

Selective Targeting of Muscarinic Receptors: Novel Therapeutic Approaches for Psychotic Disorders

Cyrille Sur^{1,*} and Gene G. Kinney²

¹Department of Imaging and ² Neuroscience Drug Discovery, Merck Research Laboratories, West Point, PA 19486, USA

Abstract: Schizophrenia is a well recognized and debilitating psychiatric disorder composed of several symptoms. Despite the clinical efficacy of present typical and atypical antipsychotics to alleviate positive symptoms, negative symptoms and cognitive disorders are not optimally controlled. Thus, there is an unmet medical need to develop novel medications with improved tolerability and efficacy for the treatment of these symptoms. Clinical observations over the past four decades have accumulated to support a role of central muscarinic cholinergic neurotransmission in psychosis. Indeed, recent studies have shown that acetylcholine esterase inhibitors as well as weakly selective muscarinic agonists such as xanomeline improved neuropsychiatric symptoms and cognitive function in Alzheimer's disease patients. Preclinically, a large body of studies has highlighted the involvement of muscarinic cholinergic signaling in cognition and psychosis. However the lack of truly selective drugs for the five muscarinic receptors has prevented an unambiguous determination of the role of each receptor subtypes in these behaviors. Recent progress in behavioral studies of mice deficient for the muscarinic receptors and in the discovery of selective muscarinic agonists have started to unravel the contribution of muscarinic receptor subtypes to complex behaviors. Accordingly, this review examines the potential of selectively targeting muscarinic receptor subtypes as a therapeutic approach for the treatment of psychotic disorders.

INTRODUCTION

Schizophrenia is an old and complex psychiatric disorder with a similar worldwide prevalence rate. This debilitating disease affects around 1% of the world population and is considered the fourth leading cause of disability by the WHO. Schizophrenia is causing substantial suffering to patients and their families with 10% of schizophrenics committing suicide. Since the pioneering work of Kraepelin [43] and Bleuler [11], schizophrenia can be considered as a clinical syndrome composed of several symptoms that vary between patients and individually are not unique to schizophrenia [1]. Schizophrenia symptomatology is well described by three categories of symptoms: positive, negative and cognitive symptoms [4]. The positive symptoms include hallucinations, disorganized speech and behavior and delusions whereas negative symptoms comprise anhedonia, avolition and affective flattening. The cognitive deficits associated with schizophrenia include disordered thinking and working

memory and are thought to be a core feature of the disease. In contrast the etiology and pathophysiology of schizophrenia are complex and still poorly understood. The discovery of the first clinically effective antipsychotics chlorpromazine and haloperidol and the demonstration of their antagonism at dopamine receptors paved the way to the dopamine hyperfunction hypothesis of schizophrenia [20]. Subsequent researchers have revealed that the therapeutic efficacy of antipsychotics is correlated with their affinity for striatal D2 receptors and indirect dopamine agonists like cocaine and amphetamine have psychotomimetic effects in human [57]. Despite the effectiveness of current typical and atypical antipsychotics to control positive symptoms, negative symptoms and cognitive disorders are not optimally treated. Furthermore treatment with antipsychotics remains plagued by multiple side effects like tardive dyskinesia, sedation and metabolic disorders including weight gain. These limitations in the current pharmacopeias for psychotic disorders highlight the need to develop better medicines with novel mechanism of action and/or more benign side effects.

Acetylcholine (ACh) is a major neurotransmitter in the central nervous system (CNS) and cholinergic

*Address correspondence to this author at Merck Research Laboratories, Department of Imaging, P. O. Box 4, WP26A-3000, West Point, PA 19486, USA; Tel: 215 652 2081; Fax: 215 652 2075; E-mail: cyrille_sur@merck.com

neurotransmission plays an important role in many physiological functions such as sleep, cognition, attention and memory. Cholinergic neurons are located in several, well-defined neuronal populations with basal forebrain cholinergic cells (Ch1-Ch4) innervating cortical regions and hippocampus [74]. Another important cluster of cholinergic neurons is located in the mesopontine area (Ch5-Ch6) and these cells send projections to many subcortical regions including almost all thalamic nuclei as well as the ventral tegmental area and both parts of substantia nigra [74]. A minor cell group (Ch7) is also present in the habenula with projections to the hippocampus. Cholinergic interneurons with local projections are also present in different brain regions like the striatum. Acetylcholine predominantly exerts its central effects through its interaction with ligand-gated nicotinic receptors and muscarinic acetylcholine receptors (mAChR). The muscarinic receptors belong to the G protein-coupled receptor (GPCR) family. Five members (M1 to M5) have been cloned and their cellular signaling pathway characterized [14,15,21,38]. The M1, M3 and M5 subtypes activate phospholipase C (PLC) and stimulate phosphoinositide (PI) turnover leading to PKC activation and intracellular calcium release [21,38]. These intracellular events then induce a network of intracellular signaling pathways such as activation of mitogen-activated protein kinase (MAPK) [36]. The M2 and M4 subtypes reduce cAMP production through inhibition of adenylate cyclase activity [21,26]. Neuroanatomical studies have revealed a unique pattern of expression of these five subtypes in the periphery and central nervous system [44,46]. Peripheral receptors are responsible for the muscarinic effects of ACh on tissues innervated by the parasympathetic system such as reduction of heart rate, activation of glandular secretion and contraction of smooth muscle [16,19,72]. In the central nervous system, the M1 receptor is the predominant subtype in forebrain regions such as cortex and hippocampus and account for roughly half of muscarinic receptors [35,44,46]. M2 and M4 receptors are also well expressed in rat cortex whereas M5 was not detected by immunoprecipitation with a specific antibody [46]. Different patterns of muscarinic receptor subtype expression have been described in other CNS areas such as in striatum (M1=M2=M4) and thalamus (M2>M4>M1) [46]. At the circuit and cellular levels, expression of these receptors is well segregated as demonstrated by the work of Rouse and co-workers in the hippocampus [55]. In the cerebrum, a complex organization of muscarinic receptor expression is

evidenced by the presence of M2/M3 in septal cholinergic cells but M1/M3 in granule cells of dentate gyrus and pyramidal neurons of the entorhinal cortex. Differences in pre-synaptic expression are also demonstrated with cholinergic terminals enriched in M2 subtype and perforant pathway nerve ending containing M3 receptors [55].

Although the involvement of muscarinic receptors in multiple central and peripheral functions has long been known, it is only recently that the physiological and behavioral contribution of each subtype has started to be unraveled following the generation of M1-M5 knockout mice [5,29,31-33,35,37,41,48-50,66,68,70,-75,77].

Following a review of the clinical and preclinical evidences supporting the involvement of the acetylcholine muscarinic signaling system to various aspects of schizophrenia pathology, we examine the potential of several muscarinic receptors as novel targets for the treatment of schizophrenia. Current strategies followed by pharmaceutical and biotechnology companies in their attempt to develop novel, selective muscarinic compounds will be discussed.

IMPLICATION OF THE CHOLINERGIC SYSTEM IN PSYCHOTIC AND COGNITIVE SYMPTOMS: CLINICAL AND PRECLINICAL EVIDENCES.

Clinical observations over the past four decades have accumulated support for a role of central cholinergic neurotransmission involvement in psychosis. The non selective muscarinic blocker Ditrin has been reported to induce psychosis in healthy humans and to exacerbate symptoms in schizophrenia patients [51]. In the seventies, intoxication by alkaloid from Angel's Trumpet resulted in hallucinations, agitation and memory disturbances that were reversed by intravenous injection of the acetylcholine esterase (AChE) inhibitor physostigmine [34]. Interestingly, recently developed AChE inhibitors such as tacrine and rivastigmine have been shown to decrease hallucinations in Parkinson's patients [16,39]. This is also consistent with recent clinical trials that have shown that AChE inhibitors improve neuropsychiatric symptoms in AD patients and in patients with Lewy body dementia, in addition to providing beneficial effects on cognitive function [42,54,56]. Treatment of extrapyramidal side effects in schizophrenics with the anticholinergic antiparkinson drug, benztropine (Cogentin®) significantly arrested and even reversed thera-

peutic improvement in schizophrenia symptoms responsive to neuroleptics [62,63]. These observations pointed to an involvement of cholinergic muscarinic-mediated mechanisms in the expression of psychosis in schizophrenia subjects. More recently large, double-blind, placebo controlled clinical study in Alzheimer disease patients treated with weakly selective muscarinic agonists devoid of dopaminergic activity such as xanomeline revealed significant improvements in psychotic behavior and cognitive abilities [12,13]. Xanomeline was shown to have a statistically modest effect on cognitive function ($p = 0.06$). More strikingly, xanomeline exhibited robust therapeutic effects on psychotic symptoms and behavioral disturbances associated with AD. Thus, xanomeline induced a significant dose-dependent reduction in vocal outbursts ($p \leq 0.002$), delusions ($p \leq 0.001$), agitation ($p \leq 0.02$), hallucinations ($p \leq 0.01$), wandering ($p \leq 0.001$), fearfulness ($p \leq 0.004$) and threatening behavior ($p \leq 0.02$) for instance. Preclinically, a large body of studies has pinpointed an involvement of muscarinic cholinergic neurotransmission in cognition and psychosis [27,30,40,59, 60,65,76]. In rats, the non-selective, centrally active muscarinic antagonist scopolamine is well known to impair attention and memory [6,58] and to decrease prepulse inhibition (PPI) of the acoustic startle response [40], an operational measure of sensory gating that is dysfunctional in schizophrenia patients [30]. Conversely, the non-selective muscarinic agonist oxotremorine dose-dependently reversed scopolamine-induced disruption in PPI [40]. Additional behavioral studies conducted by several groups further supported the antipsychotic activity of muscarinic agonists as compounds like xanomeline inhibited conditioned avoidance response in rats, attenuated amphetamine-induced hyperactivity and reversed apomorphine-induced disruption of PPI in rodent and non-human primates [3,59,60,65].

Based on these clinical observations and positive outcome from preclinical studies in standard animal models used to predict antipsychotic efficacy, a small clinical study to evaluate the antipsychotic potential of xanomeline in schizophrenic patients was carried out [61]. Using Clinical Global Impression (CGI) evaluation, 3 of 10 placebo patients were defined as responders whereas 7 of 10 xanomeline patients were responders. Responders were defined as those with clinical global impression (CGI) scores of ≤ 3 . For brief psychiatric rating scale (BPRS) scores, xanomeline patients showed a change from baseline of -12 versus -6 for placebo patients. For positive and negative

symptom scale (PANSS) scores, xanomeline patients showed a change from baseline of -25 versus -5 for placebo. In PANSS positive symptom scores, xanomeline patients showed a change of -7 vs. -2 for placebo. While this was a study with a low number of patients, the response was superior to that of traditional antipsychotics. Further, significant effects on these different measures were seen in less than one week, as opposed to the multi week delay typically required for efficacy following treatment with traditional antipsychotics. Unfortunately, xanomeline suffers from side effects, the most prominent being mediated by activation of peripheral mAChRs, including bradycardia, GI distress, excessive salivation, and sweating. A large number of pharmacological, genetic, and anatomical studies suggest that M2 and M3 mAChR subtypes mediate these unwanted adverse events [19,25,31, 49,66,72]. However, these studies provided strong preclinical and clinical validation of mAChR agonists as novel therapeutic agents for treatment of schizophrenia and neuropsychiatric disturbances in demented patients.

Xanomeline lacks true M1/M4 specificity and has significant affinity and antagonist activity at M2, M3, and M5 [17,71,73]. Furthermore M2/M4 muscarinic agonists like BuTAC and PTAC have also been shown to inhibit conditioned avoidance response and antagonist apomorphine-induced climbing in mice suggesting an antipsychotic profile for BuTAC and PTAC [18,53]. The lack of absolute subtype agonism selectivity of these muscarinic drugs together with their antagonism of the other muscarinic receptors prevents an unambiguous determination of the receptor isoform(s) involved in their antipsychotic profile. The generation of transgenic mice deficient for one or two subtypes of muscarinic receptors as well as the discovery of novel more selective compounds have allowed progress to be made in our understanding of the contribution of individual muscarinic receptors to the antipsychotic profile of muscarinic agonists [10,19,64,72].

MUSCARINIC 1 RECEPTORS AND SCHIZOPHRENIA

The M1 receptor plays a prominent role in regulating function of limbic, midbrain, and cortical regions that are disrupted in schizophrenia and other psychotic states [36,47, 52]. Furthermore, transgenic mice deficient for M1 receptors displayed several neurochemical and behavioral phenotypic alterations

consistent with M1 playing a role in neuronal domains dysfunctional in schizophrenia [2,29,36,50]. M1 knockout mice exhibited deficit in cortical MAPK signaling [36], hippocampus long-term potentiation and a specific impairment in non-matching-to-sample working memory, a process requiring cortico-hippocampus communication [2]. However M1 receptors do not seem to play a critical role in memory formation and its initial stabilization in the hippocampal formation as M1 knockout mice showed normal response in contextual fear conditioning and Morris water maze tests and the amnesic effects of scopolamine treatment were present in these transgenic animals [2,50]. Although a deficit in the eight-arm radial maze test was reported under specific experimental conditions, it appears that this behavioral impairment may have resulted from the hyperactive phenotype present in this mouse model [29,50]. This hyperactivity is likely due to a 2-fold increase in extracellular levels of striatal dopamine as revealed by *in vivo* microdialysis, providing further evidence for a regulation of striatal dopaminergic transmission by M1 receptors [24,29]. Finally, clinically used typical and atypical antipsychotics, haloperidol and clozapine, respectively were shown to dose-dependently attenuate the hyperlocomotor phenotype in these transgenic mice [29]. The overall phenotype of these mice supports a role for M1 receptors in psychiatric disorders that involve a dysfunction of the dopaminergic system such as schizophrenia, Parkinson's disease and attention deficit/hyperactivity disorder.

Recent pharmacological studies aimed at discovering molecular mechanisms responsible for the well-established unique antipsychotic profile of clozapine in schizophrenia patients have highlighted M1 receptors as a potential target [67,71]. Indeed, N-desmethylclozapine, the major metabolite of clozapine has been shown to be a potent and efficacious agonist at the M1 receptor, a characteristic not shared by clozapine and other clinically used antipsychotics [67,71]. *In vitro* experiments revealed that N-desmethylclozapine potentiated N-methyl-D-aspartate receptor currents in hippocampal CA1 pyramidal cells and that systemic injection of this metabolite stimulated the phosphorylation of MAP kinase in these CA1 neurons demonstrating a specific M1 muscarinic receptor agonist activity in CNS [67,71]. Interestingly, re-analysis of two clinical trials involving neuroleptic resistant and responsive patients revealed that the N-desmethylclozapine/clozapine concentration ratio in patient serum is the best predictor of clinical response

in cognitive functioning and quality of life [71]. Despite the potential of M1 receptor agonists for the treatment of psychiatric disorders the development of highly selective M1 agonists has been largely unsuccessful because of the high level of amino acid conservation of the ACh orthosteric binding site in the five muscarinic receptor subtypes [14,15,38,73]. However, a recent pharmacological advance suggests that it may be possible to develop highly selective muscarinic agonists [64]. Scientists at Acadia Pharmaceuticals have reported the discovery of AC42, a M1 receptor subtype selective ectopic site agonist [64]. This small molecule is a modestly potent partial agonist at human M1 receptor with high selectivity for this receptor subtype as no agonist activity is detected at the other muscarinic receptor subtypes. Furthermore, pharmacological and site-directed mutagenesis studies have demonstrated that AC42 does not mediate its agonist activity by interacting with ACh orthosteric site but through an ectopic/allosteric site [64]. Recent work from our group has further validated this concept and demonstrated that N-desmethylclozapine also acts as a human M1 receptor ectopic site agonist [67]. In addition to ectopic site agonists, pharmacological studies have also described highly subtype selective muscarinic receptor allosteric potentiators [9,22,45]. The demonstration of the alkaloid brucine as an allosteric potentiator of the M1 receptor [8,45] supported this concept and allows for the possibility that novel, more potent allosteric potentiators of the M1 receptor may be identified through library screening and/or medicinal chemistry efforts.

Altogether, clinical, genetic, pharmacological, and anatomical studies suggest that M1 receptors play an important role in psychosis and cognition and represent a likely candidate for mediating xanomeline effects. At present, however, a possible role for other muscarinic receptors cannot be excluded.

MUSCARINIC 2 RECEPTORS AND SCHIZOPHRENIA

Although M2 muscarinic receptors have not been directly implicated in psychosis, their well-established role in learning and memory function and a reported antipsychotic activity of mixed M2/M4 receptor agonists ButAC and PTAC in animal models suggest that M2 receptor agonists may be useful for the treatment of cognitive disorders, a core symptom of schizophrenia. Analysis of M2 subtype knockout mice supported a beneficial effect of M2 agonists in

neuronal plasticity and cognitive processes [68,72]. M2 knockout mice exhibited reduction in hippocampal long-term potentiation and concomitant deficits in working memory [72] whereas deletion of this subtype did not significantly affect behavioral responses in animal models of psychosis. Although a potentially attractive target for cognition, the critical role of M2 receptors in the control of heart rate and body temperature may limit the successful development of a safe and potent M2 agonist [31].

MUSCARINIC 4 RECEPTORS AND SCHIZOPHRENIA

Regional and cellular distribution of M4 muscarinic receptors in the CNS and in particular their expression in the nucleus accumbens, striatal cholinergic interneurons and medium spiny neurons provided an anatomical substrate for the implication of this receptor subtype in behaviors mediated by dopaminergic neurotransmission [7,46]. A combination of clinical and preclinical studies using poorly selective drugs like xanomeline (M1/M4) and BuTAC, PTAC (M2/M4) have also suggested a potential role of M4 muscarinic receptors in psychotic disorders [12,18,53,60, 65]. Recent neurochemical and behavioral analysis of M4 knockout mice have started to reveal the function of M4 receptors [27,32,33,41,68]. *In vivo* microdialysis revealed a 2.5-fold increased basal level of dopamine and its metabolites DOPAC and HVA in the nucleus accumbens of M4 deficient mice relative to wildtype controls [68] demonstrating the involvement of M4 receptors in mediating dopamine efflux in this mesolimbic structure. Furthermore, treatment with the well-characterized psychomimetic PCP (see Kinney and Sur in this review) led to higher release of dopamine in M4 knockout mice compared to wild type littermates. Consistent with these neurochemical modifications, M4 knockout mice demonstrate a small increase in basal locomotor activity and a heightened sensitivity to PCP-induced hyperactivity [27,32]. Finally, behavioral experiments evaluating sensory gating activity in M4 knockout mice showed no apparent disruption of basal prepulse inhibition of the acoustic startle response but a sensitized response to the disruptive effect of PCP on PPI compared to wild type animals [27]. Together these observations suggest that M4 receptors through their fine monitoring of dopaminergic activity is implicated in processing sensory and motivational responses and as such may represent a novel target for the development of antipsychotic drugs.

MUSCARINIC 5 RECEPTORS AND SCHIZOPHRENIA

Until recently the function of muscarinic M5 receptors in the CNS have remained elusive principally due to a low level of expression and the lack of specific pharmacological tools [15,44,46]. Nonetheless, the high expression of M5 receptors by dopamine-containing neurons of the ventral tegmental area, the major source of dopaminergic innervation of several limbic structures as well as the direct cholinergic activation of monoaminergic neurons by Ch5/Ch6 cells, led to the hypothesis that blockade of tegmental M5 receptors could alleviate positive symptoms of schizophrenia [69,76]. Transgenic knockout mice lacking the muscarinic M5 receptors displayed no obvious phenotype and behaved similar to their wild type littermates in several behavioral assays, including locomotor activity and motor coordination tasks [5,75]. However, recent neurochemical studies revealed a significant unbalance in the cholinergic control of dopamine release in the nucleus accumbens of these mice due to an absence of M5 receptor-mediated excitation of ventral tegmental area dopaminergic neurons [28]. Consistent with this neurochemical defect, M5 mutant mice were less sensitive to amphetamine-induced locomotor effects [70]. Further, testing of M5 receptor knockout mice in a latent inhibition paradigm, a behavioral parameter impaired in schizophrenic patients, demonstrated that M5 receptor knockout mice exhibited a significant increase in latent inhibition [70]. Collectively, these observations strengthened the involvement of M5 receptors in mesolimbic neuronal circuitry relevant to schizophrenia. Interestingly, a recent linkage study in 82 Canadian families with schizophrenic patients suggested that the cholinergic M5 muscarinic receptor gene in combination with 7 nicotinic receptor genes on human chromosome 15q13 may be linked to schizophrenia [23]. Further, two highly efficacious anti-psychotic drugs in clinical use, olanzapine and clozapine, are potent (30-60 nM) antagonists of M5 muscarinic receptors. These findings suggest that modulation of M5 receptors represents an attractive target for the development of novel antipsychotic drugs.

CONCLUSION

Despite major progresses in antipsychotic drugs since the introduction of haloperidol, several inadequacies remain. Among these, modest efficacy of current medicines in treating negative and cognitive

symptoms and the existence of a significant pool of non-responders patients are probably the most pressing. Modulation of the complex muscarinic-dependent cholinergic circuitry through the development of muscarinic receptor subtype selective drug offers novel therapeutic opportunities. In this review we present a body of clinical and preclinical evidence supporting an involvement of central muscarinic receptors in different pathological aspects of schizophrenia and discuss the potential of several muscarinic receptor subtypes as targets for the development of novel antipsychotic therapeutics. While clinical evidence of the efficacy of the mixed M1/M4 agonist xanomeline is available, the potential therapeutic utility of potent and selective M1 and M4 agonists is further supported by an array of genetic, behavioral and neurochemical observations. Rationale for an M2 muscarinic receptor agonist also exists for the treatment of cognitive symptoms, but the role of M2 receptors in several critical physiological functions may decrease the attractiveness of this target. Recent behavioral and genetic observations also suggest some potential role for M5 receptor antagonist in schizophrenia.

Following the genetic delineation of muscarinic receptor subtype function, the appreciable challenge to develop highly selective drugs remains. Past failures and advances in our understanding of muscarinic receptor structure and pharmacology suggests that the development of allosteric ligands (i.e., ectopic agonists and allosteric potentiators) represent reasonable paths forward for future medicinal chemistry endeavors.

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