonist at MOR and to a lesser extent KOR and DOR, attenuates reward from food [52], physical activity [53], music [54] and drugs of abuse including cocaine [55], alcohol and nicotine [56].

4.2.2 DOR: DOR does not seem to be essential for the rewarding effects of opioids, as morphine self-administration in DOR knockout mice is similar to wild-type mice [57]. However, DOR knockout mice show enhanced depressive-like behavior and DOR agonists show antidepressant effects in animal models of depression suggesting that DOR may be related to mood-regulating effects of opioids [58].

4.2.3 KOR: Dynorphin A is the main endogenous KOR agonist. Dynorphin, through KOR activation, counteracts the rewarding effects of MOR in the ventral tegmental area and reduces DA release in the nucleus accumbens [59]. As a result, activation of KOR induces dysphoric effects and blocks rewarding effects from drugs of abuse including nicotine, alcohol, cocaine and tetrahydrocannabinol [60–62].

4.2.4 NOP: NOP (also known as ORL1) are not blocked by opioid antagonists, naloxone or naltrexone. The main endogenous ligand for NOP is a peptide named N/OFQ [35]. Similar to other opioid receptors, when activated, NOP inhibit adenylate cyclase, increase potassium conductance and reduce calcium conductance [63]. These inhibitory effects result in reduced neuronal activity and neurotransmitter release [64]. N/OFQ has complex effects on pain: it blocks morphine-induced anti-nociception and conditioned place preference when administered supraspinally [65], but has analgesic effects at the spinal cord in mice [66].

4.3 Molecular mechanisms of opioid effects:

Exposure to opioids leads to upregulation and desensitization of the opioid receptors [67]. Desensitization is a likely mechanism for development of opioid tolerance and includes several processes including receptor phosphorylation, uncoupling of receptors to G proteins, and internalization of receptors [67]. Phosphorylation of opioid receptors by G-protein-coupled receptor kinases increases their affinity for intracellular β-arrestin molecules. β-arrestin-opioid receptor complex formation results in uncoupling of G-proteins and facilitates opioid receptor internalization[68]. The internalization process disrupts the heterodimer between MOR and DOR, resulting in reduced function of opioid receptors.

Opioid agonists differ in their ability to induce internalization resulting in different level of tolerance in response to chronic exposure [69].

4.4 Adaptations to chronic opioid exposure:

Chronic exposure to opioids leads to tolerance and withdrawal as the main components of physical dependence to opioids[68]. Multiple mechanisms for the development of opioid tolerance have been discussed, including uncoupling of MOR to co-effectors like G-proteins [70] and changes in secondary signaling cascades (e.g. upregulate of cyclic adenosine monophosphate-dependent signaling)[71]. Tolerance development leads to dose increases by the individuals in order to receive the desired effects from opioids (e.g., pain relief or euphoria). In the presence of physical dependence, abstinence (or significant reduction in opioid intake) leads to opioid withdrawal syndrome, which includes both physical and affective components [72]. Physical signs and symptoms include abdominal pain, diarrhea, vomiting, nasal discharge, enlarged pupils, pain and chills. Affective symptoms include dysphoria, anhedonia, anxiety/irritability, and craving. Depending on the elimination half-life of the particular opioid drug, physical components of opioid withdrawal syndrome subside within 1 to 2 weeks [73].

4.5 Insights into the opioid system from human genetics studies:

Genetic variation in genes that encode opioid receptors and endogenous opioid peptides has received much attention. The genes that encodes MOR, DOR, KOR and NOP in humans are *OPRM1, OPRD1, OPRK1 and OPRL1*, respectively. Genetic studies can be useful for understanding how certain genes function in humans, for prioritizing molecules as therapeutic targets, and for stratifying patients for precision medicine approaches. Genomewide association studies (GWAS), which interrogate most common variants in the genome without bias, are the current “gold-standard” method for conducting genetic association studies [74]. GWAS and other genetic studies of OUD and OUD treatment have been more extensively reviewed elsewhere by Jensen [75], and more recently by Crist and Berrettini [76, 77]. Among these genetic studies are several that highlight an important role of opioid receptors for OUD (Table 2). As discussed in prior sections, opioid receptors are important for several processes such as reward, pain, emotion, cognition, and immune response. MOR, in particular, is essential for the rewarding effects of drugs of abuse, including opioids and genetic variation in *OPRM1*, the gene encoding MOR, has been studied intensely. A nonsynonymous single nucleotide polymorphism (SNP), rs1799971, that causes an asparagine to aspartic acid substitution (Asn40Asp) in MOR, has been widely studied due to its possible effects on MOR function, although some *in vitro* studies suggest that effects on protein function are modest [78, 79] and might involve alternative mechanisms, such as effects on *OPRM1* mRNA expression [80]. Moreover, genetic association studies of rs1799971 to OUD have yielded conflicting results, indicating that research on other variations encoded within *OPRM1*, and elsewhere in the genome, is warranted. For example, a recent study of heroin dependence that focused on 103 *OPRM1* cis-eQTLs (i.e. SNPs associated with *OPRM1* mRNA expression in brain tissue) identified a robust association ($p = 4.3 \times 10^{-8}$) with rs3778150, a SNP in the first intron of *OPRM1*[81]. This association was based on a meta-analysis that included 16,729 subjects and it was noted that rs1799971 was not associated with heroin dependence in any cohort or in the meta-

analysis [81]. In a GWAS of methadone dose requirements by Smith et al., the most statistically robust association ($p = 2.8 \times 10^{-8}$) identified was for a SNP 5' the *OPRM1* transcription start site in an African American sample (n= 383) with OUD [82]. The SNP was also associated to morphine dose in a separate sample of opioid-naïve African American pediatric subjects (n=241) [82]. Crist et al. tested the association of several *OPRM1* haplotypes to OUD treatment response and identified a SNP, rs10485058, in the *OPRM1* 3' untranslated region that was associated with response to methadone in two European ancestor samples of OUD [83]. In an important extension of this work, they found that the SNP modified microRNA regulation of gene expression in a cell culture system [83]. In a separate study of variation in *OPRD1*, the gene encoding DOR, Crist et al. tested the association of on OUD treatment response to an *OPRD1* SNP, rs678849, which had been previously linked to opioid dependence risk. They observed an association to treatment response in an African American sample with OUD that differed in terms of the effect direction based on the medication group (methadone or buprenorphine) [84]. Many association signals have emerged for GWAS of OUD-related phenotypes (e.g. OUD symptom count, sensitivity to opioids) that have implicated genes with no clear link to opioid signaling, and functional studies to elucidate the biological mechanisms are required [20–22, 85].

5. Overview of Acetylcholine

ACh, the first discovered neurotransmitter, contributes to multiple CNS functions including sensory and motor processing, sleep, nociception, mood, stress response, attention, arousal, memory, motivation and reward [10–14]. A large body of evidence supports the role of ACh in initiation and maintenance of addictive processes as well. It has been suggested that the DA/ACh balance in the nucleus accumbens may affect reward and aversion spectrum such that an increased ratio facilitates reward and decreased ratio generates an aversive state [86]. For example, drugs that increase ACh levels reduce self-administration of drugs of abuse including stimulants and opioids [87, 88]. Conversely, drug withdrawal states for opioids, cocaine and nicotine are associated with reduced DA and increased ACh levels [89–91].

5.1 Endogenous Cholinergic System in the CNS:

In the cytoplasm of cholinergic neurons, ACh is synthesized from acetyl-coenzyme A and choline by choline acetyltransferase (ChAT) [92]. Following its release into the synaptic cleft, ACh signals through two classes of receptor: nicotinic (nAChR) or muscarinic (mAChR) type cholinergic receptors, which are both described in more detail below. ACh is rapidly inactivated by an enzyme, acetylcholinesterase (AChE), which is inhibited by a range of toxins and medications as well [93].

5.2 ACh Biosynthesis and distribution in the CNS:

Cholinergic neurons consist of two types: cholinergic interneurons and cholinergic projection neurons [94]. Cholinergic interneurons are located mainly in the striatum and modulate output from the basal ganglia. A subgroup of these neurons, the tonically-active striatal cholinergic interneurons, has important roles in stimulus salience and orienting functions [95].

The cholinergic projection neurons are located in the brainstem and the basal forebrain. The brainstem cholinergic neurons are located in pedunculopontine tegmental and the laterodorsal tegmental nuclei and project to the ventral tegmental area and thalamus [94]. These neurons modulate the sleep/wake cycle [96]. The basal forebrain cholinergic neurons are located in the nucleus basalis of Meynert, medial septal nucleus and vertical and horizontal limb nuclei of Broca [97]. These cholinergic neurons project to the hippocampus, amygdala, and cerebral cortex and modulate memory and attention functions [98]

5.3. Muscarinic receptors:

mAChRs are ACh receptors that are activated by muscarine. There are 5 types of muscarinic receptors that are classified into two groups: M_1, M_3 and M_5 vs. M_2 and M_4 [99, 100]. Among these, M_1, M_2 and M_4 are the main mAChR expressed in the CNS. M_1, M_3, and M_5 mAChR are Gq-coupled and largely post-synaptic. They activate phospholipase C, intracellular calcium, inositol triphosphatase, and mitogen-activated protein kinase [100]. M_1 mAChR, the predominant mAChR in the CNS, is implicated in learning and memory processes. Consistent with their functions, they are distributed in the cerebral cortex, hippocampus and striatum [101]. M_3 mAChR are sparsely distributed in the CNS and their functions are not well-known [102] M_5 receptors, expressed on the DA neurons in the ventral tegmental area and substantia nigra, facilitate DA release in the nucleus accumbens [103].

M_2 and M_4 mAChR are usually presynaptic and inhibit adenylyl cyclase and voltage-operated calcium channels and activate mitogen-activated protein kinases and G-protein-activated inwardly rectifying potassium channels M_2 receptors are expressed in the brainstem, thalamus, cortex, hippocampus and striatum and inhibit ACh and DA release [101, 102, 104]. M_4 mAChR are found in the midbrain, cortex, hippocampus, and striatum [101, 102]. Stimulation of M_4 mAChR inhibits ventral tegmental area DA neurons, leading to reduced DA release in the nucleus accumbens [105].

The net effect of mAChR signaling is to reduce the number of synaptic inputs that neurons receive, resulting in increased responsivity for the remaining synaptic inputs [106]. This is achieved by increased membrane resistance and input sensitivity, through activation of M_1, M_3 and M_5 receptors, and reduced neurotransmitter release, through activation of M_2 and M_4 mAChR. These effects are consistent with enhanced specificity of neuronal communication and memory encoding function of mAChRs [107, 108].

5.3.2. Nicotinic receptors: nAChRs are ACh receptors that are activated by nicotine. nAChRs are ligand-gated ion channels arranged around a central pore, which is permeable to sodium, potassium, and calcium ions. nAChRs are comprised of pentameric combinations of α subunits (α2-α10) and β subunits (β2-β4) [109–111]. They can be either homomeric nAChRs that consist of one type of α subunits (e.g., α6 or α7) or heteromeric nAChRs that consist of a combination of α and β subunits (e.g. α4β2, α3β4). α4β2 and α7 subtypes represent the majority of nAChRs in the brain [109–111]. Most nAChRs in the brain are located presynaptically and increase the release of ACh, DA, serotonin, glutamate, GABA, and norepinephrine[112–115]. Stimulation of α4β2 nAChR located on the DA cell bodies in

the ventral tegmental area shifts these cells from tonic to phasic firing mode, which results in increased DA release in both the nucleus accumbens and the prefrontal cortex. β_2-containing receptors are critical for the addictive as well as cognitive performance-enhancing properties of nicotine [116, 117]. Nicotine withdrawal has been shown to reduce brain reward function in rats, which may also be mediated by α_4, β_2, α_7 subunits [118].

5.4 Insights into the cholinergic system from human genetics studies:

GWAS have highlighted some important functions for certain genes within the cholinergic system. There are 5 genes within the human genome that encode mAChRs (abbreviated as; *CHRM1*, *CHRM2, CHRM3, CHRM4* and *CHRM5*), and 16 genes that encode nAChRs, including multiple alpha and multiple beta subunits (abbreviated as; *CHRNA1*-7,9,*10*, *CHRNB1-4*, *CHRNE*, *CHRND*, *CHRNG*). AChE, is encoded by the *Acetylcholinesterase* gene, which is abbreviated as *AChE*, and ChAT is encoded by the *Choline O-acetyltransferase* gene, which is abbreviated as *CHAT*. Among the most robust and well-characterized associations based on GWAS is genetic variation within the *CHRNA5–A3–B4* gene cluster on chromosome 15 and measures of nicotine intake, such as the number of cigarettes smoked per day and cotinine levels [119, 120]. However, it is unclear whether genetic variation encoded within these genes is related to substance use disorder phenotypes like OUD. It is important to note that sample sizes for genetic studies of OUD have been smaller than many genetic studies of medical traits that have yield highly informative genetic associations. This likely limits the statistically power that is required to detect genetic effects associated with OUD. Also, whether genetic variants in the cholinergic system, (including SNPs with strong statistical links to clinically-relevant traits) affect the response to medication is a topic of ongoing research.

6. Interactions between opioids and ACh

Several lines of evidence suggest a close functional coupling between ACh and opioid transmission. An earlier study demonstrated that morphine administration in mice increased ACh concentration in striatum that coincided with the time-course of the analgesic effects of morphine [121]. Similar increases in ACh levels have also been observed in the spinal cord following morphine administration in monkeys [122]. In another study conducted in rats, ACh administration increased the release of beta-endorphin, Leu-enkephalin and dynorphin in the spinal cord [123]. Consistent with these findings, an increase in ACh level in the CNS produced by systemic administration of acetylcholine esterase inhibitors enhanced the analgesic effects of opioids (see next section for details). In contrast to the actions of MOR agonists on ACh release, N/OFQ reduced ACh release in cortical and hippocampal slices and NOP knock-out mice showed increased ACh release in hippocampus [124, 125]. These findings support the possible role of NOP receptors in hippocampal cholinergic function.

In a recent study, chronic morphine treatment increased ACh transmission in the laterodorsal tegmental nucleus (LDTg)/pedunculopontine tegmentum, which provide stimulatory cholinergic inputs to the ventral tegmental area DA cells [126]. This effect may represent one potential mechanism by which ACh transmission modulates neuroadaptation to chronic morphine exposure. On the other hand, chronic nicotine exposure attenuated the analgesic

effects of opioids, suggesting a cross-tolerance between opioids and nicotine. In humans, opioids and nicotine products (e.g., tobacco cigarettes) are commonly abused together and smoking status is an important predictor for using higher doses of prescription opioids and misuse of prescription opioids [127, 128]. Together, these studies suggest that ACh and opioids may play an important role in modulating the pharmacological effects of each other as well as impact ongoing use and addiction to opioids and nicotine. The role of nAChR and mAChR in mediating the analgesic and rewarding effects of opioids remains controversial, partly due to lack of pharmacological specificity of currently available drugs targeting these receptors [126, 129]. The neural circuits mediating the effects ACh on opioid analgesia, reward, withdrawal and behavioral sensitization remain to be elucidated.

7. ACh as treatment target for OUD: Current Evidence

We performed a PubMed search between January to March 2018 to identify preclinical and clinical publications relevant to clarifying the role of the cholinergic system as a potential treatment target for OUD. The search was limited to English language articles. Preclinical studies were included 1) if they included outcomes related to OUD including opioid self-administration, conditioned place preference, opioid sensitization, opioid withdrawal and opioid analgesia and 2) used a cholinergic medication. Clinical studies were included if they examined opioid use, opioid withdrawal and opioid adverse effects. We included studies published between 1999 and 2018, as previous studies have been reviewed elsewhere [130, 131].

Preclinical and clinical studies examining the effects of cholinergic compounds on opioid-related outcomes are summarized in Table 3. The highlights from the table are described below. The majority of studies are preclinical. Regarding opioid reward, morphine self-administration was decreased by arecoline (AREC), a non-selective partial mAChR agonist, and by scopolamine, a mAChRs antagonist [132, 133]. Morphine-induced conditioned place preference was inhibited by donepezil or rivastigmine, which are both AChE inhibitors, and by scopolamine [134–136]. Morphine-induced sensitization to locomotor effects was attenuated by mecamylamine, a nAChR antagonist [126], while morphine-induced behavioral sensitization was attenuated by huperzine A, an AChE inhibitor [137]. AREC also reduced reinstatement of drug seeking for morphine [133]. Regarding effects on analgesia, both donepezil or rivastigmine increased morphine's acute analgesic effects [138]. Donepezil, rivastigmine and scopolamine each attenuated the development of tolerance to morphine's analgesic effects [134, 138, 139]. In contrast, mecamylamine did not affect tolerance to morphine's analgesic effects [126].

Naloxone-induced opioid withdrawal symptoms were attenuated by diisopropylflurophosphate (DFP; an AChE inhibitor which acts both centrally and peripherally), echothiophate (a selective peripherally-acting AChE inhibitor), or AREC [140], or scopolamine [141]. Nicotine and the nAChR antagonist, lobeline, attenuated opioid-withdrawal symptoms induced by naloxone, but mecamylamine was not effective [142].

In human studies, donepezil reduced opioid-induced sedation without affecting analgesia in a sample of cancer patients (n=6) that were receiving high doses of opioids [143]. In another study of in-patients undergoing opioid taper, varenicline was well-tolerated. In one clinical study, a non-significant trend for decreased opioid withdrawal symptoms compared to placebo was noted [144]. In a separate clinical study, scopolamine was tested in 91 opioid-dependent patients undergoing opioid taper. Scopolamine, compared to placebo, reduced anxiety, depression, craving and prolonged time to relapse [145]. Overall, there are currently very limited clinical data to support a role of cholinergic agents in treating OUD.

8. Future directions for medication development

The preclinical and clinical studies summarized above support the promise of medications targeting the cholinergic system for the treatment of OUD. These include AChE inhibitors and medications targeting nAChR and mAChR. The adverse effects of opioid agonists (i.e., methadone or buprenorphine) include nausea, vomiting, constipation, endocrine disturbances, decreased libido and increased pain sensitivity or hyperalgesia, and possible cognitive deficits [31–34]. Naltrexone's adverse effects include nausea, headaches, insomnia, injection site pain (injectable form), elevation of transaminases, hypertension, nasopharyngitis, and influenza, possible depression or anhedonia [146, 147]. The most common adverse effects of AChE inhibitors include nausea, vomiting, diarrhea, loss of appetite, headache and dizziness [148]. For intravenous scopolamine, common adverse effects include reduced sweating, dry skin, dry mouth, amnesia, somnolence and less commonly hallucinations and confusion [149]. Overall, cholinesterase inhibitors have a long-established safety profile and their use for OUD is feasible. The more serious adverse effects of scopolamine (e.g., confusion or hallucinations) require closed monitoring of the adverse effects at the time and after infusion. Recent technological advances, such as in genomics, can facilitate the development of new medications by identifying and prioritizing drug targets, helping improve outcomes for established treatments (i.e., by patient stratification), and capturing in-depth treatment responses (e.g. biomarkers) to assist in evaluating efficacy [150, 151]. As such, it is a promising time to develop and evaluate new medications for OUD treatment.

8.1 Medications Targeting AChE

AChE inhibitors rivastigmine, donepezil, and galantamine, are marketed for the treatment of dementia [93, 152, 153]. AChE inhibitors differ in their pharmacological profiles although their efficacy for treatment of dementia is comparable [154]. Galantamine is a positive allosteric modulator of nAChRs, resulting in increased synaptic DA and glutamate levels [155, 156]. Donepezil and rivastigmine are more potent AChE inhibitors than galantamine [157, 158]. Rivastigmine may also modulate a glutamatergic transporter [159]. In contrast to donepezil and rivastigmine, galantamine also increases the activity of nAChR via allosteric effects [155]. As summarized in Table 3, in preclinical studies AChE inhibitors attenuated opioid reinforcement, enhanced the analgesic effects of opioids, reduced tolerance to opioid analgesia and attenuate opioid withdrawal. In human studies, galantamine showed promising results as a treatment for alcohol or tobacco use disorder [160, 161]. In a recent randomized clinical trial, galantamine, compared to placebo, reduced cocaine use among cocaine and

opioid addictive individuals that were stabilized on methadone [162]. AChE inhibitors have not been examined for the treatment of OUD in humans. Clinical trials testing the potential efficacy of AChE inhibitors on OUD are warranted.

8.2 Medications Targeting nAChR

Although there is some evidence that medications targeting nAChR have efficacy for treating substance use disorders, the evidence specifically for OUD is mixed. In preclinical studies, the nAChR antagonists lobeline and mecamylamine did not show consistent efficacy across outcomes [126, 136, 138, 142]. Varenicline, a partial agonist at the $\alpha_4\beta_2$ nAChR, is marketed for smoking cessation and has also shown promise for alcohol use disorder [163–165]. Varenicline also reduced rates of smoking in opioid addicted individuals maintained on methadone, however there was no effect on opioid use [166]. Functional genetic variation encoded in nAChRs, such as the well-characterized and common variants in the *CHRNA5–A3–B4* gene cluster, might affect the response to some medications. For smoking cessation, reports on differential responses to varenicline based on *CHRNA5–A3–B4* gene cluster variants have been mixed [167–169]. As noted above, other nicotinic receptor genes encode variants with strong trait associations (e.g. *CHRNA4*) that might affect nACHR function and response to treatment. As research in this area progresses, it will be important to consider these known genetic effects and how they might shape the response to medication that targets nAChRs.

8.3 Medications targeting mAChR

As outlined in Table 1, in preclinical studies, both mAChR agonists (e.g., AREC) and the mAChR antagonist scopolamine showed promising effects. AREC reduced both opioid self-administration and reinstatement of opioid self-administration and blocked naloxone-induced opioid withdrawal [133]. Scopolamine also prevented development of tolerance to morphine and attenuated naloxone-induced opioid withdrawal [138]. These findings seem to be contradictory as both an antagonist and an agonist of mAChR have similar effects. It is important to note that AREC is a non-selective partial mAChR agonist, which also has significant agonist effects in multiple nAChR subtypes [170]. Similarly, scopolamine influences nAChR and N-Methyl-D-aspartate (NMDA) receptors in addition to its mAChR antagonist effects [171]. Several mAChR agonists, including AREC, carbachol, and cevimeline have been examined in clinical trials, although not for OUD[172]. Development of these compounds have been abandoned due to their adverse effects, like nausea and diarrhea, which are likely mediated by the drug acting at peripheral M_2 and M_3 mAChRs. Scopolamine, however, is currently used for post-operative nausea and motion sickness. It has rapid anti-depressant effects with some reports showing efficacy for treating major depressive disorder [173]. It is noteworthy that the Liu et al. study reported that compared to methadone treatment, scopolamine reduced depression and anxiety during opioid tapering [145]. Depression is elevated among those seeking treatment for substance use disorders [174] and common in the population, with 40% of an individual's risk for depression attributed to their genetics [175]. Scopolamine could be most effective for those that are at increased risk for depression during detoxification, either based on their genetics or a pre-existing condition (e.g. major depressive disorder or bipolar). Further studies testing the efficacy of scopolamine for OUD are warranted.

9. Conclusion

New strategies are needed to meet the challenges associated with the current OUD epidemic facing the United States and other countries. MAT, using opioid-based pharmacotherapies (methadone, buprenorphine and naltrexone), are the only current US Food and Drug Administration-approved treatments for OUD. MAT effectiveness is limited by high drop-out rates and the adverse effects associated with long-term treatment with opioid medications, such as cognitive deficits, endocrine disturbances, decreased libido and increased pain sensitivity or hyperalgesia. The cholinergic system shows promise as a treatment target for OUD. The cholinergic and opioid systems are tightly linked, with ACh involved in CNS functions that are relevant to the development and maintenance of OUD. Several cholinergic medications show promise in clinical and preclinical studies and further studies testing the efficacy of cholinergic medication for OUD are warranted.

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Key Points

- Opioid use disorder is increasing in the population and new medications could help reduce the negative impact to global health.
- Medications that target the brain cholinergic system show promise in clinical and preclinical studies of opioids.
- Further studies on cholinergic medications for opioid use disorder are warranted.

Table 1:

Endogenous opioid peptides and their high affinity receptors

PRECURSOR	PEPTIDES	RECEPTOR ACIVITY
POMC	β-endorphin	delta, mu
PENK	leucine-enkephalin methionine -enkephalin	delta, mu
PDYN	dynorphins A and B, and neoendorphins	delta, kappa, mu
PNOC	nociceptin	NOP

POMC, proopiomelanocortin; PENK, preproenkephalin; PDYN, preprodynorphin; PNOC, prepronociception; NOP, nociception opioid peptide receptor.

Table 2:

Potentially clinically relevant genetic polymorphisms in opioid receptor genes

Gene	SNP	Potential Functional Effect	Association findings
OPRM1	rs1799971	Missense Mutation (N40D) and *OPRM1* mRNA levels	Mixed for OUD and OUD treatment
	rs3778150	*OPRM1* mRNA levels	Heroin dependence
	rs73568641	None observed	Methadone dose
	rs10485058	Disrupts microRNA regulation of *OPRM1*	OUD treatment response
OPRD1	rs678849	None observed	OUD treatment response

OUD, opioid use disorder; SNP, single nucleotide polymorphism; *OPRM1*, mu opioid receptor gene; *OPRD1*, delta opioid receptor gene. N, asparagine; D, aspartic acid.

Table 3:

Clinical and preclinical studies of ACh targeting medications and opioid outcomes.

Study type	Reference	Drugs	Procedures	Outcome	Key findings
Rodent	Bajic et al. 2015 [126]	Saline, morphine, mecamylamine	The locomotor effects of chronic morphine either paired with saline or mecamylamine: The test day treatments included, saline or morphine paired with either saline or acute mecamylamine. Withdrawal induced by single injection of naloxone (20mg/kg ip) immediately after locomotor testing Antinociceptive effects of morphine compared among the following treatment groups: morphine, mecamylamine, morphine + mecamylamine, and saline. Drug exposure for each group was twice daily for 6.5 days.	Sensitization to locomotor effects	Mecamylamine (acute and chronic) reduced morphine-induced locomotor sensitization.
				Pain perception	There was no effect of mecamylamine on morphine induced antinociceptive tolerance.
				Withdrawal (naloxone-induced)	Mecamylamine did not reduce withdrawal symptoms overall.
	Buccafusco et al 2000 [140]	Morphine, DFP, echothiophate, AREC, naloxone	Morphine dependence induced using morphine paired with saline, DFP (50 or 250 μg/kg daily), AREC (1 mg/kg, twice daily; s.c.), or echothiophate (2.5 μg/kg daily; s.c.).	Withdrawal (naloxone-induced)	DFP, AREC and echothiophate each separately reduced severity of withdrawal symptoms.
	Buccafusco et al. 2007 [133]	Morphine, arecoline hydrobromide (AREC), scopolamine	Morphin SA and reinstatement during protracted morphine withdrawal comparing the following morphine SA phase treatments: saline, twice daily AREC (0.25 or 1 mg/kg s.c.), twice daily scopolamine (0.5 mg/kg) + AREC (1 mg/kg), and saline (days 1–9) + twice daily AREC (1 mg/kg) starting day 10.	Self-administration	AREC reduced morphine SA. Scopolamine did not modify the effect of AREC.
				Reinstatement of drug-seeking	AREC reduced ‘drug seeking’ lever presses during reinstatement. This effect was blocked by scopolamine co-treatment. AREC inhibited withdrawal symptoms (weight loss). This effect was reversed by scopolamine (i.e., scopolamine + AREC was similar to saline).
	Gawel K et al. 2014 [136]	Donepezil, rivastigmine, mecamylamine, scopolamine	The effect of saline, donepezil or rivastigmine on CPP to morphine (5 mg/kg).	Motivational Effects of Opioids	Donepezil and rivastigmine attenuated acquisition and expression of mCPP and inhibited reinstatement of mCPP. Inhibition of reinstatement was reversed by mecamylamine but not scopolamine.
	Gawel K et al. 2017 [138]	Donepezil, rivastigmine, mecamylamine, Scopolamine	Hot plate test with morphine (5 mg/kg) or saline + treatment with saline, donepezil or rivastigmine	Tolerance to antinociceptive effects	Donezepil and Rivastigmine each increased morphine’s antinociceptive effects. Effects reversed by scopolamine but not mecamylamine. Not influence

Study type	Reference	Drugs	Procedures	Outcome	Key findings
					analgesia in already morphine tolerant mice.
	Hikida et al. 2003 [135]	Morphine, donepezil, galantamine	CPP and anti-nociceptive effects to morphine (1.0, 5.0 mg/kg), morphine + naloxone, and cocaine.	Motivational and anti-nociceptive effects	Ablation of cholinergic neurons increased acquisition of morphine CPP, mCPA following naloxone, withdrawal (jumping) after naloxone, anti-nociception effects. Donepezil reduced morphine CPP
	Li et al. 2010[132]	Morphine, scopolamine, nicotine	Morphine (3.2–10 mg/kg), scopolamine (0.032–1.0 mg/kg) and nicotine (0.1–1 mg/kg) were administered alone or in combination to assess effect on lever pressing (previously paired with food reward).	Drug reinforcement	A single acute dose of scopolamine dose-dependently decreased responding to morphine (only significant at highest 1.0 dose)
	Mukae T et al. 2015 [176]	Pilocarpine, pirenzepine, donepezil, atropine	Thermal paw withdrawal (latency); mechanical paw pressure test; electrical stimulation paw withdrawal	Antinociceptive effects	Pilocarpine (1 mg/kg) and donepezil (10 μg/kg) increased pain threshold and reduced allodynia. The effects of pilocarpine and donepezil were blocked by pirenzepine and atropine, respectively.
	Neugebauer et al. 2013 [142]	Saline, nicotine, lobeline, mecamylamine, morphine, and naloxone	Morphine-tolerant mice, following their last morphine dose, were administered either saline, nicotine (0.2 or 0.4mg/kg), or lobeline (3 or 10 mg/kg) or mecamylamine (1 mg/kg).	Withdrawal (naloxone-induced)	Nicotine (0.4 mg/kg) or lobeline (3 mg/kg) decreased naloxone-induced jumping. No effect of mecamylamine.
	Sharifipour M et al. 2014 [139]	Donepezil	The effects of donepezil on tail flick latency in morphine tolerant mice.	Tolerance to antinociceptive effects	Donepezil delayed tolerance to morphine's analgesic effects and reduced the number of apoptotic cells on the cortex and lumbar spinal cord.
	Sun et al. 2017 [137]	huperzine A	Effects of huperzine A (0.2, 0.3 or 0.4 mg/kg) or saline on morphine−induced behavioral sensitization.	Sensitization to locomotor effects	High doses of huperzine A when administered daily prior to morphine during the initial development of morphine dependence, attenuated morphine-induced locomotor activity
	Xiang et al. 2006 [141]	Morphine, scopolamine, naloxone	Morphine (10, 20, 30, 40, or 50 mg/kg/injection) for 7 days, with or without scopolamine pre-treatment (0.5 mg/kg), or followed by 7 days of scopolamine (0.5 mg/kg). Withdrawal induced with naloxone.	Withdrawal (naloxone-induced)	Each scopolamine treatment condition reduced some symptoms of withdrawal. However, an increase in 'wet dog shakes' was noted for the pre-treatment condition.
				Behavioral effects and drug clearance	Each scopolamine treatment condition increased water consumption, urine volume and total volume of morphine excreted in urine.

Study type	Reference	Drugs	Procedures	Outcome	Key findings
	Zhou et al. 1999 [134]	Scopolamine, morphine, naloxone	The morphine conditioning phase consisted of morphine (10 mg/kg) daily for 3 days. Animals received scopolamine (0.5 mg/kg) twice daily for 7 days prior to the morphine conditioning phase, or with scopolamine (0.5 mg/kg) 15 min before or 1 h after daily morphine treatment during the conditioning phase.	Conditioned place preference	Scopolamine, administered prior to or during the morphine conditioning phase, attenuated morphine-induced conditioned place preference
			Animals were treated for 9 days with saline, 0.5 mg/kg scopolamine, or	Pain perception	Scopoloamine, prior to and alongside repeated morphine administrations, attenuated tolerance to morphine's analgesic effects.
			10 mg/kg morphine twice daily, or treated with scopolamine for 7 days prior to morphine treatment at 0.5 mg/kg 15 min before each morphine injection, or at 1.0 mg/kg once daily between two morphine injections.		
			The effect of pretreatment with scopolamine or saline on Morris Water Maze (prior to test, for 7 consecutive days).	Cognition	Scopolamine treated animals performed worse on Morris water maze (a measure of spatial learning and memory).
			Saline, scopolamine (0.5 mg/kg), or morphine (10 mg/kg) twice daily for 9 days, or scopolamine (0.5 or 1.0 mg/kg) twice daily for 7 days prior to morphine treatment at 0.5 mg/kg 15 min before each morphine injection or at 1.0 mg/kg once daily between morphine injections.	Withdrawal (naloxone-induced)	Scopolamine attenuated naloxone-induced withdrawal symptoms.
Human	Liu S et al. 2013 [145]	Scopolamine, chlorpromazine	Opioid dependent subjects were randomized to scopolamine detox (SD; n=46, 7 female) or methadone detox (MD, n=45, 5 female). SD given IV scopolamine + chlorpromazine under anesthesia.	Withdrawal	SD attenuated withdrawal and delayed relapse. The most common SD treatment adverse events was a confused state in 54% of participants.
	Hooten WM et al. 2015 [144]	Varenicline, placebo	Randomized, single blind pilot study including chronic pain patients comparing varenicline to placebo. 11/11 placebo completers and 7/10 varenicline completers.	Withdrawal	No significant varenicline effects on withdrawal, pain or depression symptoms. Varenicline well-tolerated.
	Slatkin NE et al. 2001 [143]	Donepezil	Cancer patients (n=6) receiving oral morphine (> 200 mg).	Antinociceptive effects	Donepezil reduced opioid-induced sedation and was well-tolerated without negatively impacting analgesia.
	Poling et al. 2010 [166]	Varenicline and placebo	Cocaine, tobacco and opioid use was evaluated in a sample of 31 methadone-maintained tobacco smokers with opioid and cocaine dependence	Tobacco, cocaine and opioid use	No effect of varenicline on opioid positive urines compared to placebo. Varenicline reduced the number of cigarettes smoked per day.

AREC, arecoline; DFP, diisopropylflurophosphate; SA, self-administration; CPP, conditioned place preference; mCPP, morphine, conditioned place preference; CPA, conditioned place aversion; mCPA, morphine conditioned place aversion