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REVIEW

GPCR, a rider of Alzheimer's disease

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Abstract **Alzheimer's disease (AD) is the most common type of dementia that affects thinking, learning, memory and behavior of older people. Based on the previous studies, three pathogenic pathways are now commonly accepted as the culprits of this disease namely, amyloid-β pathway, tauopathology and cholinergic dysfunction. This review focuses on the current findings on the regulatory roles of G protein-coupled receptors (GPCRs) in the pathological progression of AD and discusses the potential of the GPCRs as novel therapeutic targets for AD.**

Keywords Alzheimer's disease (AD), G protein-coupled receptors (GPCRs), secretase, amyloid-β, tau

Alzheimer's disease

In 2010, about 35.6 million people worldwide lived with dementia. This number is estimated to nearly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050 (Martin Prince, 2010). Alzheimer's disease (AD) is the most common type of dementia and accounts for 50 to 70 percent of dementia cases (Goedert and Spillantini, 2006). The total estimated worldwide cost of dementia was US$604 billion in 2010, which accounts for around 1% of the world's gross domestic product (Martin Prince, 2010). Thus, in addition to developing cost-effective medical and social care strategies, there is also an urgent need to seek novel and effective ways to treat and prevent the disease based on further understanding of the pathogenesis of AD.

AD is a progressive brain disorder that leads to loss of memory and, eventually, the deterioration of all brain functions caused by neuron loss. Several hallmarks have been identified in the brains of those with AD, including plaques, tangles, loss of synapses among neurons, inflammation, neuron death and severe tissue shrinkage (LaFerla et al., 2007). Three major pathogenic pathways are now widely noted as the major causes of the disease.

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Amyloid-β pathway

Proteinaceous aggregates comprising amyloid-β (Aβ) peptides are found in AD patients' brains (Selkoe, 2001). Aβ is the product of the sequential processing of amyloid-β precursor protein (APP) by β- and γ-secretases. On the other hand, when APP is processed by α-secretase that cleaves inside the Aβ peptides, no Aβ peptide is generated (Roberson and Mucke, 2006). Aβ peptides are 39 to 43 amino acids in length. The major form is 40 amino acids long ($A\beta_{40}$), accounting for ~90% of total Aβ in the patients' brains. Another longer form, $A\beta_{42}$ (the 42-residue variant), that accounts for ~10%, is more hydrophobic, aggregates faster and is more toxic (Selkoe, 2001; Goedert and Spillantini, 2006). Therefore, it is commonly accepted that $A\beta_{42}$ is one of the main causes of AD, initiating a neurotoxic cascade and leading to the pathogenesis of AD. Genetic mutations in *APP*, *presenilin* (the enzymatic component of γ-secretase) and *apolipoprotein E* (*APOE*, the only well-established genetic risk factor for sporadic AD) have been identified as causes of the disordered accumulation of Aβ, leading amyloid-β plaque-mediated neurotoxicity (Goate et al., 1991; Chartier-Harlin et al., 1991; Murrell et al., 1991; Strittmatter et al., 1993; Sherrington et al., 1995; Rogaev et al., 1995). On the other hand, two enzymes, insulin-degrading enzyme (IDE) and neprilysin (NEP), in neurons have been reported to effectively enhance the proteolysis of Aβ in *APP* transgenic mice, preventing amyloid plaque formation and its associated cytopathology(Leissring et al., 2003).

Tauopathology

Neurofibrillary tangles (NFTs) are proteinaceous aggregates composed of hyperphosphorylated microtubule-associated protein tau (MAPT), which functions to stabilize and facilitate the polymerization of microtubules (Lee et al., 2001; Ballatore et al., 2007). The NFT density correlates well with the cognitive decline in AD patients (Arriagada et al., 1992; Gomez-Isla et al., 1997; Blalock et al., 2004), suggesting that tau plays a key role in the observed clinical symptoms and AD pathology. There are two isoforms of tau generated by alternative splicing. These two isoforms of tau contain three (3R) or four (4R) repeats of the microtubule-binding domain, which determine their affinity to microtubules (Brunden et al., 2009). Normally, the amounts of 3R and 4R tau are equal in neurons, while in AD patients' brains, the level of 4R tau is dramatically increased (Ballatore et al., 2007; Hanger et al., 2009). Under normal physiological conditions, tau must be phosphorylated at multiple serine and threonine residues to function properly. In AD patients' brains, tau is hyperphosphorylated, which reduces the binding of tau to microtubules and may enhance aggregation, causing tau deposition in brains (Alonso et al., 1994, 1996; Necula and Kuret, 2004). In normal neurons, tau functions and is confined in axons. But in neurodegenerative tauopathies, tau is mis-distributed to the cell body and dendrites and forms neurofibrillary tangles (NFTs) there (Shahani and Brandt, 2002; Ballatore et al., 2007). Furthermore, the dendritic role of tau has recently been identified to confer Aβ toxicity at the postsynapses (Ittner et al., 2010), highlighting tau as an attractive drug target, in addition to Aβ (Ashe, 2007).

Cholinergic hypothesis

Cholinergic neurons are the primary basis of the hippocampus, which is the major area related to memory and is the first and primary area affected during pathogenesis of AD (Mesulam et al., 1983). Cholinergic neurons are thought to be involved in memory, cognition and attention (Baxter and Chiba, 1999). Since the cholinergic dysfunction is mainly caused by a reduction in acetylcholine (Ach) synthesis due to the reduced choline acetyltransferase (ChAT) activity and decreased choline uptake (Fisher, 2008), people are now developing drugs that slow the degradation of acetylcholine after its release at synapses; acetylcholinesterase inhibitors and cholinergic muscarinic receptor agonists are also being developed (Doraiswamy and Xiong, 2006; Lleo et al., 2006). Further, the loss and dysfunction of cholinergic neurons correlate well with Aβ plaques, NFTs and clinical observation of AD (Ladner and Lee, 1998), suggesting an interplay among the above three theories. Nevertheless, the causal relationship among them remains unclear.

G protein-coupled receptors

More than 1000 genes in the human genome encode a superfamily of receptors characterized by seven transmembrane (7TM) configurations. These 7TM receptors couple with heterotrimeric GTP-binding proteins (G proteins) to regulate the activities of diverse downstream effector molecules. This superfamily of receptors is named G protein-coupled receptors (GPCRs). GPCRs detect extracellular signal molecules including many hormones, neurotransmitters, chemokines and calcium ions as well as various odorants and even photons of light (Pierce et al., 2002). The heterotrimeric G protein contains three subunits (α, β and γ). Gα binds to and hydrolyzes GTP to GDP, whereas the Gβ and Gγ subunits are tightly associated (Gilman, 1987). Heterotrimeric G proteins are classified based on the different Gα subunits.

Stimulation of GPCRs modulates the level of second messengers, including cAMP/cGMP, inositol 1,4,5-trisphosphate (IP3), diacylglycerol (DAG) and Ca^{2+}. The Gαs and Gαi/o activate or inhibit adenylyl cyclase, resulting in an increased or reduced cAMP level, respectively, leading to the up- or down-regulation of cAMP-dependent protein kinase (PKA). The Gαq-coupled GPCRs will activate phospholipase Cβ, generating IP3 and DAG, resulting in a change in the cytosolic Ca^{2+} level, followed by the activation of calcium-dependent protein kinase (PKC). PKA and PKC then initiate downstream phosphorylation cascades to transduce and amplify the original signal (Lefkowitz, 2007), which leads to the regulation of cell proliferation, differentiation, inflammation and apoptosis. Once the GPCRs are activated, they will be phosphorylated by a G protein-coupled receptor kinase (GRK). Then, the β-arrestins bind to the phosphorylated GPCRs and initiate the desensitization and internalization of GPCRs.

In addition to the G protein-dependent signaling, β-arrestin-mediated biased GPCR signaling pathways have recently been intensively studied. A typical example of recent findings is the discovery of the interaction between β-arrestin-1 and c-Src; this β-arrestin/c-Src interaction facilitates the β_2-adrenergic receptor (β_2-AR)-dependent activation of the ERK/MAPK cascade (Lefkowitz and Shenoy, 2005). Thus, by interacting with diverse proteins, the β-arrestins function to scaffold various signalosomes, including the ERK1/2, P38, JNK3, AKT and PI3 kinase cascades (Lefkowitz and Shenoy, 2005; Lefkowitz, 2007). This list of β-arrestin-mediated GPCR signaling is sure to increase in the upcoming years.

GPCRs in AD pathogenesis

In 2004, Blalock et al. reported an alteration of the gene expression profile in AD patients' postmortem brains by

cDNA microarray analysis. Analysis of the gene expression profile of AD patients with different pathological severity vs. normal age-matched controls showed that the levels of transcripts from a number of GPCR genes changed, among which were inflammation associated GPCRs (e.g., leukotriene B4 receptor, chemokine (C-X-C motif) receptor 4, histamine receptor H3), hormone receptors (e.g., angiotensin II receptor, parathyroid hormone receptor 1, prostaglandin E receptor 3), neurotransmitter receptors (dopamine receptor D2, D4, gamma-aminobutyric acid (GABA) B receptor 1, cholinergic receptor, muscarinic 3 and 5), orphan GPCRs (GPR17, GPR22, GPR55) and some others (e.g., purinergic receptor P2Y G-protein-coupled 10, sphingolipid G-protein-coupled receptor, 1). Given that the change in expression levels of the GPCRs should have an effect on the related biological processes, these observations suggest a potential role of GPCR in the pathological progression of AD, which requires further investigation.

GPCRs and amyloid-β

Studies have demonstrated that GPCRs can modulate the amyloidogenic and non-amyloidogenic processing pathways of APP via the regulation of three critical secretases: α-, β- and γ-secretase (Vassar et al., 1999; Asai et al., 2003; Takasugi et al., 2003). β-secretase, also called BACE (β-site APP-cleaving enzyme), is a transmembrane aspartic protease that mediates the β-site cleavage of APP, generating a 99-residue C-terminal fragment called C99, which is the direct substrate of γ-secretase (Vassar et al., 1999; Sinha et al., 1999; Takasugi et al., 2003). Subsequent cleavage of C99 by γ-secretase at the γ-site then generates Aβ and AICD (Takasugi et al., 2003). AICD can function in the nucleus (Budde, 2006). The β-site cleavage of APP has been regarded as the rate-limiting step of Aβ generation (Sinha and Lieberburg, 1999). Studies reveal that the processing of APP by BACE and γ-secretase is directly regulated by GPCRs, such as delta-opioid receptor (DOR), β_2-AR and GPR3. Moreover, several studies have provided evidence that some GPCRs are involved in the regulation of Aβ degradation and in Aβ clearance.

δ-opioid receptor and β- and γ-secretase

Opioid receptors are a subfamily of Gαi/o-coupled GPCRs that includes delta-(DOR), mu-(MOR) and kappa-(KOR) opioid receptors. Opioid receptors are highly expressed in the central nervous system, including the hippocampus and the cortex, which are vulnerable to AD pathogenesis. Early in 1980s, opioid receptors were demonstrated to function in learning, memory and synaptic activation (Gallagher et al., 1983; Bramham et al., 1991). Moreover, the expression of opioid receptors has been found to be dysregulated in AD patients' brains (Mathieu-Kia et al., 2001). Recently, Teng et al. (2010) reported that activated DOR forms a protein complex with β- and γ-secretases to mediate the intracellular trafficking of the secretases/GPCR complex into late endosome and lysosome (LEL), where the specific processing of APP to Aβ is facilitated. However, activated MOR, which quickly recycles back to the membrane, has no such effect. Furthermore, *in vivo* administration of the DOR antagonist NTI to an AD mouse model can significantly rescue learning memory deficits and mitigate the AD-like pathology, while the MOR antagonist β-FNA fails to provide any benefit. More importantly, the modulation of secretase activities by DOR is Aβ-generation specific. Neither activation nor blockage of DOR affects the processing of other substrates of these secretases, including Notch, N-cadherin or APLP1, which play important physiological roles in many biological processes. These findings suggest that some GPCRs may be novel potential therapeutic targets for specific inhibition of Aβ production to protect against Alzheimer's disease.

β_2-adrenergic receptor and γ-secretase

The γ-site cleavage of C99 has been regarded as pivotal because it generates Aβ directly and determines the ratio of $A\beta_{40}$ and $A\beta_{42}$ (Sisodia and St George-Hyslop, 2002). Therefore, the γ-secretase has been a hot target in the AD therapeutics field (Citron, 2010). γ-secretase is a multi-protein complex containing four essential components: APH1, nicastrin (NCT), presenilin (PS) and Pen2. APH1 and NCT form a pre-complex to initiate the following holo-complex assembly (Hu and Fortini, 2003). After recruiting presenilin, the catalytic domain of γ-secretase (Takasugi et al., 2003), Pen2 will be added to the complex to facilitate the maturation and stabilization of the holo-γ-secretase complex (Watanabe et al., 2005).

β_2-AR is a well-studied typical Gαs-coupled GPCR that is expressed in the hippocampus and the cortex (Russo-Neustadt and Cotman, 1997). In 2006, we reported that β_2-AR interacts with PS1 at the plasma membrane. The activation of β_2-AR enhances the γ-secretase activity and Aβ production as the results of co-endocytosis and co-trafficking of β_2-AR/γ-secretase complex to the LEL, where APP processing is elevated (Ni et al., 2006). Similar to DOR, the administration of the β_2-AR antagonist ICI 118,551(Ni et al., 2006) or propranolol (our unpublished data) also decreases cerebral amyloid plaques in an AD mouse model. However, it remains important to determine whether this β_2-AR-mediated γ-secretase activity-enhancing effect is specific to Aβ generation, because a severe side effect of targeting γ-secretase has been reported.

The orphan GPR3 and γ-secretase

G protein-coupled receptor 3 (GPR3) is a constitutively active orphan GPCR that is highly expressed in the central nervous system, especially in the hippocampus, cortex and amygdale

(Iismaa et al., 1994). In 2009, a high-throughput functional genomics screen by Thathiah et al. identified GPR3 as a modulator of Aβ production. Unlike that of β_2-AR and DOR, the overexpression of GPR3 will enhance the γ-secretase holo-complex assembly *in vivo* and *in vitro*. Furthermore, genetic ablation of GPR3 in an AD mouse model reduces the Aβ production in the mouse brain. Interestingly, GPR3 specifically mediated activities for Aβ production without obvious effect on notch processing (Thathiah et al., 2009), suggesting that, in addition to mediating γ-secretase holo-complex assembly, GPR3 might modulate γ-secretase activity by other mechanisms.

GPCRs and α-secretase

In contrast to β- and γ-secretases, the α-secretase-mediated APP processing occurs inside the Aβ peptide, generating a soluble N-terminal fragment (sAPPα) and an 83-residue C-terminal fragment (C83), which is then hydrolyzed by γ-secretase to generate AICD and a short fragment called P3. Thus α-secretase-mediated APP processing prevents the production of Aβ (Roberson and Mucke, 2006). To date, three enzymes, ADAM9, ADAM10 and ADAM17, have been identified as putative α-secretases (Asai et al., 2003; Tian et al., 2008; Liu and Chang, 2010).

The α-site processing of APP has been demonstrated to be mediated by the PI3-kinase pathway (Solano et al., 2000) and the MAPK pathway, the activities of which are under tight regulation by GPCRs (Mills et al., 1997). Consistently, administration of AF267B, a Muscarinic AChR agonist to a transgenic AD mouse model (3xTg-AD), reduces Aβ plaque and NFTs in the hippocampus and cortex and rescues the cognitive deficits. Further, Caccamo et al. (2006) found the activation of M1AChR by AF267B selectively activate ADAM17 in an ERK1/2 and PKC activation-dependent manner, suggesting a potential ERK1/2 and PKC-mediated activation of α-secretase by M1AChR stimulation. Studies further show that GPCRs, such as metabotropic glutamate receptors and 5-hydroxytryptamine receptors, regulate the Aβ production and the sAPPα level simultaneously through the activation of the MAPK or PI3K pathways (Nitsch et al., 1996; Phillips et al., 1998; Ferraguti et al., 1999; Arjona et al., 2002). However, the pathological role of α-secretase in AD progress, as well as the more specific regulatory mechanism, requires further close investigation.

GPCRs and Aβ degradation

Neprilysin (NEP) has been identified as a physiological Aβ-degrading peptidase and has been shown to regulate the steady-state levels of both $A\beta_{40}$ and $A\beta_{42}$ *in vivo* (Iwata et al., 2001), suggesting that promoting Aβ degradation via enhancing neprilysin activity could be an alternative therapeutic strategy against AD.

The neuropeptide somatostatin is the endogenous ligand for the somatostatin receptor, which is a Gαi-coupled GPCR, expressed in the central nervous system. Saito et al. (2005) reported that somatostatin facilitates the metabolism of Aβ via upregulation of neprilysin activity. Moreover, genetic ablation of somatostatin downregulates the hippocampal neprilysin activity and localization, increasing the $A\beta_{42}$ level, suggesting that the somatostatin receptor is a pharmacological target candidate for Alzheimer's disease therapy.

GPCRs and Aβ clearance

Microglial cells are the principal immune cells of the brain. These cells secrete enzymes that degrade Aβ (Qiu et al., 1997) and express receptors that promote the clearance and phagocytosis of Aβ. CCR2 is a chemokine receptor (GPCR) expressed on microglia that mediates the infiltration of mononuclear phagocytes to sites of inflammation. El Khoury et al. (2007) found that CCR2 deficiency in an AD mouse model accelerates early disease progression and significantly impairs microglial accumulation for Aβ clearance. Furthermore, CCR2 knockout leads to a severe premature death of AD mice in a gene dose-dependent manner, suggesting that CCR2-mediated microglial infiltration plays a protective role in the early stages of Alzheimer's disease pathogenesis by promoting Aβ clearance (El Khoury et al., 2007).

GPCRs and tauopathies

Tau is phosphorylated on multiple residues under physiological conditions. The extent of tau phosphorylation is considerably increased in the AD patients' brains (Matsuo et al., 1994). Hyperphosphorylation of tau decreases its binding to microtubules and accelerates its aggregation, NFT formation and AD pathogenesis. Caccamo et al. reported that M1AChR activation decreases GSK3β activity, which is responsible for the hyperphosphorylation of tau (Caccamo et al., 2006; Gong and Iqbal, 2008). Thus, administration of the M1AChR agonist AF267B might enhance not only the ADAM17 activity but also the tau phosphorylation, which then reduces the tau pathology in the cortex and hippocampus of the 3XTg AD mice (Caccamo et al., 2006). mGluR2 and AT_2R have also been reported to be involved in tauopathies and to mediate activation of the kinases responsible for tau hyperphosphorylation (Lee et al., 2004; AbdAlla et al., 2009). However, the understanding of the detailed regulatory mechanisms underlying these observations requires more in-depth investigations.

GPCRs and immune responses in AD

Although the role of inflammation in AD progression remains elusive, nonsteroidal anti-inflammatory drugs, especially indomethacin, have been associated with protection against

Alzheimer's disease (Tabet and Feldman, 2002; Wyss-Coray, 2006). In 2007, Ray et al. reported that a group of 18 signaling proteins were dysregulated in AD patients' plasma. The levels of these proteins can be used to classify blinded samples from Alzheimer's and control subjects with close to 90% accuracy. Moreover, these signaling proteins could be used to identify potential patients with mild cognitive impairment who might progress to AD in 2–6 years. Interestingly, several of these 18 signaling proteins are ligands for chemokine receptors, including CCL5 (chemokine that contains a C-C motif), CCL7, CCL15, CCL18 and CXCL8 (chemokine that contains a C-X-C motif). The chemokine receptors are important GPCRs in inflammatory reactions. This evidence is in line with the reports showing that a number of chemokine receptors and corresponding ligands are dysregulated in AD patients (Hesselgesser and Horuk, 1999; Blalock et al., 2004), which suggests a potential role of GPCRs in AD pathogenesis via the regulation of immunoreactions.

Perspective

Although specific anti-dementia drugs are available for patients with Alzheimer's disease, they provide, at best, only modest symptomatic improvement. None of these drugs is considered to have disease-modifying effects. Thus, there is an urgent need to address the underlying pathology of Alzheimer's disease for developing new therapeutic strategies. To date, late-phase clinical trials of several modulators for treating AD that function to inhibit Aβ generation via direct target the β- and γ-secretases have been discontinued, even with strong support from the results of cellular and animal testing (Francis et al., 2002; Martin Prince, 2010). Regarding the essential physiological roles of the reported kinases cascades that are responsible for tau hyperphosphorylation, such as ERK2, GSK3, CDK5, PKA, casein kinase 1 and MARK1 (Brunden et al., 2009), direct inhibition will result in inevitable side effects. In addition to the novel functions of GPCRs discussed above, i.e., mediation of the APP procession by α-, β- and γ-secretases, degradation and clearance of Aβ, the phosphorylation level of tau, and the critical regulatory roles of GPCR in AD pathogenesis still need further investigation. Nevertheless, it is of note that the GPCRs have been proven to be the most drugable targeting sites; thus, a feasible screening model based on newly defined mechanisms may provide alternative strategies for effectively modulating AD pathogenesis with fewer side effects.

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