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FEATURE REVIEW

Towards a muscarinic hypothesis of schizophrenia

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Although the neurotransmitter dopamine plays a prominent role in the pathogenesis and treatment of schizophrenia, the dopamine hypothesis of schizophrenia fails to explain all aspects of this disorder. It is increasingly evident that the pathology of schizophrenia also involves other neurotransmitter systems. Data from many streams of research including preclinical and clinical pharmacology, treatment studies, post-mortem studies and neuroimaging suggest an important role for the muscarinic cholinergic system in the pathophysiology of schizophrenia. This review will focus on evidence that supports the hypothesis that the muscarinic system is involved in the pathogenesis of schizophrenia and that muscarinic receptors may represent promising novel targets for the treatment of this disorder.
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Introduction

Schizophrenia is a severe psychiatric illness with a lifetime prevalence of ~1% that imposes a huge toll on patients, their families and public health services worldwide. The diagnosis of schizophrenia is still based on the presence of a typical symptom-constellation and time course. The peak onset of symptoms occurs most frequently in early adulthood and in a significant number of cases the disorder is life-long.

Delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior and negative symptoms (such as apathy, anhedonia and social withdrawal) constitute the core symptoms of schizophrenia. These core clinical symptoms of schizophrenia are frequently complicated by cognitive deficits, mainly in the areas of attention, memory, executive functioning and intelligence[1,2] and the presence of affective disturbances.[3] Although negative and cognitive symptoms markedly impact on the functional outcome in schizophrenia,[4] they do not respond well to existing treatments. Therefore, the treatment of negative and cognitive symptoms in schizophrenia is a pressing unmet need.

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Neuropsychopharmacological studies have focused on the role of different neurotransmitter systems in schizophrenia and led to hypotheses as to the causes of this disorder. The dopamine hypothesis of schizophrenia (for a review see Carlsson[5]) is based on the observation that stimulation of the endogenous dopaminergic system with drugs such as amphetamine frequently leads to transient psychotic symptoms. By contrast, blockade of the dopamine D_2-receptor with antipsychotic drugs leads to a reduction of the positive symptoms of schizophrenia.[6–8] From this it has been concluded that overactive dopaminergic pathways in the central nervous system (CNS) are a major contributor to the positive symptoms associated with schizophrenia. This hypothesis has been validated by recent neuroimaging data from positron emission tomography (PET) and single photon emission computed tomography (SPECT). Studies have shown that unmedicated subjects with schizophrenia release more dopamine after stimulation with amphetamine than healthy controls[9–11] and that subjects with schizophrenia have a higher fraction of dopamine D_2 receptors occupied by endogenous dopamine than normal controls.[12] The consequences of a hyperdopaminergic state are complex. In mice, a dopamine-excess in the striatum results in working memory deficits as well as an impact on dopamine levels, dopamine turnover and activation of dopamine D_1-receptors in the prefrontal cortex.[13,14] Thus, there is still a significant amount of knowledge required to fully understand the outcomes of an overactive dopaminergic system in the human CNS.

Despite these data, the dopamine hypothesis cannot entirely explain the whole range of psychopathology associated with schizophrenia. Therefore, research has also focused on the role of other neurotransmitter systems such as glutamate,[15,16] γ-amino-*n*-butyric acid (GABA),[17] serotonin[18] and nicotinic acetylcholine[19,20] in schizophrenia. A growing body of evidence also suggests that changes in muscarinic cholinergic neurotransmission contribute to the pathology of schizophrenia. Muscarinic cholinergic neurotransmission is a part of cholinergic neurotransmission, which constitutes a crucial factor for different cognitive processes including sensory perception, memory and learning. It is therefore attractive to posit that these receptors are involved in the deficits in cognition and reality-orientation associated with psychiatric disorders such as schizophrenia. This review focuses on the hypothesis that the muscarinic receptor system plays a role in the pathophysiology of schizophrenia.

Acetylcholine

Since the beginning of the last century, acetylcholine has been recognized as a neurotransmitter both in the CNS as well as the peripheral nervous system.[21] Acetylcholine is synthesized in neurons from acetyl-CoA and choline in a reaction catalyzed by the enzyme choline acetyltransferase, an enzyme that is almost exclusively located in high concentrations in cholinergic neurons. Glucose and citrate serve as a source for acetyl-CoA, whereas choline is transported into the brain from the blood stream. Choline is also recycled after acetylcholine hydrolysis in the synaptic cleft by choline transporters on neurons and neuroglia.[22] Despite these two mechanisms, the availability of choline appears to be the rate-limiting step of acetylcholine synthesis. After synthesis, acetylcholine is stored in synaptic vesicles, from where it is released into the synaptic cleft following the activation of the neuron. In the synaptic cleft, acetylcholine either binds to pre- and post-synaptic receptors (see below) or is inactivated through hydrolysis by the enzyme cholinesterase. Once acetylcholine is hydrolyzed, choline is transported through a specific choline transporter back into the pre-synaptic neuron, where it is again synthesized into acetylcholine. Different substances (e.g. organophosphates, physostigmine, acetylcholinesterase inhibitors) inhibit the enzymatic inactivation of acetylcholine in the synaptic cleft and thus increase the concentration of acetylcholine.

In the peripheral nervous system, acetylcholine is the neurotransmitter of the autonomic ganglia and the neuromuscular junction. In the CNS, there are both cholinergic interneurons and cholinergic projection neurons. Cholinergic interneurons are mainly located in the striatum and nucleus accumbens, whereas most cholinergic projection neurons are located in the basal forebrain and the brainstem. Based on their anatomical location and pattern of innervation, the following two principal cholinergic cell groups can be differentiated:[23–25]

- Basal forebrain cholinergic neurons: these cell groups are located in the medial septum, diagonal band of Broca and the nucleus basalis of Meynert, and innervate primarily the cerebral cortex and hippocampus. The innervation of the cortex follows a topographic distribution. The highest densities of cholinergic innervation are found in the limbic system.
- Brainstem cholinergic neurons: these neurons can be found in the laterodorsal and pedunculopontine tegmental nuclei and project primarily to the midbrain and brainstem.

Cholinergic neurotransmission

Cholinergic neurotransmission plays a crucial role in a variety of CNS functions including sensory perception, motor function, cognitive processing, memory, arousal, attention, sleep, nociception, motivation, reward, mood and psychosis. Besides its activity in the CNS, acetylcholine is also involved in different peripheral functions such as heart rate, blood flow, gastrointestinal tract motility, sweat production and smooth muscle activity. Thus, targeting pharmacological treatments to the CNS without affecting the peripheral functions of acetylcholine has been a difficult challenge. In understanding the function of acetylcholine in the brain, a special emphasis has been placed on the importance of acetylcholine for memory and learning[26,27] with a focus on a specific role of the cholinergic forebrain system in attention.[21,28] A deficit in the function of the cholinergic system is thus likely to result in cognitive impairment. As neurocognitive impairment is frequently associated with schizophrenia,[1,2] and has been shown to be worsened by exposure to muscarinic antagonists,[29,30] an involvement of the cholinergic system in the pathophysiology of this illness seems possible.

An increasing clarification of the effects of different agonists and antagonists on the cholinergic system is helping to better understand the mechanism of cholinergic neurotransmission. This led to the initial discovery of two families of acetylcholine receptors, one of which binds muscarine (muscarinic receptors), whereas the other binds nicotine (nicotinic receptors).[31] Muscarinic and nicotinic cholinergic receptors differ with regards to their function as well as their receptor structure. Nicotinic receptors are composed of five subunits, made up from 17 different subunits that can combine in various sequences, to form a ligand-gated ion channel.[32] The binding of acetylcholine to the nicotinic receptor leads to an activation of the ion channel, resulting in an inflow of sodium ions,[33,34] causing a rapid neural response. By contrast, muscarinic receptors are G-protein coupled receptors (see below).[35] Activation of the muscarinic receptors results in a slower but potentially more sustained response than activation of nicotinic ion channels.

The understanding of the cholinergic system is complicated by the fact that both nicotinic and muscarinic cholinergic neurotransmission contribute to its function.[36] In addition, both systems do not function in isolation but closely interact with each other and with other neurotransmitter systems including dopamine, glutamate and GABA.[37–41] The interactions between the muscarinic cholinergic system and the nicotinic cholinergic system as well as other neurotransmitter systems are complex and bi-directional. Given the central role of dopamine in schizophrenia, the interactions between the muscarinic cholinergic system and the dopaminergic system will be reviewed in more detail.

Muscarinic receptors

Muscarinic cholinergic receptors belong to the superfamily of G-protein coupled receptors[42–44] that either activate or inhibit message transduction systems, thus having an effect on the intracellular second messengers such as cyclic AMP (cAMP) or inositol triphosphate (IP_3). Muscarinic receptors consist of seven transmembrane-spanning domains and are composed of 460–590 amino acids.[45] The link between muscarinic receptors and the G-protein is thought to involve the third intracellular domain of the receptor. Muscarinic receptors can be found on cholinergic and noncholinergic cells, both as auto- and heteroreceptors.[46–49]

Molecular cloning strategies revealed five different muscarinic receptors (M_1–M_5) that can be distinguished pharmacologically[50] and that are encoded by five different genes (m1–m5).[51–54] All five subtypes of the muscarinic receptors are found in the human CNS, albeit in regionally varying concentrations.[55,56] For example, the basal ganglia and cortex predominantly express M_1 and M_4 receptors, whereas M_2 receptors predominate in the thalamus and brainstem.[57–61] Overall, the M_1, M_3 and M_4 subtype are found abundantly in the brain,[62] whereas the M_5-subtype is the least abundant.[63,64] However, the M_5-subtype may be relevant to schizophrenia as it is located in the brainstem and midbrain, where it has an effect on dopamine release.[65]

Based on their functional activity, muscarinic receptors can be subdivided into two groups (M_1, M_3 and M_5 vs M_2 and M_4) with differing effects on the G protein system. M_1, M_3 and M_5 receptors are expressed post-synaptically. Activation of M_1, M_3 and M_5 muscarinic receptors results in an activation of phospholipase C and mitogen-activated protein kinase and increases intracellular concentrations of Ca^{2+} and inositol triphosphate. By contrast, M_2 and M_4 receptors are localized pre- and post–synaptically, where they function as autoreceptors and heteroreceptors. M_2 and M_4 muscarinic receptors are negatively coupled to adenylyl cyclase. Activation of the M_2 and M_4 muscarinic receptors decreases the formation of cAMP and also reduces neurotransmitter release.[66–69]

A better understanding of the physiological role of the different subtypes of the muscarinic receptors has been gained from the study of knockout animals that lack one or more of these receptors;[70–75] for a review see Bymaster *et al.*[76]). Depending on the muscarinic receptor subtype involved, cholinergic activation can have different effects on the peripheral and central nervous function.

Role of muscarinic receptors in schizophrenia

The availability of specific tools to study the family of muscarinic receptors has produced data to suggest that these receptors may play a crucial role in the pathology and treatment of schizophrenia. An involvement of muscarinic cholinergic receptors in schizophrenia is supported by data from post-mortem, neuropsychopharmacological and neuroimaging studies.

Post-mortem CNS studies

Evidence for an involvement of the muscarinic cholinergic receptors in schizophrenia has been gained from the study of CNS tissue obtained postmortem. Few studies have so far assessed the distribution of cholinergic neurons in schizophrenia. A reduced number of cholinergic interneurons was described in the ventral striatum in schizophrenia,[77,78] but the distribution of mesopontine cholinergic neurons has yielded conflicting results.[79–81]

Analyzing the density of muscarinic receptors, an early study using ^{3}H-QNB reported a significant reduction in the level of muscarinic receptor binding in the frontal cortex of subjects with schizophrenia compared to healthy controls.[82] This result was not replicated in a later study also using ^{3}H-QNB, which reported a reduced affinity and increased muscarinic receptor number in orbitofrontal and medial frontal cortex in medicated subjects with schizophrenia, whereas unmedicated subjects with schizophrenia did not differ from controls.[83] These results were interpreted to represent the result of long-term treatment with antipsychotic drugs. The finding of increased muscarinic receptor density was replicated in the frontal cortex from subjects who had received antipsychotic medication until death.[84]

More recent studies, using more selective radioligands such as [^{3}H]pirenzepine, suggested that levels of muscarinic M_1 and M_4 receptors are decreased in the caudate and putamen from subjects with schizophrenia[85,86] (see Table 1). Similar findings of decreased levels of muscarinic M_1 and M_4 receptors in schizophrenia have been reported in the hippocampus[87] and the prefrontal cortex,[88,89] but not in the parietal cortex.[89] Using the same cohort of subjects, no changes were seen in the levels of muscarinic M_2 and M_3 receptor protein and muscarinic M_2 and M_3 mRNA in the prefrontal cortex.[90] More recent data have shown that decreased levels of [^{3}H]pirenzepine binding in the hippocampus of subjects with schizophrenia are associated with decreased levels of M_4-, but not M_1-receptor mRNA.[91] Thus, at least in some areas of the cortex, the decrease in muscarinic receptor

Table 1 Neuropathological studies of the muscarinic system in schizophrenia

Authors	*Muscarinic receptor subtype*	*Brain area*	*Result*
Scarr *et al*.[90]	M_2, M_3	Dorsolateral prefrontal cortex	No change in schizophrenia
Zavitsanou *et al*.[92]	M_2, M_4	Anterior cingulate cortex	No changes in schizophrenia, depression and bipolar disorder
Katerina *et al*.[93]	M_1, M_4	Anterior cingulate cortex	Significant decrease of M_1 and M_4 receptors in schizophrenia but not in bipolar disorder or depression
Deng and Huang[94]	M_1, M_2, M_4	Superior temporal gyrus	Significant decrease of M_1 and M_4 receptors; trend reduction in M2 and M4 receptors
Mancama *et al*.[95]	M_1	Frontal cortex	mRNA decreased
Dean *et al*.[89]	M_1, M_4	Dorsolateral prefrontal cortex	Significant decrease of M1 receptors in schizophrenia
Crook *et al*.[88]	M_1, M_4	Prefrontal cortex	Significant decrease of M1 and M4 receptors in schizophrenia with and without pre-treatment with anticholinergics
Crook *et al*.[87]	M_1, M_4	Hippocampus	Significant decrease in schizophrenia
Dean *et al*.[96]	M_1 and M_2 mRNA	Caudate, putamen	No differences in mRNA
Crook *et al*.[86]	M_2, M_4	Caudate, putamen	Significant decrease in schizophrenia
Dean *et al*.[85]	M_1	Caudate, putamen	Significant decrease in schizophrenia

levels in schizophrenia appears to be subtype-specific.

Using AF-DX 384 as a marker of M_2 and M_4 muscarinic receptors, a study failed to find differences in the anterior cingulate cortex between patients with schizophrenia, bipolar disorder or major depression.[92] However, this group did report a significant reduction in the levels of M_1 and M_4 muscarinic receptors in the anterior cingulate cortex in schizophrenia.[93] These changes were shown to have some disease-specificity as the density of M_1 and M_4 muscarinic receptors was not altered in the same CNS region from subjects with bipolar disorder or major depression.[93] In the superior temporal gyrus, another relevant brain region for schizophrenia, the density of M_1 and M_4 muscarinic receptors (using pirenzepine) was significantly decreased in schizophrenia. M_2 and M_4 muscarinic receptor levels in the same brain region (using AF-DX 384) showed a decrease that failed to reach significance.[94] These results lend further support to the concept of subtype-specific decreases in muscarinic receptor density in schizophrenia.

Levels of mRNA for muscarinic M_1 receptors are significantly decreased in the superior prefrontal gyrus[95] and dorsolateral prefrontal cortex[96] in subjects with schizophrenia. However, whereas pirenzepine binding was significantly decreased in the caudate and putamen in subjects with schizophrenia, levels of mRNA for muscarinic M_1 receptors did not differ between subjects with schizophrenia and healthy controls.[96] Further evidence for subtype-specific changes in muscarinic receptor density comes from the observation that both M_1 receptor protein and M_1 receptor mRNA are significantly decreased in the dorsolateral prefrontal cortex in schizophrenia, whereas both M_4 receptor protein levels and M_4 receptor mRNA levels are unchanged.[89]

In summary, the results from different post-mortem studies suggest that decreased muscarinic receptor density in schizophrenia may be disease-specific with evidence showing that the decrease is not apparent in bipolar disorder and major depression. The decreased muscarinic receptor density in schizophrenia is not found throughout the human cortex but is region-specific and appears to be subtype-specific, involving in particular the muscarinic M_1-receptor subtype.

The interpretation of these data showing a decrease of muscarinic receptors in schizophrenia is hampered by several limitations. Some of the ligands used in the neuropathological studies are not specific for one single subtype of the muscarinic receptor but interact with different muscarinic receptor subtypes. Pirenzepine, for example, binds to M_1 and M_4 receptors whereas AF-DX 384 labels M_2 and M_4 receptors. Ongoing neuropathological studies using other techniques such as *in situ* hybridization or Western blot are warranted to further clarify the specificity of changes in muscarinic receptor subtypes.

Neuroimaging studies

To date, there is only one imaging study that has evaluated the muscarinic receptor availability *in vivo* in unmedicated subjects with schizophrenia. This study used [I-123]IQNB (quinuclidinyl benzilate) as a SPECT-ligand that binds with very high affinity to all five subtypes of the muscarinic receptor, making it possible to study muscarinic receptors in the CNS *in vivo*. Twelve subjects with schizophrenia (mean duration of illness 12 years) were studied with

IQNB-SPECT after being off antipsychotic and anticholinergic medication for a mean of 18 days. This cohort was compared to an age- and sex-matched group of healthy controls. This study reported a significant decrease of muscarinic receptor availability in the cortex and basal ganglia in the unmedicated subjects with schizophrenia. Compared to the healthy controls, the muscarinic receptor occupancy in the subjects with schizophrenia was decreased by 20–35%.[97]

IQNB was also used to assess the effects of antipsychotic medications on muscarinic receptors *in vivo*. The second-generation antipsychotic olanzapine reduced the availability of muscarinic cholinergic receptors *in vivo*, reflecting binding of olanzapine to the muscarinic receptor. At a daily dose of 20 mg of olanzapine, the muscarinic receptor occupancy was estimated to be 28% in the basal ganglia and 38% in the cortex.[98] In another study using [I-123]-iododexetimide, a different SPECT-ligand for the muscarinic receptors, a substantial occupancy of the muscarinic receptors was confirmed in the striatum and cortex after treatment with olanzapine.[99] Both studies found no relationship between muscarinic receptor availability and side effects.

A reduction of muscarinic receptor availability *in vivo* was also shown with IQNB after treatment with clozapine, another second-generation antipsychotic. After treatment with a daily dose of at least 200 mg of clozapine (mean 275.0 mg/day), the muscarinic receptor occupancy was 45% for the basal ganglia and 58% for the cortex.[100] In direct comparison of these data, clozapine results in a significantly lower availability of the muscarinic receptor than olanzapine.[101] These results of decreased muscarinic receptor availability *in vivo* after treatment with clozapine and olanzapine are consistent with *in vitro* studies, in which both antipsychotic drugs showed high affinity to all subtypes of the muscarinic receptor.[102]

Several limitations should be included in the interpretation of these neuroimaging studies. The SPECT-ligand IQNB binds very selectively and with high affinity to all subtypes of the muscarinic receptors and thus does not allow a discrimination between the different subtypes of the muscarinic receptors. At the same time, IQNB allows an assessment of the availability of muscarinic receptors, but not of the function or the affinity states of these receptors. With regards to pharmacological studies, IQNB does not allow to distinguish between agonist and antagonist properties of medications binding to the muscarinic receptor. Some of these shortcomings of IQNB may be overcome with newly developed SPECT- and PET-ligands that bind selectively to specific subtypes of the muscarinic receptor.[103,104]

Neuropsychopharmacological studies

Muscarinic receptor antagonists (anticholinergics) such as atropine and scopolamine cause cognitive dysfunction in healthy controls[29] and, at higher doses, can induce delirium as well as vivid hallucinations in healthy controls.[105] Despite these potential effects, treatment of subjects with schizophrenia with anticholinergics has been a common practice for many years to alleviate motor side effects caused by first-generation antipsychotics. However, it was also noted that treatment with anticholinergic drugs resulted in a worsening of psychosis but a modest improvement of negative symptoms of schizophrenia.[106–110] These effects could be secondary to an increased dopamine release associated with the application of anticholinergic agents.[111] Consistent with these findings, subjects with schizophrenia frequently report an activating effect of higher doses of anticholinergics, which occasionally results in an abuse of these medications.[112] Another important observation from the clinical use of muscarinic cholinergic receptor antagonists is that these drugs worsen cognitive impairment associated with schizophrenia.[30]

Other effects associated with the cholinergic system in schizophrenia include a significant shortening of rapid eye movement (REM) latency during acute exacerbations,[113,114] greater shortening of REM latency following a muscarinic agonist[115] and a lesser prolongation of REM latency following a muscarinic antagonist.[116] In addition, a cholinergic challenge-test, using the cholinesterase inhibitor pyridostigmine, showed that the growth hormone response was increased in unmedicated subjects with schizophrenia.[117] These data from sleep and endocrine studies were interpreted as being indicative of an increased cholinergic tone in schizophrenia.

Based on these clinical findings, different hypotheses have proposed a role of the cholinergic system in schizophrenia and include the general concept of an alteration of the muscarinic cholinergic system in schizophrenia.[118] Yeomans suggested that schizophrenia involves an overactivation of cholinergic neurons in the pedunculopontine nucleus (Ch5) and the laterodorsal tegmental nucleus (Ch6). These cholinergic neurons provide cholinergic input to the dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra. An overactivation of the Ch5 and Ch6 cholinergic neurons thus leads to an overactivation of the dopaminergic neurons with the subsequent development of the symptoms of schizophrenia.[119]

The newer antipsychotic agents are found to be moderately more effective than the older antipsychotics in improving cognitive function,[120] which may in part be explained by their lower propensity to cause extrapyramidal side effects and associated lower use of anticholinergic agents.[121,122] However, the overall effects of antipsychotic medications on cognitive functioning are modest.

Use of cholinesterase inhibitors in schizophrenia. Cholinesterase inhibitors (e.g. tacrine, donepezil, rivastigmine and galantamine) increase the synaptic levels of acetylcholine through an inhibition of the enzyme cholinesterase. Enhancing the synaptic levels

of acetylcholine is viewed as a key step in restoring cognitive function. Cholinesterase inhibitors are a standard treatment for Alzheimer's disease and have moderate effects on the cognitive functioning.[123]

In a similar approach, cholinesterase inhibitors have been assessed as a potential treatment to improve cognitive deficits in schizophrenia.[124] Most studies have so far used donepezil as an add-on medication in the treatment of schizophrenia (Table 2). The addition of donepezil (5 mg) to the standard antipsychotic treatment in a small cohort of elderly subjects with schizophrenia was shown to cause a modest improvement in cognitive measurements.[125] In a subsequent study, the addition of donepezil to clozapine in a double-blind crossover design showed no overall effect. Still, three out of eight subjects improved in the total Positive and Negative Syndrome Scale-score during the donepezil-phase.[126] In another open-label trial, the addition of donepezil to a stable dose of olanzapine resulted in moderate improvement in memory and processing speed.[127] In an open-label 8-week study of 12 schizophrenia patients refractory to treatment with risperidone or olanzapine, the addition of 10 mg donepezil resulted in a 20% reduction in positive symptoms in four patients.[116] In another randomized, double-blind, crossover study, the addition of donepezil to standard antipsychotics led to a modest improvement in positive and negative symptoms and verbal learning.[128] Despite these moderate effects in clinical studies, the addition of donepezil resulted in an increase in left frontal lobe and cingulate activity in a functional magnetic resonance imaging study.[129] Moreover, the addition of donepezil to a stable antipsychotic regimen has been found to ameliorate signs of tardive dyskinesia.[130]

In contrast to these studies, several other studies failed to find a beneficial effect of the adjunctive use of donepezil in schizophrenia. In a double-blind placebo-controlled study in 36 subjects treated with risperidone, the addition of 5 or 10 mg of donepezil produced no significant improvement in cognitive measures when compared to placebo.[131] This latter finding was supported by a study in which the addition of donepezil in 12 subjects with schizophrenia, who were treated with high-potency typical antipsychotics, failed to show a significant improvement in cognitive measures.[132] Likewise, the addition of up to 10 mg of donepezil to ongoing antipsychotic treatment did not improve cognitive or psychopathological measures in 36 subjects with schizophrenia.[133]

Fewer studies have yet been conducted with the other cholinesterase inhibitors. A 12-month study using low-dose rivastigmine, another cholinesterase inhibitor, showed a significant improvement in quality of life and neuropsychological measures in subjects with schizophrenia with predominant residual symptoms.[134] Similarly, Aasen *et al*.[135] observed an improvement in sustained attention following the addition of rivastigmine. However, a recent double-blind study of adjunctive rivastigmine failed to show any improvement.[136] In a small case-series, the addition of galantamine to clozapine resulted in an improvement in sustained attention, selective attention and psychomotor speed.[137]

Although the current body of data about the utility of adding a cholinesterase inhibitor to an antipsychotic in the treatment of schizophrenia is not entirely conclusive, it suggests that cholinesterase augmentation may at best lead to a modest improvement in cognitive function, positive symptoms and tardive dyskinesia in some patients. The benefits of such an augmentation may depend on the antipsychotics being used as well as the phase of illness.

Muscarinic agonists in schizophrenia. Different muscarinic agonists have been studied in schizophrenia. Betel nut chewing is a common practice in some Asian and Pacific cultures. Arecoline, an active ingredient of betel nut, is a potent muscarinic agonist. The recreational use of betel nut has been associated with fewer positive and negative symptoms in schizophrenia.[138] Xanomeline, an arecoline derivative, is an M_1/M_4 muscarinic receptor agonist and has been evaluated in schizophrenia.[139] In animals, xanomeline results in behavioral responses similar to those seen after treatment with traditional antipsychotics.[140–142] Similar to traditional antipsychotic compounds, treatment with xanomeline inhibited the behavioral and motor effects of amphetamine and apomorphine in monkeys.[143] Recent data suggest that xanomeline is also an antagonist at the M_5 receptor.[144] As muscarinic neurons carrying M_5 receptors have synaptic contact with dopaminergic neurons in the brainstem, the functional antagonism of xanomeline at M_5 receptors may offer an additional modulatory pathway on dopaminergic cell-firing.

In humans, the efficacy and tolerability of xanomeline has been demonstrated in clinical studies in dementia. A surprising result of these studies was that xanomeline showed a dose-dependent efficacy against psychotic symptoms (agitation, delusions and hallucinations) in Alzheimer's disease.[145] More recently, monotherapy with xanomeline resulted in an improvement in positive symptoms as well as in cognitive function in 20 subjects with schizophrenia.[146]

Muscarinic effects of antipsychotics. Clozapine remains the gold standard of antipsychotic treatment[147] and was traditionally considered to be a potent muscarinic receptor antagonist. In seeming contrast to this assumption, treatment with clozapine results in some improvement in cognitive function.[148,149] The clinical observation that higher doses of clozapine frequently result in hypersalivation that can be effectively treated with anticholinergics such as pirenzepine[150,151] and that clozapine is also the only antipsychotic agent that increases REM sleep activity[152] raised further questions about the functional effects of clozapine at muscarinic receptors *in vivo*. These observations

Table 2 Pharmacological studies of cholinesterase-inhibitors in schizophrenia

Author	*Subjects*	*Design*	*Duration*	*Cholinesterase-inhibitor*	*Antipsychotic medication*	*Result*
Erickson *et al.*[128]	15	Double blind	18 weeks	Donepezil	Standard antipsychotics	Modest improvement in psychiatric symptoms and verbal learning
Freudenreich *et al.*[133]	36	Double blind placebo-controlled	8 weeks	Donepezil 5–10 mg	Standard antipsychotics	No improvement in cognitive or psychopathological measures
Stryjer *et al.*[126]	8	Double blind cross-over	18 weeks	Donepezil 5–10 mg	Clozapine	No overall change in PANSS, three patients improved during donepezil
Tugal *et al.*[132]	12	Double blind placebo-controlled	12 weeks	Donezepil 5 mg	High potency typical AP	No changes in PANSS or cognitive measures
Stryjer *et al.*[125]	6	Single blind	4 weeks	Donepezil 5 mg	Standard antipsychotic medication	Improvement in MMSE, CGI and PANSS
Buchanan *et al.*[127]	15	Open label	6 weeks	Donepezil	Olanzapine	Improvement in memory and processing speed
Friedman *et al.*[131]	36	Double blind placebo-controlled	12 weeks	Donepezil 5–10 mg	Risperidone	No significant improvement
Tandon[116]	12	Open label	8 weeks	Donepezil 10 mg	Risperidone/ olanzapine	Improvement in positive symptoms
Aasen *et al.*[135]	20	Double blind	12 weeks	Rivastigmine	Standard antipsychotics	Nonsignificant improvement in sustained attention
Lenzi *et al.*[134]	16	Open	12 months	Rivastigmine 6 mg b.i.d.	Not specified	Improvement in quality of life and cognition
Sharma *et al.*[136]	21	Double blind placebo-controlled	24 weeks	Rivastigmine	Not specified	No significant improvement
Bora *et al.*[137]	5	Case-series	8 weeks	Galantamine 16 mg	Clozapine	Improvement in sustained attention, selective attention, psychomotor speed

Abbreviations: CGI, Clinical Global Impression severity scale; MMSE, Mini-Mental State Examination; PANSS, Positive and Negative Syndrome Scale.

favor the concept that clozapine could act as a muscarinic agonist. This presumed muscarinic agonist activity of clozapine has been implicated in its unique benefits in treatment-refractory schizophrenia.[153] This position gained support from *in vitro* studies using functional assays in human muscarinic receptors expressed in cell cultures that suggested that clozapine is a full agonist at the muscarinic M_4-receptor.[154–156] These findings were questioned by another study that failed to show an agonist effect of both clozapine and olanzapine on the M_4 receptor[157] and the failure to show agonist activity of clozapine at muscarinic M_4-receptors in animal brain tissue.[155,158] The picture is further complicated by other data that suggest that clozapine is also a partial agonist at the M_1, M_2, and M_3 receptor.[156,159,160]

Although these *in vitro* studies of the cholinergic properties of clozapine did not yield a clear outcome, *N*-desmethylclozapine (NDMC), the major metabolite of clozapine, has been shown to be a potent partial agonist at cloned human M_1 receptors.[161] NDMC is the only currently available antipsychotic with M_1 agonist activity.[162] At the same time, NDMC is also a partial agonist at the dopamine D_2 and D_3 receptors.[163] In addition, NDMC, but not clozapine, leads to an increased release of dopamine and acetylcholine in the prefrontal cortex and the hippocampus.[164] NDMC also potentiates NMDA-receptor activity in the hippocampus,[165] which constitutes an alternative mechanism that could contribute to cognitive enhancement. Thus, the cognitive enhancement observed with clozapine could be due to its metabolite NDMC rather than due to the parent compound.

Looking at the effects of different antipsychotics on the release of acetylcholine, atypical antipsychotics, but not typical antipsychotics, selectively increase the release of acetylcholine in the medial prefrontal cortex. This effect is not observed in other brain regions such as the striatum and nucleus accumbens.[166] Clozapine and NDMC also increase the release of acetylcholine in the ventral hippocampus, another brain region with a crucial importance for memory.[164,167]

The interpretation of the effects of pharmacological interventions on dopamine release is complicated by the fact that the regulation of the basal dopamine release is poorly understood. This makes it difficult to unequivocally identify medication effects on dopamine release. At the same time, little is known about the regulation of basal acetylcholine release and even less about the effects of medications on activated neuronal symptoms. Still, clozapine and other atypical antipsychotics may facilitate cognition through an increase in cholinergic and dopaminergic neurotransmission. This result is of potential relevance, as a deficit in dopaminergic neurotransmission in the frontal cortex is thought to play a role in negative symptoms and cognitive deficits associated with schizophrenia.[168] Like atypical antipsychotics, xanomeline has also been shown to increase extracellular concentrations of dopamine in the prefrontal cortex.[169] The data on xanomeline, along with the findings on NDMC, support the proposition that muscarinic receptor agonists may offer a new therapeutic approach in schizophrenia.

Interactions between the muscarinic cholinergic system and dopamine: a potential mechanism of action for muscarinic receptor agonists

The importance of maintaining the exquisite balance between the muscarinic and the dopaminergic system is well established in the striatum for movement control[170,171] (for a review see Zhou *et al*.[172]). Consistent with this concept, antimuscarinic agents have been used to pharmacologically re-establish the balance between these two neurotransmitter systems in movement disorders[173] and schizophrenia.[118]

The interactions between the muscarinic and the dopaminergic systems have been studied and occur directly and indirectly (via other neurotransmitter systems such as the GABAergic neurotransmitter system[174]) as well as at different levels in the brain. In the substantia nigra, cholinergic fibers have synaptic contact with dopaminergic neurons.[175] Functionally active muscarinic receptors are located on midbrain dopaminergic neurons.[176,177] Muscarinic receptors on dopaminergic neurons in the substantia nigra and the VTA are predominantly of the M_5 receptor subtype.[64,178] The activation of muscarinic receptors on VTA dopamine neurons receptors stimulates the release of dopamine.[179] At the same time, dopaminergic projections also have a modulating effect on the muscarinic system as the release of acetylcholine in the striatum is stimulated through the release of dopamine.[170]

Studies of the functional interaction between the muscarinic and the dopaminergic neurotransmitter systems have yielded varying results. The effects of a muscarinic stimulation of the dopaminergic system depend on the muscarinic receptors involved as well as the brain regions involved. Activation of muscarinic receptors in the striatum can result in both an increase in dopamine release[46,176,180] as well as a decrease in dopamine release.[176] The firing rate of the mesostriatal dopamine system increases when muscarinic agonists are applied to midbrain dopaminergic neurons.[177,179] This muscarinic activation of midbrain dopaminergic cells involves M_1 receptors.[181] At the same time, the functional effect of the application of muscarinic agonists on dopaminergic neurons is influenced by the temporal pattern of activation. Although a brief activation of muscarinic receptors results in hyperpolarization of the dopaminergic neurons, prolonged activation of the muscarinic receptors leads to their desensitization.[182] The effects of muscarinic stimulation on dopamine release are also region-specific. The stimulation of M_1/M_4 muscarinic receptors leads to a strong dopamine release in the cortex, whereas the dopamine release is less pronounced in the nucleus accumbens.[169,183]

So far only little is known about the effects of the different muscarinic receptor subtypes on the regulation of dopamine. Knockout mice are helpful to clarify the physiological role of muscarinic receptor subtypes on the release of dopamine. In M_1 and M_2 knockout mice, cholinergic stimulation has no effect on dopamine release in the striatum.[41] In a seeming contrast to these results, another study showed that M_1 knockout mice have significantly elevated extracellular dopamine levels in the striatum as measured by microdialysis. These results were interpreted to reflect a lack of inhibition of striatal dopamine release through extrastriatal M_1 receptors.[184] In M_3 knockout mice, the release of dopamine in the striatum is increased after cholinergic stimulation, whereas the release of dopamine is completely eliminated in M_4 knockout mice and significantly reduced in M_5 knockout mice.[41]

In M_4 knockout animals, basal levels of dopamine are elevated by a factor of two in the nucleus accumbens. At the same time, these M_4 knockout animals also show a significant increase in dopamine release in the nucleus accumbens after the administration of D-amphetamine, a substance known to release dopamine. M_2 knockout animals do not differ from wild-type animals in any of these experiments.[185] These results suggest that M_4 but not M_2 muscarinic receptors exert a crucial control over dopamine levels and dopamine release in the nucleus accumbens. As M_4 muscarinic receptors serve as autoreceptors and thus regulate cholinergic activity in the midbrain, changes in the muscarinic feedback loop can result in increased dopamine release.

Further evidence to highlight the functional interaction between muscarinic receptors and dopamine comes from new muscarinic ligands. PTAC ((5R,6R) 6-(3-propylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1] octane) and BuTAC ((5R,6R)-6-(3-butylthio-1,2,5-thiadiazol-4-yl)-1-azabicyclo[3.2.1]octane) are partial agonists at muscarinic M_2 and M_4 receptors and antagonists at M_1, M_3 and M_5 receptors. Behavioral studies suggest that these drugs behave functionally as antipsychotic agents whereas they have no affinity for dopamine D_2-receptors.[186,187]

Prepulse inhibition (PPI) of the acoustic startle reflex is a sensorimotor gating process that is frequently impaired in schizophrenia. It is well established that the muscarinic system plays a major role in PPI.[188] PPI can be used as an animal model for schizophrenia.[189] Both BuTAC and xanomeline reverse a pharmacological disruption of the PPI in a way similar to dopamine D_2-antagonists.[190]

Sarter *et al*.[191] recently suggested that an abnormal increase in the reactivity of the cholinergic neurotransmission results in an impaired regulation of the mesolimbic dopaminergic neurotransmission and thus in the symptoms of schizophrenia. It should be kept in mind that these effects are not unidirectional, as the release of dopamine also has an effect on the regulation of the muscarinic cholinergic system. An abnormally regulated dopaminergic system in schizophrenia could result in a dysregulation of the cholinergic system, including the forebrain cholinergic system crucial with its crucial role in attention. In view of the exquisite balance between the muscarinic and the dopaminergic system, muscarinic ligands may offer a novel approach to pharmacologically modify an abnormal release of dopamine.

Concluding remarks

This review focuses on the role of the muscarinic cholinergic system in the pathophysiology and treatment of schizophrenia. Although clinical, pharmacological, post-mortem and brain-imaging studies support an involvement of the muscarinic cholinergic system in the pathophysiology and treatment of schizophrenia, many questions remain unanswered. It also remains unclear, if these changes in the muscarinic cholinergic system in schizophrenia are of a primary or of a secondary nature.[78,192] However, the 'muscarinic hypothesis of schizophrenia' should not be seen in isolation but as an addition to existing theories on schizophrenia.

Strong support for a role of the muscarinic cholinergic system in schizophrenia comes from post-mortem and brain-imaging studies. Several post-mortem studies have consistently shown a significant decrease of muscarinic receptor density in different brain regions that are considered to be of crucial importance in the pathophysiology of schizophrenia (e.g. frontal cortex, basal ganglia and hippocampus) (see Table 1). These results include significant decreases in specific subtypes of the muscarinic receptor (in particular M_1). This decrease in muscarinic receptor density as seen in post-mortem studies in schizophrenia is not uniform across all brain regions but is region-specific. These post-mortem results were confirmed by the only currently available *in vivo* brain-imaging study in which the muscarinic cholinergic receptor availability was measured with SPECT.[97]

At the same time, these neuropathological and brain-imaging studies cannot solve the question of the pathomechanism underlying the decrease in muscarinic receptor density in schizophrenia. The interpretation of receptor studies is further complicated by potential residual effects of antipsychotic or anticholinergic treatments. Reduced muscarinic receptor density can be due to a primary reduction in the number of muscarinic receptors, an increased occupancy of the muscarinic receptor through the endogenous neurotransmitter acetylcholine or through exogenous substances (e.g. pharmaceutical agents), or a muscarinic receptor downregulation secondary to a hypercholinergic state.

The finding of reduced M_1-receptor protein and M_1-receptor mRNA in the dorsolateral prefrontal cortex in the presence of unchanged M_4 receptor-protein and M_4-receptor mRNA levels[89] does not support a general increase of acetylcholine levels in schizophrenia. The finding of decreased muscarinic

receptor availability in unmedicated subjects with schizophrenia speaks against a mere residual effect of pharmacological treatment. The pharmacological data as presented above do not endorse a generalized hypercholinergic state. Therefore, a primary reduction of the number of muscarinic receptors in schizophrenia seems a likely factor. However, these different mechanisms are not necessarily mutually exclusive, but can combine to an overall effect.

Recent studies have reported circulating antibodies against different neurotransmitter receptors, including M_1 and M_2 muscarinic receptors, in the serum from patients with schizophrenia.[193,194] These antibodies are functionally active as they activate muscarinic receptors on astrocytes[195] and induce an increase in M_1 muscarinic receptor mRNA.[196] These findings, while still preliminary, suggest an interesting possible link between the immune system and muscarinic receptors in schizophrenia.

Genetic studies have not proven helpful in elucidating the role of the muscarinic cholinergic system in the treatment of schizophrenia.[197] Genetic studies of the cholinergic system in schizophrenia have focused on the nicotinic cholinergic system with a special interest in the α_7 nicotinic receptor gene (e.g. Leonard and Freedman[198]). Looking at the genetics of the muscarinic cholinergic system in schizophrenia, a combined effect was found for the muscarinic M_5 receptor gene and the α_7 nicotinic receptor gene on the risk of schizophrenia.[199] A polymorphism of the M_1 muscarinic receptor gene was associated with a better score on the Wisconsin Card Sorting Test in schizophrenia.[200] No other genetic studies have been reported on the muscarinic cholinergic system in schizophrenia so far.

Pharmacological studies of the muscarinic system in schizophrenia have yielded varying results. The stimulation of cholinergic neurotransmission through the use of cholinesterase inhibitors has shown very little effect on cognitive function in schizophrenia. Beyond their antagonistic effects on dopamine D_2-receptors, clozapine and olanzapine are the two antipsychotics with the strongest antagonistic effect on the muscarinic cholinergic system *in vitro* and *in vivo*. Comparing the *in vivo* binding, clozapine has a stronger effect on the muscarinic receptor availability.[101] In addition to antagonistic effects, clozapine and in particular its active metabolite NDMC also have a dose-dependent agonist effect on subtypes of the muscarinic receptor.

When considering the potential consequences of a pharmacological manipulation of the muscarinic cholinergic system in schizophrenia, improvement in cognitive function should be differentiated from antipsychotic effects. M_1 agonists have proven moderately effective in improving cognitive function in neuropsychiatric disorders associated with a loss in cognitive function. M_1 agonists may also help to reverse some of the cognitive deficits seen in schizophrenia. Only very few of the currently available antipsychotic drugs have potent M_1 agonistic effects. NDMC, the active metabolite of clozapine, is a potent M_1 agonist. The M_1 agonist properties of clozapine have been associated with its unique clinical profile[161] and N-desmethylclozapine is currently being evaluated as a potential new pharmacological agent for the treatment of schizophrenia. Other M_1 receptor agonists (e.g. sabcomeline) are currently undergoing phase II and phase III studies as potential treatments of cognitive dysfunction in schizophrenia.

Besides an improvement in cognitive function through M_1 agonistic properties, muscarinic agents also carry the potential of having antipsychotic effects. Muscarinic agonists were tested positive in animal models predictive of antipsychotic activity and have functional dopamine antagonist activity. So far, animal and human studies have focused on agonistic properties at the M_1 and M_4 receptor. However, the relevance of agonistic vs antagonistic effects on the different muscarinic subtypes for these putative antipsychotic effects remains unclear. The strongest data currently available support antipsychotic effects for M_1 and M_4 agonists.

Finally, it remains unclear if the potential antipsychotic effects of muscarinic agents are due to direct muscarinic effects (independent of dopamine) or if these antipsychotic effects are mediated through a modulatory effect on the dopaminergic system. Animal studies support both dopamine-dependent and dopamine-independent effects of muscarinic agents.[40,190] The muscarinic and dopaminergic system interact bidirectionally at different levels in the brain and the nature of these interactions is not fully understood. Depending on the brain region and muscarinic receptor subtype involved, stimulation and inhibition of the muscarinic system can result in different effects on the dopaminergic system. These assumptions of a dopamine-dependent and a dopamine-independent mechanism of action for the antipsychotic effects of muscarinic agents are also not necessarily mutually exclusive and could combine to additional efficacy. It seem possible that novel muscarinic agents will exert their effects on positive symptoms of schizophrenia primarily via an interaction with the dopaminergic system, whereas the effects on neurocognitive functioning may be primarily through direct (non-dopaminergic) effects.

The lack of sufficient information about the function of the subtypes of the muscarinic receptor renders a rational development of novel pharmaceutical agents for the treatment of schizophrenia difficult. Yet, as a substantial proportion of subjects with schizophrenia do not respond adequately to treatment with currently available medications or suffers from severe side effects,[201] muscarinic agents may at some point represent a new therapeutic approach for the treatment of schizophrenia and other psychotic disorders. More basic and clinical studies seem warranted to evaluate the muscarinic hypothesis of schizophrenia, which will hopefully translate into better diagnostic and therapeutic tools for this illness.

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