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Targeting Muscarinic Receptors to Treat Schizophrenia

Daniel J. Foster[1,2], **Zoey K. Bryant**[1,2], **P. Jeffrey Conn**[1,2]

[1]Department of Pharmacology, Vanderbilt University, Nashville, TN 37232

[2]Department of Warren Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, TN 37232

Abstract

Schizophrenia is a severe neuropsychiatric disorder characterized by a diverse range of symptoms that can have profound impacts on the lives of patients. Currently available antipsychotics target dopamine receptors, and while they are useful for ameliorating the positive symptoms of the disorder, this approach often does not significantly improve negative and cognitive symptoms. Excitingly, preclinical and clinical research suggests that targeting specific muscarinic acetylcholine receptor subtypes could provide more comprehensive symptomatic relief with the potential to ameliorate numerous symptom domains. Mechanistic studies reveal that M1, M4, and M5 receptor subtypes can modulate the specific brain circuits and physiology that are disrupted in schizophrenia and are thought to underlie positive, negative, and cognitive symptoms. Novel therapeutic strategies for targeting these receptors are now advancing in clinical and preclinical development and expand upon the promise of these new treatment strategies to potentially provide more comprehensive relief than currently available antipsychotics.

Keywords

Muscarinic; Schizophrenia; Dopamine; Acetylcholine

1.1 Overview of schizophrenia and current treatments

Schizophrenia is a severe neurological disorder characterized by a complex and wide array of symptoms that can cause immense suffering for affected patients and their families. The term schizophrenia comes from the Greek for “splitting of the mind”; however, we have learned a lot about this disorder over the years with our current understanding reflecting that schizophrenia is a complex disorder that impacts multiple brain systems and has numerous genetic and environmental components [1]. Current antipsychotics differ in their pharmacological profiles but all share the common feature of reducing signaling of the neurotransmitter dopamine (DA) through the D2 subtype of DA receptor, a mechanism that

Correspondence should be addressed to P. Jeffrey Conn, Vanderbilt School of Medicine, 1205 Light Hall, Nashville, TN 37232. jeff.conn@vanderbilt.edu.

may be necessary for antipsychotic efficacy of currently used therapeutics [2]. These medications broadly show efficacy in improving symptoms in the positive domain of the disorder, which includes hallucinations, disorganized speech, and delusions. However, these strategies often have profound side effect liabilities including undesirable metabolic and motor effects [3]. In addition to unwanted side effects, the inability of current therapies to treat negative and cognitive symptoms is another major drawback of both typical and atypical antipsychotics, as the anhedonia, lack of motivation, social withdrawal, and deficits in attention and working memory can be very difficult for patients to cope with [4].

Due to the wide array of symptoms, it should be no surprise that there are multiple theories and frameworks from which to view the development and treatment of schizophrenia. Views on how to best categorize schizophrenia patients have changed over the years but now many clinicians believe that schizophrenia is a syndrome spanning a spectrum of underlying etiologies [5]. The DA hypothesis of schizophrenia has been an important model for understanding how dysregulations of DA signaling relate to different symptoms. Briefly, this model posits that dysregulation of presynaptic DA release leads to 1.) DA hyperactivity in the striatum, which plays a key role in mediating positive symptoms and 2.) DA hypoactivity in cortical structures, which plays a key role in mediating negative and cognitive symptoms [6].

Typical (or first-generation antipsychotics, e.g. Haloperidol) are D2 receptor antagonists that can provide efficacy in treating positive symptoms. However, in some patients, the therapeutic window between antipsychotic efficacy and motor side effects is very narrow which greatly limits the utility of these treatments. The advent of atypical (or second-generation antipsychotics, e.g. Risperidone, and Clozapine) has provided relief for some of the patients that do not respond well to first-generation drugs [7]. Individual atypical antipsychotics have unique pharmacological profiles but are generally distinguished by less robust activity at the D2 receptor, as well as by their ability to modulate numerous other receptors including serotonin receptors (e.g. 5HT2A), adrenergic receptors, histamine receptors, and acetylcholine (ACh) receptors [8]. Some atypical antipsychotics even have weak agonist activity at the D2 receptor and this mechanism of action may offer advantages by providing some level of D2 receptor activity rather than complete blockade. However, these partial agonists are still thought to mediate their antipsychotic activity by reducing the ability of the full agonist DA to bind and signal through these receptors [9]. Due to these differences in pharmacology, atypical antipsychotics are generally characterized by reduced motor side effects, but they are also more likely to produce undesirable metabolic side effects [10, 11]. Similar to typical antipsychotics, the second-generation or atypical antipsychotics tend to show little to no efficacy in providing relief for negative and cognitive symptoms, and in some cases may even worsen symptoms [7, 12]. Given both the devastating consequences of negative and cognitive symptoms on patients' quality of life, as well as the large number of patients that are resistant to currently available therapies, it is critical that we identify novel therapeutic strategies that move beyond D2 antagonism and provide more extensive relief that spans across multiple symptom domains.

1.2 Clozapine efficacy in treatment-resistant psychosis and effects on muscarinic acetylcholine receptor (mAChR) signaling

Many schizophrenia patients have treatment-resistant psychosis as defined by being non-responsive to at least two antipsychotic medications. This treatment-resistant clinical phenotype is a large problem and represents up to 30% of the schizophrenia patient population [13]. Clozapine has shown superior efficacy as a therapy for treatment-refractory psychosis compared to other antipsychotics [14], and shows some degree of efficacy in treating 60–70% of patients who are refractory to other atypical antipsychotic drugs [15]. In addition, clozapine is somewhat uncommon in its ability to improve cognitive symptoms in some subsets of patients [16], although this effect is inconsistent as a lack of efficacy or deleterious effects are observed in other patients [17]. What pharmacologically distinguishes clozapine from other antipsychotics to create this particular clinical profile? Several theories, including the balance of activity at 5HT2A, 5HT6, D2, and D4 receptors have been proposed, but do not appear to be unique to clozapine [18–20]. As discussed below, one possible explanation that differentiates clozapine from olanzapine and other treatments with seemingly similar pharmacological profiles is through a complicated bidirectional modulation of mAChRs by clozapine and its metabolites.

Clozapine itself has a high affinity for all 5 mAChR subtypes and has been observed to have either weak partial agonist activity (6–25% of a full agonist) [21, 22] or competitive antagonist activity [23, 24] in over-expressing cell lines. However, in striatal tissue (with endogenous receptor expression that is lower than over-expressing cell lines) clozapine acts as a competitive mAChR antagonist [25]. Regardless of the ability of clozapine to act as a true mAChR antagonist, or as a weak partial agonist, the net effect of clozapine in physiological tissue is a reduction in ACh signaling through mAChRs. However, the properties of clozapine as an antagonist / weak agonist of mAChRs does not distinguish clozapine from other antipsychotic medications, as many first-generation and second-generation antipsychotics can also reduce mAChR signaling.

Interestingly, it may be not clozapine *per se* that distinguishes this therapeutic from other antipsychotics, but the primary metabolite N-Desmethylclozapine (norclozapine). Following systemic administration, clozapine is readily metabolized to norclozapine in the liver by cytochrome P450 1A2 (CYP1A2), CYP3A4, and to a lesser extent by CYP2C9 and CYP2D6 [26]. Surprisingly, norclozapine is a robust agonist of mAChRs with particularly strong activity at the M1 receptor subtype [24, 27]. This M1 agonist activity is mediated via binding at an allosteric site and is observed both in over-expressing cells, and in acute rodent brain slices, where it can induce mAChR-dependent increases in *N*-methyl-D-aspartate (NMDA) receptor-mediated currents and MAPK signaling in hippocampal tissue [24, 27]. No other currently used antipsychotics, or their metabolites, are known to act as mAChR agonists, which raises the possibility that this agonist activity of norclozapine may be one of the unique properties that distinguish the parent drug clozapine from other atypical antipsychotics.

Several lines of evidence suggest that activation of M1 receptors plays a key role in the ability of clozapine to modulate schizophrenia-related circuitry and mediate behavioral

efficacy in rodents. The ability of clozapine and norclozapine to reverse PCP-mediated deficits in novel object recognition are blocked by the inclusion of either scopolamine or an M1-selective antagonist, whereas mAChR blockade did not affect the efficacy of the atypical antipsychotic lurasidone [28]. Interestingly, sub-efficacious doses of clozapine in reversing MK-801-induced deficits in sensorimotor gating are potentiated by co-administration of an M1 positive allosteric modulator (PAM), an effect that is not observed in M1 knockout (KO) mice [29]. On a circuit level, local administration of norclozapine can alter extracellular DA content as assessed via microdialysis in the PFC and hippocampus without altering DA in the nucleus accumbens. While clozapine has no effects on DA in the PFC alone, local co-administration of clozapine completely blocks the effects of norclozapine [30], consistent with these molecules having opposing effects on M1 activation.

Interestingly, lower clozapine:norclozapine ratios in schizophrenia patients (which would be associated with a greater mAChR agonist profile) are associated with improvements in working memory and executive function while higher ratios are associated with cognitive deficits [31, 32]. The ratio of clozapine:norclozapine appears to be much more predictive of cognitive effects than either measure alone, which could be attributable to the conflicting effects of these compounds on mAChR activity. In recent clinical studies, it was found that norclozapine is well tolerated in patients [33]; however, a phase 2b trial of norclozapine was discontinued due to lack of efficacy [34, 35]. While the lack of efficacy for norclozapine in the clinic is disappointing, it is consistent with results from preclinical studies showing that direct administration of norclozapine alone does not have efficacy in preclinical models predictive of antipsychotic-like activity [36]. However, it is still unclear if norclozapine would show effects in the subset of patients with treatment-resistant schizophrenia patients, as the enrollment for the clinical trial was not restricted to this patient sub-group. It is also possible that the presence of the parent drug clozapine may be required to observe the beneficial effects of norclozapine. In addition to mediating beneficial effects, it is also possible that norclozapine could be responsible for some of the side effects seen following clozapine administration such as hyper-salivation [37], which is consistent with mAChR activation. While there is currently limited enthusiasm for advancing norclozapine as a stand-alone therapy, it is not yet clear if co-dosing clozapine/norclozapine with M1 PAMs, or combining clozapine administration with modulators of CYP1A2 metabolism to obtain a more favorable norclozapine:clozapine ratio [38] could improve outcomes. However, these data collectively fit with the hypothesis that mAChR activation (and M1 activation in particular) could mediate beneficial effects in ameliorating cognitive deficits in schizophrenia and could be a key signaling pathway through which clozapine exerts its unique clinical profile.

1.3 Cholinergic signaling and schizophrenia

While the link between DA and schizophrenia has been extensively studied, it is clear that many other neurotransmitter systems are dysregulated in this disease. The psychotomimetic effects of NMDA receptor antagonists, as well as postmortem studies in schizophrenia patients [39], have led to a glutamatergic hypothesis of schizophrenia that highlights the importance of glutamatergic signaling, especially through NMDA receptors, as a key regulator of schizophrenia-related circuitry [40]. There is also growing evidence that

inhibitory γ-aminobutyric acid (GABA) signaling is dysregulated in schizophrenia, particularly in the cortex [41]. Dopaminergic, GABAergic, and glutamatergic signaling can all be strongly modulated by the activation of the cholinergic system, and below we review clinical and preclinical data suggesting that modulation of mAChRs represents an exciting new strategy with the potential of providing broad symptomatic relief to schizophrenia patients that is not currently provided by available treatment strategies (Figure 1).

The cholinergic system is comprised of two families of receptors that are activated by binding of the neurotransmitter acetylcholine (ACh). This includes a family of ion channels called nicotinic acetylcholine receptors (nAChRs), and a family of G-protein coupled receptors called muscarinic acetylcholine receptors (mAChRs). Both nAChRs and mAChRs have been found to modulate brain circuitry dysregulated in schizophrenia [42]. While nAChRs are primarily targeted for cognitive enhancement, the effects of mAChRs appear to have greater potential for providing relief for a wide range of schizophrenia symptoms. There are five subtypes of muscarinic receptors (M1-M5). M1, M3, and M5 typically couple to the G-protein $G\alpha_q$ leading to activation of phospholipase C, IP_3 production, and Ca^{2+} mobilization. In contrast, M2 and M4 receptors couple to the G-protein $G\alpha_i$ resulting in a decrease in adenylyl cyclase function and reduced cAMP formation. It should be noted that these receptors are more complex than simply being an on/off switch for these canonical signaling pathways and can engender important physiological effects through numerous signal transduction pathways [43]. mAChR antagonists showing little to no selectivity can induce schizophrenia-like symptoms in healthy controls [44], and worsen positive symptoms while alleviating negative symptoms in schizophrenia patients[45]. Collectively, these early studies suggesting that targeted activation of specific mAChRs could be a novel therapeutic strategy for treating schizophrenia. As discussed in detail below and highlighted in Figure 1, the M1, M4, and M5 mAChR subtypes can all robustly modulate schizophrenia-related circuitry and are intriguing novel targets for moving schizophrenia treatments beyond the currently available dopamine receptor based approaches.

1.4 The mAChR agonist xanomeline shows efficacy in treating positive, negative, and cognitive symptoms

Pharmaceutical companies have been targeting mAChRs as potential cognitive enhancers for decades, owing to the robust pro-cognitive effects seen in both clinical and preclinical settings with compounds that either directly activate mAChRs or indirectly activate mAChRs by increasing cholinergic signaling (ie acetylcholinesterase inhibitors). Using traditional therapies that lack specificity among mAChR subtypes requires tailoring a therapy to hit a therapeutic window between beneficial effects (e.g. pro-cognitive efficacy) and classical cholinergic side effects such as salivation, lacrimation, urination, defecation, and emesis. Many of these side effects are mediated by peripherally expressed M2 and M3 receptors [46], making direct targeting of M1, M4, and M5 therapeutically attractive. The orthosteric binding pocket, or site where mAChRs bind ACh, is a highly conserved region of the different mAChR subtypes. This high level of conservation makes it challenging to design molecules targeting this site with appreciable subtype-selectivity. However, some agonists have been developed that, while lacking complete subtype-selectivity, show profiles of

preferential activation of certain subtypes over others. One of the best-studied mAChR agonists in clinical studies of CNS disorders is the M1- and M4-preferring orthosteric agonist Xanomeline. When xanomeline was given to Alzheimer's patients, there was a trend towards improving cognitive measures, but also an unexpected reduction in behavioral disturbances such as hallucinations, vocal outbursts, and agitation [47, 48]. This promising efficacy at reducing behavioral disturbances and improving cognitive deficits lead to a Phase II clinical trial in schizophrenia patients, where efficacy was examined in all symptom clusters by evaluating the Positive and Negative Syndrome Scale (PANSS) and the Brief Psychiatric Rating Scale (BPRS), Clinical Global Impression Scale (CGI), and a wide battery of tests to assess cognitive function. The 4-week, double-blind and placebo-controlled study discovered that Xanomeline significantly improved BRPS and PANSS scores in schizophrenia patients, as well as facilitated improvement in verbal learning and short-term memory function [49], which provides clinical evidence that targeting mAChR activation can provide broad symptomatic relief to schizophrenia patients. Unfortunately, in both trials xanomeline treatment also resulted in gastrointestinal distress in the form of nausea, indigestion, vomiting, sweating, and salivation, which led to a large discontinuation rate and ultimately caused the removal of this treatment from clinical consideration.

The gastrointestinal side effects seen with xanomeline fit with symptoms of classical cholinergic toxicity and are likely due to the activity of xanomeline at M2 and M3 receptors [46]. Recently, Karuna pharmaceuticals have adopted a novel clinically strategy for maintaining the clinical efficacy of xanomeline while mitigating the side effect profile. Their candidate, KarXT, is a co-formulation of xanomeline along with trospium, a non-selective mAChR antagonist. Trospium has been clinically used for decades in the treatment of overactive bladder syndrome and the safety and tolerability of this compound are well-documented [50]. Importantly, trospium has a quaternary amine in its structure that results in this drug being peripherally-restricted following oral dosing since it does not readily cross the blood-brain barrier [51]. By blocking the peripheral receptors with trospium, and activating the central M1 and M4 receptors with xanomeline, this strategy has the potential to activate the receptors mediating antipsychotic efficacy while avoiding activation of the peripheral receptors that mediate undesirable side effects. Critical to the success of this strategy is finding a therapeutic window of trospium that is below the level at which this compound can induce anticholinergic side effects [50], while simultaneously achieving an exposure that can competitively displace efficacious doses of xanomeline from peripheral mAChRs. Excitingly, Karuna announced results from a Phase II trial showing that KarXT was well tolerated and significantly reduced PANSS scores in schizophrenia patients [52]. While additional large-scale clinical studies are needed to further determine the efficacy of KarXT in schizophrenia patients, this is a very exciting and novel therapeutic strategy with great promise.

2.1 Allosteric modulators target specific mAChR subtypes

Xanomeline is referred to as "M1- and M4-preferring agonist" due to the increased potency and efficacy of this compound at M1 and M4 receptors compared to other mAChR subtypes. However, this compound can bind and activate all mAChR subtypes at higher concentrations [53, 54]. While recent rodent studies indicate that consequences of M4 receptor activation *in*

vivo can be observed prior to activation of other mAChR subtypes [55], the therapeutic window between antipsychotic activity at M1 / M4 and gastrointestinal side effects seen with M2 / M3 activation is not sufficient for xanomeline to be considered as a standalone therapy. While the clinical efficacy of xanomeline serves as a proof-of-concept for targeting mAChRs for the treatment of schizophrenia, there is still a great need for developing compounds that are highly selective for M1 and/or M4 receptors and avoid activity at other mAChR subtypes. Several efforts have been made and have resulted in agonists that have slightly more favorable selectivity profiles than xanomeline [56]; however, finding highly selective agonists by targeting the orthosteric binding pocket has proved challenging. Recent advances in allosteric pharmacology (targeting binding pockets that are outside of the orthosteric binding pocket that binds to ACh) have provided great advances in achieving subtype-selectivity and are showing great promise potential as novel therapeutics for treating schizophrenia.

2.2 Allosteric modulation of M4 receptors for treatment of schizophrenia

One of the best-studied mAChR targets for schizophrenia is the M4 receptor subtype. In preclinical studies, the antipsychotic-like efficacy of xanomeline is lost in M4 KO mice [57], suggesting a key role for this subtype in mediating xanomeline efficacy related to positive symptoms. Several groups have had great success in discovering highly selective M4 compounds by targeting allosteric sites that are distinct from the ACh binding pocket [43]. This has led to the discovery of several PAMs from numerous chemical scaffolds that can robustly modulate M4 receptor signaling with little to no activity at other mAChR subtypes. M4 PAMs have been found to have robust efficacy in numerous preclinical assays that are predictive of antipsychotic efficacy including amphetamine- and apomorphine-disrupted prepulse inhibition, amphetamine- and MK-801-induced hyperlocomotion, and conditioned avoidance responding [58–62]. Importantly, the antipsychotic-like efficacy of M4 PAMs are absent in M4 KO mice and are not accompanied by side effect profiles in rodents that are seen with non-selective mAChR agonists [58]. Collectively, these studies suggest that selectively targeting M4 may confer the beneficial effects of xanomeline without the dose-limiting side effects.

An abundance of preclinical and clinical evidence supports the hypothesis that hyperactive DA signaling in the striatum is associated with positive symptoms of schizophrenia [6], and the cholinergic system can robustly modulate striatal DA signaling through the activation of both mAChRs and nAChRs [63]. Studying the contribution of different mAChRs to DA signaling is complicated by the fact that all 5 mAChR subtypes are expressed in the striatum [64–69], and that cholinergic effects are highly dependent on the receptor subtype being activated as well as by the mode of cholinergic signaling [63, 70, 71]. In the striatum, the M4 subtype is primarily expressed postsynaptically on direct pathway spiny projection neurons [65], on cholinergic interneurons where it acts as an autoreceptor [66], as well as on glutamatergic inputs where it acts as a heteroreceptor [72]. The M4 receptor populations on cholinergic interneurons and spiny projection neurons can both robustly modulate DA signaling, although through very different mechanisms. Activation of M4 autoreceptors on cholinergic interneurons can reduce ACh release and subsequent nAChR-dependent effects on DA [69], while activation of M4 receptors expressed on spiny projection neurons leads to

an endocannabinoid-dependent inhibition of DA release that is nAChR-independent [60]. Selective deletion of M4 receptors from D1-expressing neurons (D1-M4 knockout mice) [73] results in the loss of functional M4 receptors from direct pathway SPNs and endocannabinoid-mediated reductions in DA release [60]. Interestingly, the antipsychotic-like efficacy of both xanomeline and M4 PAMs are absent in these mice [60, 74], suggesting that this receptor subpopulation is critical to the behavioral efficacy of these compounds. The ability of M4 PAMs to reduce DA release in the striatum via locally mobilized endocannabinoid signaling provides a mechanism whereby striatal DA signaling can be reduced without altering DA release in other brain regions such as the cortex and hippocampus. This provides M4 PAMs with a key advantage over currently utilized antipsychotics that antagonize D2 receptors throughout the brain.

In addition to exerting important modulator effects on striatal signaling, the M4 receptor can also robustly modulate hippocampal circuitry involved in cognitive processes. Activation of the M4 receptor with numerous highly selective M4 PAMs from different chemical scaffolds can robustly inhibit excitatory potentials at the Schaffer collateral inputs to CA1 without affecting either temporoammonic inputs or inhibitory inputs in *ex vivo* slices [75, 76]. Further *in vivo* studies have shown that M4 PAMs can reduce CA1 pyramidal activity *in vivo* and reverse deficits in spatial learning and memory as assessed using the Morris Water maze [77]. Imaging studies have shown that M4 PAM administration can normalize amphetamine-induced changes in hippocampal activity [78], and M4 PAM administration can dose-dependently reverse MK-801-induced deficits in both a visual pairwise discrimination task and contextual fear conditioning [58]. Interestingly, chronic administration of M4 PAMs can also enhance the rate of acquisition in pairwise discrimination in WT, but not M4 KO mice, suggesting that M4 PAMs can enhance cognition under both basal conditions and MK-801 disrupted conditions [79]. These exciting findings suggest that M4 PAMs have the potential to not only treat positive symptoms in schizophrenia, but to alleviate cognitive deficits as well.

Collectively, these preclinical studies have generated great enthusiasm about targeting the M4 receptor for the treatment of schizophrenia. The recent development of a radiolabeled M4 PAM PET tracer has opened up the possibility of assessing receptor occupancy in both non-human primates and patients [80], which will greatly facilitate efforts to determine relevant dose ranges. In addition, a phase 1b study of the M4 PAM CVL-231 was recently initiated in schizophrenia patients [35], which will provide important information on the safety and tolerability of M4 PAMs. The next few years promise to be an exciting time for M4 PAM research as we continue to learn more about M4 receptor function in relation to schizophrenia as well as the safety and utility of M4 PAMs in the clinic.

2.2 Allosteric modulation of M1 receptors for treatment of schizophrenia

The M1 receptor subtype is a well-studied therapeutic target for providing cognition-enhancing effects, particularly via modulation of memory and attentional processes. One of the first M1-selective PAMs discovered was BQCA, which has been demonstrated to have robust pro-cognitive efficacy in rodents where it can improve the rate of acquisition in a pairwise discrimination task, reverse deficits in memory tasks and contextual fear

conditioning, and modulate sleep-wake architecture in rodents and nonhuman primates [81, 82]. Several M1-selective PAMs and agonists have been discovered that possess more desirable physiochemical properties than BQCA and can robustly facilitate novel object recognition [83–85] and improve performance in sustained attention tasks [86] in rodents. In non-human primates M1 PAM administration has been shown to improve paired associates learning, continuous-performance task performance, and working memory [87, 88], further demonstrating the pro-cognitive effects of M1 receptor potentiation. M1 agonists and PAMs can also reverse deficits in reversal learning, social interaction, novel object recognition, and fear conditioning in rodents [84, 89, 90], providing exciting evidence that targeting M1 may be able to provide efficacy in treating both cognitive and negative symptoms.

The behavioral efficacy of M1-selective compounds in the preclinical studies described above are thought in large part to be mediated via M1 receptors that are expressed in the cortex and hippocampus. Both of these brain regions are highly involved in memory processing and top-down cognitive processing and express M1 receptors that can robustly modulate neuronal function. In the hippocampus, M1 receptor activation can increase the excitability of CA1 pyramidal neurons [75, 91], and can potentiate NMDA receptor-dependent long-term potentiation of glutamatergic signaling onto CA1 neurons [92, 93]. In the prefrontal cortex (PFC), M1 activation can induce long-term depression (LTD) of glutamatergic signaling [89] via a mechanism that depends on endocannabinoid signaling [94] and phospholipase D signaling [95]. M1-mediated plasticity of inputs to PFC pyramidal neurons is input-specific with robust LTD observed on hippocampal and amygdala inputs, with no LTD observed at thalamic terminals [96]. Interestingly, M1 mediated LTD can be observed with M1 PAMs but not with M1 agonists [83], suggesting that avoiding agonist activity may be key to modulating critical cognitive circuitry as well as in avoiding undesirable side effects [84]. Importantly, M1 PAMs can reverse deficits in mAChR-LTD induced by subchronic administration of NMDA receptor antagonist [89] and deficits in mAChR-LTD observed in a model of NMDA receptor hypofunction [97], suggesting that normalization of disrupted PFC plasticity may play a key role in the ability of M1 PAMs to reverse cognitive and social deficits in these models.

While the M1 receptor can robustly modulate cognitive related circuits, accumulating data suggests that M1 receptor expression is reduced in a subset of schizophrenia patients. Post-mortem studies have shown decreased M1 protein, RNA, and radioligand binding in the cortex of schizophrenia patients compared to controls [98], with no significant changes observed with other mAChR subtypes [99]. This downregulation of M1 receptors is only observed in ~25% of schizophrenia patients, who are collectively referred to as a muscarinic receptor deficit sub-group (MRDS; for review see [100]). The deficits in M1 receptor expression are brain region-specific as M1 receptor-expressing neurons are reduced in the cortex, but not thalamus or hippocampus, of schizophrenia patients compared to controls [101]. In particular, cortical pyramidal neurons in laminae III and V appear to be vulnerable to changes in M1 expression, as the number of M1 receptor-positive neurons in these laminae were reduced in MRDS patients, and to a lesser, but still significant, effect reduced in non-MRDS patients [101]. Changes in M1 receptor expression on specific neuronal populations could profoundly impact the efficacy of compounds targeting this receptor as agonist and allosteric modulators require the receptor to be present in order to mediate their

effects. However, a recently developed M1-selective PET-ligand suitable for use in non-human primates [102] is now under evaluation in human studies and has great potential to assist in identifying both MRDS patients and populations of patients who may be most likely to respond to M1-targeted therapies.

M1-selective compounds can be divided in ago-PAMs (compounds that show both agonist activity and allosteric modulator activity) and pure PAMs that do not directly activate the M1 receptor in the absence of acetylcholine. This distinction is especially important when considering the important temporal nature of cholinergic signaling with regards to cue detection in the prefrontal cortex and how this timing regulates PFC synchrony and cognitive processing [103, 104]. Pure PAMs would retain the pattern and timing of receptor activation to coincide with the activity of cholinergic projections to the PFC, while Ago-PAMs will activate the receptors even when cholinergic activity is silent [43]. Consistent with the idea that the temporal nature of cholinergic signaling must be preserved for optimal cognitive enhancement, it was found that ago-PAMs did not have the robust cognitive enhancing efficacy in rodent models that was observed with pure PAMs [83]. Compound with ago-PAM activity such as MK-7622 have also failed to demonstrate efficacy in the clinic [105], further supporting the idea that pure PAMs may provide an optimal profile for cognition enhancing efficacy and avoiding adverse side effects [83].

In addition to the cognitive enhancing properties, some M1-selective modulators have also shown antipsychotic-like efficacy in rodents. Administration of the M1 PAM TAK-071 can reverse MK-801-induced hyperlocomotion but does not reduce methamphetamine-induced hyperlocomotion [106], whereas the M1 ago-PAM PF-06767832 can reverse both amphetamine-induced hyperlocomotion and amphetamine-induced deficits in sensorimotor gating [107]. While the M1 PAM BQCA shows little to no efficacy in reversing MK-801-induced disruptions in sensorimotor gating, it can potentiate submaximal effects seen with atypical antipsychotics in an M1-dependent manner [29]. While M1 is still primarily considered as a target for enhancing cognition, the antipsychotic-like activity seen with some M1 PAMs and the possible importance of M1 modulation to the clinical efficacy of clozapine / norclozapine discussed above, raise the possibility that M1 receptors may be able to provide symptomatic relief across positive, negative, and cognitive symptom domains of schizophrenia.

2.3 Allosteric modulation of M5 receptors for treatment of schizophrenia

The M5 receptor is a $G\alpha_q$ coupled receptor with a unique expression pattern in the brain that makes it an interesting target for treating dopaminergic disorders including schizophrenia. While M5 expression in the brain accounts for less than 2% of total mAChR expression [108], it is the only mAChR that is expressed on DA neurons in both the substania nigra pars compacta and ventral tegmental area [67]. Accordingly, targeting of M5 could provide a strategy for targeting dopaminergic neuron activity that is devoid of direct effects in other brain regions.

The majority of studies using M5-selective compounds to date have identified the M5 receptor as a promising target for treating substance abuse [109, 110]. However, several lines

of evidence suggest that M5 expressed in the midbrain DA could also play a key role in modulating schizophrenia symptom clusters. The mAChR subtypes expressed in the midbrain include the M5 subtype that is expressed on midbrain DA neurons [67], mAChR autoreceptors expressed on cholinergic inputs arising from the pedunculopontine nucleus and lateral dorsal tegmental nucleus [111], and M4 heteroreceptors expressed on GABAergic projections from the striatum [112]. Administration of non-selective mAChR antagonists such as scopolamine can induce psychosis in humans and exacerbate positive, negative, and cognitive symptoms in schizophrenia patients [44, 113]. In rodents, local injections of mAChR agonists and antagonists into the midbrain is sufficient to induce bidirectional changes in locomotion, stereotypy, reward, and immobility in the forced swim test [114–116], suggesting that this brain region plays a key role in the mediating many of the schizophrenia-like behavioral effects induced by systemic mAChR antagonist administration. Activation of somatic M5 receptors on DA neurons can induce robust physiological changes including the generation of a large inward current, mobilization of Ca^{2+} release, and increased excitability [68]. Interestingly, local application of an M5-selective negative allosteric modulator (NAM) into the ventral tegmental area can normalize disruptions in the forced swim test induced by a hyper-cholinergic state, suggesting that M5 neurons on DA neurons can robustly modulate depressive-like behaviors [117]. These exciting findings suggesting that M5 could be an interesting target for treating negative symptoms in schizophrenia.

In addition to effects at the level of DA cell bodies, activation of M5 receptors can also modulate DA release at the level of DA neuron terminals. Potentiation of M5 signaling with the M5 PAM VU0238429 can lead to an inhibition of DA release in the dorsal striatum, an effect that is paradoxically opposite to the excitatory effects seen with M5 expressed in DA cell bodies [68]. Interestingly, M5 activation can lead to an enhancement of DA release in the ventral striatum [69], suggesting that M5 effects on DA release are region-dependent. While the mechanism(s) whereby M5 activation can lead to different effects in different striatal sub-regions still need to be dissected, the ability of M5 to robustly modulate DA signaling via actions at both DA terminals and DA cell bodies makes it an interesting target in the context of positive symptoms in schizophrenia. One major drawback has been a lack of systemically available PAMs and NAMs with high selectivity for the M5 receptor. However, with the advent of highly M5-selective NAMs with good CNS penetration, we are starting to learn a lot of about this receptor and its potential to regulate substance abuse disorders [109, 118]. As medicinal chemistry efforts continue to discover new improved compounds targeting M5 [119, 120], these tools will be critical in elucidating the potential efficacy for M5-selective NAMs and PAMs in rodent models of positive and negative symptoms of schizophrenia.

3.1 Summary

Cholinergic signaling through mAChRs can robustly modulate circuitry that is involved in mediating positive, negative, and cognitive symptoms of schizophrenia. Several important landmarks have recently been passed as compounds targeting these receptors move into the clinic with the potential to provide desperately needed therapeutic strategies capable of providing more comprehensive relief from schizophrenia symptoms. One interesting area

that has received relatively little attention has been potential sex differences with regards to mAChR regulation of schizophrenia related circuitry. Recent studies have highlighted how schizophrenia associated circuitry such as dopaminergic signaling is regulated in a sex-specific manner [121], and these changes could play a key role in influencing symptom severity, adverse effect liability, and potential therapeutic efficacy between male and female patients [11, 122]. While no studies to our knowledge have directly looked at sex differences of mAChR-specific modulators in schizophrenia patients, it has been reported that females respond more poorly to clozapine [123], a finding that could potentially be explained by differences in clozapine to norclozapine ratio in females [124] which could effect mAChR activity as discussed above. Collectively, these findings point to the need for more pre-clinical and clinical research into potential sex-specific differences that could be observed with different mAChR targeted therapies.

As discussed above, compounds targeting M1 have shown great efficacy in improving cognition in rodent models and some efficacy in mediating antipsychotic-like effects. As M1 allosteric modulators move into the clinic for the first time [125], we hope to learn more about the therapeutic potential of selectively targeting M1 for mediating cognitive enhancement in numerous disorders. Given the potential involvement of M1 receptors in the etiology of schizophrenia [100], it will be important to develop strategies to help classify patients and identify those that are most likely to benefit from M1-targeted therapies. As discussed above, the M4 receptor also shows great promise in modulating both the positive and cognitive symptoms in preclinical studies. The movement of allosteric compounds targeting this receptor into the clinic [35] represents an exciting opportunity to learn about the therapeutic efficacy of targeting M4. In addition, targeting M1 and M4 with xanomeline has already shown efficacy in ameliorating a diverse array of schizophrenia symptoms but has been limited by adverse side effects. Current trials pairing xanomeline with a peripherally restricted antagonist could provide an exciting new strategy for maintaining the efficacy of xanomeline without the dose-limiting peripheral side effects [52].Collectively, the studies described above highlight several new avenues to unlocking the exciting therapeutic potential of targeting mAChRs. Advancing beyond DA-centric schizophrenia therapies is a critical need and there is hope that targeting mAChRs may provide a path to improved clinical outcomes.

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Abbreviations

(DA)	Dopamine
(mAChRs)	Muscarinic acetylcholine receptors
(Ach)	Acetylcholine
(nAChRs)	Nicotinic acetylcholine receptors

(PAM)	Positive allosteric modulator
(PANSS)	Positive and Negative Syndrome Scale
(BPRS)	Brief Psychiatric Rating Scale
(CGI)	Clinical Global Impression Scale
(PFC)	Prefrontal cortex
(LTD)	Long-term depression
(GABA)	γ-aminobutyric acid
(NMDA)	*N*-methyl-D-aspartate

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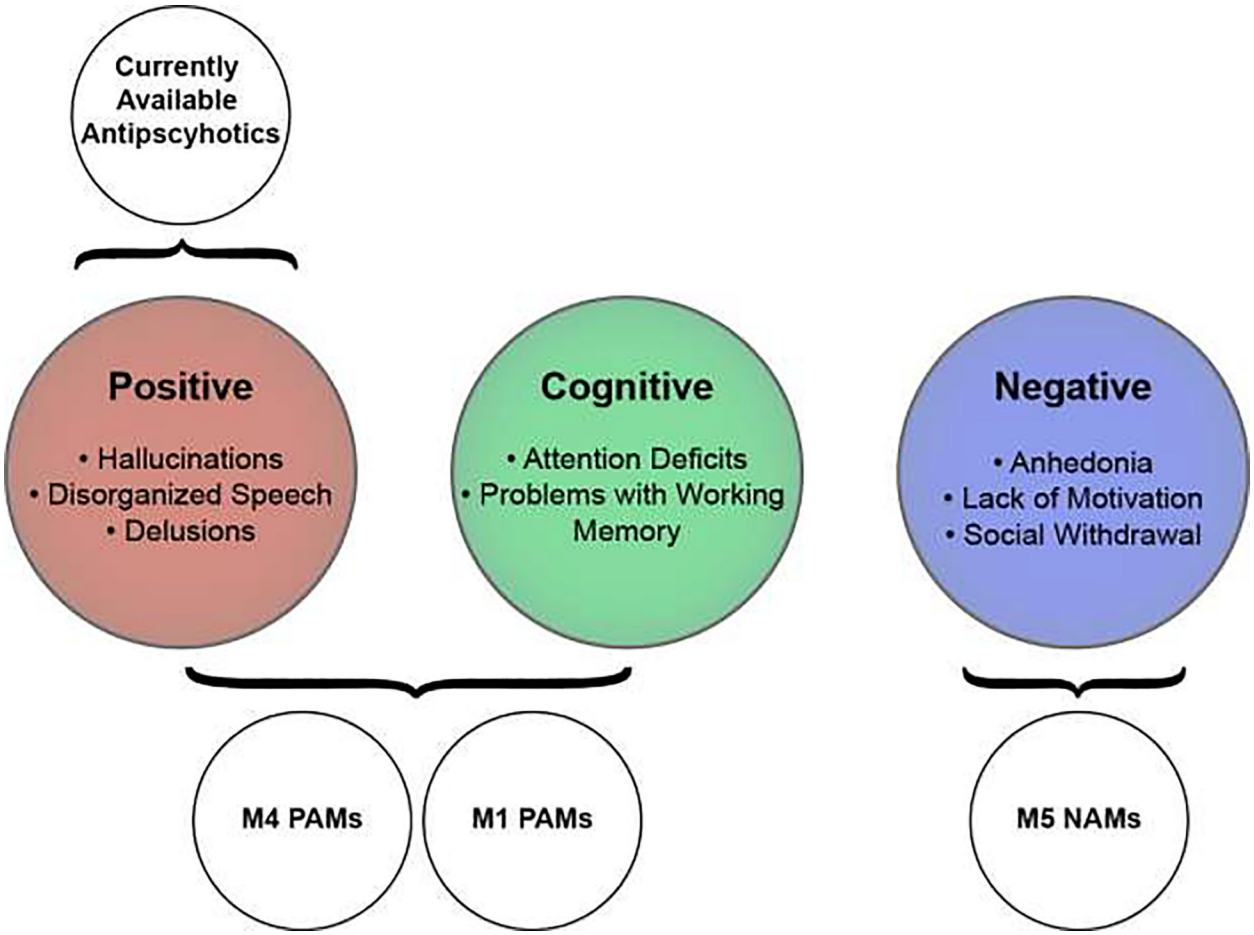

Figure 1: Muscarinic receptor based therapies have the potential to provide more comprehensive relief to schizophrenia patients than currently available dopamine receptor-based approaches.

Positive allosteric modulators (PAMs) targeting M4 and M1 receptor subtypes can mediate pro-cognitive and antipsychotic-like activity in preclinical animal models, while M5-selective negative allosteric modulators (NAMs) have potential in treating antihedonic and depressive symptoms. Collectively, targeting muscarinic receptors has the potential to alleviate all three symptom clusters in schizophrenia patients including cognitive and negative symptom clusters that are currently not alleviated by currently used antipsychotics.