·Review·

M1 muscarinic acetylcholine receptor in Alzheimer's disease

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The degeneration of cholinergic neurons and cholinergic hypofunction are pathologies associated with Alzheimer's disease (AD). Muscarinic acetylcholine receptors (mAChRs) mediate acetylcholine-induced neurotransmission and five mAChR subtypes (M1–M5) have been identified. Among them, M1 mAChR is widely expressed in the central nervous system and has been implicated in many physiological and pathological brain functions. In addition, M1 mAChR is postulated to be an important therapeutic target for AD and several other neurodegenerative diseases. In this article, we review recent progress in understanding the functional involvement of M1 mAChR in AD pathology and in developing M1 mAChR agonists for AD treatment.

Keywords: agonist; Alzheimer's disease; amyloid; cholinergic hypofunction; M1 muscarinic acetylcholine receptor; tau

Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder afflicting millions of people. It is diagnosed by the progressive loss of cognitive function and behavioral deficits and is characterized by the presence of neurofibrillary tangles (NFTs), senile plaques, cholinergic neuron loss, and neuronal atrophy at autopsy[1, 2].

Senile plaques and NFTs are major pathological hallmarks of AD in the brain. Senile plaques consist of deposits of small peptides called β-amyloid (Aβ). Multiple lines of evidence suggest that the overproduction/aggregation of neurotoxic Aβ in vulnerable brain regions is the primary cause of AD[3-6]. NFTs are formed by accumulation of hyperphosphorylated tau protein[7, 8]. Tau is a microtubule-binding protein whose function is to stabilize microtubules and facilitate fast axonal transport. Once highly phosphorylated, tau dissociates from microtubules and is prone to aggregate, forming paired helical filaments that aggregate into NFTs[9, 10].

The third important hallmark of AD is cholinergic hypofunction. The neurotransmitter acetylcholine (ACh) exerts its physiological functions by activating either ionotropic nicotinic ACh receptors (nAChRs) or metabotropic muscarinic ACh receptors (mAChRs). It has been reported that in AD brains there are (1) reduced choline acetyltransferase levels accompanied by decreased ACh synthesis; (2) significant loss of cholinergic neurons; (3) reduction in the numbers of postsynaptic neurons accessible to ACh; (4) cholinergic neuronal and axonal abnormalities; and (5) reduction in nAChR levels[11-19]. Recent evidence indicates that cholinergic hypofunction is closely linked to Aβ and tau pathologies[20]. As a major receptor group for ACh, mAChRs have also been implicated in the pathophysiology of AD. In the present review, we focus on M1 mAChR, the dominant mAChR subtype involved in learning and memory, and discuss its involvement in AD.

Overview of the mAChR Family

mAChRs are seven-transmembrane G-protein-coupled receptors. Upon binding to the endogenous neurotransmitter ACh, mAChRs couple to G proteins to transduct signals[21-23]. So far, five mAChR subtypes (M1–M5) have been identified and are divided into two categories based on the manner of signal transduction: M1, M3, and M5 subtypes preferentially interact with the $G_{q/11}$ family of G proteins, activating phospholipase C and mobilizing intracellular calcium, while M2 and M4 subtypes couple to the $G_{o/i}$ family, inhibiting adenylate cyclases and reducing intracellular cAMP levels[24-26]. The amino-acid sequences of the five mAChRs are highly conserved, with an average sequence consensus of 56.6% (Fig. 1A, B). Phylogenetic analysis indicates that the relationship between the M2 and M4 subtypes is much closer than those among the M1, M3,

and M5 subtypes (Fig. 1C). These mAChRs share a highly-conserved pocket deep within the transmembrane regions, and ACh binds to amino-acid residues on the outer region of the binding pocket with a critical asparagine (Asp105) residue[23, 27]. The similarity in ligand-binding sites across all five subtypes makes it difficult to design subtype-selective ligands. In addition, mAChR subtypes possess numerous allosteric sites at which compounds modulate the function of the receptor upon binding[28-30]. Importantly, most of the allosteric sites differ greatly among mAChR subtypes and thus provide opportunities to design highly subtype-selective allosteric modulators of mAChRs[30].

Members of the mAChR family are widely expressed in various regions in the central nervous system (CNS) and in the peripheral system. They play crucial roles in diverse physiological processes such as memory, attention, nociception, motor control, sleep-wake
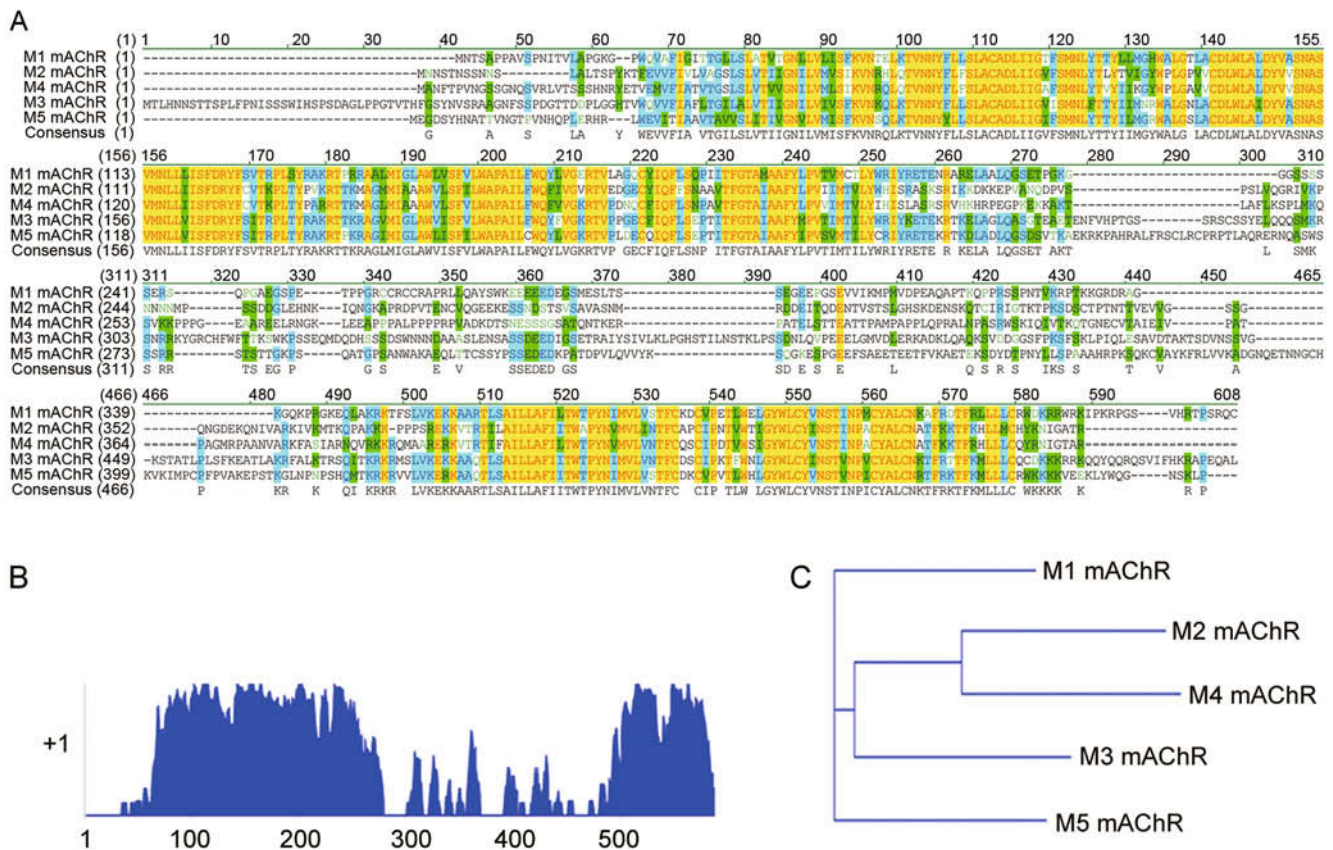

Fig. 1. Sequence comparison of mAChR subtypes. A: Amino-acid sequence alignment of the five subtypes. B: Conservation distribution pattern of mAChR sequences. X axis indicates the amino-acid numbering, and Y axis indicates the sequence consistency ratio. C: Phylogenetic analysis of the five subtypes.

cycles, and cardiovascular, renal, and gastrointestinal functions[24-26, 31-35]. Studies using *in situ* hybridization or immunohistochemistry with highly-specific antibodies to individual mAChR subtypes have revealed a unique yet somewhat overlapping distribution of these subtypes throughout the nervous system, being expressed both pre- and post-synaptically.

Among the mAChR family members, the M1 subtype makes up 50–60% of the total and is predominantly expressed in all major areas of the forebrain, including the hippocampus, cerebral cortex, corpus striatum, and thalamus[36-38]. M1 mAChR-knockout mice show a series of cognitive deficits and impairments in long-term potentiation, indicating that the M1 subtype is physiologically linked to multiple functions such as synaptic plasticity, neuronal excitability, neuronal differentiation during early development, and learning and memory[38-44]. At the cellular level, M1 mAChR is highly expressed in striatonigral, striatopallidal, and glutamatergic pyramidal neurons, especially in extrasynaptic regions. This localization of M1 mAChR is consistent with the cholinergic modulation of glutamatergic neurotransmission[45, 46].

M2 mAChR is expressed throughout the brain, including the hippocampus and neocortex, and is abundant in non-cholinergic neurons that project to these areas. In the caudate-putamen, M2 mAChR acts as an inhibitory modulator on dopaminergic terminals[46-48]. Therefore, selectively blocking M2 mAChR may provide an approach for the treatment of schizophrenia, a neuronal disorder associated with excessive dopamine neurotransmission. Mice deficient in M2 mAChR also show a striking reduction in muscarinic-dependent antinociceptive responses[49], suggesting a general antinociceptive effect.

M3 mAChR is widely distributed in the CNS, although at a lower level than other mAChR subtypes. M3 mAChR is expressed at a relatively high level in the hypothalamus, but is also found in many other regions including the hippocampus[47]. Mice lacking M3 mAChR appear hypophagic and lean, suggesting a general function of M2 mAChR in regulating food intake[50]. Consistently, mice with conditional knockout of M3 mAChR in the brain exhibit a dwarf phenotype. They also exhibit hypoplasia of the anterior pituitary gland and significantly decreased hormones including pituitary prolactin and growth hormone[51]. These findings indicate that M3 mAChR plays a critical role in promoting body growth.

M4 mAChR is mainly expressed in the corpus striatum in the CNS and on various prejunctional nerve terminals in the periphery. M4 mAChR has been suggested to play a role in psychosis and to be a promising target for the treatment of schizophrenia[52]. Indeed, the mixed M1/M4 mAChR agonist xanomeline has antipsychotic effects, and M4 mAChR-knockout mice display increased sensitivity to the disruptive effects of phencyclidine, a drug of abuse[53, 54]. M4 mAChR is also involved in the pathology of Parkinson's disease, which is associated with the loss of dopaminergic neurons projecting to the striatum and an imbalance between cholinergic and dopaminergic systems. In the corpus striatum, M4 mAChR is closely co-localized with dopamine receptors on striatal-projecting neurons and the striatal M4 mAChR inhibits dopamine D1 receptor function. Mice lacking M4 mAChR show increased locomotor activity and enhanced dopamine D1 receptor-mediated effects[55]. Consequently, selective M4 mAChR antagonists, such asbenzoxazines, have been developed for the treatment of Parkinson's disease[56].

M5 mAChR is predominantly distributed in the pars compacta of the substantia nigra, a structure that provides dopaminergic innervation to the striatum, and in the ventral tegmental area, a structure providing dopaminergic innervation to the nucleus accumbens and other limbic areas[26, 57]. These areas are well known to play a critical role in the rewarding effects of several drugs of abuse. M5 mAChR-knockout mice are less sensitive to addictive drugs such as morphine and cocaine[58]. Therefore, M5 mAChR antagonists may be important candidates for the treatment of drug addiction.

M1 mAChR in Alzheimer's Disease

Aβ, an important player in AD, is derived from β-amyloid precursor protein (APP) through sequential cleavages by β- and γ-secretases: APP is cleaved by β-secretase (BACE1) to generate the large secreted derivative sAPPβ and the membrane-bound APP C-terminal fragment-β; the latter can be further cleaved by γ-secretase to generate Aβ and APP intracellular domain. Alternatively, APP can be cleaved by α-secretase within the Aβ domain, which precludes Aβ production and instead generates secreted sAPPα that has been shown to be neuroprotective[59, 60]. Interestingly,

stimulation of M1 mAChR by agonists has been found to enhance sAPPα generation and reduce Aβ production[61-70]. Protein kinase C (PKC) is well-known to be activated upon stimulation of M1 mAChR. PKC may promote the activity of α-secretase[71] and the trafficking of APP from the Golgi/trans-Golgi network to the cell surface[72]. Some studies suggest that M1 mAChR stimulation also leads to activation of ERK1/2, which can modulate α-secretase activity and APP processing[67, 73], though there are contradictory findings showing that the α-secretase-mediated APP processing *via* M1 mAChR stimulation is not modulated by the ERK1/MEK cascade[71]. On the other hand, loss of M1 mAChR increases amyloidogenic APP processing in neurons and promotes brain Aβ plaque pathology in a mouse model of AD[74].

M1 mAChR also affects BACE1, the rate-limiting enzyme for Aβ generation[75, 76]. When APP/PS1/tau triple transgenic (3×Tg) AD mice are treated with the selective M1 mAChR agonist AF267B, the endogenous level of BACE1 decreases *via* an unclear mechanism, accompanied by a decreased Aβ level[77]. However, another study found that stimulation of M1 mAChR upregulates BACE1 levels in SK-SH-SY5Y cells *via* the PKC and MAPK signaling cascades[78]. We recently found that M1 mAChR directly interacts with BACE1 and mediates its proteasomal degradation[79]. These results suggest that M1 mAChR modulates BACE1 in a mixed manner.

In addition to inhibiting Aβ generation, activation of M1 mAChR counteracts Aβ-induced neurotoxicity through the Wnt signaling pathway, as Aβ impairs the Wnt pathway and M1 mAChR stimulation inactivates GSK-3β *via* PKC activation, stabilizes β-catenin, and induces the expression of Wnt-targeting genes engrailed and cyclin-D1 for neuron survival[80].

The involvement of M1 mAChR in AD is also manifested by its amelioration of tau pathology. Stimulation of M1 mAChR by two agonists, carbachol and AF102B, time- and dose-dependently decreases tau phosphorylation in PC12 cells[81]. Chronic treatment with AF267B also alleviates tau pathology in 3×Tg AD mice, possibly by activating PKC and inhibiting GSK-3β[77, 82].

Activation of M1 mAChR also protects against apoptotic factors in human neuroblastoma SH-SY5Y cells, such as DNA damage, oxidative stress, caspase activation, and mitochondrial impairment[83]. In addition, apoptosis induced by serum deprivation is blocked by M1 mAChR activation in a phosphoinositide 3-kinase- and MAPK/ERK-independent manner[84].

The M1 mAChR cascade may also be involved in counteracting decreased cerebral blood flow, which is one of the most consistent characteristics in pathological conditions such as AD, ischemic brain injury, intracerebral hemorrhage, and cognitive dysfunction[85, 86]. In mice with scopolamine-induced deficits, PQCA, a selective M1 mAChR positive allosteric modulator[87], improves not only recognition memory, spatial working memory, and executive function, but also blood-flow in the frontal cortex, though the mechanism is not yet clear.

Although the post-synaptic M1 mAChR level is relatively unaltered in AD[88-90], there are reports suggesting an uncoupling of M1 mAChR from G-protein in the postmortem brains of AD patients, especially in the hippocampal area, which is the most affected by Aβ[91-95]. In fact, Aβ has been shown to induce the uncoupling of M1 mAChR from G-protein, antagonizing the function of M1 mAChR under the pathological conditions of AD[96, 97]. Such an uncoupling may result in decreased signal transduction, reduced levels of sAPPα, and increased production of Aβ, triggering a vicious cycle. Although the mechanism by which Aβ disrupts mAChR-G-protein coupling is unclear, this uncoupling is palliated by antioxidants, implicating the involvement of free radicals[96]. A summary of the involvement of M1 mAChR in AD is illustrated in Fig. 2.

M1 mAChR Drugs

Because M1 mAChR plays a crucial role in learning and memory and is closely associated with AD, it has long been postulated as a therapeutic target. However, although stimulation of M1 mAChR is advantageous for cognitive improvement in AD patients, co-activation of other mAChR subtypes leads to side-effects[98]. Hence, an ideal M1 mAChR agonist should possess a high selectivity for the M1 subtype, desirable pharmacological properties, and favorable CNS penetration. So far, three types of M1 mAChR-targeting drugs have been developed: orthosteric agonists, M1 positive allosteric modulators (M1 PAMs), and allosteric agonists.

Orthosteric Agonists
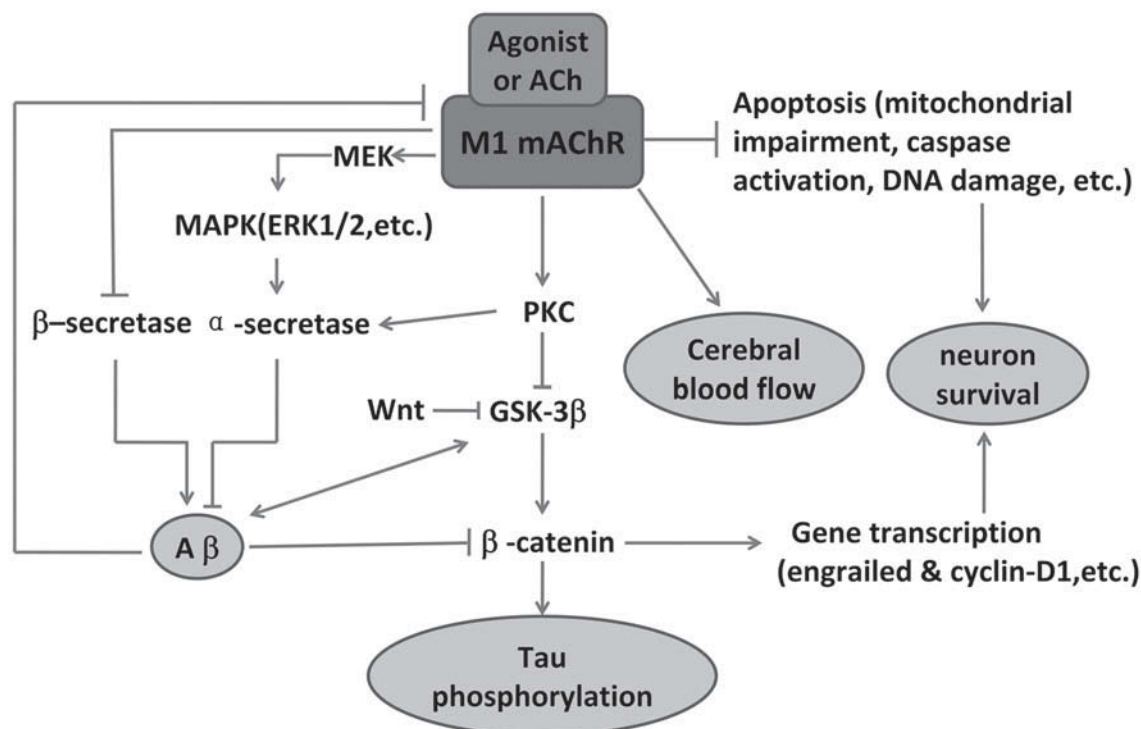
Orthosteric agonists, the first-generation M1 mAChR-

Fig. 2. The involvement of M1 mAChR in AD. Stimulation of M1 mAChR by agonists or ACh enhances sAPPα generation and reduces Aβ production. PKC and MAPKs (such as ERK1/2) are reported to be involved in this process by activating α-secretase. M1 mAChR activation also alters β-secretase (BACE1) levels *via* uncertain mechanisms. In addition, M1 mAChR stimulation counteracts Aβ-induced neurotoxicity through the Wnt signaling pathway, as Aβ impairs this pathway by destabilizing β-catenin, and M1 mAChR stimulation inactivates GSK-3β *via* PKC activation, thus stabilizing β-catenin and inducing the expression of the Wnt-targeting genes engrailed and cyclin-D1 for neuronal survival. In contrast, Aβ may induce the uncoupling of M1 mAChR from G-protein, antagonizing the function of M1 mAChR under the pathological conditions of AD. As GSK-3β is a major kinase for tau phosphorylation, stimulation of M1 mAChR inhibits tau phosphorylation as well. Moreover, activation of M1 mAChR protects cells against apoptotic factors such as DNA damage, mitochondrial impairment, caspase activation, and oxidative stress. Finally, the involvement of M1 mAChR in AD is indicated by its amelioration of decreased cerebral blood flow that has been reported as one of the most consistent characteristics in AD.

selective agonists, bind directly to the highly-conserved orthosteric ACh-binding site. Unfortunately, the similarity of orthosteric agonist-binding sites among all five mAChR subtypes makes it difficult to develop compounds that specifically target M1 mAChR. This may at least partly explain the failures of such agonists in clinical trials[99, 100]. One example is xanomeline, an mAChR agonist with selectivity for the M1 and M4 subtypes. Xanomeline improves working memory in rodents and improves cognition and reduces psychotic episodes in AD patients, but it failed during phase-II clinical trial because of serious side-effects, probably due to simultaneous activation of M1 and M4 mAChRs (M4 > M1)[53, 101-106]. AF267B and

AF102B provide another example. Chronic treatment with AF267B reduces Aβ plaques and tau hyperphosphorylation and rescues learning and memory impairments in 3×Tg AD mice[77]. However, although AF267B is a selective M1 mAChR agonist and has ~30-fold selectivity for M1 *versus* the M2, M4, and M5 subtypes, it has no better selectivity for M1 *versus* the M3 subtype[107]. Administration of AF267B and AF102B (Cevimeline, EVOXAC™), an M1 mAChR-selective agonist once prescribed for the treatment of Sjogren's syndrome, decreases Aβ42 levels in the cerebral spinal fluid (CSF) of rabbits without affecting APP[108]. Moreover, AF102B administration decreased the total CSF Aβ levels by 22% in 14 of 19 AD patients without

affecting sAPPα levels. However, AF102B has serious side-effects including gastrointestinal symptoms, diaphoresis, confusion, diarrhea, and asthenia[109, 110]. Another M1 mAChR-selective agonist, talsaclidine, enhances non-amyloidogenic processing of APP, resulting in increased sAPPα release from both a transfected human astrocytoma cell line and rat brain slices in a dose-dependent manner, as well as significantly decreasing CSF Aβ in AD patients[111]. However, talsaclidine at high doses had several side-effects such as sweating and salivation[101]. Similarly, the M1 agonist WAY-132983 at a low dose improves cognitive status in animal models but at a high dose causes side-effects such as salivation and hypothermia[102, 112]. The advantages and disadvantages of various M1 mAChR agonists are listed in Table 1 and their chemical structures are shown in Figure 3.

Allosteric Compounds

An alternative approach to design selective M1 mAChR agonists is to develop compounds that bind to the less-conserved allosteric or ectopic binding sites. Since these regions are not highly conserved among mAChR subtypes and are topographically distinct from the orthosteric binding site[28-30], allosteric compounds may have better selectivity for the M1 subtype. Based on their activation mechanisms, allosteric compounds can be further classified into regular agonists and M1 PAMs. PAMs cannot activate receptors directly. Instead, their binding modifies the receptor conformation and changes the ligand-binding and functional properties of M1 mAChR[110]. Thus, PAMs are inactive in the absence of the endogenous neurotransmitter ACh and only exert their effect in its presence. On the other hand, regular allosteric agonists activate the receptor directly, independent of the presence of the endogenous agonist[110]. Since cholinergic neurons degenerate in specific brain areas and thus cause a decrease of presynaptic ACh release in AD, M1 mAChR allosteric agonists may have unique advantages for AD treatment because they selectively activate the M1 subtype when the endogenous ligand Ach is insufficient. Over the years, major advances have been made in developing selective allosteric agonists and PAMs of M1 mAChR. These molecules are now being optimized for use and tested in animal models.

Brucine, the first reported M1-PAM, when it binds to M1 mAChR simultaneously with orthosteric ligands, potentiates the binding affinity of the ligands. However, brucine only induces a modest increase in ACh affinity and has its effect at relatively high doses[113, 114]. Brucine failed in a preclinical test for further application. Nevertheless, the high selectivity of brucine for M1 mAChR shed light on the possibility of developing agents with absolute selectivity for mAChR subtypes. After brucine, several other M1-PAMs have been discovered, including VU0029767, VU0090157, and benzyl quinolone carboxylic acid (BQCA)[115-117]. These compounds do not activate M1 mAChR directly but greatly increase the affinity of ACh for the M1 subtype. In addition, BQCA is effective in restoring discrimination reversal learning in a mouse model of AD and regulating non-amyloidogenic APP processing[117]. These positive roles of M1-PAMs make them potentially useful for AD treatment.

Besides M1-PAMs, several allosteric agonists of M1 mAChR have been discovered. A novel compound, AC-42, was found to be able to activate M1 mAChR at a region clearly distinct from the orthosteric ACh-binding site and this region is not conserved among mAChR subtypes, explaining its unprecedented selectivity for M1 mAChR. The highly anticipated AC-42 was also confirmed to be active in cell lines by monitoring intracellular calcium release and inositolphosphate accumulation. Unfortunately, AC-42 failed to activate M1 mAChR in more complex systems such as brain slices[118, 119]. Other disadvantages of many allosteric agonists developed earlier include off-target activity and poor solubility in physiological buffer systems, preventing their application *in vivo*[110]. Nevertheless, a compound developed later, TBPB, selectively activates M1 mAChR in cell lines and shows no agonist activity in any other mAChR subtype. Interestingly, TBPB also potentiates the NMDA-evoked current in hippocampal pyramidal neurons, which is considered to be important for the effect of M1 mAChR on improving cognition. In addition, TBPB shifts the processing of APP in the non-amyloidogenic direction and thereafter decreases neurotoxic Aβ production *in vitro*[120]. Further studies demonstrated that TBPB is also systemically active and crosses the blood-brain barrier[110]. All these encouraging data support the potential of using M1 allosteric agonists in the treatment of AD. The allosteric agonists 77-LH-28-1 and AC-260584 were synthesized as structural analogs of AC-42. Compound 77-LH-28-1 shows relatively higher selectivity for the M1 than for the M2, M4, and M5 subtypes, but retains weak agonist activity for M3 mAChR

Table 1. M1 mAChR-based drugs for AD treatment

Orthosteric agonists of M1 mAChR

Drugs	Potential roles in AD	Disadvantages
Xanomeline[53, 101-105]	Improves cognition and reduces psychotic symptoms in both preclinical and clinical studies.	Failed during Phase II clinical test due to dose-dependent side-effects - nausea and diarrhea.
Talsaclidine[101, 111]	Enhances non-amyloidogenic processing of APP and decreases CSF Aβ level in AD patients.	Discontinued due to side-effects - sweating and salivation.
AF102B[108-110]	Reverses cognitive impairments at low dose and reduces CSF Aβ in AD patients. The first drug ever shown to have such an effect in human patients.	Discontinued due to side-effects - gastrointestinal symptoms, diaphoresis, confusion, diarrhea, and asthenia.
AF267B[77, 98, 107, 108]	Improves cognitive function, decreases Aβ, hyperphosphorylated tau, and BACE1 levels in AD mice.	Inactive in clinical trial and discontinued.
WAY-132983[102, 112]	Enhances performance memory and cognition in animal models.	Discontinued.

M1 positive allosteric modulators

Drugs	Potential roles in AD	Disadvantages
Brucine[113, 114]	The first reported M1-PAM. Potentiates ACh affinity to M1 mAChR.	Only induces a modest increase of ACh affinity. Relatively high dose required to elicit effect.
VU0029767[115]	Increases ACh affinity to M1 mAChR.	Unreported
VU0090157[115]	Increases ACh affinity to M1 mAChR.	Unreported
BQCA[115-117]	Reverses learning impairment in an AD mouse model.	Unreported

Allosteric agonists of M1 mAChR

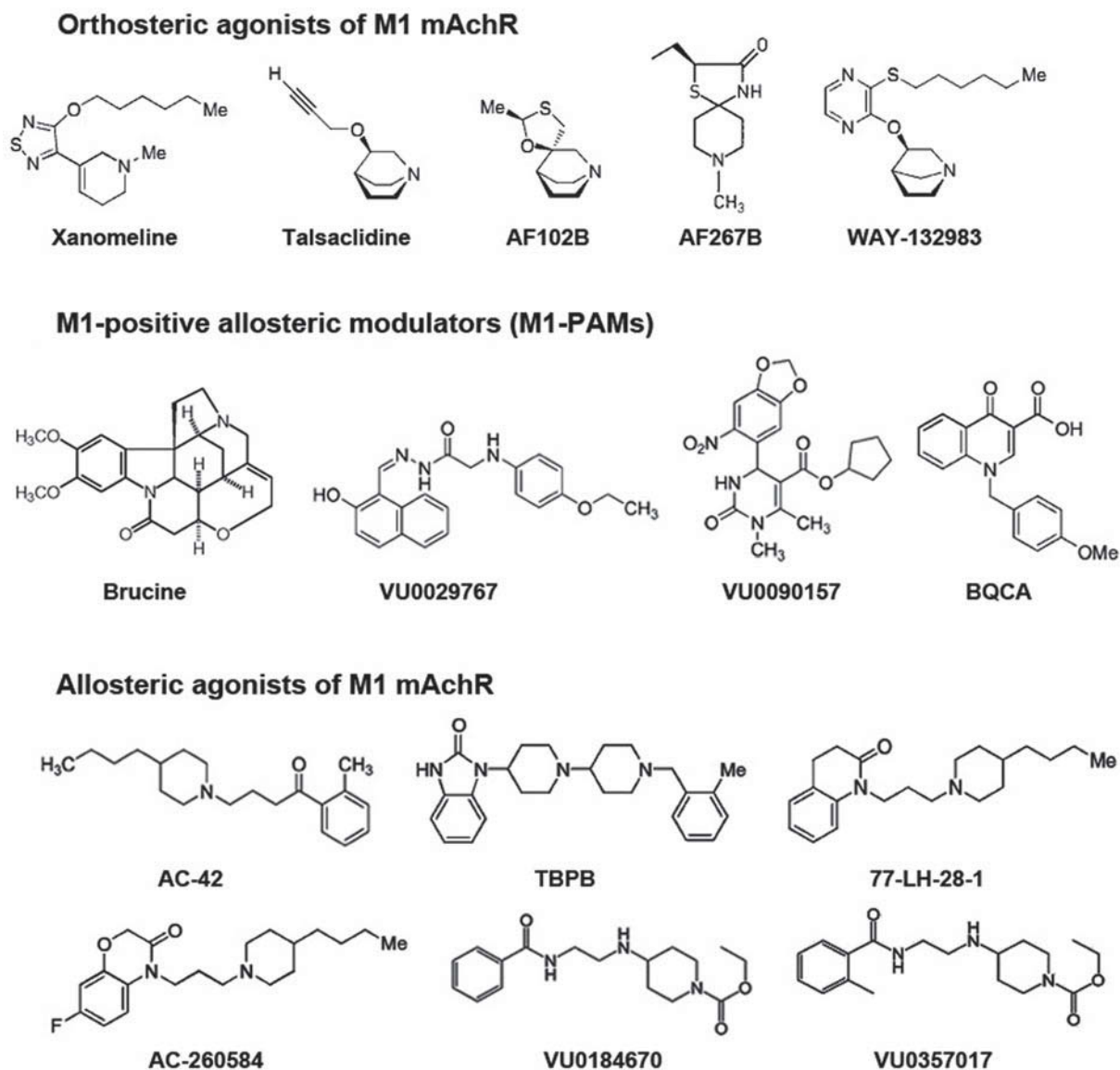| Drugs	Potential roles in AD	Disadvantages
AC-42[118, 119]	The first confirmed allosteric agonist of M1 mAChR and selectively activates M1 mAChR at an allosteric binding site in cell lines.	Failed to activate M1 mAChR in brain slices.
TBPB[110, 120]	Highly selective agonist for M1 mAChR subtype. Shifts APP processing toward the non-amyloidogenic pathway in cells and appears to have antipsychotic-like effects.	May also bind allosteric sites shared by other G protein-coupled receptors.
77-LH-28-1[121]	Highly selective and highly efficient agonist of M1 mAChR. Promotes several physiological functions related to cognition.	Unreported
AC-260584[118, 122]	Orally bioavailable with favorable antipsychotic and cognitive enhancing effect.	Not specifically selective to M1 mAChR.
VU0184670[110, 123]	Highly selective to M1 mAChR and excellent pharmacokinetic profile. Also potentiates NMDA receptor-mediated current in hippocampal neurons.	Unreported
VU0357017[110, 123]	Highly selective to M1 mAChR and excellent pharmacokinetic profile. Reverses cognitive deficits induced by mAChR antagonist scopolamine in animal models.	Unreported

Orthosteric agonists of M1 mAchR

Xanomeline   Talsaclidine   AF102B   AF267B   WAY-132983

M1-positive allosteric modulators (M1-PAMs)

Brucine   VU0029767   VU0090157   BQCA

Allosteric agonists of M1 mAchR

AC-42   TBPB   77-LH-28-1

AC-260584   VU0184670   VU0357017

Fig. 3. Chemical structures of M1-selective agonists.

at high doses. Electrophysiological studies indicate that 77-LH-28-1 increases the activity of hippocampal CA1 pyramidal cells both *in vitro* and *in vivo*. Interestingly, unlike other normal orthosteric agonists, 77-LH-28-1 appears to selectively activate M1 mAChR in a distinct signaling pathway[121]. Such a difference requires more caution in determining the potential of 77-LH-28-1 for the treatment of AD. The M1 mAChR agonist AC-260584 was recently reported to be orally bioavailable with favorable antipsychotic and cognition-enhancing effects[118, 122].

However, the lack of absolute M1 mAChR selectivity of AC-260584 and other related compounds may limit their therapeutic use. During the past few years, the M1 mAChR allosteric agonists VU0184670 and VU0357017 have been screened out, and have more exciting properties. Both compounds have high solubility in aqueous solutions as well as good CNS penetration, without any agonist or antagonist activity for the M2 and M5 subtypes. Moreover, VU0184670 potentiates neuronal NMDAR-mediated currents in hippocampal brain slices and VU0357017

reverses the cognitive deficits induced by an mAChR antagonist in a contextual fear conditioning paradigm, exhibiting improvement of hippocampus-dependent learning[110, 123]. These results implicate the two compounds as a highly potent, selective, and systemically active new generation of M1 allosteric agonists.

Conclusion

M1 mAChR plays a crucial role in cognitive functions like learning and memory. Dysregulation of M1 mAChR contributes to AD and the specific activation of the M1 subtype is considered to be a promising strategy for AD treatment. A full elucidation of the network interactions between the M1 mAChR and other AD core factor-mediated signaling pathways will facilitate the development of effective therapeutics.

REFERENCES

[1]   Richter JA, Perry EK, Tomlinson BE. Acetylcholine and choline levels in post-mortem human brain tissue: preliminary observations in Alzheimer's disease. Life Sci 1980, 26: 1683–1689.

[2]   Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science 1982, 215: 1237–1239.

[3]   Pakaski M, Kalman J. Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. Neurochem Int 2008, 53: 103–111.

[4]   Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 2005, 120: 545–555.

[5]   Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992, 256: 184–185.

[6]   Koffie RM, Hyman BT, Spires-Jones TL. Alzheimer's disease: synapses gone cold. Mol Neurodegener 2011, 6: 63.

[7]   Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001, 81: 741–766.

[8]   Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Brain Res Rev 2000, 33: 95–130.

[9]   Iqbal K, Alonso AD, Gondal JA, Gong CX, Haque N, Khatoon S, *et al.* Mechanism of neurofibrillary degeneration and pharmacologic therapeutic approach. J Neural Transm Suppl 2000, 59: 213–222.

[10]   Mudher A, Lovestone S. Alzheimer's disease-do tauists and baptists finally shake hands? Trends Neurosci 2002, 25: 22–26.

[11]   Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet 2006, 368: 387–403.

[12]   Salloway S, Mintzer J, Weiner MF, Cummings JL. Disease-modifying therapies in Alzheimer's disease. Alzheimers Dement 2008, 4: 65–79.

[13]   Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev 2006: CD005593.

[14]   Ringman JM, Cummings JL. Current and emerging pharmacological treatment options for dementia. Behav Neurol 2006, 17: 5–16.

[15]   Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol 2000, 163: 495–529.

[16]   Geula C, Nagykery N, Nicholas A, Wu CK. Cholinergic neuronal and axonal abnormalities are present early in aging and in Alzheimer disease. J Neuropathol Exp Neurol 2008, 67: 309–318.

[17]   Ladner CJ, Lee JM. Pharmacological drug treatment of Alzheimer disease: the cholinergic hypothesis revisited. J Neuropathol Exp Neurol 1998, 57: 719–731.

[18]   Perry E, Court J, Goodchild R, Griffiths M, Jaros E, Johnson M, *et al.* Clinical neurochemistry: developments in dementia research based on brain bank material. J Neural Transm 1998, 105: 915–933.

[19]   Chen GJ, Xiong Z, Yan Z. Abeta impairs nicotinic regulation of inhibitory synaptic transmission and interneuron excitability in prefrontal cortex. Mol Neurodegener 2013, 8: 3.

[20]   Fisher A. Cholinergic modulation of amyloid precursor protein processing with emphasis on M1 muscarinic receptor: perspectives and challenges in treatment of Alzheimer's disease. J Neurochem 2012, 120 Suppl 1: 22–33.

[21]   Nathanson NM. Molecular properties of the muscarinic acetylcholine receptor. Annu Rev Neurosci 1987, 10: 195–236.

[22]   Caulfield MP. Muscarinic receptors--characterization, coupling and function. Pharmacol Ther 1993, 58: 319–379.

[23]   Wess J. Molecular biology of muscarinic acetylcholine

receptors. Crit Rev Neurobiol 1996, 10: 69–99.

[24] Bonner TI, Buckley NJ, Young AC, Brann MR. Identification of a family of muscarinic acetylcholine receptor genes. Science 1987, 237: 527–532.

[25] Bonner TI, Young AC, Brann MR, Buckley NJ. Cloning and expression of the human and rat m5 muscarinic acetylcholine receptor genes. Neuron 1988, 1: 403–410.

[26] Felder CC, Bymaster FP, Ward J, DeLapp N. Therapeutic opportunities for muscarinic receptors in the central nervous system. J Med Chem 2000, 43: 4333–4353.

[27] Hulme EC, Lu ZL, Saldanha JW, Bee MS. Structure and activation of muscarinic acetylcholine receptors. Biochem Soc Trans 2003, 31: 29–34.

[28] Wess J. Allosteric binding sites on muscarinic acetylcholine receptors. Mol Pharmacol 2005, 68: 1506–1509.

[29] Presland J. Identifying novel modulators of G protein-coupled receptors via interaction at allosteric sites. Curr Opin Drug Discov Devel 2005, 8: 567–576.

[30] Conn PJ, Christopoulos A, Lindsley CW. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat Rev Drug Discov 2009, 8: 41–54.

[31] Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol 2006, 16: 710–715.

[32] Briand LA, Gritton H, Howe WM, Young DA, Sarter M. Modulators in concert for cognition: modulator interactions in the prefrontal cortex. Prog Neurobiol 2007, 83: 69–91.

[33] Wess J, Eglen RM, Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat Rev Drug Discov 2007, 6: 721–733.

[34] Bymaster FP, McKinzie DL, Felder CC, Wess J. Use of M1-M5 muscarinic receptor knockout mice as novel tools to delineate the physiological roles of the muscarinic cholinergic system. Neurochem Res 2003, 28: 437–442.

[35] Birdsall NJ, Farries T, Gharagozloo P, Kobayashi S, Lazareno S, Sugimoto M. Subtype-selective positive cooperative interactions between brucine analogs and acetylcholine at muscarinic receptors: functional studies. Mol Pharmacol 1999, 55: 778–786.

[36] Hamilton SE, Loose MD, Qi M, Levey AI, Hille B, McKnight GS, *et al*. Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. Proc Natl Acad Sci U S A 1997, 94: 13311–13316.

[37] Gerber DJ, Sotnikova TD, Gainetdinov RR, Huang SY, Caron MG, Tonegawa S. Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. Proc Natl Acad Sci U S A 2001, 98: 15312–15317.

[38] Miyakawa T, Yamada M, Duttaroy A, Wess J. Hyperactivity and intact hippocampus-dependent learning in mice lacking the M1 muscarinic acetylcholine receptor. J Neurosci 2001, 21: 5239–5250.

[39] VanDeMark KL, Guizzetti M, Giordano G, Costa LG. The activation of M1 muscarinic receptor signaling induces neuronal differentiation in pyramidal hippocampal neurons. J Pharmacol Exp Ther 2009, 329: 532–542.

[40] Hohmann CF, Potter ED, Levey AI. Development of muscarinic receptor subtypes in the forebrain of the mouse. J Comp Neurol 1995, 358: 88–101.

[41] Shideler KK, Yan J. M1 muscarinic receptor for the development of auditory cortical function. Mol Brain 2010, 3: 29.

[42] Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM, *et al.* Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci 2003, 6: 51–58.

[43] Shinoe T, Matsui M, Taketo MM, Manabe T. Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse hippocampus. J Neurosci 2005, 25: 11194–11200.

[44] Wess J. Muscarinic acetylcholine receptor knockout mice: novel phenotypes and clinical implications. Annu Rev Pharmacol Toxicol 2004, 44: 423–450.

[45] Mrzljak L, Levey AI, Goldman-Rakic PS. Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: morphological evidence for cholinergic modulation of excitatory neurotransmission. Proc Natl Acad Sci U S A 1993, 90: 5194–5198.

[46] Levey AI. Muscarinic acetylcholine receptor expression in memory circuits: implications for treatment of Alzheimer disease. Proc Natl Acad Sci U S A 1996, 93: 13541–13546.

[47] Levey AI. Immunological localization of m1-m5 muscarinic acetylcholine receptors in peripheral tissues and brain. Life Sci 1993, 52: 441–448.

[48] Hersch SM, Gutekunst CA, Rees HD, Heilman CJ, Levey AI. Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. J Neurosci 1994, 14: 3351–3363.

[49] Chen SR, Wess J, Pan HL. Functional activity of the M2 and M4 receptor subtypes in the spinal cord studied with muscarinic acetylcholine receptor knockout mice. J Pharmacol Exp Ther 2005, 313: 765–770.

[50] Gautam D, Jeon J, Li JH, Han SJ, Hamdan FF, Cui Y, *et al.* Metabolic roles of the M3 muscarinic acetylcholine receptor studied with M3 receptor mutant mice: a review. J Recept Signal Transduct Res 2008, 28: 93–108.

[51] Gautam D, Jeon J, Starost MF, Han SJ, Hamdan FF, Cui Y, *et al.* Neuronal M3 muscarinic acetylcholine receptors are essential for somatotroph proliferation and normal somatic growth. Proc Natl Acad Sci U S A 2009, 106: 6398–6403.

[52] Trendelenburg AU, Gomeza J, Klebroff W, Zhou H, Wess J. Heterogeneity of presynaptic muscarinic receptors

mediating inhibition of sympathetic transmitter release: a study with M2- and M4-receptor-deficient mice. Br J Pharmacol 2003, 138: 469–480.

[53] Mirza NR, Peters D, Sparks RG. Xanomeline and the antipsychotic potential of muscarinic receptor subtype selective agonists. CNS Drug Rev 2003, 9: 159–186.

[54] Chan WY, McKinzie DL, Bose S, Mitchell SN, Witkin JM, Thompson RC, *et al.* Allosteric modulation of the muscarinic M4 receptor as an approach to treating schizophrenia. Proc Natl Acad Sci U S A 2008, 105: 10978–10983.

[55] Gomeza J, Zhang L, Kostenis E, Felder C, Bymaster F, Brodkin J, *et al.* Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M(4) muscarinic acetylcholine receptor knockout mice. Proc Natl Acad Sci U S A 1999, 96: 10483–10488.

[56] Bohme TM, Augelli-Szafran CE, Hallak H, Pugsley T, Serpa K, Schwarz RD. Synthesis and pharmacology of benzoxazines as highly selective antagonists at M(4) muscarinic receptors. J Med Chem 2002, 45: 3094–3102.

[57] Eglen RM, Nahorski SR. The muscarinic M(5) receptor: a silent or emerging subtype? Br J Pharmacol 2000, 130: 13–21.

[58] Fink-Jensen A, Fedorova I, Wortwein G, Woldbye DP, Rasmussen T, Thomsen M, *et al.* Role for M5 muscarinic acetylcholine receptors in cocaine addiction. J Neurosci Res 2003, 74: 91–96.

[59] Zheng H, Koo EH. Biology and pathophysiology of the amyloid precursor protein. Mol Neurodegener 2011, 6: 27.

[60] Zhang YW, Thompson R, Zhang H, Xu H. APP processing in Alzheimer's disease. Mol Brain 2011, 4: 3.

[61] Haring R, Gurwitz D, Barg J, Pinkas-Kramarski R, Heldman E, Pittel Z, *et al.* Amyloid precursor protein secretion via muscarinic receptors: reduced desensitization using the M1-selective agonist AF102B. Biochem Biophys Res Commun 1994, 203: 652–658.

[62] Haring R, Gurwitz D, Barg J, Pinkas-Kramarski R, Heldman E, Pittel Z, *et al.* NGF promotes amyloid precursor protein secretion via muscarinic receptor activation. Biochem Biophys Res Commun 1995, 213: 15–23.

[63] Eckols K, Bymaster FP, Mitch CH, Shannon HE, Ward JS, DeLapp NW. The muscarinic M1 agonist xanomeline increases soluble amyloid precursor protein release from Chinese hamster ovary-m1 cells. Life Sci 1995, 57: 1183–1190.

[64] Farber SA, Nitsch RM, Schulz JG, Wurtman RJ. Regulated secretion of beta-amyloid precursor protein in rat brain. J Neurosci 1995, 15: 7442–7451.

[65] Muller DM, Mendla K, Farber SA, Nitsch RM. Muscarinic M1 receptor agonists increase the secretion of the amyloid precursor protein ectodomain. Life Sci 1997, 60: 985–991.

[66] Pittel Z, Heldman E, Barg J, Haring R, Fisher A. Muscarinic control of amyloid precursor protein secretion in rat cerebral cortex and cerebellum. Brain Res 1996, 742: 299–304.

[67] Haring R, Fisher A, Marciano D, Pittel Z, Kloog Y, Zuckerman A, *et al.* Mitogen-activated protein kinase-dependent and protein kinase C-dependent pathways link the m1 muscarinic receptor to beta-amyloid precursor protein secretion. J Neurochem 1998, 71: 2094–2103.

[68] Hung AY, Haass C, Nitsch RM, Qiu WQ, Citron M, Wurtman RJ, *et al.* Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. J Biol Chem 1993, 268: 22959–22962.

[69] Nitsch RM, Slack BE, Wurtman RJ, Growdon JH. Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science 1992, 258: 304–307.

[70] Wolf BA, Wertkin AM, Jolly YC, Yasuda RP, Wolfe BB, Konrad RJ, *et al.* Muscarinic regulation of Alzheimer's disease amyloid precursor protein secretion and amyloid beta-protein production in human neuronal NT2N cells. J Biol Chem 1995, 270: 4916–4922.

[71] Cisse M, Braun U, Leitges M, Fisher A, Pages G, Checler F, *et al.* ERK1-independent alpha-secretase cut of beta-amyloid precursor protein via M1 muscarinic receptors and PKCalpha/epsilon. Mol Cell Neurosci 2011, 47: 223–232.

[72] Xu H, Greengard P, Gandy S. Regulated formation of Golgi secretory vesicles containing Alzheimer beta-amyloid precursor protein. J Biol Chem 1995, 270: 23243–23245.

[73] Bigl V, Rossner S. Amyloid precursor protein processing *in vivo*--insights from a chemically-induced constitutive overactivation of protein kinase C in Guinea pig brain. Curr Med Chem 2003, 10: 871–882.

[74] Davis AA, Fritz JJ, Wess J, Lah JJ, Levey AI. Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology *in vitro* and *in vivo*. J Neurosci 2010, 30: 4190–4196.

[75] Chami L, Checler F. BACE1 is at the crossroad of a toxic vicious cycle involving cellular stress and beta-amyloid production in Alzheimer's disease. Mol Neurodegener 2012, 7: 52.

[76] Cole SL, Vassar R. The Alzheimer's disease beta-secretase enzyme, BACE1. Mol Neurodegener 2007, 2: 22.

[77] Caccamo A, Oddo S, Billings LM, Green KN, Martinez-Coria H, Fisher A, *et al.* M1 receptors play a central role in modulating AD-like pathology in transgenic mice. Neuron 2006, 49: 671–682.

[78] Zuchner T, Perez-Polo JR, Schliebs R. Beta-secretase BACE1 is differentially controlled through muscarinic acetylcholine receptor signaling. J Neurosci Res 2004, 77: 250–257.

[79] Jiang S, Wang Y, Ma Q, Zhou A, Zhang X, Zhang YW. M1 muscarinic acetylcholine receptor interacts with BACE1 and regulates its proteosomal degradation. Neurosci Lett 2012, 515: 125–130.

[80] Farias GG, Godoy JA, Hernandez F, Avila J, Fisher A, Inestrosa NC. M1 muscarinic receptor activation protects neurons from beta-amyloid toxicity. A role for Wnt signaling pathway. Neurobiol Dis 2004, 17: 337–348.

[81] Sadot E, Gurwitz D, Barg J, Behar L, Ginzburg I, Fisher A. Activation of m1 muscarinic acetylcholine receptor regulates tau phosphorylation in transfected PC12 cells. J Neurochem 1996, 66: 877–880.

[82] Forlenza OV, Spink JM, Dayanandan R, Anderton BH, Olesen OF, Lovestone S. Muscarinic agonists reduce tau phosphorylation in non-neuronal cells via GSK-3beta inhibition and in neurons. J Neural Transm 2000, 107: 1201–1212.

[83] De Sarno P, Shestopal SA, King TD, Zmijewska A, Song L, Jope RS. Muscarinic receptor activation protects cells from apoptotic effects of DNA damage, oxidative stress, and mitochondrial inhibition. J Biol Chem 2003, 278: 11086–11093.

[84] Leloup C, Michaelson DM, Fisher A, Hartmann T, Beyreuther K, Stein R. M1 muscarinic receptors block caspase activation by phosphoinositide 3-kinase- and MAPK/ERK-independent pathways. Cell Death Differ 2000, 7: 825–833.

[85] Hanyu H, Shimizu T, Tanaka Y, Takasaki M, Koizumi K, Abe K. Regional cerebral blood flow patterns and response to donepezil treatment in patients with Alzheimer's disease. Dement Geriatr Cogn Disord 2003, 15: 177–182.

[86] Bateman GA, Levi CR, Schofield P, Wang Y, Lovett EC. Quantitative measurement of cerebral haemodynamics in early vascular dementia and Alzheimer's disease. J Clin Neurosci 2006, 13: 563–568.

[87] Uslaner JM, Eddins D, Puri V, Cannon CE, Sutcliffe J, Chew CS, et al. The muscarinic M1 receptor positive allosteric modulator PQCA improves cognitive measures in rat, cynomolgus macaque, and rhesus macaque. Psychopharmacology (Berl) 2013, 225: 21–30.

[88] Svensson AL, Alafuzoff I, Nordberg A. Characterization of muscarinic receptor subtypes in Alzheimer and control brain cortices by selective muscarinic antagonists. Brain Res 1992, 596: 142–148.

[89] Mulugeta E, Karlsson E, Islam A, Kalaria R, Mangat H, Winblad B, et al. Loss of muscarinic M4 receptors in hippocampus of Alzheimer patients. Brain Res 2003, 960: 259–262.

[90] Volpicelli LA, Levey AI. Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. Prog Brain Res 2004, 145: 59–66.

[91] Ferrari-DiLeo G, Mash DC, Flynn DD. Attenuation of muscarinic receptor-G-protein interaction in Alzheimer disease. Mol Chem Neuropathol 1995, 24: 69–91.

[92] Tsang SW, Lai MK, Kirvell S, Francis PT, Esiri MM, Hope T, et al. Impaired coupling of muscarinic M1 receptors to G-proteins in the neocortex is associated with severity of dementia in Alzheimer's disease. Neurobiol Aging 2006, 27: 1216–1223.

[93] Potter PE, Rauschkolb PK, Pandya Y, Sue LI, Sabbagh MN, Walker DG, et al. Pre- and post-synaptic cortical cholinergic deficits are proportional to amyloid plaque presence and density at preclinical stages of Alzheimer's disease. Acta Neuropathol 2011, 122: 49–60.

[94] Ladner CJ, Lee JM. Reduced high-affinity agonist binding at the M(1) muscarinic receptor in Alzheimer's disease brain: differential sensitivity to agonists and divalent cations. Exp Neurol 1999, 158: 451–458.

[95] Shiozaki K, Iseki E. Decrease in GTP-sensitive high affinity agonist binding of muscarinic acetylcholine receptors in autopsied brains of dementia with Lewy bodies and Alzheimer's disease. J Neurol Sci 2004, 223: 145–148.

[96] Kelly JF, Furukawa K, Barger SW, Rengen MR, Mark RJ, Blanc EM, et al. Amyloid beta-peptide disrupts carbachol-induced muscarinic cholinergic signal transduction in cortical neurons. Proc Natl Acad Sci U S A 1996, 93: 6753–6758.

[97] Janickova H, Rudajev V, Zimcik P, Jakubik J, Tanila H, El-Fakahany EE, et al. Uncoupling of M1 muscarinic receptor/G-protein interaction by amyloid beta(1-42). Neuropharmacology 2013, 67: 272–283.

[98] Fisher A. Cholinergic treatments with emphasis on m1 muscarinic agonists as potential disease-modifying agents for Alzheimer's disease. Neurotherapeutics 2008, 5: 433–442.

[99] Fisher A. Therapeutic strategies in Alzheimer's disease: M1 muscarinic agonists. Jpn J Pharmacol 2000, 84: 101–112.

[100] Eglen RM, Choppin A, Watson N. Therapeutic opportunities from muscarinic receptor research. Trends Pharmacol Sci 2001, 22: 409–414.

[101] Clader JW, Wang Y. Muscarinic receptor agonists and antagonists in the treatment of Alzheimer's disease. Curr Pharm Des 2005, 11: 3353–3361.

[102] Bartolomeo AC, Morris H, Buccafusco JJ, Kille N, Rosenzweig-Lipson S, Husbands MG, et al. The preclinical pharmacological profile of WAY-132983, a potent M1 preferring agonist. J Pharmacol Exp Ther 2000, 292: 584–596.

[103] Cui YH, Si W, Yin L, An SM, Jin J, Deng SN, et al. A novel derivative of xanomeline improved memory function in aged mice. Neurosci Bull 2008, 24: 251–257.

[104] Si W, Zhang X, Niu Y, Yu H, Lei X, Chen H, et al. A novel derivative of xanomeline improves fear cognition in aged mice. Neurosci Lett 2010, 473: 115–119.

[105] Bodick NC, Offen WW, Levey AI, Cutler NR, Gauthier SG, Satlin A, et al. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. Arch Neurol 1997, 54: 465–473.

[106] Bodick NC, Offen WW, Shannon HE, Satterwhite J, Lucas R, van Lier R, *et al.* The selective muscarinic agonist xanomeline improves both the cognitive deficits and behavioral symptoms of Alzheimer disease. Alzheimer Dis Assoc Disord 1997, 11 Suppl 4: S16–22.

[107] Langmead CJ, Watson J, Reavill C. Muscarinic acetylcholine receptors as CNS drug targets. Pharmacol Ther 2008, 117: 232-243.

[108] Beach TG, Walker DG, Potter PE, Sue LI, Fisher A. Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. Brain Res 2001, 905: 220–223.

[109] Fisher A. M1 muscarinic agonists target major hallmarks of Alzheimer's disease--the pivotal role of brain M1 receptors. Neurodegener Dis 2008, 5: 237–240.

[110] Digby GJ, Shirey JK, Conn PJ. Allosteric activators of muscarinic receptors as novel approaches for treatment of CNS disorders. Mol Biosyst 2010, 6: 1345–1354.

[111] Hock C, Maddalena A, Raschig A, Muller-Spahn F, Eschweiler G, Hager K, *et al.* Treatment with the selective muscarinic m1 agonist talsaclidine decreases cerebrospinal fluid levels of A beta 42 in patients with Alzheimer's disease. Amyloid 2003, 10: 1–6.

[112] Sullivan NR, Leventhal L, Harrison J, Smith VA, Cummons TA, Spangler TB, *et al.* Pharmacological characterization of the muscarinic agonist (3R,4R)-3-(3-hexylsulfanyl-pyrazin-2-yloxy)-1-aza-bicyclo[2.2.1]heptane (WAY-132983) in *in vitro* and *in vivo* models of chronic pain. J Pharmacol Exp Ther 2007, 322: 1294–1304.

[113] Jakubik J, Bacakova L, El-Fakahany EE, Tucek S. Positive cooperativity of acetylcholine and other agonists with allosteric ligands on muscarinic acetylcholine receptors. Mol Pharmacol 1997, 52: 172–179.

[114] Lazareno S, Birdsall B, Fukazawa T, Gharagozloo P, Hashimoto T, Kuwano H, *et al.* Allosteric effects of four stereoisomers of a fused indole ring system with 3H-N-methylscopolamine and acetylcholine at M1-M4 muscarinic receptors. Life Sci 1999, 64: 519–526.

[115] Marlo JE, Niswender CM, Days EL, Bridges TM, Xiang Y, Rodriguez AL, *et al.* Discovery and characterization of novel allosteric potentiators of M1 muscarinic receptors reveals multiple modes of activity. Mol Pharmacol 2009, 75: 577–588.

[116] Ma L, Seager MA, Wittmann M, Jacobson M, Bickel D, Burno M, *et al.* Selective activation of the M1 muscarinic acetylcholine receptor achieved by allosteric potentiation. Proc Natl Acad Sci U S A 2009, 106: 15950–15955.

[117] Shirey JK, Brady AE, Jones PJ, Davis AA, Bridges TM, Kennedy JP, *et al.* A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. J Neurosci 2009, 29: 14271–14286.

[118] Spalding TA, Ma JN, Ott TR, Friberg M, Bajpai A, Bradley SR, *et al.* Structural requirements of transmembrane domain 3 for activation by the M1 muscarinic receptor agonists AC-42, AC-260584, clozapine, and N-desmethylclozapine: evidence for three distinct modes of receptor activation. Mol Pharmacol 2006, 70: 1974–1983.

[119] Langmead CJ, Fry VA, Forbes IT, Branch CL, Christopoulos A, Wood MD, *et al.* Probing the molecular mechanism of interaction between 4-n-butyl-1-[4-(2-methylphenyl)-4-oxo-1-butyl]-piperidine (AC-42) and the muscarinic M(1) receptor: direct pharmacological evidence that AC-42 is an allosteric agonist. Mol Pharmacol 2006, 69: 236–246.

[120] Jones CK, Brady AE, Davis AA, Xiang Z, Bubser M, Tantawy MN, *et al.* Novel selective allosteric activator of the M1 muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats. J Neurosci 2008, 28: 10422–10433.

[121] Langmead CJ, Austin NE, Branch CL, Brown JT, Buchanan KA, Davies CH, *et al.* Characterization of a CNS penetrant, selective M1 muscarinic receptor agonist, 77-LH-28-1. Br J Pharmacol 2008, 154: 1104–1115.

[122] Bradley SR, Lameh J, Ohrmund L, Son T, Bajpai A, Nguyen D, *et al.* AC-260584, an orally bioavailable M(1) muscarinic receptor allosteric agonist, improves cognitive performance in an animal model. Neuropharmacology 2010, 58: 365–373.

[123] Lebois EP, Bridges TM, Lewis LM, Dawson ES, Kane AS, Xiang Z, *et al.* Discovery and characterization of novel subtype-selective allosteric agonists for the investigation of M(1) receptor function in the central nervous system. ACS Chem Neurosci 2010, 1: 104–121.