European Review for Medical and Pharmacological Sciences 2016; 20: 2389-2403

Therapeutic approaches to Alzheimer's disease through stimulating of non-amyloidogenic processing of amyloid precursor protein

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Abstract. – Amyloid beta (Aβ) plaques are pathological hallmarks of neurodegenerative Alzheimer's disease (AD) that is predominantly characterized by clinical symptoms of dementia. Therapies targeting Aβ are essential for preventing and treating AD. This review focuses on the non-amyloidogenic pathways that prevent the generation of Aβ peptide and thereby plaque formation in AD. An a-secretase-dependent cleavage of Amyloid Precursor Protein (APP) precludes the amyloidogenic pathway of Aβ generation. This non-amyloidogenic a-secretase activation thereby secretes sAPPa with prominent neurotrophic and memory-enhancing properties. Several "A Disintegrin and Metalloprotease" (ADAM) proteins, specifically ADAM17, ADAM10 and ADAM9, comprise active members of this a-secretase family. It is conventionally accepted that whereas ADAM10 executes constitutive APP cleavage, ADAM17 and ADAM9 are dedicated towards the regulated processing. Therefore, promoting a-secretase activity offers thorough neuroprotection against AD, and emerges as a pertinent strategy in attenuating Aβ. We discuss signaling pathways, particularly those mediated by protein kinase C and phorbol esters, that enhance ADAM functioning and sAPPa release. We also elaborate upon the associated M1 and M3-muscarinic acetylcholine receptors, ERK-MAP kinase, tyrosine kinase and calcium signaling pathways. Clinical studies suggest that regulated hormone and cholesterol levels are essential for restricting neurodegeneration, and here we illustrate the role of estrogen and testosterone and that of cholesterol-attenuating statins in generating sAPPa. We also emphasize the need for novel ADAM activators that may be screened for targeting the interleukin-1-responsive mRNA 5'-untranslated region of APP. This review offers an in-depth insight into pathways, strategies and probable therapies restricting AD pathology via its non-amyloidogenic route.

Key Words:
APP, ADAM, PKC, Estrogen, IL-1, APP5'UTR.

Introduction

Amyloid beta (Aβ) plaque deposition is a major pathological feature of the neurodegenerative Alzheimer's disease (AD)[1]. The amyloid precursor protein (APP), via stimulation of amyloidogenic processing, undergoes sequential proteolytic cleavage by β-secretase and γ-secretase to generate Aβ[2]. Alternatively, a non-amyloidogenic pathway involving α-secretase activation generates sAPPα[3,4]. The advantage of this α-secretase pathway is that it causes proteolytic cleavage within Aβ peptide sequence of APP and, therefore, competitively inhibits activation of the detrimental amyloidogenic pathway[5]. Also, sAPPα is proven to possess neuroprotective and memory-enhancing properties, often being compared to cerebral growth stimulants[6,7]. Thus, these two features of reduced Aβ generation and sAPPα-induced neuroprotection point towards APP non-amyloidogenic pathway as a suitable therapeutic target for AD. Intriguingly, although amyloidogenic pathway is fairly well explored in relation to AD therapy, the non-amyloidogenic neuroprotective pathway remained mostly ignored. This review will bring forth the neuroprotective properties of sAPPα and α-secretase proteins and will focus on important signaling pathways and therapeutic targets that elevate non-amyloidogenic APP processing. We will also highlight the need for new therapies and strategies that may promote α-secretase activation.

APP Processing: Towards the Non-Amyloidogenic Pathway

sAPPa: Mode of Action

The 695-amino acid sAPPα protein represents the N-terminal domain of APP. It is functio-

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nally marked by 12-cysteine residues, active disulfide bonds and heparin-binding sites which bind to copper, zinc and growth factors[8]. sAPPα knock-in mice and transgenic mice mutated at sAPPα activation sites demonstrate aberrant neurobehavior and loss in memory and long-term potentiation (LTP)[9]. Particularly, the amino acid 319-335 sequences of sAPPα proved essential in preventing the neuronal loss and promoting neurite outgrowth[10,11]. sAPPα's heparin-binding sites that interact with extracellular heparan sulfate proteoglycans participates in neuronal cell adhesion and dendritic and axonal outgrowth of the brain[8,12]. Interestingly, sAPPα alone could rescue electrophysiological aberrations detected in the APP knock-out mice, supporting its significant contribution in restoring the cognition[9]. Its effects include increased synaptic density and memory retention via stimulation of N-methyl-D-aspartate currents[13]. Heparin-binding sites of sAPPα also block the proximal copper and Zinc-binding sites, and inhibit generation of oxidative stress[8,14]. The non-amyloidogenic pathway is involved in the reduction of toxic calcium signaling, activation of K^+ channel, decrease of glutamate excitoxicity and attenuation of glucose deprivation[6,7,15]. sAPPα also degrades Aβ aggregates through the lymphocyte immune defense mechanism, reduces coagulation factor and enhances cytokine release from activated astrocytes and microglia[8]. Furthermore, sAPPα mimics epidermal growth factor (EGF) functioning that promotes neuronal proliferation and protects against AD pathology[16].

A-secretase Members

Zinc metalloproteases, mainly the members of disintegrin and metalloprotease (ADAM) families, such as ADAM17, ADAM10 and ADAM9, are considered as α-secretases that activate the non-amyloidogenic APP processing[17]. ADAMs belong to type-1 integral membrane protein family, characterized by a multi-domain structure consisting of (1) pro-domain, (2) catalytic metalloprotease domain, (3) disintegrin domain, (4) cysteine-rich domain, and (5) cytoplasmic tail for binding adaptor proteins[18] (Figure 1). Through knock-out, knock-down and gene silencing studies, the functional and comparative efficacy of ADAM members in reducing Aβ pathology has been investigated[19,20].

ADAM17

ADAM17, also known as Tumor Necrosis Factor-α-converting enzyme (TACE), is believed to promote the regulated cleavage of APP towards sAPPα[7]. ADAM17 knock-down cell lines and ADAM17-deficient mice could not promote non-amyloidogenic processing of APP, whereas ADAM17 over-expression enhanced sAPPα, suggesting a regulated mode of action[21,22]. In support of this idea, ADAM17 inhibition in the Chinese Hamster Ovary cells (CHO) influences protein kinase C (PKC)-mediated sAPPα generation[23-25]. In addition, ADAM17 over-expression in PKC-deficient LoVo cell lines fails to influence constitutive sAPPα secretion[26].

Apart from APP, ADAM 17 influences proteolytic cleavage of other substrates that directly or indirectly reduce Aβ. Notably, ADAM17-mediated shedding of microglial pro-inflammatory mediators, like tumor necrosis factor-α, fractalkine and interleukin-8 (IL-8), and stimulation of IL-1 and IL-6 receptors prompts phagocytosis that degrades Aβ[18,27]. In addition, ADAM17-dependent activation of Epidermal Growth Factor (EGF) family members[28], especially heparin-binding EGF-like growth factor, promotes neuronal proliferation and reduces cerebral damage in AD [29].

However, as evident from studies on neuroblastoma cells and mice in situ hybridization experiments, ADAM17 only partially functions as α-secretase, and an overall non-amyloidogenic APP processing essentially required the other

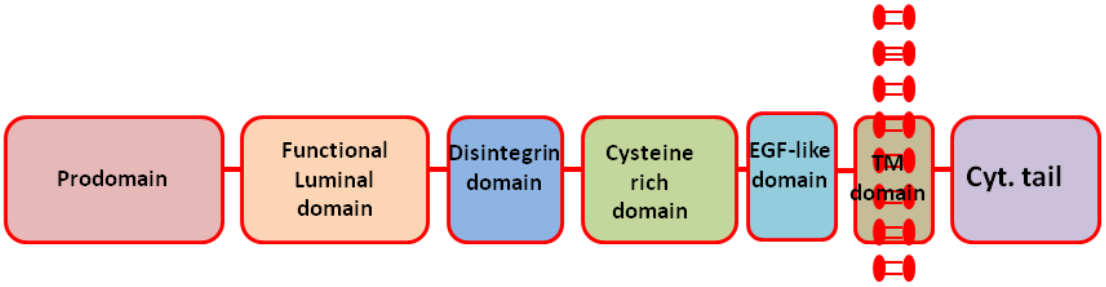

Figure 1. Structure of ADAM.

ADAM family members as well[7,30]. Moreover, the reduced ability of ADAM17 to influence the constitutive sAPPα release supports the view that other ADAM members are required for α-secretase functioning.

ADAM 10

Unlike ADAM17, ADAM10 functions through a constitutive mode[31]. Enhanced sAPPα in ADAM10 over-expressed cells and attenuated Aβ plaque deposition in ADAM10 knock-out mice brain support this constitutive concept[32].

ADAM10 exists as a pro-enzyme in Golgi apparatus, and following cleavage and N-glycosylation acquires a protease activity at the plasma membrane[32-34]. ADAM10-mediated APP cleavage involves an altered APP interaction with adhesion proteins and extracellular matrix[35]. In addition, the clathrin adaptor AP2-mediated endocytosis regulates ADAM10 expression[36], and synapse-associated protein-97 (SAP-97) stimulates APP cleavage by targeting the excitatory synapses in AD brain[37,38]. Also, ADAM10, in association with AP2 and SAP-97, improves LTP and synaptic activity in AD patients[36].

The over-expression of ADAM10 in primary neurons of knock-down and ADAM10-dominant-negative mutants reduce sAPPα, with a simultaneous increase in BACE-1[21,39]. *In vivo* studies revealed that Q170H and R181G mutations in ADAM10 gene are responsible for this reduced α-secretase activity[40]. Consistently, over-expression of this mutated gene inhibits Aβ deposition even in the APP transgenic animal models[39], proving the importance of these two amino acid residues for the ADAM10 functioning. ADAM10 also limits the AD vascular pathology, as evident from the shedding of lipoprotein receptor related protein-1 (LRP1) that regulates Aβ transport and clearance across the blood-brain barrier[41].

ADAM9

Like ADAM17, ADAM9 participates in the non-constitutive, regulated sAPPα processing[42]. ADAM9 activity mainly appears to be PKC-dependent[43], and its cleavage sites were identified at His13-Lys16 and His14-Gln15 within Aβ peptide sequence[43]. Nonetheless, a constitutive role of ADAM9 where it closely mimicked the ADAM10 functions was also reported[44,45]. This report also claims that ADAM9 functions in the microglial cells only, targeting APP-HHQK sequence specific for microglia[46]. Comparative data on the three ADAM proteins demonstrate that initial activation of ADAM17 and ADAM9 culminates in constitutive ADAM10 functioning at the cell surface[44]. This is indicative of an overlap or interaction of these important ADAM family members. Clinical trials are being carried out to verify specific role of ADAM9 and its connection with ADAM17 and ADAM10[7]. Overall, ADAM9 is relatively less investigated and is believed to be of lesser significance in AD compared to ADAM17 and 10[22]. Rather, ADAM9 mRNA having been first isolated from lungs is considered more important for the ectodomain shedding of lung epithelia[7].

It is interesting to note that distribution and activation of the ADAM metalloproteases determine their specific activities[7]. Active ADAM17 is expressed at the cell surface and in perinuclear intracellular compartment[7]. Biotinylated ADAM10 was found to be located at cell surface and in intracellular Golgi region[32], and active ADAM9 is mainly localized in the Golgi apparatus[47]. However, distribution and localizations generally guide the basal activities of ADAMs, and their regulated activities are governed distinctly[48]. It is thoroughly proven that ADAM members fail to function in isolation, and their combined impact culminates in the ultimate α-secretase functions.

Stimulation of the non-amyloidogenic Pathway

sAPPα promotes synaptic plasticity and neuronal survival by stimulating several neurotrophic signaling pathways. The most prominent among them is the protein kinase C (PKC) signaling (Figure 2) that results in increased level of anti-apoptotic BCl2 and Bcl-xL proteins and attenuated caspase-mediated apoptosis in the AD brain [49]. Altered tyrosine kinase (TK), mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) signaling, and Ca^{2+} signaling contributes to the anti-apoptotic mechanism of sAPPα[50]. In addition, acetylcholine, serotonergic and glutamatergic receptors, hormones and cholesterol-lowering statins enhance sAPPα release. In the last few years, targeting of IL-1 and its responsive element on the APP gene also emerged as a useful strategy towards augmenting the non-amyloidogenic APP processing (Figure 3).

Promoting Signaling Pathways of sAPPa

PKC pathway

Activation of PKC signaling emerged as the first promising strategy to manipulate and promote α-secretase cleavage of APP[24]. In fact, in-

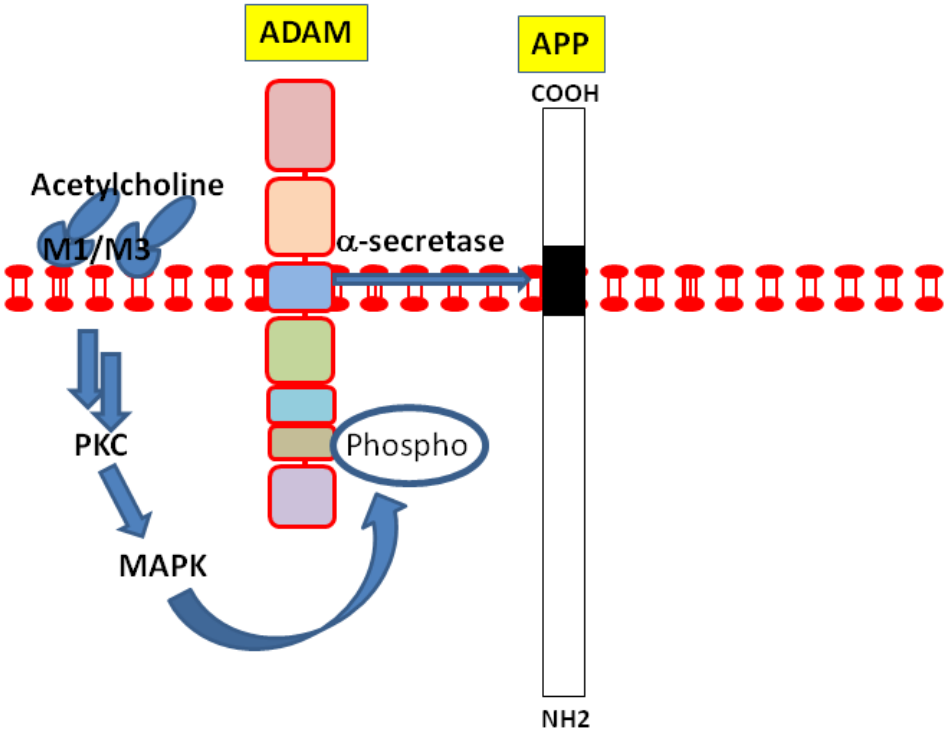

Figure 2. Acetylcholine binding to M1/M3 receptors activates PKC and downstream MAPKs. The MAPKs phosphorylate ADAMs along the transmembrane that stimulates α-secretase cleavage of Alzheimer's Amyloid Precursor Protein (APP).

formation on the role of PKC in sAPPα release appeared prior to characterization of ADAMs[45]. Supportively, almost all pharmacological pathways promoting sAPPα release converged upon PKC activators[51].

Phorbol esters that are prominent PKC activators are well-recognized stimulants of α-secretase pathway[52]. These PKC activators, in association with cell-surface receptors and neurotransmitters stimulate the activity of α-secretase ADAM family members[51]. Phorbol 12-myristate 13-acetate (PMA) and phorbol-12, 13-dibutyrate (PDBu), even at nanomolar levels, increase PKCε synthesis, and inhibiting of PKCε activation blocks cholinergic modulation of APP metabolism towards sAPPα[53]. Thus, a direct or indirect regulation of PKCϵ appears to be promising in reducing the Aβ via the α-secretase pathway. However, the use of PMA and PDBu is problematic due to their tumor promoting properties[54]. Thus, PKC activators, such as benzolactam (BL) and its related compound, LQ12, as well as marine natural macrocyclic polyketide, bryostatin, that causes a significant increase in sAPPα in clinical AD cases, were investigated further[55]. Among all these compounds, bryostatin was the safest and showed a consistent increasing effect on sAPPα release [56]. Bryostatin-1, even at sub-nanomolar concentrations, promoted sAPPα secretion and attenuated Aβ deposition in transgenic mice without generating tumors[56]. However, differing from the ubiquitous PKC concept, some findings indicate that PKC signaling preferentially promotes regulated sAPPα release and influences only 30-40% of basal or constitutive secretion[51]. This disparate view on PKC in terms of constitutive and regulated α-secretase activity awaits in-depth characterization[57].

Muscarinic (M1/M3) acetylcholine receptors emerged as functional activators of PKC signaling that work in a dose and time-dependent manner[45]. Existing evidence proves that PKC coupled to M1 and M3-muscarinic receptors stimulate sAPPα release[58,59]. *In vitro* observations revealed that this sAPPα and muscarinic receptor association preferentially involves PKCα and PKCε, rather than PKCδ[60]. In support of this view, PKCα and predominantly PKCε rather than PKCδ isoforms demonstrated extensive activation at the pre-synaptic domain of central nervous system[61].

Blocking of acetylcholinesterase activity increases PKC signaling and enhances sAPPα release thus helping in restoring the cognitive performances in AD[57]. Likewise, supplementing a selective M1-muscarinic agonist, AF267B, through intraperitoneal route reduces acetylcholinesterase activity and stimulates ADAM17, culminating in reduced AD pathology in a transgenic AD mice model[62]. A very similar M1 agonist, AF102B, inhibits neuronal apoptosis via increased sAPPα[58,63]. A third M1/M3 receptor agonist, RS86, elevates the sAPPα level in cerebrospinal fluid, with concomitant decrease in cortical and hippocampal full-length APP and improved cognitive performance[45]. Here, fibroblast growth factor (FGF) and EGF-activated serine/threonine-phosphorylation of ADAM17 appear to mediate the α-secretase functions[64,65]. Phospholipase-C (PLC), diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) participation in the PKC, M1/M3-muscarinic and sAPPα pathways has also been proposed[66].

TK Pathway

A combined action of the EGF, platelet-derived growth factor (PDGF), FGF and brain-derived neurotrophic factor (BDNF) that activate TK signaling contributes to the Aβ suppression via α-secretase pathway[66]. Suppressed TK signaling was reported in the frontal cortex and hippocampus of AD brain[66]. Conversely, activated TK signaling via stimulated non-amyloidogenic APP-processing was shown to protect against AD pathology[67-69]. A synergistic PKC and TK signaling are also known in sAPPα release, where tyrosine phosphate agonists appear to activate the M1/M3 muscarnic receptors[70]. Similarly, inhibiting the non-receptor Src-TKs by protein phosphatase-1 (PP1) suppresses the PKC-induced sAPPα release[71], and inhibiting the EGFR trans-activation via AG1478 blocks the EGF-induced sAPPα[72]. A combined application of FGF with PMA also results in higher sAPPα release compared to their additive effects in human neuroblastoma cells, thus supporting TK and PKC synergism[73]. PDGF participates in sAPPα generation in astrocytes, and suppressing the PDGF expression by using genistein blocks TK activation as well as sAPPα release[74]. BDNF-Trkb interaction promotes the APP non-amyloidogenic pathways, whereas exogenous retinoic acid stimulation offers a significant protection in AD[75].

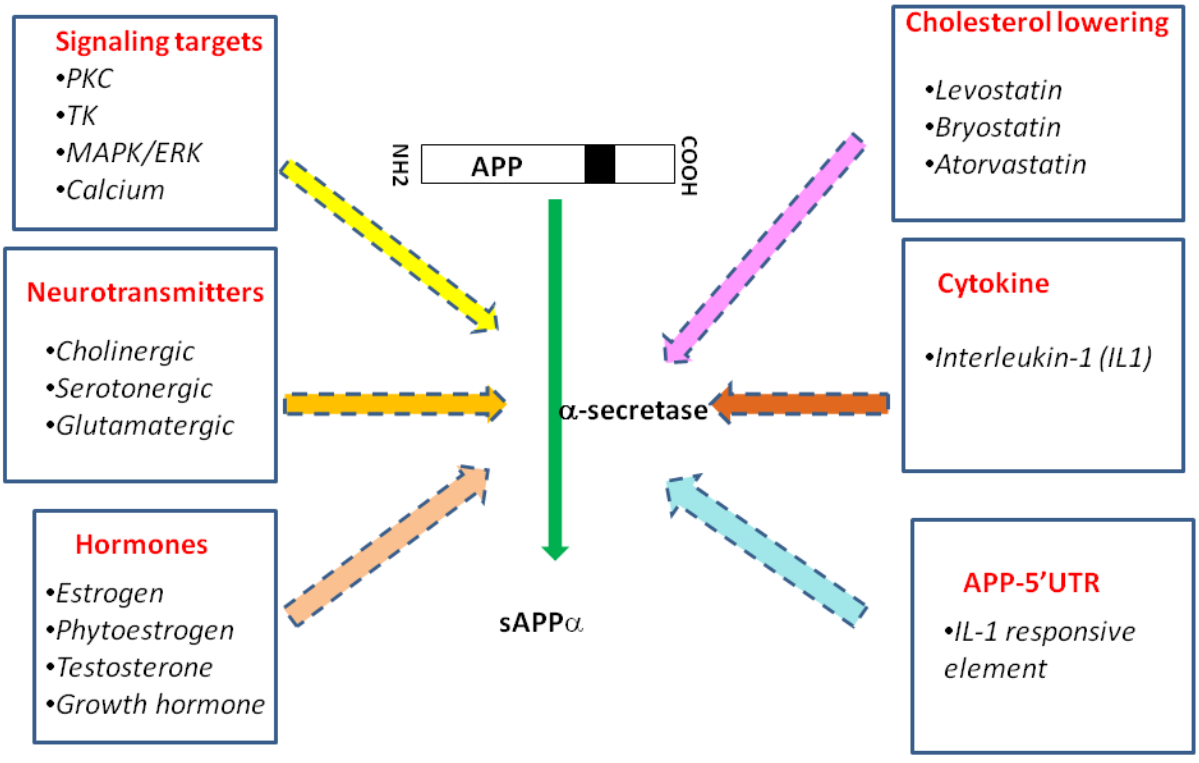

Figure 3. Factors stimulating α-secretase cleavage and therapeutic targets in sAPPα release.

MAPK Pathway

Several independent experimental studies demonstrated that MAPK-ERK pathway is an important regulator of α-secretase activity[76,77]. It was demonstrated that ERK1 interaction with threonine-735 residue of ADAM17 promotes ADAM17 translocation along plasma membrane, linking the two pathways, i.e. ERK and non-amyloidogenic APP processing[65]. An intricate association is also thought to exist between PKC-regulated α-secretase processing and ERK, where MAPK inhibitor, PD 98059, blocks the impact of phorbol esters[78]. Similarly, pituitary adenylate cyclase-activating polypeptide (PACAP)-regulated α-secretase activity can be disrupted via synergistic interaction of PKC inhibitor, chelerythrine, and ERK inhibitor, PD 98059[79]. The neuroprotective and anti-apoptotic monoamine-oxidase inhibitor, rasagiline, also requires PKC and ERK activation for stimulating the sAPPα secretion[80]. Cisse et al[45] showed that the PKC-regulated α-secretase pathway depends significantly on the ERK and ADAM17 interaction. However, this PKC and ERK-ADAM interaction link need to be validated further.

Notably, unlike the PKC pathway, the ERK pathway is proven to regulate equally both constitutive and regulated sAPPα release[45]. The discrepant ERK and PKC responses could be explained by the fact that membrane lipid-rafts are key factors in choosing between regulated and constitutive ADAM activities[45]. PKC activation is generally localized in the cholesterol-rich domain, whereas constitutive ADAM activation demands non-lipid sites[45]. ERK undergoes activation at both low and high-cholesterol micro-domains, rationalizing its role in regulated as well as constitutive α-secretase functioning[81].

The ERK inhibitor, PD 98059, also inhibits Nerve growth factor (NGF)-induced TK signaling and sAPPα release, proving ERK participation in the TK-mediated α-secretase activation[82]. Overall, the studies of these signaling pathways highlighted the importance of PKC, MAPK, and TK second-messenger pathways for α-secretase activation, where PKC and TK signals congregate at ERK and ultimately drive the enhanced sAPPα release[74,80].

Ca^{2+} Signaling

Calcium homeostasis is essential for the normal sAPPα functioning, as it influences neuronal plasticity and development and the release of neurotransmitters[83]. The calcium ionophore, A23187, causes increased sAPPα release with concomitant decrease in Aβ expression in primary neurons and B104 neuroblastoma cells[84,85]. The phorbol ester-mediated ADAM17 increase also involves enhanced cytoplasmic Ca^{2+} signaling and activation of the calcium-dependent cysteine protease, calpain[86]. Likewise, inhibiting calpain activity blocks α-secretase functioning. Based on this strong link between ADAMs and calpain, it is presumed that calpain signaling mimics the α-secretase activities to certain extent[87,88].

Acetylcholine, serotonergic and Glutamatergic receptors, and sAPPa

Acetylcholine Receptor

The loss of cholinergic neurons results in cognitive deficiencies, a major pathological feature of AD[89]. Thus, cholinesterase inhibition is one of the pharmacologic approaches that stimulate non-amyloidogenic APP processing and thereby restore cognition[89]. It is reported that the three main acetylcholinesterase inhibitors, physostigmine (PHY), heptyl-physostigmine (HEP) and 2,2-dichlorovinyldimethyl phosphate (DDVP) significantly enhance the sAPPα release[90]. In this study, the electrical stimulation frequency of acetylcholine neurotransmission was used to determine the cholinergic activity that influences sAPPα[90].

Loss of cholinergic primary cortical neurons suppressed the non-amyloidogenic processing of APP, and thereby up-regulated Aβ levels in the brain. Likewise, mutation at the muscarinic acetylcholine receptor in APP transgenic mice reduced sAPPα and increased Aβ[91]. Clinical studies also confirmed this acetylcholine receptor's participation in sAPPα release[62]. Investigating the mechanism of action revealed that acetylcholine targets membrane trafficking of the ADAMs[91]. It is assumed that muscarinic acetylcholinesterase inhibitors function by drawing ADAM10 and APP substrate together on the plasma membrane[59]. For instance, treating SH-SY5Y neuroblastoma cells with the muscarinic acetylcholinesterase inhibitor, donepezil, not only increased sAPPα release, but also led to the accumulation of ADAM10 and APP active forms in the close vicinity to plasma membrane[59]. Co-immunolabeling experiments validated this functional ADAM10 and APP association[59]. However, it was proven that along with muscarinic receptors, n-acetylcholine receptors, or a combination of the two, participate in the sAPPα formation. In support of this view, the

muscarinic-anticholinergic drug, atropine, failed to suppress fully sAPPα in experiments and needed involvement of n-acetylcholine receptor antagonist too[92].

Serotoninergic agonists, via cyclic adenosine monophosphate (cAMP)-dependent pathways or through coupling with IP3 and PLC pathways increases sAPPα secretion[93]. Likewise, serotonin-specific reuptake inhibitors suppress sAPPα, which could be recovered via serotoninergic 5-hydroxytryptamine receptor activation[94]. In hippocampal neurons, the metabotropic glutamatergic pathway also accelerates non-amyloidogenic APP processing[95]. Here, the hippocampal and cortical PKC pathways play a major role, as evident from the reduced sAPPα levels upon PKC inhibition, even in the presence of the glutamatergic agonists[96,97]. Glutamatergic interplay with Ca^{2+} signaling also appears to be responsible for glutamate receptor activation in sAPPα release [96,97].

Hormonal Regulation and sAPPa

Clinical findings reveal that steroid hormones have significant pharmacological relevance in AD. Estrogen is one such hormone that alters APP metabolism, promoting the shift towards sAPPα[98]. Estrogen attenuates Aβ-induced apoptosis via reduced oxidative stress and neuroinflammation[98]. Studies revealed the PKC pathway's involvement as well, as apparent from the suppression of estrogen-induced sAPPα by PKC inhibitor, calphostin C[99,100]. Participation of MAPK-ERK1/2 in estrogen-regulated α-secretase activation is also evident in hypothalamic HT22 cells[101]. MAPK and PKC together could also promote estrogen-induced sAPPα in the hypothalamic gonadotrophin, GT-17 cells[102].

Polyphenolic flavonoid from green tea, epigallocatechin gallate (EGCG), activates ADAM10, enhances sAPPα release and suppresses Aβ in SH-SY5Y and PC12 cell lines, as well as in N2a/APPsw cells[103-106]. Analysis of the mechanism of EGCG action revealed that the gallate group of EGCG mimicks 7α-estrogen site, thereby allowing EGCG binding to estrogen receptor-1-alpha (ERlα)[107]. This gallate group was found to function by promoting maturation of ADAM10 protein[107]. Further investigation proved that gallate-dependent ERlα activation involves PKC signaling[107]. Other phenolic compounds bearing this gallate domain, including octyl gallate and atranorin, could also activate sAPPα generation via ERlα and PKC[108]. Thus, pharmacologically safe EGCG and gallate compounds emerged as reliable protectors against AD development via activation of non-amyloidogenic pathway[109]. EGCG mode of functioning via PKC also involves binding to the furin protein convertase enzyme that stimulates ADAM10 activation [110]. Here, up-regulation of ER1α/MAPK/ERK signaling rather than the PI3k/AKT pathway has been hypothesized[110].

The phytoestrogen ginsenoside Rg1 promotes non-amyloidogenic APP processing[111]. PKC, MAPK and PI3K inhibitors block ginsenoside Rg1-mediated sAPPα release, indicating the participation of these signaling pathways in the phytoestrogens action[111]. Interestingly, although ginsenoside Rg1 failed to bind ERα directly, the PKC, MAPK and PI3K pathways phosphorylated AF-1 (Ser118) domain on the receptor, thereby arbitrating estrogen-mediated α-secretase activation[112]. Via enhanced α-secretase activity, Rg1 also inhibited ER withdrawal-mediated Aβ accumulation in ovariectomized rats[111].

A highly selective β1-adrenoceptor antagonist, Nebivolol, stimulated ADAM9 and reduced Aβ upon co-treatment with E2 in N2Aswe cells, implying combined estrogen and inactivated β1-adrenoceptor-mediated functioning[113]. Co-treatment with ER inhibitor, ICI182780, blocks Nebivolol and E2-mediated effects on Aβ, verifying estrogen receptor's involvement in Nebivolol-induced anti-amyloidogenicity[113].

Apart from estrogen, the steroid hormone testosterone influenced sAPPα in the GT1-7 cells and in N2a neuronal cell lines, as well as primary neurons. However, a testosterone to estrogen conversion involving MAPK activation is presumed to be actually responsible for neuroprotection and sAPPα increase[100,114].

Suppression of luteinizing hormone (LH) at the hypothalamic-pituitary-gonadal axis promotes α-secretase functioning, and thereby suppresses cognitive deficits and Aβ deposition in Tg 2576 AD mice, indicating negative effects of LH on sAPPα[115]. Growth hormones associated with growth factor signaling participates in non-amyloidogenic pathway activation too[116]. Insulin-like growth factor-1 is one such growth stimulator that coordinates with growth hormones in order to metabolize APP towards the non-amyloidogenic pathway[117]. Melatonin and thyroid hormones have also been linked to APP metabolism; however, in-depth studies are needed to investigate their exact participation in the α-secretase activity[118,119].

Statins and sAPPa

Cholesterol level affects the APP processing, and epidemiological data demonstrated that cholesterol-suppressing statins play a beneficial role by suppressing the amyloidogenic pathway[7,120]. Reduced cholesterol disintegrates the functional lipid rafts that promote Aβ formation, resulting in a shift from amyloidogenic to non-amyloidogenic APP processing and thereby sAPPα generation[121]. Spatial separation of ADAM-10 and its physiological inhibitor, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), in the glycosylphosphatidylinositol-rich lipid rafts governs the statin-mediated sAPPα generation[19]. Supporting this view, lovastatin and atorvastatin were demonstrated to separate RECK and ADAM significantly and promote ADAM10 availability[122]. The PKC stimulator, bryostatin, also behaves in a similar manner[19]. Docosahexaenoic acid (DHA), a well-known polyunsaturated fatty acid (PUFA) that reduces hypercholesterolemia and cholesterol de novo, attenuates ADAM17 protein degradation in lipid rafts[123]. DHA was shown to promote the ADAM17 stability and the corresponding induction of α-secretase activity in SH-SY5Y cells[123].

Statins that block 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and reduce hypercholesterolemia also enhances sAPPα[124-128]. HMG-CoA inhibition involves the isoprenoid and Rho-GTPase pathways of sAPPα modulation[129,130]. Rho-GTPase and its effector molecule, Rho-associated kinase (ROCK) ultimately appears to be responsible for the statin-mediated sAPPα shedding[129]. Supporting this idea, farnesyl transferase inhibitor, FTI-1, that inhibits ROCK enhances sAPPα and attenuates Aβ in the N2a mouse cell line[131]. In addition, arachidonic acid that stimulates ROCK expression attenuates sAPPα[131]. Activated PI3K/Akt pathway, coupled to the insulin receptor, could also regulate statin-mediated neuroprotection in AD[132]. Reduced calpain activation and calcium flux also demonstrate certain involvement[133].

Targeting IL-1 and the APP 5′-Untranslated Region (APP5′UTR) Towards sAPPa

The GC-rich APP mRNA 5'UTR-stem loop structure is a significant regulator of APP gene expression. The loop bears an amyloid-specific CAGA sequence (+83/+86), IL-1 responsive element (+55 to +144) and an iron responsive element (+51 to +94). IL-1 binding to its responsive element significantly impacts the functioning of APP5'UTR that affects APP metabolism and thus sAPPα release[134].

As reported for U373MG astrocytoma cell line, short exposure to IL-l causes a dose-dependent increase in ADAM10 and ADAM17, both at mRNA and protein levels that led to APP5'UTR-dependent Aβ reduction[77]. Supporting prior observations on these α-secretase members, IL-1 increased ADAM10 constitutively and ADAM17 in a regulated manner in U373MG astrocytoma cells[77]. Further delving into the mechanism demonstrated that IL-1 significantly stimulates P38 and ERK pathways and partially the PI3K/AKT pathway[77]. Overall, in this study the activated P38 pathway appeared proximal to IL-1 that regulates other kinases, proving the IL-1-mediated P38®ERK®PI3K/AKT pathway of non-amyloidogenic APP processing[77]. A similar observation was reported in U251 neuroglioma cells, where intermediate ERK and JNK pathway activations stimulated α-secretase activity[135,136].

Drug screen assays targeted towards APP5'UTR mRNA proved effective in identifying therapeutics for AD[134]. High Throughput Screening (HTS) of 1200 FDA-approved drugs identified several serotonin reuptake inhibitors, metal chelators, N-acetyl cysteine antioxidants, macrolide antibiotics and anticholinesterases as APP suppressors[137-139]. Of these, both *in vitro* and *in vivo* investigations revealed M1 muscarinic agonist, AF102B, as an enhancer of α-secretase activity[140]. The compound not only promoted sAPPα generation, but also stimulated expression of neurotrophins and growth factors[140]. AF102B caused significant recovery in cholinergic functions, and restored cognitive performances such as escape latency and reversal learning in mice[140,141]. The mechanism of AF10B action involved a synergistic association with NGF and EGF, leading to inhibition of oxidative stress and neuronal apoptosis[140].

Stable SH-SY5Y cell transfectants expressing the APP5′UTR-dependent luciferase reporter also served as a target for 110,000 compounds from the FDA drug library[142]. Green fluorescent protein under control of the viral ribosomal entry site was used as an internal specificity control[142]. Around twenty compounds from the screen modulated the APP5'UTR-driven luciferase reporter expression. Prion protein-5′UTR that served as a negative control was unaffected, that prove specificity of the screened compounds towards APP[142]. These compounds are presently being explored for further understanding of their efficacies in sAPPα generation.

Conclusions and Future Perspectives

Molecular pathways stimulating the non-amyloidogenic APP processing were widely investigated during the last several years. ADAMs, particularly ADAM10, 17 and 9, are the key α-secretase members that are being targeted for enhancing the sAPPα generation. Nonetheless, further studies are needed to distinctly identify specific activations and molecular mechanism of action of these ADAM members.

A plausible approach towards stimulating the α-secretase activity could be by promoting ADAM-trafficking along the plasma membrane. Since sAPPα behaves like neurotrophins, drugs directly targeting its increase may also be screened. G-protein-coupled receptors (GPCR) are good drug screen targets; however, they are less investigated for α-secretase activity. Thus, a new strategy or screening program for identifying α-secretase activators may aim at targeting the GPCRs. PKC, TK, PI3K and MAPK activators may also be screened for sAPPα generation. In addition, the compound AF267B needs to be further developed, so that it can emerge as a novel drug attenuating AD via stimulation of APP non-amyloidogenic pathway. Besides, HTS may also be carried out in recognizing α-secretase pharmacological activators targeting IL-1 pathway[142]. Natural compounds, such as flavonoids, PUFA, phytonutrients, etc. could be screened for this purpose. Isoflavones, lignans and phytoestrogens that are well-recognized in preventing the neurodegeneration[143] may be examined for their effects on APP metabolism. Based on its favorable role in preventing cognitive damage, garlic containing S-allycisteine and allicin may be tested for sAPPα[144]. In addition, carotenoids and antioxidants, like strawberry, blueberry, *Ginkgo biloba*, curcumin, etc. that have been proven to have beneficial effects on aging may also be screened[145]. Thus, screening of these natural products may lead to novel and less toxic α-secretase activators culminating in reduced detrimental cerebral Aβ deposition.

Acknowledgements

This study was supported by Provincial Training Program of Science and Technology for Innovative Talents of Jilin (No. 20130521002JH).

Conflicts of interest

The authors declare no conflicts of interest.

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