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2.1. Alzheimer’s disease

World Health Organization and Alzheimer’s Disease International. 2012 Overviewed the report “Dementia: a public health priority” has been jointly developed by WHO and AD International. The report was to raise awareness of dementia as a public health priority, to articulate a public health approach and to advocate for action at international and national levels. Dementia is a syndrome that affects memory, thinking, behaviour and ability to perform everyday activities. The number of people living with dementia worldwide is currently estimated at 35.6 million. This number will double by 2030 and more than triple by 2050. Dementia is overwhelming not only for the people who have it, but also for their caregivers and families. There is lack of awareness and understanding of dementia in most countries, resulting in stigmatization, barriers to diagnosis and care, and impacting caregivers, families and societies physically, psychologically and economically. The report is expected to facilitate governments, policy-makers, and other stakeholders to address the impact of dementia as an increasing threat to global health. It was hoped that the report will promote dementia as a public health and social care priority worldwide.

Thies et al. 2013 provide the report about information to increase understanding of the public health impact of AD, including incidence and prevalence, mortality rates, health expenditures and costs of care, and effect on caregivers and society in general. It also explores the roles and unique challenges of long-distance caregivers, as well as interventions that target those challenges. An estimated 5.2 million Americans have AD. Approximately 200,000 people younger than 65 years with AD comprise the younger onset AD population; 5 million comprise the older onset AD population. Throughout the coming decades, the baby boom generation is projected to add about 10 million to the total number of people in the United States with AD. Today, someone in America develops AD every 68 seconds. By 2050, one new case of AD is expected to develop every 33 seconds, or nearly a million new cases per year, and the total estimated prevalence is expected to be 13.8 million. AD is the sixth leading cause of death in the United States and the fifth leading cause of death in Americans age 65 years or older. Between 2000 and 2010, the proportion of deaths resulting from heart disease, stroke, and prostate cancer decreased 16%, 23%, and 8%, respectively, whereas the proportion resulting from AD increased 68%. The number of deaths from AD as determined by official death certificates (83,494 in 2010) likely underrepresents the number of AD-related deaths in the United States. A projected 450,000 older Americans with AD will die in 2013, and a large proportion will die as a
result of complications of AD. In 2012, more than 15 million family members and other unpaid caregivers provided an estimated 17.5 billion hours of care to people with AD and other dementias, a contribution valued at more than $216 billion. Medicare payments for services to beneficiaries age 65 years and older with AD and other dementias are three times as great as payments for beneficiaries without these conditions, and Medicaid payments are 19 times as great. Total payments in 2013 for health care, long-term care, and hospice services for people age 65 years and older with dementia are expected to be $203 billion (not including the contributions of unpaid caregivers). An estimated 2.3 million caregivers of people with AD and other dementias live at least 1 hour away from the care recipient. These "long-distance caregivers" face unique challenges, including difficulty in assessing the care recipient's true health condition and needs, high rates of family disagreement regarding caregiving decisions, and high out-of-pocket expenses for costs related to caregiving. Out-of-pocket costs for long-distance caregivers are almost twice as high as for local caregivers.

Potter et al. 2010 reviewed investigational medications for treatment of patients with AD. Development of effective treatments for patients with AD has been challenging. Currently approved treatments include AChEIs and the N-methyl-D-aspartate receptor antagonist memantine hydrochloride. To investigate treatments in development for patients with AD, the author conducted a review of the literature. New approaches for treatment or prevention focus on several general areas, including cholinergic receptor agonists, drugs to decrease Aβ and tau levels, anti-inflammatory agents, drugs to increase nitric oxide and cyclic guanosine monophosphate levels, and substances to reduce cell death or promote cellular regeneration. The author focuses on medications currently in clinical trials. Cholinergic agents include orthostatic and allosteric muscarinic M1 agonists and nicotinic receptor agonists. Investigational agents that target Aβ include vaccines, antibodies, and inhibitors of Aβ production. Anti-inflammatory agents, including nonsteroidal anti-inflammatory drugs, the natural product curcumin, and the tumor necrosis factor α inhibitor etanercept, have also been studied. Some drugs currently approved for other uses may also show promise for treatment of patients with AD. Results of clinical trials with many of these investigational drugs have been disappointing, perhaps because of their use with patients in advanced stages of AD. Effective treatment may need to begin earlier-before neurodegeneration becomes severe enough for symptoms to appear.
2.2. Concept of allosteric modulation

**Wenner et al. 2009** reviewed a new kind of drug target as an emerging class of medicines works its magic by targeting unusual sites on biological molecules with key concepts such as a new drug discovery approach focuses on a property known as allosterism. Allosteric drugs attach to biological molecules at binding sites distinct from those usually targeted by medications. Instead of activating or inhibiting the bound molecules, as classic drugs do, allosteric types can act more like dimmer switches and might, at times, cause fewer side effects. Such agents may be able to treat disorders that lack drug therapies today.

**Raddatz et al. 2007** overviewed about allosteric approaches to the targeting of GPCRs for novel drug discovery: A critical assessment. In recent years, the concept of allosteric modulation of GPCRs has matured and now represents an increasingly viable approach to drug discovery. This is evident in the fact that allosteric modulators have been reported for every class of GPCR, and several are currently in clinical trials with one drug example approved and launched. The allosteric approach has been highlighted for the potential of identifying highly selective compounds with a minimal propensity to produce adverse effect. While much has been written regarding the promises of this approach, important challenges, caveats, and pitfalls exist that are often overlooked. Therefore, a balanced overview of the field that describes both the promises and the challenges of discovering allosteric modulators of GPCRs as novel drugs is presented.

**Conn et al. 2009** discussed of allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. GPCRs being among the most fruitful targets for marketed drugs, intense discovery efforts for several GPCR subtypes have failed to deliver selective drug candidates. Historically, drug discovery programmes for GPCR ligands have been dominated by efforts to develop agonists and antagonists that act at orthosteric sites for endogenous ligands. However, in recent years, there have been tremendous advances in the discovery of novel ligands for GPCRs that act at allosteric sites to regulate receptor function. These compounds provide high selectivity, novel modes of efficacy and may lead to novel therapeutic agents for the treatment of multiple psychiatric and neurological human disorders.

**Engers et al. 2013** described about the allosteric modulation of Class C GPCRs: a novel approach for the treatment of CNS disorders. Allosteric modulation has emerged as an innovative pharmacological approach to selectively activate or inhibit several Class C GPCRs. Of the Class C GPCRs, mGlu receptors represent the most promising candidates for clinical success, and both PAMs and NAMs of mGluRs have demonstrated therapeutic
potential for a range of psychiatric and neurological disorders such as pain, depression, anxiety, cognition, Fragile X syndrome, PD and SZ.

Nickols et al. 2014 summarized the development of allosteric modulators of GPCRs for treatment of CNS disorders. The discovery of allosteric modulators of GPCRs provides a promising new strategy with potential for developing novel treatments for a variety of CNS disorders. Traditional drug discovery efforts targeting GPCRs have focused on developing ligands for orthosteric sites which bind endogenous ligands. Allosteric modulators target a site separate from the orthosteric site to modulate receptor function. These allosteric agents can either potentiate (PAM) or inhibit (NAM) the receptor response and often provide much greater subtype selectivity than orthosteric ligands for the same receptors. Experimental evidence has revealed more nuanced pharmacological modes of action of allosteric modulators, with some PAMs showing allosteric agonism in combination with positive allosteric modulation in response to endogenous ligand (ago-potentiators) as well as "bitopic" ligands that interact with both the allosteric and orthosteric sites. Drugs targeting the allosteric site allow for increased drug selectivity and potentially decreased adverse side effects. Promising evidence has demonstrated potential utility of a number of allosteric modulators of GPCRs in multiple CNS disorders, including neurodegenerative diseases such as AD, PD, and Huntington's disease, as well as psychiatric or neurobehavioral diseases such as anxiety, SZ, and addiction.

2.3. M1 mAChR as potential treatments for AD

Hock et. 2003 suggested that treatment with the selective muscarinic M1 agonist talsaclidine decreases cerebrospinal fluid levels of Aβ42 in patients with AD. The Aβ-peptides Aβ40 and Aβ42 are highly amyloidogenic constituents of brain Aβ plaques in AD. Lowering their formation may be achieved by modulating the activities of proteases that cleave the APP, including α-β-, and γ-secretases. Talsaclidine is a functionally selective muscarinic M1 agonist that stimulates non-amyloidogenic α-secretase processing in vitro. Fisher et al. 2008a have demonstrated that the M1 muscarinic agonists target major hallmarks of AD--the pivotal role of brain M1 receptors. The M1 mAChR is a therapeutic target in AD and the M1-selective muscarinic agonists AF102B, AF150(S) and AF267B are cognitive enhancers and potential disease modifiers. Notably, AF267B decreased cerebrospinal fluid Aβ proteins (Aβ40) and Aβ42) in rabbits, decreased brain Aβ levels in hypercholesterolemic rabbits and vascular Aβ42 deposition from the cortex in cholinotoxin-treated rabbits. In triple transgenic AD mice, AF267B reduced cognitive deficits and decreased Aβ42 and tau pathologies in the cortex and hippocampus (not
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amygdala), via M1 mAChR activation of protein kinase C and a disintegrin and metalloproteinase domain 17 (ADAM17 or TACE) and decreased β-site APP-cleaving enzyme 1 and glycogen synthase kinase 3beta, respectively. AF267B is the first reported low-molecular-weight therapy that targets the major AD hallmarks.

Espinoza-Fonseca et al. 2006 described the existence of a second allosteric site on the M1 mAChR and its implications for drug design. Fully flexible docking of KT5720, an allosteric modulator of the mAChRs, was performed on a dynamic model of the M1 mAChR. The results confirmed the existence of a second allosteric site, located on the intracellular face of the receptor. These results would be beneficial for the design of modulators of this receptor to be used as an effective alternative against the AD.

Fisher et al. 2008b explores the cholinergic modulation of APP processing with emphasis on M1 mAChR: perspectives and challenges in treatment of AD. The prescribed drugs for treatment of cognitive deficits in AD patients are regarded as symptomatic drugs. Effective disease modifying therapies are not yet prescribed in AD patients. Three major hallmarks of AD (e.g. cholinergic hypofunction, Aβ and tau neuropathologies) are closely linked raising the expectation that restoring the cholinergic hypofunction to normal, in particular via selective activation of M1 mAChR, may alter the onset or progression of AD dementia. This review is focused mainly on modulation of amyloid precursor processing and Aβ levels in the brain via cholinergic treatment strategies based on M1 muscarinic agonists versus other cholinergic treatments (e.g. cholinesterase inhibitors prescribed for treatment of AD, M2 antagonists and nicotinic agonists). Advantages and potential drawbacks of these treatment modalities are reviewed versus the notion that due to an elusive etiology of AD, future disease modifiers should address comprehensively most of these AD hallmarks (e.g. Aβ pathology, tau and tangle pathologies, as well as the cholinergic hypofunction and cognitive impairments). This major requirement may be fulfilled with M1-selective muscarinic agonists and less with other reviewed cholinergic treatments.

Foster et al. 2014 studied activation of M1 and M4 mAChRs as potential treatments for AD and SZ. AD and SZ are neurological disorders with overlapping symptomatology, including both cognitive deficits and behavioral disturbances. Current clinical treatments for both disorders have limited efficacy accompanied by dose-limiting side effects, and ultimately fail to adequately address the broad range of symptoms observed. Novel therapeutic options for AD and SZ are needed to better manage the spectrum of symptoms with reduced adverse-effect liability. Substantial evidence suggests that activation of mAChRs has the potential to treat both cognitive and psychosis-related symptoms.
associated with numerous CNS disorders. However, use of nonselective modulators of mAChRs is hampered by dose-limiting peripheral side effects that limit their clinical utility. In order to maintain the clinical efficacy without the adverse-effect liability, efforts have been focused on the discovery of compounds that selectively modulate the centrally located M1 and M4 mAChR subtypes. Previous drug discovery attempts have been thwarted by the highly conserved nature of the ACh site across mAChR subtypes. However, current efforts in their laboratory and others have now focused on modulators that bind to allosteric sites on mAChRs, allowing these compounds to display unprecedented subtype selectivity. Over the past couple of decades, the discovery of small molecules capable of selectively targeting the M1 or M4 mAChR subtypes has allowed researchers to elucidate the roles of these receptors in regulating cognitive and behavioral disturbances in preclinical animal models. Here, they provided an overview of these promising preclinical and clinical studies, which suggest that M1- and M4-selective modulators represent viable novel targets with the potential to successfully address a broad range of symptoms observed in patients with AD and SZ.

Melancon et al. 2013 overviewed Allosteric modulation of the M1 mAChR: improving cognition and a potential treatment for SZ and AD. Allosteric modulation of AMPA, NR2B, mGlu2, mGlu5 and M1, targeting glutamatergic dysfunction, represents a significant area of research for the treatment of SZ. Of these targets, clinical promise has been demonstrated using muscarinic activators for the treatment of AD and SZ. These diseases have inspired researchers to determine the effects of modulating cholinergic transmission in the forebrain, which is primarily regulated by one of five subtypes of mAChR, a subfamily of GPCRs. Of these five subtypes, M1 is highly expressed in brain regions responsible for learning, cognition and memory. Xanomeline, an orthosteric muscarinic agonist with modest selectivity, was one of the first compounds that displayed improvements in behavioral disturbances in AD patients and efficacy in schizophrenics. Since these initial clinical results, many scientists, including those in our laboratories, have strived to elucidate the role of M1 with compounds that display improved selectivity for this receptor by targeting allosteric modes of receptor activation. A survey of selected compounds in this area will be presented.
2.4. Benzyl quinolone carboxylic acid

Wittmann et al. 2008 examined in vivo pharmacodynamic effects of BQCA, a novel selective allosteric m1 receptor modulator. M1 mAChRs are expressed in neurons in the cortex and hippocampus and are believed to play a central role in cognition. Therefore, Wittmann et al. 2008 developed a selective allosteric M1 potentiator, BQCA, as a novel treatment approach for AD and demonstrated that BQCA activates M1 receptors in vivo in three pharmacodynamic assays with relevance for cognition. Hence, they suggest that BQCA activates M1 mAChRs in the brain in a way that indicates a cognitive enhancing effect of the compound making BQCA a promising new approach for the treatment of AD.

Ray et al. 2008 demonstrated that allosteric potentiation of the M1 mAChR provides unprecedented selectivity and a novel therapeutic strategy for the treatment of AD. They were screened a chemical library for positive allosteric modulators of the M1 receptor and assessed chemical leads for selectivity in vitro and efficacy in rodent AD models in vivo. They were identified BQCA, a small molecule potentiator selective for M1. In CHO cells expressing recombinant human receptor, BQCA sensitizes M1 to ACh 83.9-fold (inflection point = 449 nM), while having no potentiation agonist, or antagonist, effect on M2, M3, or M4 receptors up to 100 µM. In the mouse contextual fear conditioning model of episodic-like memory, BQCA fully reversed the cognitive impairment caused by the non-selective muscarinic antagonist scopolamine, demonstrating a critical role for M1 in this type of memory. Importantly BQCA does not show signs of unwanted peripheral cholinergic stimulation at doses that produce central physiological responses. In vivo BQCA and other muscarinic agonists can specifically reduce cortical A42 in some experiments. However, this effect is likely complex and indirect, since it does not occur in cultured neurons and is not consistently observed across species and disease models. BQCA is a highly selective pharmacological reagent for understanding the normal physiology of the M1 receptor and its potential as a therapeutic target for AD.

Ma et al. 2009 focused on selective activation of the M1 mAChR achieved by allosteric potentiation. The forebrain cholinergic system promotes higher brain function in part by signaling through the M1 mAChR. During AD, these cholinergic neurons degenerate, therefore selectively activating M1 receptors could improve cognitive function in these patients while avoiding unwanted peripheral responses associated with non-selective muscarinic agonists. They described here BQCA, a highly selective allosteric potentiator of the M1 mAChR. BQCA reduces the concentration of ACh required to activate M1 up to 129-fold with an inflection point value of 845 nM. No potentiation, agonism, or antagonism
activity on other mAChRs is observed up to 100 μM. Furthermore studies in M1\(^{(-/-)}\) mice demonstrates that BQCA requires M1 to promote inositol phosphate turnover in primary neurons and to increase c-fos and arc RNA expression and ERK phosphorylation in the brain. Radioligand-binding assays, molecular modeling, and site-directed mutagenesis experiments indicate that BQCA acts at an allosteric site involving residues Y179 and W400. BQCA reverses scopolamine-induced memory deficits in contextual fear conditioning, increases blood flow to the cerebral cortex, and increases wakefulness while reducing delta sleep. In contrast to M1 allosteric agonists, which do not improve memory in scopolamine-challenged mice in contextual fear conditioning, BQCA induces beta-arrestin recruitment to M1, suggesting a role for this signal transduction mechanism in the cholinergic modulation of memory. In summary, BQCA exploits an allosteric potentiation mechanism to provide selectivity for the M1 receptor and represents a promising therapeutic strategy for cognitive disorders.

Shirey et al. 2009 studied a selective allosteric potentiator of the M1 mAChR increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. M1 mAChRs may represent a viable target for treatment of disorders involving impaired cognitive function. However, a major limitation to testing this hypothesis has been a lack of highly selective ligands for individual mAChR subtypes. They now report the rigorous molecular characterization of a novel compound, BQCA which acts as a potent, highly selective positive allosteric modulator (PAM) of the rat M1 receptor. This compound does not directly activate the receptor, but acts at an allosteric site to increase functional responses to orthosteric agonists. Radioligand binding studies revealed that BQCA increases M1 receptor affinity for ACh. They found that activation of the M1 receptor by BQCA induces a robust inward current and increases spontaneous EPSCs in medial prefrontal cortex (mPFC) pyramidal cells, effects which are absent in acute slices from M1 receptor knock-out mice. Furthermore, to determine the effect of BQCA on intact and functioning brain circuits, multiple single-unit recordings were obtained from the mPFC of rats that showed BQCA increases firing of mPFC pyramidal cells in vivo. BQCA also restored discrimination reversal learning in a transgenic mouse model of AD and was found to regulate non-amyloidogenic APP processing in vitro, suggesting that M1 receptor PAMs have the potential to provide both symptomatic and disease modifying effects in AD patients. Together, these studies provide compelling evidence that M1 receptor activation induces a dramatic excitation of PFC neurons and suggest that selectively activating the M1 mAChR subtype may ameliorate impairments in cognitive function.
Amarante et al. 2010 synthesized and examined Mechanism of pharmacologically active quinolones from Morita–Baylis–Hillman adducts. The synthesis of quinolones from Morita–Baylis–Hillman adducts is reported. The quinolone skeleton is formed via a TFA-mediated cyclization of the MBH adduct, and a mechanism study using ESI(+)-MS(ESI) has indicated the role played by TFA in this key reaction step. The total syntheses of Norfloxacin and a BQCA derivative are described. Norfloxacin is a fluoroquinolonic antibacterial drug whereas BQCA is M1 receptor positive allosteric modulator and seem to provide access to new potential drugs for AD, pain, and sleep disorders. The syntheses of these two important quinolones exemplify the versatility and potentiality of the approach.

Jakubík et al. 2010 describes an allosteric modulator is a ligand that binds to an allosteric site on the receptor and changes receptor conformation to produce increase (positive cooperativity) or decrease (negative cooperativity) in the binding or action of an orthosteric agonist (e.g., ACh). Since the identification of gallamine as the first allosteric modulator of mAChRs in 1976, this unique mode of receptor modulation has been intensively studied by many groups. This review summarizes over 30 years of research on the molecular mechanisms of allosteric interactions of drugs with the receptor and for new allosteric modulators of mAChRs with potential therapeutic use. Identification of positive modulators of ACh binding and function that enhance neurotransmission and the discovery of highly selective allosteric modulators are mile-stones on the way to novel therapeutic agents for the treatment of SZ, AD and other disorders involving impaired cognitive function.

Basso et al. 2011 investigated Muscarinic M1 positive allosteric modulators as potential target for the treatment of AD and SZ. Despite promising signs, adverse effects due to insufficient mAChR subtype selectivity have led to termination of xanomeline development. Consensus opinion indicates that targeting the orthosteric site will not lead to sufficiently selective M1 agonists. It has been shown recently that it is possible to target allosteric sites within the M1 receptor, reaching higher selectivity versus other mAChRs while maintaining agonistic activity in vivo. BQCA, a M1 PAM, has shown activity in animal models for psychosis, cognition and AD (Aβ and tau-pathology). The goal of this study was to evaluate BQCA and to compare its profile to xanomeline in models of cognition and SZ. BQCA (15-20 mg/kg) increased the latency to cross to the dark/punished side of the chamber in the mouse 24 h inhibitory avoidance (IA) and decreased interaction time with a familiar juvenile in rat social recognition (SR), thereby indicating enhancement of memory consolidation and short term social memory. BQCA also demonstrated
antipsychotic-like activity in the mouse PCP-induced locomotor hyperactivity (10-30 mg/kg). Xanomeline improved memory consolidation in mouse IA (0.3-10 mg/kg) and seemed to have memory enhancing properties in SR (6-10 mg/kg), although this last effect appears to be nonspecific. Xanomeline attenuated mouse PCP-induced locomotor hyperactivity (10-30 mg/kg) though the effect was observed at doses that also decreased spontaneous locomotion. Furthermore, immediate early gene expression studies suggest the induction of mAChR activation in the cortex and hippocampus with xanomeline as well as BQCA. The data demonstrate that a M1 PAM has the selectivity necessary to sufficiently amplify and/or stimulate the M1-mediated response and induce similar level of efficacy as an orthosteric agonist, suggesting it has the potential as a therapeutic treatment for AD and/or SZ. Future studies should address potential liability issues of M1 PAM compounds.

Waepenaert et al. 2011 evaluated a BQCA on phosphorylation of CREB in mouse brain regions using Alphascreen® technology and focused microwave irradiation. BQCA, a well validated compound was used as tool compound. To circumvent the laborious and high time-consuming western blot they optimized the use of Alphascreen® technology with brain homogenates. They demonstrated that focused microwave irradiation is necessary to preserve the protein phosphorylation state of CREB and show increased pCREB in hippocampus and cortex of C57BL/6JCrI after administration of BQCA. Maximal levels of pCREB were reached 15 min after subcutaneous administration of 15 mpk BQCA. These results add to the evidence that BQCA activates the PKA/CREB signaling pathway in the hippocampus known to play an important role in spatial memory formation. Furthermore, this ex vivo measurement can be used as a screening system to investigate the central effect of M1 receptor agonists. The Alfascreen® technique combined with microwave irradiation, can be applied to the measurement of other phosphoproteins involved in signaling pathways.

Ma et al. 2011 suggested that the unique binding site of the M1 mAChRs selective allosteric potentiator, BQCA. Modeling studies and site directed mutagenesis were used to further clarify the roles of specific residues in these binding sites. A potential extracellular binding site of BQCA was identified near the ACh binding site, close to residue Y179, which is in the extracellular loop between transmembrane domains IV and V, and W400, which precedes transmembrane domain VII. In contrast, the activities of TBPB, NDMC and gallamine were generally unaffected by the point mutation investigated in these studies. Chimeric M1 and M3 receptor constructs defined the first intracellular loop and TMII of M1 as being critical to TBPB agonist activity, but did not affect BQCA, brucine or
gallamine activity. These results suggest that the M1 mAChR selective allosteric potentiator, BQCA, binds to a unique site distinct from orthosteric, allosteric agonist and antagonist binding sites.

**Kuduk et al. 2011** discovered selective allosteric M1 receptor modulator with suitable development properties based on a quinolizidine carboxylic acid scaffold. One approach to ameliorate the cognitive decline in AD has been to restore neuronal signaling from the basal forebrain cholinergic system via the activation of the M1 mAChR. A number of nonselective M1 muscarinic agonists have previously shown positive effects on cognitive behaviors in AD patients, but were limited due to cholinergic adverse events thought to be mediated by the activation of the M2 to M5 subtypes. One strategy to confer selectivity for M1 is the identification of positive allosteric modulators, which would target an allosteric site on the M1 receptor rather than the highly conserved orthosteric ACh binding site. Quinoline carboxylic acids have been previously identified as highly selective M1 positive allosteric modulators with good pharmacokinetic and in vivo properties. Herein is described the optimization of a novel quinolizidine carboxylic acid scaffold with 4-cyanopiperidines being a key discovery in terms of enhanced activity. In particular, modulator 4i gave high plasma free fractions, enhanced CNS exposure, was efficacious in a rodent in vivo model of cognition, and afforded good physicochemical properties suitable for further preclinical evaluation.

**Canals et al. 2012** demonstrated A MWC mechanism can explain GPCR allosteric modulation. The MWC model was initially proposed to describe the allosteric properties of regulatory enzymes and subsequently extended to receptors. Yet despite GPCRs representing the largest family of receptors and drug targets, no study has systematically evaluated the MWC mechanism as it applies to GPCR allosteric ligands. They reveal how the recently described allosteric modulator, BQCA, behaves according to a strict, two-state MWC mechanism at the M1 mAChR. Despite having a low affinity for the M1 mAChR, BQCA demonstrated state dependence, exhibiting high positive cooperativity with orthosteric agonists in a manner that correlated with efficacy but negative cooperativity with inverse agonists. The activity of BQCA was significantly increased at a constitutively active M1 mAChR but abolished at an inactive mutant. Interestingly, BQCA possessed intrinsic signaling efficacy, ranging from near-quiescence to full agonism depending on the coupling efficiency of the chosen intracellular pathway. This latter cellular property also determined the difference in magnitude of positive cooperativity between BQCA and the orthosteric agonist, carbachol, across pathways. The lack of additional, pathway-biased,
allosteric modulation by BQCA was confirmed in genetically engineered yeast strains expressing different chimeras between the endogenous yeast G(pa1) protein and human Ga subunits. These findings define a chemical biological framework that can be applied to the study and classification of allosteric modulators across different GPCR families. Kuduk et al. 2012 reviewed a patent on novel M1 allosteric ligands. There is substantial evidence from preclinical and early proof-of-concept studies suggesting that selective modulation of the M1 mAChR is efficacious in cognitive models of AD and antipsychotic models of SZ. For example, a number of nonselective M1 muscarinic agonists have previously shown positive effects on cognitive function in AD patients, but were limited due to cholinergic adverse events thought to be mediated by pan activation of the M2 to M5 subtypes. Thus, there is a need to identify selective activators of the M1 receptor to evaluate their potential in cognitive disorders. One strategy to confer selectivity for M1 is the identification of allosteric agonists or positive allosteric modulators, which would target an allosteric site on the M1 receptor rather than the highly conserved orthosteric ACh binding site. This review discusses the M1 mAChR and its potential therapeutic value in the treatment of CNS disorders such as AD and SZ. Specifically, novel allosteric ligands that activate or positively modulate the M1 receptor are examined and peer-reviewed articles associated with these patents publications are also described. There is substantial evidence supporting activation of the M1 receptor might be effective in treating symptoms of AD and SZ, but therapeutic success has been elusive and is hypothesized to be due to the lack of selectivity among orthosteric agonists. During the past decade, allosteric modulation of GPCRs has evolved as a viable strategy toward generating subtype selective molecules. A number of novel, selective ligands in the form of allosteric agonists and positive allosteric modulators of the M1 receptor have been identified offering the potential for clinical evaluation of M1-specific receptor activation. Abdul-Ridha et al. 2013 demonstrated Allosteric modulation of a chemogenetically modified G protein-coupled receptor. DREADDs are chemogenetically modified mAChRs that have minimal responsiveness to ACh but are potently and efficaciously activated by an otherwise inert synthetic ligand, CNO. DREADDs have been used as tools for selectively modulating signal transduction pathways in vitro and in vivo. Recent comprehensive studies have validated how the pharmacology of a CNO-bound DREADD mirrors that of an ACh-bound WT mAChR. However, nothing is known about whether this equivalence extends to the allosteric modulation of DREADDs by small molecules. To address this, they investigated the actions at an M1 DREADD of BQCA, a PAM of ACh
binding and function that is known to behave according to a simple two-state mechanism at the WT receptor. They found that allosteric modulation of the CNO-bound DREADD receptor is not equivalent to the corresponding modulation of the ACh-bound WT receptor. They also found that BQCA engenders stimulus bias at the M1 DREADD, having differential types of cooperativity depending on the signaling pathway. Furthermore, the modulation of ACh itself by BQCA at the DREADD is not compatible with the two-state model that they previously applied to the M1 WT receptor.

**Decker et al. 2013** reviewed of M1 mAChR allosteric modulators as potential therapeutic opportunities for treating AD. In this review the various chemical structures of allosteric agonists and modulators of the mAChR subtype 1 (mAChR1 or M1) and their relevance for possible treatment of AD are discussed. Furthermore, their design principles, common structural properties and their unique features with regard to structure–activity relationships (SARs), such as ‘molecular switches’ and ‘steep/flat’ SARs, are highlighted. M1 bitopic ligands and hybrid molecules are also considered. All these differently designed M1 allosteric modulators open exciting new paths in medicinal chemistry with most promising therapeutic opportunities to fight AD.

### 2.5. BBB and Nanotechnology

**Banks et al. 2012** reviewed of Drug delivery to the brain in AD: consideration of the BBB. The successful treatment of AD will require drugs that can negotiate the BBB. However, the BBB is not simply a physical barrier, but a complex interface that is in intimate communication with the rest of the CNS and influenced by peripheral tissues. This review examines three aspects of the BBB in AD. First, it considers how the BBB may be contributing to the onset and progression of AD. In this regard, the BBB itself is a therapeutic target in the treatment of AD. Second, it examines how the BBB restricts drugs that might otherwise be useful in the treatment of AD and examines strategies being developed to deliver drugs to the CNS for the treatment of AD. Third, it considers how drug penetration across the AD BBB may differ from the BBB of normal aging. In this case, those differences can complicate the treatment of CNS diseases such as depression, delirium, psychoses, and pain control in the AD population.

**Sharma et al. 2012** overview the BBB in AD: novel therapeutic targets and nanodrug delivery. Treatment strategies for AD are still elusive. Thus, new strategies are needed to understand the pathogenesis of AD in order to provide suitable therapeutic measures. Available evidences suggest that in AD, passage across the BBB and transport exchanges for AβP between blood and the CNS compartments play an important regulatory role for
the deposition of brain AβP. New evidences suggest that BBB is altered in AD. Studies favoring transport theory clearly show that AβP putative receptors at the BBB control the level of soluble isoform of AβP in brain. This is achieved by regulating influx of circulating AβP into brain via specific receptor for advanced glycation end products (RAGE) and gp330/megalin-mediated transcytosis. On the other hand, the efflux of brain-derived AβP into the circulation across the vascular system via BBB is accomplished by low-density receptor-related protein-1 (LRP1). Furthermore, an increased BBB permeability in AD is also likely since structural damage of endothelial cells is quite frequent in AD brain. Thus, enhanced drug delivery in AD is needed to induce neuroprotection and therapeutic success. For this purpose, nanodrug delivery could be one of the available options that require active consideration for novel therapeutic strategies to treat AD cases. This review is focused on these aspects and provides new data showing that BBB plays an important role in AD-induced neurodegeneration and neurorepair.

Sharma et al. 2012 reviewed recent developments in nanomedicine resulted in targeted drug delivery of active compounds into the CNS either through encapsulated material or attached to nanowires. Nanodrug delivery by anymeans is supposed to enhance neuroprotection due to rapid accumulation of drugs within the target area and a slow metabolism of the compound. These two factors enhance neuroprotection than the conventions drug delivery. However, this is still uncertain whether nanodrug delivery could alter the pharmacokinetics of compounds making it more effective or just longer exposure of the compound for extended period of time is primarily responsible for enhanced effects of the drugs.

Wilson et al. 2008a developed PS 80 coated PBCA NPs for the targeted delivery of rivastigmine into the brain to treat AD. Rivastigmine is a reversible cholinesterase inhibitor used for the treatment of AD. CNS drug efficacy depends upon the ability of a drug to cross the BBB and reach therapeutic concentrations in brain following systemic administration. The clinical failures of most of the potentially effective therapeutics to treat the CNS disorders are often not due to a lack of drug potency but rather shortcomings in the method by which the drug is delivered. Hence, considering the importance of treating AD, they made an attempt to target the anti-Alzheimer's drug rivastigmine in the brain by using PBCA NPs. The drug was administered as a free drug, bound to NPs and also bound to NPs coated with PS 80. In the brain a significant increase in rivastigmine uptake was observed in the case of PBCA NPs coated with 1% PS 80 compared to the free drug. In conclusion that the present study demonstrates that the brain concentration of intravenously
injected rivastigmine can be enhanced over 3.82 fold by binding to PBCA NPs coated with 1% nonionic surfactant PS 80.

**Wilson et al. 2008b** investigated targeted delivery of tacrine into the brain with PS 80-coated PBCA NPs. The purpose of the present study was to investigate the possibility of targeting an anti-Alzheimer's drug tacrine in the brain using polymeric NPs. Rats obtained 1mg/kg of tacrine by intravenous injection in the form of three preparations: (1) a simple solution in phosphate buffered saline, (2) bound to PBCA NPs, and (3) bound to PBCA NPs coated with 1% PS 80. After 1h of post injection the rats were killed by decapitation and tacrine concentration in brain, liver, lungs, spleen and kidneys was analyzed by HPLC. A higher concentration of drug tacrine was observed in liver, spleen and lungs with the NPs in comparison to the free drug. The accumulation of drug tacrine in the liver and spleen was reduced, when NPs were coated with 1% PS 80. In the brain a significant increase in tacrine concentration was observed in the case of PBCA NPs coated with 1% PS 80 compared to the uncoated NPs and the free drug. In conclusion, the present study demonstrates that the brain concentration of intravenously injected tacrine can be enhanced by binding to PBCA NPs, coated with 1% the nonionic surfactant PS 80.

**Wilson et al. 2009** evaluated significant delivery of tacrine into the brain using magnetic chitosan microparticles for treating AD. Tacrine is a reversible cholinesterase inhibitor used for treating mild to moderate AD. In the present study, an attempt was made to target the anti-Alzheimer's drug tacrine in the brain by using magnetic chitosan microparticles. The magnetic chitosan microparticles were prepared by emulsion cross-linking. The formulated microparticles were characterized for process yield, drug loading capacity, particle size, in vitro release, release kinetics and magnetite content. The particle size was analyzed by scanning electron microscope. The magnetite content of the microparticles was determined by atomic absorption spectroscopy. For animal testing, the microparticles were injected intravenously after keeping a suitable magnet at the target region. The concentrations of tacrine at the target and non-target organs were analyzed by HPLC. The magnetic chitosan microparticles significantly increased the concentration of tacrine in the brain in comparison with the free drug.

**Wilson et al. 2010** evaluated Chitosan NPs as a new delivery system for the anti-Alzheimer drug tacrine. Tacrine-loaded chitosan NPs were prepared by spontaneous emulsification. The particle size and zeta potential was determined by scanning probe microscopy and Zetasizer, respectively. The prepared particles showed good drug-loading capacity. The in vitro release studies showed that after the initial burst, all the drug-loaded batches provided

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a continuous and slow release of the drug. Coating of NPs with PS 80 slightly reduced the drug release from the NPs. Release kinetics studies showed that the release of drug from NPs was diffusion-controlled, and the mechanism of drug release was Fickian. The biodistribution of these particles after intravenous injection in rats showed that of NPs coated with 1% PS 80 altered the biodistribution pattern of NPs.

**Joshi et al. 2010** developed Rivastigmine-loaded PLGA and PBCA NPs and investigated *in vitro* and pharmacodynamic studies. Sustained release nanoparticulate formulations of Rivastigmine tartrate (RT) were prepared, optimized (using factorial design) and characterized using the biodegradable polymers, PLGA and PBCA as carriers. The pharmacodynamic performances of the NPs were evaluated for brain targeting and memory improvement in scopolamine-induced amnesic mice using Morris Water Maze Test. PLGA NPs were prepared by nanoprecipitation technique, while PBCA NPs were prepared by emulsion polymerization technique. Pharmacodynamic study demonstrated faster regain of memory loss in amnesic mice with both PLGA and PBCA NPs when compared to RT solution. This indicates rapid and higher extent of transport of RT into the mice brain and thus shows the suitability of both NPs as potential carriers for providing sustained brain delivery of RT.

**Wilson et al. 2011** designed and evaluated of chitosan NPs as novel drug carrier for the delivery of rivastigmine to treat AD. Chitosan NPs containing rivastigmine were prepared by spontaneous emulsification. The mean size of the particles was 47 ± 4 nm. Zeta potential analysis demonstrated a positive charge for the particles and coating with PS 80 slightly reduced the surface charge of the particles. A biphasic release pattern was observed for the release of drug from the NPs. Release of the drug from NPs was diffusion controlled and the mechanism of drug release was Fickian. The biodistribution studies demonstrated that coating of NPs with 1% PS 80 altered the uptake of NPs by different organs.

**Mittal et al. 2011** evaluated polymer NPs for oral delivery of estradiol to rat brain in a model of Alzheimer's pathology. The purpose of this study was to develop PS 80 coated PLGA NPs that can deliver estradiol to the brain upon oral administration. Estradiol containing NPs were made by a single emulsion technique and PS 80 coating was achieved by incubating the re-constituted NPs at different concentrations of PS 80. The process of PS 80 coating on the NPs was optimized and the pharmacokinetics of estradiol NPs was studied as a function of PS 80 coating. The NPs were then evaluated in an ovariectomized (OVX) rat model of AD that mimics the postmenopausal conditions. The NPs bound PS 80 were found to proportionally increase from 9.72 ± 1.07 mg to 63.84 ± 3.59 mg with an

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increase in the initial concentration PS 80 from 1% to 5% and were stable in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Orally administered PS 80 coated NPs resulted in significantly higher brain estradiol levels after 24h (1.969 ± 0.197 ng/g tissue) as compared to uncoated ones (1.105 ± 0.136 ng/g tissue) at a dose of 0.2 mg/rat, suggesting a significant role of surface coating. Moreover, these brain estradiol levels were almost similar to those obtained after administration of the same dose of drug suspension via 100% bioavailable intramuscular route (2.123 ± 0.370 ng/g tissue), indicating the increased fraction of bioavailable drug reaching the brain when administered orally. Also, the nanoparticle treated group was successful in preventing the expression of Aβ-42 immunoreactivity in the hippocampus region of brain. Together, the results indicate the potential of NPs for oral delivery of estradiol to brain.

Doggui et al. 2012 evaluated neuronal uptake and neuroprotective effect of curcumin-loaded PLGA NPs on the human SK-N-SH cell line. Curcumin, a natural polyphenolic pigment present in the spice turmeric, is known to possess a pleiotropic activity such as antioxidant, anti-inflammatory, and anti-amyloid-β activities. However, these benefits of curcumin are limited by its poor aqueous solubility and oral bioavailability. In the present study, a polymer-based nanoparticle approach has been utilized to deliver drugs to neuronal cells. Curcumin was encapsulated in biodegradable PLGA based-nanoparticulate formulation (NPs-Cur). Dynamic laser light scattering and transmission electronic microscopy analysis indicated a particle diameter ranging from 80 to 120 nm. The entrapment efficiency was 31% with 15% drug-loading. In vitro release kinetics of curcumin from Nps-Cur revealed a biphasic pattern with an initial exponential phase followed by a slow release phase. Cellular internalization of Nps-Cur was confirmed by fluorescence and confocal microscopy with a wide distribution of the fluorescence in the cytoplasm and within the nucleus. The prepared nanoformulation was characterized for cellular toxicity and biological activity. Cytotoxicity assays showed that void PLGA-NPs and curcumin-loaded PLGA NPs (Nps-Cur) were nontoxic to human neuroblastoma SK-N-SH cells. Moreover, Nps-Cur was able to protect SK-N-SH cells against H2O2 and prevent the elevation of reactive oxygen species and the consumption of glutathione induced by H2O2. Interestingly, Nps-Cur was also able to prevent the induction of the redox-sensitive transcription factor Nrf2 in the presence of H2O2. Taken together, these results suggest that Nps-Cur could be a promising drug delivery strategy to protect neurons against oxidative damage as observed in AD.
2.6. Streptozotocin induced AD model

Kosaraju et al. 2013 evaluated DPP-4 inhibitor ameliorates STZ induced AD. The present study examines the efficacy of Saxagliptin, a DPP-4 inhibitor in a STZ induced rat model of AD. Three months following induction of AD by intracerebral administration of streptozotocin, animals were orally administered Saxagliptin (0.25, 0.5 and 1 mg/kg) for 60 days. The effect of the DPP-4 inhibitor on hippocampal GLP-1 levels, Aβ burden, tau phosphorylation, inflammatory markers and memory retention were evaluated. The results reveal an attenuation of Aβ, tau phosphorylation and inflammatory markers and an improvement in hippocampal GLP-1 and memory retention following treatment. This remarkable therapeutic effect of Saxagliptin mediated through DPP-4 inhibition demonstrates a unique mechanism for Aβ and tau clearance by increasing GLP-1 levels and reverses the behavioural deficits and pathology observed in AD.

Kosaraju et al. 2013b investigated an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced AD. Three months following the induction of AD by intracerebral injection of STZ, animals were orally administered with vildaglaptin (2.5, 5 and 10 mg/kg) for 30 days. Dose-dependent and time-course effects of vildaglaptin on memory retention were investigated during the course of treatment. Following treatment, the animals were sacrificed, and brain tissues were used to evaluate the effects of vildaglaptin on hippocampal and cortical GLP-1 levels, Aβ burden, tau phosphorylation and inflammatory markers.

Kosaraju et al. 2014 evaluated DPP-4 inhibition by Pterocarpus marsupium and Eugenia jambolana ameliorates streptozotocin induced AD. The present study, the neuroprotective roles of PM and EJ for ameliorating the STZ induced AD have been tested in rat model. Experimentally, PM and EJ extracts, at a dose range of 200 and 400mg/kg, were administered orally to STZ induced AD Wistar rats and cognitive evaluation tests were performed using radial arm maze and hole-board apparatus. Following 30 days of treatment with the extracts, a dose- and time-dependent attenuation of AD pathology, as evidenced by decreasing Aβ 42, total tau, phosphorylated tau and neuro-inflammation with an increase in GLP-1 levels was observed. Therefore, PM and EJ extracts contain cognitive enhancers as well as neuroprotective agents against STZ induced AD.