

# Muscarinic Agonists for the Treatment of Cognition in Schizophrenia

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## ABSTRACT

It is widely accepted that cholinergic activity at muscarinic receptors is required to maintain cognitive functions, including learning and memory. Memory domains are especially impaired in schizophrenia, which may explain difficulties in psychosocial rehabilitation of individuals with this illness. However, little is known about the mechanism of this impairment. To understand our current knowledge, we reviewed the literature since 1990 via a PubMed search for the terms “muscarinic”, “schizophrenia”, “cognition”, “memory”, “learning”, and “agonist” in combination. We found 89 basic science/laboratory studies, case reports/series, case-control studies, cross-sectional studies, standardized controlled animal trials, standardized controlled human trials, and reviews. Although further research is required to fully understand the neuropharmacology of the cholinergic system in cognitive function in schizophrenia, we have examined the data currently available. In general,

### Needs Assessment

Cognitive dysfunction in schizophrenia is a common problem that is commonly overlooked in clinical practice. There have been several clinical trials addressing this problem recently. This article reviews the theoretical basis of this cognitive dysfunction and addresses potential solutions.

### Learning Objectives

At the end of this activity, the participant should be able to:

- Name the three core domains in schizophrenia.
- List the cognitive dimensions affected in schizophrenia.
- List the actions of the M<sub>1</sub> through M<sub>5</sub> muscarinic receptors.
- Be able to discuss the potential of muscarinic M<sub>1</sub> agonists in cognition in schizophrenia.

**Target Audience:** Neurologists and psychiatrists

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**Faculty Disclosures:** Dr. Sellin does not have an affiliation with or financial interest in any organization that might pose a conflict of interest. Dr. Shad has worked in various capacities with Abbott, Bristol-Myers Squibb, Eli Lilly, Hoechst-Roussel, Janssen, Lundbeck, Merck, Organon, the National Institute of Mental Health, Pfizer, Upjohn, and Wyeth. Dr. Tamminga is a consultant for Acadia; has been provided grant funding for a study of NDMC in schizophrenia; is an ad hoc consultant for Alexia, Astella, AstraZeneca, Eli Lilly, Lundbeck, Neurogen, and Orexin; and is on the Clinical Advisory Board of Intracellular Therapies and Acadia. She is an unpaid volunteer for the International Congress on Schizophrenia Research.

**Funding/Support:** The authors do not have an affiliation with or financial interest in any organization that might pose a conflict of interest.

Submitted for publication: May 20, 2008; Accepted for publication: October 1, 2008.

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these data suggest that agonist activity at acetylcholine muscarinic type 1 ( $M_1$ ) receptors would enhance memory and learning in schizophrenia. We present an overview of likely side effects of muscarinic agonists. We outline the anticholinergic activity of several available antipsychotics and review the available  $M_1$  muscarinic agonists.

*CNS Spectr.* 2008;13(11):985-996

## INTRODUCTION

Cognitive dysfunction is one of the three core symptom domains in schizophrenia. While research has recently focused on this aspect of the disorder,<sup>1</sup> the clinical world continues to focus on its flashier cousins, the positive and negative symptoms. There are many different types of memory (eg, visual, spatial, emotional, seminal, declarative, working, procedural, semantic, verbal, and short- and long-term), only some of which are altered in schizophrenia. It is primarily working and declarative memory that are disrupted in schizophrenia.<sup>2-6</sup> However, measures used to assess cognitive dysfunction in schizophrenia (eg, standardized neuropsychological assessments) may not be specific enough to capture discrete cognitive lesions, leaving the core dysfunctions improperly identified.<sup>7,8</sup> Trials investigating the impact of antipsychotics on cognitive function in schizophrenia have not shown a robust therapeutic effect.<sup>6,9,10</sup> Recent studies of the action of the second-generation antipsychotic drugs (SGAs) on cognition show only a small effect size.<sup>11</sup> Therefore, the cognitive deficits remain a therapeutic challenge for schizophrenia.<sup>12,13</sup>

Efforts have been underway to standardize cognitive measures across clinical trials. The Measurement And Treatment Research to Improve Cognition in Schizophrenia<sup>14</sup> group reviewed previous research, determined seven separable cognitive dimensions in schizophrenia (Figure) and has developed a cognitive battery to test these domains.<sup>15-17</sup>

We understand very little about the neurobiology of cognitive deficits, but several neurotransmitter systems have been shown to be partially involved in mediating these deficits in human cognition. One of the most important is acetylcholine, specifically in relation to the various muscarinic receptor subtypes. This system is widely recognized to mediate some aspects

of learning and memory, which are impaired in schizophrenia.<sup>10,18,19</sup> The discussion of other neurotransmitter systems affecting attention and memory, in which there has been significant research, is beyond the scope of this review.

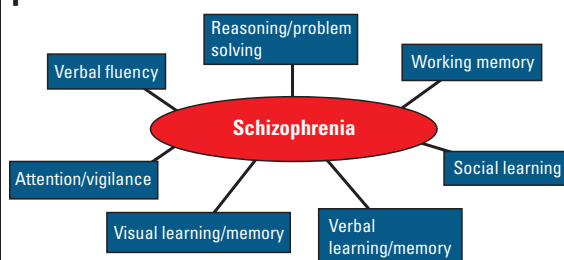
This paper will specifically explore the evidence regarding the alteration of muscarinic receptors in schizophrenia, their relationship with cognitive dysfunction, and some putative muscarinic-targeted drug candidates for treatment of altered cognition. To understand our current knowledge, we reviewed the literature since 1990 via a PubMed search for the terms "muscarinic", "schizophrenia", "cognition", "memory", "learning", and "agonist" in combination. We found 89 articles of various study types (Table 1).

## ANIMAL DATA

Due to the paucity of pharmacologic studies of specific muscarinic receptor subtypes in human subjects, we rely on animal studies. Muscarinic receptors are encoded by genes with high sequence homology among species,<sup>20</sup> so mouse and rat muscarinic receptors are very similar to human receptors. Muscarinic receptor strains knockout mice have been bred for each receptor subtype; study of these genetically engineered mice helps elucidate the function of each subtype. The  $M_1$  knockout mouse demonstrates disturbed learning and memory.<sup>21,22</sup>

$M_1$  knockout mice showed enhanced context conditioning, normal water maze learning, and severely impaired win-shift and social discrimination learning.<sup>23</sup> The first two tasks rely

**FIGURE.**  
**Neuropsychological deficits in schizophrenia.<sup>16</sup>**



The MATRICS group determined seven separable cognitive dimensions that are affected in schizophrenia.

MATRICS=Measurement and Treatment Research to Improve Cognition in Schizophrenia.

Sellin AK, Shad M, Tamminga C. *CNS Spectr.* Vol 13, No 11. 2008.

on declarative memory, require only invariant trial-independent information, and can be computationally solved; the latter two rely on working memory, require both trial-independent and non-matching to sample information, and may necessitate inhibition against generalization.<sup>23</sup> Non-matching to sample and working memory tasks require the prefrontal cortex (PFC), while matching tasks do not; thus, this pattern of deficit in the M<sub>1</sub> knockout mice suggests that the M<sub>1</sub> receptor is important in cortical memory function and the interaction between the cortex and hippocampus rather than memory acquisition by the hippocampus alone.<sup>23</sup>

Wess and colleagues<sup>24</sup> showed that M<sub>1</sub> knockout mice perform poorly in non-matching to sample tasks, which involve working memory and memory consolidation. These mice also have decreased hippocampal long-term potentiation and a two-fold increase in extracellular striatal dopamine concentration.<sup>22,24</sup> This increase in dopamine may help explain the hyperactivity and increased amphetamine-induced stimulation.<sup>22,25</sup> In later trials, Wess and colleagues<sup>26</sup> found that neuronal cultures from cortex or CA1 hippocampus in M<sub>1</sub> knockout mice have almost no mitogen-activated protein kinase-signaling pathway, which is considered crucial in enabling synaptic plasticity. The M<sub>1</sub> knockout mice were found to have significant impairments in non-matching-to-sample memory tasks, but normal matching-to-sample performance. M<sub>1</sub> knockout mice displayed only selective and attenuated cognitive deficits when exposed to non-selective muscarinic antagonists. M<sub>1</sub> knockout mice also had an ~2-fold increase in extracellular stri-

atal dopamine concentrations, associated with significant increase in motor activity. In terms of peripheral effects, M<sub>1</sub> knockout mice had decreased salivary flow.

Alteration in the intrinsic function of the muscarinic receptors impacts learning and memory, but extrinsic modification of muscarinic receptors with muscarinic agonists and antagonists also effects cognition. Returning to basic science, muscarinic receptor activation in a certain cell line has been shown to stimulate outgrowth of neurite-like processes and induction of marker genes for neuronal differentiation,<sup>27</sup> as well as inhibit the apoptotic death of growth-factor-deprived cells and block progression of the cell cycle.<sup>28</sup>

Animal studies demonstrate the positive effects of muscarinic agonists on cognition. The level of muscarinic receptors in the hippocampus of rats trained in a hippocampal dependent spatial learning task reflects the degree of training in that task.<sup>29</sup> Rabbits who learned an eyeblink task faster showed more muscarinic receptors in their hippocampal pyramidal cells than the slow-learning rabbits, who in turn had higher receptor levels than controls who had not learned the task.<sup>29</sup> These data suggest that improved learning may be related to higher density of muscarinic receptors.

It also seems to be true that increased muscarinic receptor density improves learning. An increase in muscarinic receptors facilitates long-term potentiation (the tissue representation of memory) in the hippocampus, and somewhat less so in the amygdala.<sup>29</sup> Indeed, M<sub>1</sub> agonists, such as methoctramine and 3-(3-S-n-pentyl-1,2,5-thiadiazol-4-yl)-1,2,5, 6-tetrahydro-1-methylpyridine, improve working memory in animals.<sup>30,31</sup> Low doses of talsclidine, an M<sub>1</sub> agonist, led to a modest increase in working memory in elderly rhesus monkeys.<sup>32</sup>

In addition, AF-150(s), an M<sub>1</sub> selective partial agonist, ameliorates memory impairment and central acetylcholine deficiency induced by apolipoprotein E deficiency in experimental animals.<sup>33</sup> It increased performance in old and young rats and decreased the rate of forgetting.<sup>34</sup> AF-150(s) also attenuates deficits in reference, working, and passive avoidance memory induced by an anticholinergic drug in rats.<sup>35</sup> With this basis in animal data, the remainder of the review will examine the human data. The prevalence of case series and lack of randomized controlled trials illustrates the need for further research.

**TABLE 1.**  
**Types of Studies Used in This Review**

<i>Type of Study</i>	<i>Number of Papers</i>
Basic science/laboratory	12
Case reports/case series	4
Case-control or cohort	14
Cross-sectional	5
Standardized controlled trials—animal	11
Standardized controlled trials—human	12
Review	30

Sellin AK, Shad M, Tamminga C. *CNS Spectr*. Vol 13, No 11. 2008.

## MUSCARINIC RECEPTORS MAY BE ALTERED IN SCHIZOPHRENIA

There is growing evidence that central nervous system (CNS) muscarinic receptors may be altered in schizophrenia. Furthermore, this alteration may be due to neither the inherent antimuscarinic properties of many antipsychotics, especially prominent in first-generation antipsychotics (FGAs), nor to the concomitant use of anticholinergic drugs used to treat antipsychotic side effects. The complexity of the muscarinic system is underscored by the fact that each of the subtypes ( $M_1$  through  $M_5$ ) has unique distribution throughout the body. Several reviews have examined and summarized these distributions.<sup>19,24,25,36,37</sup>  $M_1$  receptor distribution is highest in the more rostral areas of the brain: neocortex, hippocampus (especially the CA1 region), striatum, and nucleus accumbens;  $M_1$  receptors are post-synaptic, located on cell bodies and proximal dendrites. In the CNS,  $M_2$  receptors are located in the basal forebrain, thalamus, and brainstem; whereas peripherally, they are concentrated in gastrointestinal, genitourinary, optic, and exocrine smooth muscle and in the heart.  $M_2$  is a pre-synaptic inhibitory autoreceptor or heteroreceptor. CNS  $M_3$  receptors are in the hip-

poampus, thalamus, neocortex, and basal forebrain; peripherally, they are in gastrointestinal, genitourinary, optic, and exocrine smooth muscle.  $M_4$  receptors are located alongside  $M_1$  in the striatum, hippocampus, and neocortex; they are inhibitory autoreceptors in the hippocampus and post-synaptic modulators in the striatum and neocortex.  $M_5$  receptors are in the lowest density of all, and are localized with dopaminergic neurons in the substantia nigra and hippocampus. Table 2 summarizes the location and function of these muscarinic receptor subtypes.<sup>19-22,24,25,36-45</sup>

There are several postmortem studies of muscarinic receptor density in human brain. Many of these studies use ligands that bind selectively to specific muscarinic receptor subtypes, such as [ $^3H$ ]pirenzepine (which binds  $M_1$  and  $M_4$ ), as a measure of receptor availability or quantity. One study found a 28% reduction in  $M_1$  receptors in superior frontal gyrus in schizophrenia compared with normal cases.<sup>42</sup> Control cases showed expected variation in radioligand binding to muscarinic receptors between different regions in the brain, with the highest levels in CA1 in hippocampus. Schizophrenia cases showed no significant differences between brain regions, suggesting a

**TABLE 2.**  
**Location and Function of Muscarinic Receptors**<sup>18-21,23,24,35-44</sup>

	<u>Location</u>	<u>Cellular Function</u>	<u>Systemic Function</u>	<u>Changes in Schizophrenia</u>
$M_1$	Striatum, cortex, hippocampus	Gq; Postsynaptic, modulate fast transmission and metabolic function	Learning, memory, possible role in some types of epilepsy, cleave amyloid precursor protein, IL-2 production	Down in hippocampus, striatum, PFC, NAc
$M_2$	Basal forebrain, thalamus, heart, brainstem, pupil, exocrine glands, spinal cord	Gi; Presynaptic inhibitory auto/heteroreceptor	Salivation, akinesia, bradycardia, smooth muscle contractility, bronchoconstriction, tremor, hypothermia, analgesia, axonal growth	Down in striatum
$M_3$	Brain (evenly distributed), pupil, hypothalamus, exocrine glands, peripheral arteries	Like $M_1$	Salivation, smooth muscle contractility, vasorelaxation, NO release, appetite	Unknown
$M_4$	Striatum, cortex, hippocampus, spinal cord	Like $M_2$ plus inhibitory postsynaptic	Regulate striatal DA release, modulate PPI, analgesia, keratinocyte migration	Down in hippocampus, striatum, PFC, NAc
$M_5$	DA neurons, basal ganglia, brain vasculature	Like $M_1$	Cerebral arterial vasorelaxation	Unknown

M=muscarinic; Gq=G protein that activates phospholipase C; Gi=inhibitory G protein; PFC=prefrontal cortex; DA=dopaminergic; NAc=nucleus accumbens; PPI=pre-pulse inhibition.

Sellin AK, Shad M, Tamminga C. *CNS Spectr*. Vol 13, No 11. 2008.

decreased density of  $M_1$  and  $M_4$  receptors in hippocampus, dentate gyrus, CA1-4, subiculum, and parahippocampal gyrus in tissue from patients with schizophrenia.<sup>39</sup> [3H]pirenzepine binding is not altered in postmortem tissue from patients with bipolar disorder or major depressive disorder,<sup>43</sup> suggesting that this alteration may be unique to schizophrenia.

Although schizophrenia tissue has increased muscarinic binding in the orbital frontal cortex and putamen, there is decreased binding in the frontal, parietal, and temporal cortices,<sup>36</sup> as well as the hippocampus and striatum.<sup>19,39</sup> Schizophrenia patients have decreased  $M_2$  receptor binding in the striatum.<sup>36</sup> Zavitsanou and colleagues<sup>45</sup> reported decreased  $M_1$  and  $M_4$  receptor binding in the nucleus accumbens of schizophrenia tissue. Due to the lack of relationship between study measures and last recorded antipsychotic dose, these differences may not be due simply to antipsychotics.<sup>19</sup>

Dean and colleagues<sup>21</sup> report decreased [3H]pirenzepine binding in the frontal cortex, hippocampus, and caudate-putamen. A Western blot survey of the PFC revealed decreased  $M_1$  protein in Brodmann area (BA) 9 and decreased  $M_1$  mRNA in BA 9 and BA 40. Furthermore, in situ hybridization described a decrease in  $M_1$  mRNA in BA 9 and BA 40, and a decrease in  $M_4$  mRNA in BA 40 in schizophrenia tissue. Li and colleagues<sup>22</sup> found decreased  $M_1$  receptor cDNA in the frontal cortex of schizophrenia subjects. Additional experiments showed no change in  $M_2$  or  $M_3$  receptor proteins, or  $M_3$  receptor mRNA in either BA 9 or BA 40 in schizophrenia tissue versus controls.<sup>21,43</sup> These results suggest that, of all the muscarinic receptors,  $M_1$  may be the subtype most often altered in schizophrenia.

In vivo imaging studies of muscarinic receptors in schizophrenia are less common, but one SPECT study with [123I]idoquinuclidinyl benzilate shows widespread non-specific decreases in muscarinic receptor binding in the brains of schizophrenia patients.<sup>19</sup> Another shows a regionally selective 20% to 33% decrease in muscarinic receptors in unmedicated schizophrenia patients.<sup>46</sup> Finally,  $M_1$  mRNA was increased (as opposed to decreases seen in schizophrenia patients) in rats treated with antipsychotics in hippocampus, substantia nigra compacta, and nucleus accumbens.<sup>47</sup> The latter two studies suggests that the changes in muscarinic receptors are not likely to be due to antipsychotics.

## **ALTERATION IN MUSCARINIC RECEPTORS CONTRIBUTES TO DYSFUNCTION IN LEARNING AND MEMORY**

Alterations in muscarinic receptors, as described above, are accompanied by other neurotransmitter abnormalities in schizophrenia, and therefore are thought to be part of, and possibly represent, a system-wide dysfunction in these regions. It has been established that acetylcholine in the neocortex and hippocampus is involved in attention, learning, and memory, and facilitates the ability to focus on relevant information and maintain an appropriate stream of consciousness.<sup>19,48</sup> Also, the septohippocampal cholinergic pathway is associated with working memory and hippocampal declarative memory; the nucleus basalis-neocortical pathway is thought to be associated with long-term storage of memories in neocortex.<sup>45</sup> These data help demonstrate the role of the  $M_1$  acetylcholine receptor in these functions. It is possible that frequently observed cognitive deficits in learning and memory in schizophrenia are mediated by alterations in cholinergic transmission.

In a study of cognitive function and muscarinic receptor polymorphisms in schizophrenia and normal volunteers, schizophrenia subjects with the 267C/A polymorphism of the  $M_1$  receptor had more correct responses and fewer perseverative errors on the Wisconsin Card Sorting Test (which primarily tests prefrontal function) than subjects with the 267C/C gene; this is a silent mutation that may affect translation.<sup>49</sup> Because the alleles were equally distributed in both schizophrenia and control groups, and because within the schizophrenia group there was no significant difference between genotypes in age of onset or Brief Psychiatric Rating Scale (BPRS) scores, this allele appears to be unrelated to risk for or severity of schizophrenia.

## **THAT ALTERATION IS SIMILAR TO DEFICITS SEEN IN SCHIZOPHRENIA**

Individuals with schizophrenia have deficits in several cognitive domains, executive function (planning, problem solving, high-level thought processing), attention, verbal memory, working memory, episodic memory, delayed recall, language/fluency, sensory perception and gating, and motor processing.<sup>3,6,50</sup> The deficit in short term memory may be due to impaired learning or poor organizational strategy, rather than forget-

ting; the absence of performance deterioration between California Verbal Learning Test short- and long-delay tasks in elderly patients supports this supposition.<sup>50</sup> Schizophrenia patients also have a deficit in the ability to actively represent and maintain context (“prior task-relevant information that supports selection of appropriate behavioral response”), and a deficit in appropriately activating the dorsolateral PFC in response to a demand for context maintenance; these deficits are present at the first onset of symptoms, before administration of antipsychotics.<sup>51</sup>

Some investigators show that patients treated with FGAs demonstrate mild to moderate improvement in multiple cognitive domains but decreased motor performance and speed,<sup>6</sup> while others show no cognitive benefit despite improvement in psychotic symptoms.<sup>10</sup> Patients on SGAs may have improved attention, delayed memory, and verbal fluency without the motor side effects.<sup>6,10</sup>

A review of 20 studies indicates that SGAs improve cognition more than FGAs, but to a modest degree and without returning patients to normal cognitive functioning.<sup>9,10,52</sup> However, the Clinical Antipsychotic Trials of Intervention Effectiveness trial showed similar cognitive effects of FGAs and SGAs at 2 months, with greater improvement in the perphenazine group at 18 months.<sup>11</sup> It is hypothesized that FGAs have a direct negative effect on cognition, possibly through their anticholinergic actions, which masks the improvement that is due to symptom relief.<sup>10</sup> It is unclear whether the small but significant cognitive improvement seen with SGAs are a direct result of the medication, or secondary to the decreased negative symptoms and reduced side effects.<sup>53</sup>

From these data it is clear that alterations in human muscarinic receptors can cause significant cognitive deficits that are similar to deficits seen in schizophrenia. If it is indeed the case that muscarinic receptors are altered in schizophrenia, this alteration would certainly contribute to the cognitive dysfunction associated with the disease. Therefore, augmenting the existing receptors with cholinergic agonists could normalize their activity and improve cognitive function. That is, a muscarinic agonist may reverse some of the cognitive deficits seen in schizophrenia.

### **MOST ANTIPSYCHOTICS HAVE ANTICHOLINERGIC ACTIVITY**

Before we address muscarinic agonists, however, we must first examine the effects that anti-

psychotics have on these receptors. No currently used psychiatric medications have significant affinity for nicotinic receptors, but many have antimuscarinic activity.<sup>54</sup>

Davies and colleagues<sup>55</sup> reviewed 71 psychoactive medications and found that most are either non-reactive or are antagonists at M<sub>1</sub> receptors; chlorprothixene, chlorpromazine, mesoridazine, olanzapine, and thioridazine are all relatively potent M<sub>1</sub> antagonists. Several antipsychotics (loxapine, clozapine, fluperlapine) are M<sub>1</sub> partial agonists with low intrinsic activity,<sup>55</sup> which means that they activate the M<sub>1</sub> receptor with partial physiologic response or have both agonist and antagonist effects (Table 3).<sup>55-58</sup>

Among the SGAs, a review by Bymaster and colleagues<sup>38</sup> found that clozapine and olanzapine had high affinity and antagonist activity for all five muscarinic subtypes in clonal cell lines (though considerably less potent than atropine); quetiapine had moderate affinity for M<sub>1</sub> receptors and risperidone and ziprasidone had lower affinity for all subtypes. Weiner and colleagues<sup>58</sup> found that clozapine is an antagonist at M<sub>1</sub>, M<sub>3</sub>, and M<sub>5</sub>, and an M<sub>2</sub>/M<sub>4</sub> partial agonist; olanzapine has antagonist activity at all muscarinic subtypes; while risperidone and ziprasidone are non-reactive at all muscarinic subtypes.

### **ANTICHOLINERGIC ACTIVITY IMPAIRS AND MUSCARINIC AGONISTS IMPROVE MEMORY AND LEARNING**

There is considerable evidence that anticholinergics impair memory and learning. For example, list-learning task deteriorates with anticholinergics,<sup>59</sup> and low brain levels of choline acetyltransferase (the enzyme that synthesizes acetylcholine) are associated with poor cognitive functioning.<sup>19</sup> Scopolamine, a muscarinic antagonist, decreases working memory, declarative memory, sustained visual attention, and psychomotor speed.<sup>60</sup> Antagonists specific to the M<sub>1</sub> receptor worsen working memory in animals, and weak antagonists may also do so in individuals with schizophrenia.<sup>22</sup>

These effects on memory also occur in schizophrenia. People with schizophrenia taking both an antipsychotic and an anticholinergic had lower scores on the cognitive section of the Cambridge Examination for Mental Disorders in the Eldery and Mini Mental Status Exam (MMSE) scores than patients taking only antipsychotics.<sup>61</sup>

Patients with chronic schizophrenia scored better on the ideational praxis and orientation subscales of the Alzheimer's Disease Assessment Scale cognitive subscale within 10 days of stopping an anticholinergic, even after >1 year of treatment.<sup>61</sup>

If antimuscarinics impair learning and memory, one might speculate that muscarinic agonists would improve those domains. The cognitive symptoms of schizophrenia are not widely considered, nor are there any currently used pharmacologic tools to improve cognition, so there are few studies of muscarinic agonists in schizophrenia. However, there are many studies in a condition characterized by impaired memory—Alzheimer's disease. While the patterns of cognitive dysfunction in the two conditions are different and the neuropathology of Alzheimer's disease is not present in schizophrenia,<sup>19,62,63</sup> they do have enough similarities for the Alzheimer's disease data to be useful in addressing the potential for cholinergic treatments in schizophrenia. Due to the side effects of direct muscarinic agonists and the lack of selective agonists, the pro-cognitive agents used in Alzheimer's disease are largely acetylcholinesterase inhibitors (AChE-Is), which indirectly increase levels of acetylcholine in the brain through reuptake blockade. AChE-Is and direct muscarinic agonists augment central acetylcholine neurotransmission and produce a pro-cognitive effect.<sup>58</sup> These medications have been shown to enhance learning in normal subjects, with the degree of change being proportional to the subject's baseline performance.<sup>19</sup> Thus, since schizophrenia patients tend to perform more poorly at baseline, they may potentially benefit even more from these drugs than would normals.

There have recently been several uncontrolled studies and case-reports on AChE-Is for cognition in schizophrenia. A case report on patients with comorbid schizophrenia and dementia taking donepezil (an AChE-I) notes that one patient had decreased withdrawal, increased motivation, and gained independence in activities of daily living; these improvements disappeared when donepezil was withdrawn. A second patient had more coherent speech, less confusion, better orientation, and was able to transfer to an unlocked unit. The third patient had increased motivation and concentration and became able to recognize her children and daily care staff again. All three patients had improved MMSE and Clinical Global Improvement scores and decreased negative symptoms; there were no changes in positive symptoms.<sup>64</sup>

In a case report of an ABAB trial of donepezil in a college educated schizophrenia patient, the patient demonstrated increased word fluency and card sorting efficiency while on donepezil; he reported subjective improvement in concentration, clarity of thought, reading ability, and performance at work.<sup>65</sup> Two case studies of schizophrenia patients on galantamine (a nicotinic agonist and AChE-I) and an antipsychotic showed improvement of both the psychotic symptoms and social skills.<sup>66</sup>

Unfortunately, there are few good randomized controlled trials of cholinergic medications in schizophrenia, and the existing data are contradictory, though more studies are becoming available. Although a double-blind trial of donepezil in schizophrenia showed no significant difference in serial verbal learning, delayed recall, or vigilance between the placebo, donepezil 5 mg and 10 mg

**TABLE 3.**  
**Affinity of Antipsychotics for Neurotransmitter Receptors<sup>\*55-58</sup>**

	<u>D<sub>2</sub></u>	<u>D<sub>1</sub></u>	<u>5-HT<sub>2A</sub></u>	<u>Muscarinic</u>
Haloperidol	+++	++	+	None/questionable
Fluphenazine	+++	+	+	None
Clozapine	+	++	+++	+++
Olanzapine	++	++	+++	+++
Risperidone	+	+	+++	None
Quetiapine	+	+	+++	Antagonist M <sub>3</sub> ; none at M <sub>1</sub> , M <sub>5</sub>
Ziprasidone	+	+	+++	None

\* These data are summarized from a profile of functional activity at human monoaminergic G-protein couple receptors by 462 clinical drugs. All antipsychotics shared the properties of D<sub>1</sub> and D<sub>2</sub> antagonism and 5-HT<sub>2A</sub> antagonism/inverse agonism; however, action at muscarinic receptors is variable.

+ = weak affinity; ++ = moderate affinity; +++ = strong affinity; D = dopamine; 5-HT = serotonin.

Sellin AK, Shad M, Tamminga C. *CNS Spectr*. Vol 13, No 11. 2008.

groups<sup>9</sup> and an open-label trial of donepezil plus an SGA showed no change in BPRS positive scale or Scale for the Assessment of Negative Symptoms total score, the donepezil group in the second trial did show a significant increase in manual dexterity and a non-significant improvement in verbal recall, processing speed, and visual memory.<sup>67</sup>

Another double-blind study of young persons with stable schizophrenia showed no treatment effect of donepezil on any cognitive functions.<sup>68</sup> A meta-analysis of AChE-Is in schizophrenia also showed non-significant effects on short- and long-term memory.<sup>69</sup> A double-blind, functional imaging study of schizophrenics performing a word-list task showed normalized frontal and mediofrontal-cingulate activation in the patients taking donepezil.<sup>70</sup> Another functional imaging study with schizophrenia patients taking an AChE-I and an SGA showed normalized brain activity on functional magnetic resonance imaging during a verbal fluency task.<sup>19</sup> A small double-blind trial<sup>71</sup> of galantamine with risperidone showed statistically significant improvement in attention and delayed memory in the galantamine group. A small double-blind trial<sup>72</sup> of galantamine with FGAs showed no significant effect on any measure of cognition tested.

A larger double-blind trial<sup>67</sup> of galantamine in older schizophrenia patients showed some benefit to processing speed in the galantamine group, but no change in overall measures of cognition. Because galantamine is an AChE-I and an allosteric potentiator of the nicotinic receptor, it is unclear whether any effects seen are due to nicotinic actions or overall increase in ACh levels. If AChE-Is serve to augment certain aspects of cognition in schizophrenia, the effects are not great enough to impact overall function in schizophrenia.

## OTHER MUSCARINIC AGONIST ACTIONS

A major concern of adding new medications to schizophrenia is the possibility of counteracting the beneficial effects of the antipsychotics. There are reports of high levels of anticholinergics increasing psychosis.<sup>44,59,73</sup> Physostigmine has been reported to decrease delusions in Alzheimer's disease with similar efficacy and fewer side effects than haloperidol.<sup>63</sup> There is one report of a cholinomimetic temporarily improving psychotic symptoms in chronic treatment-resistant schizophrenics.<sup>63</sup> Thus, muscarinic agonists would not likely exacerbate the psychotic symptoms of schizophrenia and might also be beneficial for the cognitive symptoms.

Peripheral effects of muscarinic agonists, acting mainly at the M<sub>1</sub>–M<sub>4</sub> receptors, are often represented by the mnemonic "DUMBELS" —diarrhea, urination, miosis, bradycardia/bronchospasm, emesis, lacrimation, and salivation/secretions/sweating. Peripheral response to M<sub>1</sub> agonism is not described. M<sub>2</sub> agonism is associated with bradycardia, hypothermia, bronchoconstriction, salivation, nausea, vomiting, diarrhea, urination, and miosis.<sup>25,74</sup> M<sub>3</sub> agonism is associated with hypotension, nausea, vomiting, diarrhea, urination, and miosis.<sup>25,74</sup> M<sub>4</sub> agonism is associated with modulation of striatal dopamine release, pre-pulse inhibition, and analgesia; peripherally, it regulates keratinocyte migration.<sup>25,74</sup> M<sub>5</sub> agonism induces cerebral arterial vasorelaxation; its peripheral actions are unknown.<sup>25,74</sup> Peripheral muscarinic effects that have yet to be associated with a specific receptor are lost visual accommodation, headache, atrial fibrillation in hyperthyroid patients, flushing, and belching.<sup>25,74</sup>

## AVAILABLE MUSCARINIC AGONISTS

Currently, there are no fully subtype-selective muscarinic agonists and most pro-cholinergic medications used in practice are AChE-Is. In a study of 71 psychoactive medications, the following medications were found to be M<sub>1</sub> partial agonists with overall anticholinergic activity—clozapine, fluperlapine, JL13 fumarate, loxapine, 1-(m-chorophenyl)-piperazine, melperone, MK-212, pyrilamine, and xanomeline<sup>55</sup>; N-desmethylozapine (NDMC) was found to be a M<sub>1</sub> agonist at an allosteric site.<sup>55,58</sup> The property of M<sub>1</sub> partial agonism is shared by some antipsychotics (loxapine, clozapine, fluperlapine) and some that increase psychosis (m-CPP)<sup>55</sup>; it should be noted that all of these drugs have actions at multiple other receptors. While this is a wide range of drugs, there are some partially selective muscarinic agonists currently under investigation, such as AF-150(s), WAY-132983, Lu25-109, 1,2,5-thiadiazole analogues, xanomeline, and NDMC. Table 4 provides a summary of preclinical and clinical characteristics,<sup>19,20,22,33,41,55,58,75-83</sup> and Table 5 provides a summary of side effects.<sup>53,81</sup>

In vitro tests show that AF-150(s) induces growth in neurons.<sup>33</sup> However, this compound is being studied only in animal models for Alzheimer's disease, not schizophrenia. While it may become useful in the future it is not currently in consideration for human schizophrenia use.

Derived from rational drug design and receptor modeling, WAY-132983 is an M<sub>1</sub> selective agonist that is more potent and longer acting than xanomeline.<sup>75</sup> In monkeys, it attenuated anticholinergic det-

riment on cognition, increased working memory, and decreased distractibility without limiting side effects.<sup>75</sup> However, like AF-150(s), it is currently being studied only in animal models for Alzheimer disease and is not currently a prospect for schizophrenia.

Lu25-109 has agonist activity at the M<sub>1</sub> receptor and antagonist activity at the M<sub>2</sub> and M<sub>3</sub> receptors; this profile has been hypothesized to have good effect on cognition.<sup>20</sup> However, a clinical trial in Alzheimer's disease patients demonstrated no improvement in cognition, global measures of behavior or activities of daily living, and showed worsened cognition at the highest dose of 300 mg/day.<sup>82</sup>

1,2,5-thiadiazole analogues have equally high affinity for all muscarinic receptors and appear to have agonist activity at M<sub>2</sub> and M<sub>4</sub>,<sup>78</sup> although their activity at each receptor subtype was not specifically determined. These compounds had few cholinergic side effects (eg, salivation or tremor) in rats, and evidenced efficacy in animal models predictive of antipsychotic activity (conditioned avoidance responding and inhibiting apomorphine-induced climbing). These compounds are still in development.

Xanomeline is an M<sub>1</sub>/M<sub>4</sub> agonist with a SGA profile that improves cognition and psychotic symptoms (hallucinations, agitation, delusion, and suspicion) in Alzheimer's disease.<sup>19,76,79,80,84</sup> It is a more potent agonist at the M<sub>1</sub> receptor than carbachol, the full agonist prototype,<sup>55,85</sup> and it instantly binds to the M<sub>1</sub> receptor in a manner resistant to typical washing.<sup>85</sup> In rats, it reverses amphetamine-induced hyperlocomotor activity and stereotypic behaviors with no sedation, and blocks apomorphine-induced climbing behavior.<sup>38</sup> Xanomeline is theorized to have a faster onset of symptom relief than extant antipsychotics without extrapyramidal symptoms.<sup>84</sup> A small randomized controlled trial of xanomeline<sup>86</sup> demonstrated significant improvement in total BPRS scores, verbal learning, short-term memory, and Positive and Negative Syndrome Scale total score and positive and negative symptom subscales. The cognitive improvement associated with xanomeline is dose dependent, but unfortunately, so are the adverse side effects of nausea, dyspepsia, and diaphoresis.<sup>41</sup> These effects, likely caused by action on M<sub>3</sub> receptors, were severe enough to cause a 52% drop-out rate in the high-dose arm of one study.<sup>76</sup> A transdermal formulation is in development with hopes of decreasing first-pass metabolism and side effects.<sup>84</sup>

NDMC is a metabolite of clozapine with a similar receptor profile as clozapine; it has partial agonist activity at the dopamine 2 (D<sub>2</sub>) receptor family,<sup>87</sup> but is non-reactive at D<sub>3</sub>.<sup>58</sup> N-desmethylclozapine (NDMC) is an allosteric partial agonist at the M<sub>1</sub> receptor, where clozapine is a functional antagonist or partial agonist.<sup>55,58,77,80</sup> NDMC has high agonist efficacy at M<sub>1</sub> (partial), M<sub>4</sub>, and M<sub>5</sub>, but antagonist activity at M<sub>3</sub>.<sup>55,58</sup>; this would lead to fewer adverse side effects. NDMC-induced phosphorylation of mitogen-activated protein kinase, which was dose-dependent, was blocked by scopolamine; this action is consistent with M<sub>1</sub> activation.<sup>22,58</sup> NDMC also crosses the blood-brain barrier and induces c-fos expression in rat forebrain in a manner similar to clozapine and other SGAs.<sup>22,58,80,83</sup>

**TABLE 4.**  
**Preclinical and Clinical Characteristics**  
**of Available Muscarinic**  
**Agonists**<sup>19,20,22,33,41,55,58,75-83</sup>

<i>Muscarinic Agonists</i>	<i>Preclinical Characteristics</i>	<i>Clinical Characteristics</i>
AF-150(s)	Neurotrophic	Not in human trials
WAY-132983	Potent, long-acting, increased working memory, decreased distractibility, few side-effects	Not in human trials
Lu25-109	M <sub>2</sub> /M <sub>3</sub> antagonist	No cognitive improvement in AD, worse cognition at high dose
1,2,5-thiadiazole analogues	Equal affinity for all muscarinic receptors, M <sub>2</sub> /M <sub>3</sub> antagonist, few cholinergic side effects	Not in human trials
Xanomeline	M <sub>1</sub> /M <sub>4</sub> agonist, functional DA antagonist, SGA profile	Dose-dependent cognitive improvement and GI symptoms
N-desmethylclozapine	Allosteric M <sub>1</sub> agonist, M <sub>4</sub> /M <sub>5</sub> agonist, M <sub>3</sub> antagonist, crosses blood-brain barrier	Sedation, orthostasis, diaphoresis

M=muscarinic; AD=Alzheimer's dementia, DA=dopamine; SGA=second-generation antipsychotic; GI=gastrointestinal.

Sellin AK, Shad M, Tamminga C. *CNS Spectr*. Vol 13, No 11. 2008.

NDMC activates the M<sub>1</sub> receptor in vivo, not just in vitro.<sup>58</sup> It is unknown whether NDMC shares its parent compound's risk for agranulocytosis. One study that measured NDMC plasma levels in patients taking clozapine found that a high NDMC:clozapine ratio predicts better enhancement of cognition in patients<sup>58</sup>; however, other studies have not replicated this result.<sup>88</sup> In rats, NDMC did not induce catalepsy or increase prolactin, but also only partially inhibited amphetamine-induced locomotion (a stand-in for psychosis) and inhibited conditioned avoidance response only at the highest dose of 100 mg/kg (whereas antipsychotics inhibited as early as 20 minutes).<sup>88</sup>

Another study showed that NDMC did inhibit amphetamine-induced hyperactivity.<sup>89</sup> NDMC was also noted to decrease errors in an eight-arm maze test (in which clozapine induced errors); this effect was reversed by an M<sub>1</sub> antagonist.<sup>89</sup> NDMC had no effect on a novel object recognition task, whereas clozapine and other antipsychotics worsened performance.<sup>89</sup> Early human studies of NDMC have demonstrated safety and tolerability in oral formulation and outcomes consistent with antipsychotic actions.<sup>81</sup> However, studies of this drug focused on cognitive dysfunction in schizophrenia remain to be done. NDMC appears to be a good candidate to test the therapeutic potential of M<sub>1</sub> muscarinic stimulation on cognition in schizophrenia because of its agonist potency at the M<sub>1</sub> and M<sub>5</sub> receptors.

## CONCLUSION

In this review, we have examined data regarding muscarinic receptors and their relationship to schizophrenia and cognition. Evidence suggests that muscarinic receptors (especially M<sub>1</sub>) may be reduced in schizophrenia and that this alteration might contribute to the cognitive dysfunction intrinsic to this disorder. Rodent studies have shown that M<sub>1</sub> receptor blockade produces cognitive difficulties similar to those seen in schizophrenia. It is known that most antipsychotics have anticholinergic activity, and that anticholinergics, often used for antipsychotic medication side effects, impair learning and memory. Thus, the antipsychotics used to treat schizophrenia, and the anticholinergics used to treat motor side effects, may compound the illness' cognitive burden.

It is also true that acetylcholinesterase inhibitors and muscarinic agonists improve cognition without affecting psychosis. It would then stand to reason that an antipsychotic with M<sub>1</sub> agonist properties could improve cognition in schizophrenia patients. Reviewing the available M<sub>1</sub> receptor agonists shows that NDMC has considerable potential. Studies for enhancing cognition in schizophrenia are planned.

Research in the pathophysiology of schizophrenia has not yet defined the illness. We know some of the changes in neurotransmitter and receptor concentrations and the location of many receptor subtypes. However, we are only beginning to understand the function of these subtypes.

**TABLE 5.**  
**Side Effects of Muscarinic Agents<sup>53,81</sup>**

	<i>GI (pain, nausea, emesis, dyspepsia)</i>	<i>Blood pressure changes</i>	<i>Diaphoresis</i>	<i>Sedation, confusion</i>	<i>Headache</i>	<i>Cardiac (AV block, MI, PVC, syncope, bradycardia)</i>	<i>Salivation</i>
NDMC	—	+	+	+	—	—	—
Xanomeline	++	+	+	+	—	+	+
Bethanechol	+	+	+	—	+	+	+
Cevemiline	+	—	+	—	+	—	—
Pilocarpine	+	—	+	—	+	+	—
Physostigmine	+	+	+	+	+	+	—
Neostigmine	+	+	+	—	—	+	+
Donepezil	+	+	+	+	+	+	—

GI=gastrointestinal; AV=atrio-ventricular; MI=myocardial infarction; PVC=premature ventricular contraction; —=none; +=present; ++=severe; NDMC=N-desmethyl-clozapine.

Sellin AK, Shad M, Tamminga C. *CNS Spectr*. Vol 13, No 11. 2008.

Further study of the difference between schizophrenia and control brains could elucidate aspects of the pathophysiology of schizophrenia and lead to more focused treatment options. Clinical focus on a symptom domain like cognition and knowledge of its pharmacology in animals can provide a rationale for new treatment directions in schizophrenia. **CNS**

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