The Functions of the Amyloid Precursor Protein Gene and Its Derivative Peptides: II Experimental Evidence and Clinical Studies*

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ABSTRACT

In vitro studies suggest Amyloid Precursor Protein Gene (APP) is involved in interaction with the extracellular matrix, neurite growth, adhesion, development, synaptic function, platelet function, and interaction with GTP binding proteins. In vivo experiments show a role in embryonic development, response to cerebral excitotoxicity and gliosis, response to brain injury including ischaemia, hypothalamic function, locomotor function, learning and memory. In vitro observations indicate Aβ has a role in amyloid formation, excitotopic neuronal injury, tachykinin interaction, endothelial vasoconstrictor response, calcium and oxidative stress, free radical interaction, cell membrane fluidity, apoptosis, astrocyte stimulation, and microglial interaction. Other studies suggest important roles for Aβ oligomers in synaptic function and as an antimicrobial peptide. In vivo investigations show involvement in memory function, the blood brain barrier, and tachykinin response to cerebral injury.

Keywords: Amyloid Precursor Protein Gene

1. Functions of APP

1.1. In Vitro Studies: APP

Secretion of Amyloid Precursor Protein Gene (APP) involves the action of multiple cleavages [1] and in physiological conditions the production of non-amyloidogenic products from the action of integral membrane-bound metallopeptidases (secretases) [2].

APP is soluble and has soluble derivatives in CSF and other biological fluids [3,4]. Biochemical studies suggest that APP is proteolytically cleaved by a putative enzyme termed α-secretase, this cleavage occurring at position 16/17 of the Aβ region [5]. The generated N-terminal fragment is soluble, termed sAPPα, and the C-terminal fragment remains in the membrane. APP may be internalized [6] and its proteolysis diverted towards endosomes and liposomes where a β-secretase acts at the N-terminus of Aβ to generate the sAPPβ fragment containing the Aβ domain [7,8]. The Aβ sequence is liberated from the membrane by the action of γ-secretase which acts at the C-terminus of Aβ [9].

APP is first synthesized as a membrane-bound protein carrying asparagine-linked sugar chains. It is then processed into higher molecular mass forms by the additions of sulphate, phosphate and sialic acid residues—adding about 20 kDa in molecular weight [10]. The chondroitin sulfate proteoglycan form of APP is known as APPICAN or L-APP and is an alternatively spliced form of APP lacking exon 15. This results in the formation of Asp-Xaa-Ser-Gly consensus sequence site for chondroitin sulfate chain attachment to serine 619 of L-APP which is 16 amino acids upstream of the Aβ sequence; this enhances the binding of APP to the extracellular matrix [11].

Embryonic cortical neurons are believed to have two pools of APP: one within the axons and cell bodies which is mobilized after paraformaldehyde exposure and elevation of the extracellular Ca²⁺ concentration [12]. This APP is on the cell membrane and interacts with the cytoskeleton through the C-terminal. The other pool, which is probably endosomal, is not mobilized by these treatments. The C-terminal fragment of APP has been shown to form non-selective ion channels or pores in membranes of Xenopus oocytes, a property which is modulated by metal ions including Ca²⁺, Zn²⁺, Fe²⁺/Fe³⁺.

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APP isoforms with and without KPI domains stimulate adhesion of PC12 cells to tissue culture dishes [14]. These effects were enhanced by adding NGF and basic fibroblast growth factor (bFGF). NGF induced neuronal differentiation is accompanied by differential splicing of APP on PC12 cells [15]. The neurite promoting properties of APP on PC12 cells was present at low concentrations (10−10 M) and with APP in both soluble and membrane-associated forms [16], APP increased neurite length and branching but had no effect on the number of neurites. At high doses APP was toxic [17]. Addition of APP antibodies reduced the effect of NGF, suggesting an interaction between these two substances. The effect of APP on neurite outgrowth may be mediated through an interaction with heparin sulfate proteoglycans and NGF [18]. The effect of NGF on the APP probably involves neurolphin binding to the p75 neurotrophin receptor [19]. Toxicity of APP at high concentrations was confirmed in a murine embryonal carcinoma line transfected with human APP cDNA [20]. When differentiation into postmitotic neurons was induced by retinoic acid the cells possessed large amounts of APP and died. APP may be a substrate for caspase-3 in motor neurons [21].

Transfection of APP into a cell line that does not have APP revealed greater adherence, increased neurite outgrowth, and more rapid growth in low serum [22]. These cells were protected from the toxic effects of glutamate and Aβ. Substratum bound APP stimulated neurite outgrowth in cultures of chick sympathetic ganglia and mouse hippocampal neurons and was dependent on the presence of a substrate-bound proteoglycan similar to laminin [23]. A 14 kDa N-terminal fragment of APP bound to heparin, pointing to a possible endogenous antagonist. All APP isoforms have been detected in mouse oocytes, preimplantation embryos, and in all postimplantation stages—strongly supporting a wider role for APP in cellular adhesion [39].

CHO cells transfected with APP cDNA stimulated neurite outgrowth and cell adhesion in hippocampal neurons after co-culture [24]. These effects were greater with the KPI+ isoforms APP 770 and 751, than with APP 695. Amino acids 361 - 648 mediated this effect. This suggested that two extracytoplasmic domains promoted neurite outgrowth: one common to all isoforms, and the other localized to KPI+ domain. In a clonal neuronal cell line the RERMS sequence of APP (independent of KPI+) encouraged neurite induction, cell surface binding, and inositol polyphosphate stimulation [25]. These effects were shared with Aβ if it contained this sequence. An antagonist containing the sequence RMSQ successfully inhibited these effects, pointing to a possible endogenous antagonist.

With immuno-electron microscopy APP was found to be incorporated into the extracellular matrix between pre- and post-synaptic cells [26]. The secretory N-terminal of APP (sAPPα) binds to a saturable, specific receptor and binding protein which is probably a component of cell surface microdomains [27]. The presence of APP in extracellular microparticles was demonstrated using confocal immuno-fluorescence microscopy [28]. All APP isoforms had high affinity interactions with heparin sulfate proteoglycan [29]. The APP-collagen interaction is probably mediated through a heparin bridge mechanism [30]. KPI+ isoforms had the weakest affinity, possibly related to steric or conformational effects. Specific binding of APP to the IKVAV site of laminin A has been demonstrated [31]. Laminin, heparin sulfate proteoglycan, and other extracellular matrix glycoproteins are important in advancing neurite outgrowth.

The binding of vascular heparin sulfate proteoglycan to APP may be mediated by the N-terminal region of the Aβ sequence and may influence its proteolysis and promote fibril formation [32,33]. The heparin binding neurolopathic site is probably distinct [34]. The extracellular matrix probably influences the synthesis of APP in microglial cells and influences their migration, target recognition and binding [35]. APP binding to heparin is probably increased by zinc [36]. The heparin binding domains are found in the alpha-helical regions of APP [37]. There may be four distinct heparin binding domains suggesting that APP may interact with more than one glycosaminoglycan [38]. Treatment of PC12 cells with APP antisense oligonucleotides reduced neuritic processes in the presence of laminin, confirming an interaction between APP and molecules of the extracellular matrix [31]. In neuronal cultures APP colocalizes with integrins on the cellular surface in a discontinuous manner, further supporting a role for APP in cellular adhesion [39].

All APP isoforms have been detected in mouse oocytes, preimplantation embryos, and in all postimplantation stages—strongly supporting a wider role for APP in development [40]. Induction of neuronal differentiation with retinoic acid in a murine embryonal carcinoma cell line (P19) was accompanied by the production of APP 695 [41]; this was also seen in hippocampal cultures. Another study with P19 cells showed that differentiation to neuronal phenotype with retinoic acid was accompanied by the expression of all the isoforms of APP [42]. APP 695 was the most abundant in the early stages, with APP 751 and 770 increasing in amount with greater age of the cultures.

Immunofluorescence, laser confocal microscopy, and immuno-electron microscopy showed co-localization of APP and synaptophysin—a 39 kDa synaptic vesicle protein [43]. This co-localization was demonstrated in human and rat brain, and at neuromuscular junctions. Over
expression of APP in neuronal and other cell lives has been shown to enhance toxicity and change its processing by augmenting C-terminal fragments [44]. The C-terminal fragment of APP may decrease the frequency of spontaneous postsynaptic currents through an effect on K channels via a cGMP pathway, and the N-terminal fragment may increase impulse evoked postsynaptic currents involving protein kinase [45]. APP was also found in the vesicular elements of neuronal perikarya, dendrites and axons. The triplet lysine residue of APP (Lys 724 - Lys 725 - Lys 726), which is located in the carboxyol region after the transmembrane domain, may anchor APP in the cell membrane and influence the action of secretases [46]. Using immunodetection in rat peripheral nerve preparations, it was shown that APP is synthesized in the soma and is exported by fast axonal transport like neuropeptides and enzymes [47]. APP is found in the plasma membrane and intracellular membrane residues [48].

APP is released by human platelets [49] and inhibits coagulation factor X1α [50] Calcium ionophores, phosphatase inhibitors, anti mu-chain or anti CD3 antibodies in T cells, and phorbol esters in monocytes were able to induce surface membrane APP suggesting a role as a receptor in the haemopoietic and immune system [51]. Endothelial cells have been shown to be vulnerable to oxidative injury if there is over expression of APP [52]. Heat stress increases APP on endothelial cells and endothelial cells enzymes cleave APP on platelets [53,54]. APP KPI+ isosforms are identical to protease nexin II and inhibit tissue plasminogen activators, urokinase and thrombin [55,56]. A new member of the protein X11 family, X11L2, has been shown to bind APP in its intracellular domain further supporting the concept of an interaction of APP with coagulation factors [57]. Protease inhibitors may stimulate neurite extension and may be produced by glial cells [55,58,59].

Aurintricarboxylic acid (ATA), a metabolic inhibitor, decreased APP mRNA in cultured neurons [60]. In the same study interleukin 1 (IL-1) stimulated APP mRNA. The ATA-induced reduction in APP was accompanied by loss of neuronal phenotype, reduced adhesion, and neurite retraction. Cultures of sympathetic and cortical neurons grown initially with NGF showed a reduction in APP 695; KPI+ isoforms were increased when NGF was withdrawn [61]. Heat-shock, IL-1, acetylcholine, and TGF β1 have all been shown to induce APP mRNA in neuronal and other cells [62-66]. In human glialoma cells IL-1β decreased the expression of APP [67]. Interleukin-1 may alter processing and secretion of APP to augment sAPP and decrease Aβ [68]. There may also be a cellular selectivity in the influence of cytokines on APP with 1L-6 augmenting APP mRNA in neuronal but not glial cell lives [69]. The translation of APP may be determined by 1L-1α and 1L-1β operating upon a 90 nucleotide sequence in the untranslated regions (S-UTR of APP); this translational enhancer maps +55 to +144 nucleotides from the 5’ cap site and is homologous to regulatory elements found in ferritin genes [70].

APP may interact and form protein—protein complexes with the GTP binding protein G(α) [71], the enzyme glyceraldehyde-3-phosphate dehydrogenase [72]; and APOE [73]. The APOE3 isoform was more potent than the APOE4 isoform in compromising the neuroprotective properties of sAPP. The binding of APP to APOE is probably through its N-terminal involving regions 1-207 of APP and 1-191 of APOE and is independent of the Aβ sequence [74]. APOE may therefore have a chaperone function by which the metabolism and function of APP may be modulated. The isoforms of APP may form complexes with themselves and other proteins [75]. The neuronal adapter proteins FE65 and DISABLED bind to the C-terminal of APP where they may serve as molecular scaffolds for the assembly of protein complexes [76]. Heterotrimetric protein complexes involving X11α, mLin2 and mLin7 may be important in the localization of proteins in cells and the trafficking of APP in neurons [77,78].

The interaction of APP with FE65 may mobilize APP to the cell surface [79]. The cytosolic molecular chaperone Hsc73 binds adjacent to the post-transmembrane region of APP at the KFFEQ site of the C-terminal fragment and may be the targeting signal that determines lysosomal proteolysis [80]. APP may contain a domain which inhibits a matrix metallo-proteinase known as gelatinase A [81].

Other neurotransmitter and endocrine factors have been shown to interact with APP and may be involved in its regulation. Serotonin 5HT2α and 5HT2c receptors stimulate sAPPα (and APLP2) through a mechanism involving phospholipases and kinases [82]. In the same experiments muscarinic acetylcholine receptor agonists also stimulated sAPPα. Additional experiments have confirmed that an agent that is an M1 muscarinic receptor agonist and M2 antagonist stimulates sAPP and reduces Aβ [83]. Other data reveals that M1 and M3 muscarinic stimulation of sAPP may involve coupling with protein kinase C [84]. Thyroid hormone reduces APP transcription via a negative hormone response element [85]. Prostaglandin E2 stimulates APP mRNA via cAMP and this may be a mechanism by which brain injury and inflammation activates APP [86].

Electrical stimulation of rat hippocampal slices released N-terminal fragments of APP supporting the concept of APP’s involvement in synaptic processing [87].
1.2. In Vivo Studies: APP

APP immunoreactivity is widely distributed in the brains of mammals and mostly found in neurons [88,89]. Oligonucleotide probes to APP mRNA isoforms showed that APP 695 and 751 are the most abundant types in rodent and human brain [90,91]. APP 714 is greatest in the hippocampus, and APP 770 in ependymal cells in normal conditions. Full length APP probably accumulates at presynaptic terminals along with C-terminal fragments containing the Aβ domain and probably modulates synaptic activity in both forms [92].

With aging, APP immunoreactivity is found in smaller neurites in most regions of the rat CNS and this has been attributed to a decrease in axonal transport [93]. APP in the Purkinje cells of aging rats is located in the cell body and dendrites, and exists as a full length or truncated forms [94]. Development may influence the alternative splicing of APP such that KPI—isoforms predominate in early post natal life and correlate with maximum neurotrophic responsiveness [95]. The excitotoxin kainic acid damages the hippocampus and other brain regions after systemic and intraventricular administration [96]. Increased APP immunoreactivity has been observed several days after kainic acid treatment [97]. APP KPI+ deposition was shown immunocytochemically in astrocytes three days after kainic acid [98]. With in situ hybridization, increases in KPI+ isoforms were found after one day and were associated with reductions in KPI− isoforms in the hippocampus and other brain regions [99]. This stimulation in KPI+ correlated with changes in GFAP mRNA. Another study showed increases in KPI+ isoforms 12 hours after kainic acid in the dentate gyrus, CA1, and CA3 regions [100]. Neuroprotective agents including interleukin-1 receptor antagonists may modify the effects of excitotoxicity on APP expression [101,102]. Inflammatory processes may also influence the expression of APP [103].

APP KPI+ isoforms contribute to the plasticity of hippocampal sclerosis where their expression correlates with neuronal loss and gliosis in experimental temporal lobe epilepsy [104]. The changes in APP are preceded by induction of the immediate early genes c-fos and c-jun suggesting that these molecules are a component of the cascade of events leading to changes in APP [104,105]. There is tissue culture evidence for a direct role for c-jun in the regulation of APP [106].

Striatal injections of quinolinic acid in rats reduced APP KPI— in neurons, and stimulated APP KPI+ in glial cells [107]. Chemical lesions of the nucleus basalis of Meynert, adrenergic bundle, and dorsal raphe nucleus enhanced total APP expression in the cerebral cortex [108]. Ibotenic acid and colchicine have also been shown to stimulate APP [109,110]. Retinoic acid and DMSO increased total APP expression; KPI+ isoforms constituting most of this increase [111].

Penetrating needle, local head impact and other traumatic brain injuries stimulate APP immunoreactivity in damaged axons and reactive glial cells [112-115]. Focal cerebral ischaemia in the rat enhanced APP KPI+ gene expression as measured by northern analysis [116]. Stimulation of APP after rat focal ischaemia was confirmed immunocytochemically in adult and neonatal animals [117,118]. Similar increases in APP immunoreactivity have been found after global ischaemia in gerbils [119]. This degree of immunoreactivity has been related to the duration of ischaemia in the gerbil [120]. The deposition of APP has been provisionally localized to astrocytes in the ischaemic gerbil hippocampus [121]. Propentofylline, an adenosine A2 receptor agonist, decreased APP immunoreactivity in microglial cells and protected the gerbil hippocampus from ischaemia [122]. Propentofylline may enhance the neuroprotective functions of APP in ischemia [123].

Axotomy of the sciatic nerve increased APP 695 and APP KPI+ mRNAs in dorsal root ganglia [124]. After reinnervation, APP 695 levels decreased and KPI+ isoforms remained elevated. In the spinal cord, APP 695 returned to control levels after reinnervation. Rat sciatic nerve exposed to APP KPI+ and other protease inhibitors produced axonal sprouts [125]. Heat inactivated KPI+ and KPI− isoforms did not share this property. When rat sciatic nerve was treated with KPI+ Schwann cell mitosis and demyelination were found [126]. These findings give weight to in vitro evidence of the neurite-promoting properties of protease inhibitors.

Water deprivation and hypertonic saline injections stimulated APP immunoreactivity in the supraoptic and paraventricular hypothalamic nuclei [121], suggesting that APP may have broader physiological functions in response to stress.

Brain damage augments the proteoglycan form of APP (APPICAN) suggesting a novel proteolytic processing mechanism following injury [127].

Neonatal rats given anti-NGF antibodies developed neuronal degeneration in the superior cervical ganglion, associated with reductions in APP 695 mRNA and increases in APP 751 and 770 [77]. Multiple intracerebroventricular injections of NGF into rat pups did not induce changes in APP mRNA in the septal region and striatum [128]. Aging may decrease KPI+ mRNA isoforms correlating with lower NGF in the neocortex [129].

APP deficient mice have shown reactive gliosis and decreased locomotor activity indicating a role for APP in
motor function and astrocytic responses [130,131]. When the mouse APP promoter was disrupted by insertion of an exon 2 cassette containing a transcription termination sequence, the animals produced one-tenth of the usual amounts of APP [132,133]. This led to an increased frequency of agenesis of the corpus callosum and severe impairment of spatial learning and exploratory behaviour. Mice containing the double knockout (KO) APLP2 and APP die within the first week of life and the 20% of surviving mice have low weight, poor righting reflexes, ataxia, spinning behaviour and head tilt [134]. Single APLP2 KO mice were normal indicating substitution of function with APP. Hippocampal neurons from APP knockout mice have diminished viability and decreased neurite development [135]. Mice lacking APP had enhanced age related cognitive decline, compromised long-term potentiation, and gliosis in the cerebral cortex and hippocampus [136] suggesting that APP is involved in the enhancement of synaptic function.

Infusions of sAPPα 695 and sAPPα 751 intracerebroventricularly in mice, trained to perform learning tasks assessing both short term and long term memory, had memory enhancement effects and reduced the deficits induced by pretreatment with scopolamine [137]. These effects were observed at low doses, blocked by antibodies to APP, and not a result of impaired motor performance or exploratory behaviour. These data suggests that sAPP may strengthen synapses involved in memory. The findings also support the concept of APP modulating the function of cholinergic neurons.

A 17 amino acid peptide fragment immediately C-terminal to the KPI domain is probably responsible for the memory enhancing properties of APP and may lead to an increase in the number of presynaptic terminals [138].

In order to elucidate the role of APP in AD transgenic mice have been developed which over-express normal and mutational human APP. A transgenic mouse expressing human APP 751 deposited Aβ in their brains with increasing age [139]. Production of a transgenic mouse with a human missense mutation for familial AD, with over-expression of mutant APP, led to neuronal loss, senile plaques but no neurofibrillary tangles [140]. Other transgenic models have been produced with similar findings. [10,141-157]. These transgenic mice also provide further support for a role of inflammatory mediators in APP metabolism [147]. An experimental model of autoimmune encephalomyelitis revealed an increase in APP expression in microglia providing additional evidence for the role of the cholinergic system in APP metabolism [147]. An experimental model of autoimmune encephalomyelitis revealed an increase in APP expression in microglia providing further support for a role of inflammatory mediators in APP expression [167].

Hormonal manipulations may affect the function of APP. Oestrogens may reduce APP mRNA in focal ischaemia [168], and adrenalectomy augments APP immunoreactivity in the hippocampus [169]. Oestrogens and progestagens may both influence the age related decline APP695 mRNA in the hippocampus [170].

Drosophila possesses an APP-like gene encoding a protein with over 90% homology to human APP, termed APPL. Flies delete of APPL have defective fast phototaxis. This phenomenon was reversible with transgenic flies containing an APPL deletion with a normal human
APP gene, suggesting functional homology and a broader role for APP in motor behaviour [171,172]. The development of transgenic Drosophila containing normal and mutant human APP possess a blistered-wing phenotype confirming the impression from other models that APP is involved in cell adhesion and signalling [173].

Caenorhabditis elegans also has an APP-like protein with significant homology to APP [174].

2. Functions of Aβ

2.1. In Vitro Studies: Aβ

The amyloidogenic fragment Aβ is found in soluble form in blood, CSF and supernatants from cultured human brain cells [4,7,175-178]. Phosphatases may stimulate the production of Aβ through the beta-secretase pathway [179]. Aggregation and insolubility of Aβ is accompanied by toxicity, which can be reproduced by synthetic fragments containing amino acids 29 - 35 [180]. This part of the sequence is critical for initial aggregation and formation of the beta-pleated sheet structure.

Aβ has a hydrophobic C-terminal domain, which takes a beta strand structure, and the N-terminal region adopts an alpha-helical or beta-strand conformation depending on the environmental conditions such as acidic pH, peptide concentration and incubation time [181-184]. Two dimensional nuclear magnetic resonance spectroscopy and sequence data has revealed that the helical regions of Aβ have sequence homology to the helical domains of prion proteins [185]. There may also be homology to the paired helical filaments which make up neurofibrillary tangles [186]. The hydrophilic residues in Aβ contribute to the specificity of the interactions between Aβ peptides and may influence the transformation from a beta pleated sheet structure to a random coil [187]. Aβ deposits are not found in rodent brain and this might relate to differences in the amino acid sequence between rodents, and other species—unknown factors independent of the primary structure might also influence fibril formation [188- 191]. Amyloid structures in mammalian tissues might encourage covalent polymerization of reactive small molecules into larger molecules like melanin with cytotoxic properties. Amyloid minimizes toxicity by sequestering and limiting diffusion of toxic molecules, as found in Pmel17 amyloidogenesis in melanin [192].

Insertion of proline residues at positions 17 - 23 within the Aβ sequence inhibits aggregation [193] and replacement of a glutamine for a glutamate at position 22 accelerates fibril formation [194].

Aβ interacts with other proteins. It binds with high avidity to the N-terminus of its own precursor APP which may have important functional and pathophysiological implications [195]. It also binds to immunoglobulin IgG at the hinge region of the immunoglobulin heavy chain which preserves the antigen binding site [196]. It also forms heteropolymers with the alpha 2 macroglobulin receptor and extracellular matrix molecules [197].

The vulnerability of cultured neurons to glutamate, N-methyl-D-aspartate and kainate is increased after the addition of Aβ [198]. Neurotrophic effects have been observed with low concentrations of Aβ in culture [199]. The RERMS domain of Aβ is the active region for cell surface binding and neurite extension [25]. This effect was reproduced using a fragment containing amino acids 25 - 35, a region having 90% sequence homology with substance P. Tachykinin antagonists inhibited this effect [199]. These observations require confirmation, as inconsistent reports exist on a possible interaction between Aβ and NK-1 receptors [25,200-202]. When the NK-1 receptor was expressed in Xenopus oocytes, Aβ required excitatory amino acids to activate this NK-1 receptor as measured by Ca²⁺ uptake and inositol triphosphate stimulation [199]. These observations may account for some of the negative findings of Aβ-tachykinin interactions as excitatory amino acids may need to coexist in the same experimental conditions. At high concentrations in vitro Aβ caused neuronal death in tissue culture experiments [203]. Aβ has shown to promote neuronal viability in primary cultures [204]. Using an organotypic slice culture system from the hippocampus, it was revealed that Aβ inhibits excitatory synapses in response to neuronal activity using NMDA/AMPA and Ca²⁺ dependent mechanisms [205]. Aβ was shown to act in an automous manner upon both neurons and neighbouring cells, and as part of a negative feedback loop to regulate the degree of neuronal activity. Aβ also impairs long-term potentiation—a model of synaptic plasticity useful to understanding and memory [205].

Aβ (25 - 35) has a vasconstrictor response at the level of the microvasculature operating through endothelin and protein kinase C [206]. This effect might also involve an interaction with calcitonin gene related polypeptide (CGRP), substance P, and the serpin enzyme complex (SEC) [202]. Aβ may mimic the effects of substance P on nicotine secretion [207]. Aβ disrupts muscarinic receptor coupling to G proteins which mediate carbachol inositol phosphate stimulation and Ca²⁺ release [208].

Resting Ca²⁺ levels and Ca²⁺ responses to excitatory amino acids were enhanced after Aβ treatment in human cerebral cortical cells [209]. Aβ probably destabilizes Ca²⁺ regulation through an effect on Ca²⁺ channels [210]. Aβ (25-35) may also influence Ca²⁺ levels in astrocytes [211]. In astrocytes Aβ reduces intracellular Ca²⁺ concentrations; in neurons it leads to an increase [212]. Po-
tassium channel openers such as diazoxide and levocromakalim protect neurons against Aβ toxicity [213]. Aβ (25-35) increases Ca\(^{2+}\) concentrations in microglial cells via L-type voltage gated channels similar to prion proteins [214]. Aβ stimulates the synthesis of the third component of complement in microglial cells [215].

Neurotoxic effects may be a result of oxidative injury and free radical formation [216-218]. The damaging effects of Aβ are believed to be both necrotic and apoptotic [219,220]. The cells may need to be post mitotic before being vulnerable to the effects of Aβ [221]. Calretinin containing neurons have been shown to be resistant to Aβ [222]. The mechanism of toxicity may require an interaction of tau protein kinase I, as antisense oligodeoxynucleotides to the mRNA of this enzyme prevented Aβ-induced death of hippocampal neurons [223]. Aβ has been shown to reduce cell membrane fluidity and induce membrane ruffling [224]. Apoptosis has been induced by Aβ in tissue culture of CNS neurons [220,225]. Ceramide, a membrane-permeable activator of NF kappa β, protects neurons against the toxicity of Aβ [226]. The toxicity of Aβ (25 - 35) may be mediated by phospholipase D activation [227]. Oestrogens protect neurons from Aβ induced neurotoxicity [228] and reduce the production of Aβ in neurons [229].

Stimulation of astrocytic and microglial IL-1 and bFGF was found after exposure to Aβ [230,231]. The microglia proliferated and transformed morphologically. Microglial cells were activated synergistically by Aβ (25 - 35) and γ-interferon [232]. Aβ had no obvious toxic effects on astrocytes, which envelop and probably phagocytose Aβ [231]. Macrophages may be directly stimulated by Aβ [233].

Functional Ca\(^{2+}\) channels have been formed by spontaneous association of Aβ in artificial membrane bilayers [234]. Nimodipine-sensitive Ca conductance was enhanced by Aβ in neuroblastoma cells [235].

When the low affinity NGF receptor p75 is expressed in PC12 cells, Aβ toxicity is potentiated [153]. NGF was preventive, suggesting an interaction between the neurotrophins and Aβ. The non-selective neurotrophin receptor p75 NTR, a member of the death receptor family binds Aβ and leads to neuronal death [236].

The toxicity of Aβ requires fibril formation. Trans-thyretin is the major Aβ binding protein in human and animal CSF, when added to the CSF of patients and controls it sequesters Aβ [237]. The molecular chaperones antichymotrypsin and APOE increase Aβ aggregation, with APOEε4 having the highest catalytic activity [238]. This observation providing a possible mechanism for the association between APOEε4 and late onset AD [239, 240]. The neurotoxicity of Aβ may be modified by cho-

lesterol [241].

The formation of β-pleated sheets is also promoted by aluminium and inhibited by silicates [242,243]. Metal induced oxidation favours amyloidogenesis of Aβ [244]. Zinc binds human Aβ more avidly than that from the rat; this may contribute to differences in Aβ deposition between these two species [301]. Iron ions also promote Aβ aggregation [245]. Congo red inhibits fibril formation as does thiouliav T [246-248]. The N- and C-terminal of Aβ 25-35 contribute to aggregation, but amino acids 33 - 35 seem critical [177,223].

Aβ interacts with other proteins involved in the pathogenesis of AD such as α-synuclein, which is the major constituent of the non Aβ component of AD amyloid plaques (NACP), as Aβ stimulates the oligomerization of α-synuclein via the action of transglutaminase [249-251]. Presenilin 1 mutations may enhance Aβ neurotoxicity via apoptosis [252]. Aβ inhibits phosphofructokinase and decreases glycolysis, which contribute to neuronal death through reduced energy metabolism [253]. Aβ has also been shown to cause abnormal intercellular communication and alter gap junction formation through augmentation of connexins, changes which may enhance neuronal toxicity [254].

Recent evidence has shown that the receptor for advanced glycation end products (RAGE), relevant to the pathogenesis of diabetes mellitus, binds synthetic Aβ (both 1 - 40 and 1 - 42) [255]. This receptor interaction stimulates oxidant stress in endothelial cells and neurons, leading to microglial activation. RAGE is a member of immunoglobulin superfamily of cell surface molecules which includes neural cell adhesion molecule (NCAM). RAGE may be upregulated in AD and the possibility exists that agents may be developed which block Aβ-RAGE interaction and may be of therapeutic benefit. Aβ is also a ligand for an intracellular protein known as ERAB which is found in endoplasmic reticulum and mitochondria [256]. This protein is a hydroxysteroid dehydrogenase which enhances the toxicity of Aβ; if ERAB is blocked Aβ toxicity is prevented, if ERAB expression is enhanced Aβ toxicity is increased.

2.2. Anti Microbial Functions

Recent in vitro studies with Aβ have compared its antimicrobial activities with that of LL-37, a typical human antimicrobial peptide (AMP). Aβ exerted antimicrobial activities against eight common clinically relevant microorganisms with a potency equivalent to LL-37. These workers also showed that AD whole brain homogenates delivered high antimicrobial activity than aged matched non-AD, so that AMP action correlates with tissue Aβ levels. The Aβ mediated activity was ablated by immu-
nodepletion of AD brain homogenates with anti-Aβ antibodies. This work has found that Aβ is an unrecognised AMP and suggests that Aβ might be part of the innate immune system—the body’s generic cells and chemicals to fight infection. This is in contrast to the adaptive immune system with specific lymphocytes and antibodies. The blood brain barrier functions as part of this immune system and blocks adaptive cells. Innate immunity is therefore important [257].

2.3. Synaptic Functions
Stimulated hippocampal brain slices increase Aβ secretion, probably by APP trafficking to the cell membrane with β secretase cleavage. Physiological levels of APP and Aβ secretion reduce synaptic activity. That is, under physiological conditions APP and Aβ act as neuromodulators [258]. In vitro studies have shown that stimulation of NMDA receptors inhibits secretase action, increases APP, reduces alpha secretase and enhances Aβ production [259]. These findings suggest that APP and Aβ work as a coupled peptide system involved in neuromodulation. This is supported by the finding that kainic acid induced seizures are increased in APP deficient mice [260]. That is, without the inhibitory effects of Aβ, excitotoxicity is enhanced in the central nervous system.

Aβ1-42 binds with high affinity to the α7 nicotinic acetylecholine receptor (α7 nAChR) [261,262]. Aβ1-42 is able to reversibly block neuronal α7 nAChR receptors and might be relevant to some of the cognitive impairment observed in AD.

2.4. Soluble Oligomers of Aβ
The neuritic plaques of Alzheimer’s disease, which contain insoluble Aβ, do not correlate with the severity of dementia. It has been hypothesised that soluble Aβ is important and studies have shown a correlation with cognitive impairment [263]. Soluble Aβ is defined as Aβ that remains in solution after high speed centrifugation of brain extracts. Monomers and oligomers of Aβ are secreted by neural activity. The soluble oligomers are active in the synaptic cleft. Monomers and oligomers, especially trimers, seem to be active at peak of soluble concentrations and inhibit hippocampal long-term presentation (LTP), a model of learning [264]. These observations have been extended to the effects of oligomers on long-term potentiation in vivo [265]. These in vitro and in vivo studies of LTP suggest that exposure to Aβ oligomers leads to a NMDAR blockade by either reducing NMDAR activation, impairing NMDAR calcium influx or enhancing NMDAR influence on calcineurin. LTP has an effect on neuronal spine enlargement and Aβ oligomers might impair dendritic spines and the functions of excitatory neurotransmission [266]. These conclusions have been supported by studies on the glutamate AMPA receptor in hippocampus slices, where AMPA activation by Aβ might lead to synaptic depression [267]. Understanding the functions of these oligomers is important as some of the recent interest in passive immunisation treatments for Alzheimer’s disease might have their effect on binding the soluble form of Aβ and might lead to a disturbance of synaptic function. In vitro studies found that physiological concentrations of Aβ dimers and trimers, but not monomers, of human Aβ trigger synaptic loss that can be reversed by therapeutic agents [268]. APP transgenic mice expressing the E693A mutation led to Aβ oligomerization without fibrillization. These mice had age-dependent intraneuronal Aβ without extracellular amyloid deposits. Hippocampal plasticity and memory were decreased and the presynaptic marker synaptophysin was low. Tau phosphorylation and microglial increase was observed with neuronal loss. This experimental study suggests that Aβ oligomers affect synaptic function, the processing of presynaptic proteins and of the microtubular associated protein tau [269]. An oral compound De-nantiometric peptide D3 has been shown to improve the cognitive performance of AD transgenic mice probably by binding Aβ oligomers and converting them to nonamyloidogenic, non-fibrillar and non-toxic aggregates without increasing the concentration of mononeuric Aβ [270].

PiB PET scanning highlights Aβ plaques in the brains of patients with Alzheimer’s disease which might be useful in the diagnosis and monitoring effects of treatment. It is possible that monoclonal antibodies that bind Aβ might be operational upon soluble oligomers and not Aβ deposited in neuritic plaques [271]. Bapineuzumab, a humanized anti Aβ monoclonal, reduced Aβ deposition using PiB PET imaging but seemed to have no effect on cognition suggesting that the soluble oligomers might be important in the cognitive effects of Alzheimer’s disease [272].

2.5. In Vivo Studies: Aβ
Bilateral hippocampal injection of Aβ did not influence reinforcement or delayed conditional discrimination in rats [273]. Aβ immunoactivity in rabbit brain has been induced by a high cholesterol diet and confirmed in pigs; Aβ deposition was found in neurons and accompanied by microgliosis [274]. Intrahippocampal injections of Aβ induced errors in a radial maze test in rats as a test of short-term memory [275].

Infusions of Aβ into the carotid arteries of rats have been shown to increase the permeability of the blood brain barrier (BBB) [276,277] and alter lectin binding sites on endothelial cells [276]. Aβ crosses the BBB in
primates in an aged dependent manner. These studies suggest that Aβ influences the integrity of the BBB, findings which may be important in the mechanism by which inflammatory mediators influence AD.

Fragments of Aβ (1 - 28, 12 - 28, 12 - 20) given by intracerebroventricular injection had an amnestic effect on footshock-active avoidance training in mice; impaired learning was also seen when Aβ was injected into the cerebral cortex. Aβ peptides containing the VVG domain were active for amnesia in this model; peptides with the sequences QFVG, DFFVG, AIFT blocked this effect, again suggesting the possibility of endogenous antagonists.

After injection of Aβ into the cerebral cortex of rats neuronal loss and degenerating neurites were observed; this was prevented by substance P administered systemically. Retroviral expression of a C-terminal fragment in PC12 cells, when injected into the brains of newborn mice, caused atrophy of the cerebral cortex.

Transformed cells over-expressing Aβ transplanted into the suprachiasmatic nuclei of adult rats altered their circadian rhythms suggesting a physiological role for Aβ in hypothalamic function.

2.6. Human Studies

The human APP gene is found on chromosome 21. The gene and its product have been found in most human tissues. Apart from brain and spinal cord, APP has been found in peripheral nerve, dorsal root ganglia, sympathetic ganglia, anterior nervous system, and the neuromuscular junction.

The 5' end of the promoter region of human APP has been sequenced and shares many properties with the rat APP promoter. These are: 1) The promoter has 79.5% C+G regions; 2) No TATA or CAAT box; and 3) The promoter recognizes SP1, AP1, AP2, AP4, GCF and heat-shock elements. The following elements are found in the promoter region of APP in humans and primates: APOE-β1, AP2, immediate early genes, heat shock elements, and AP-1. The Aβ region is identical in humans and microcebus monkeys.

Electrophoresis mobility shift assays showed that the promoter is heavily occupied by nuclear protein factors. The interaction of the promoter with protein extracts from HeLa cells and nuclei obtained from human cortical neurons was different, suggesting the chromatin conformation of the promoter region may vary with cell type and origin; this possibly relates to regional differences in gene expression. Only minor differences in chromatin structure were observed between AD and non-AD neocortical nuclei. This finding suggested that a post-transcriptional process, or regulatory elements elsewhere in the APP gene, caused the abnormal accumulation of the gene product. Frameshift mutations at a transcriptional level or at the post-transcriptional editing of APP RNA may be important in the development of sporadic AD and may be secondary to aging. Somatic mutations do not account for sporadic AD.

Over expression of APP due to a gene dosage effect probably explains the Aβ deposition and AD pathology seen in trisomy 21 (Down’s syndrome, DS). The over expression of APP is detectable at 21 weeks gestation. Duplication of the APP gene does not occur in familial and sporadic AD. Increased expression of APP in cell lines is associated with cytotoxicity and increased generation of C-terminal fragments containing the complete Aβ sequence.

Other studies of APP in DS have not revealed differences in its splice variants in mononuclear cells from peripheral blood. The secretory processing of APP may be heterogeneous in DS generating multiple amyloidogenic C-terminal fragments. Fibroblasts from DS patients secrete more APP and have reduced responsiveness to protein kinase stimulation. There may be an increase in KPI+ isoforms in young DS patients in comparison to old. Lymphocytes contain higher APP content from DS patients than controls.

Three point mutations of APP 770 at valine 717, 3 residues carboxyl to Aβ, account for a small proportion of familial AD patients: Val717 to isoleucine, Val717 to phenylalanine, Val717 to glycine. Human neuroblastoma cells transfected with these mutations produce more amyloidogenic Aβ (1 - 42) and less Aβ (1 - 40) than control cells. Insertion of the Val717 to isoleucine mutation into mice produced Alzheimer-like pathology. Other mutations in APP also result in familial AD and these involve the Swedish double mutation at codons 670 and 671 in which Lys-Met → Asn-Leu. A Gly-Ala substitution at codon 692 is also associated with presenile dementia and cerebral haemorrhages. A glutamine substitution at position 22 of Aβ and codon 693 of APP is associated with autosomal dominant hereditary cerebral haemorrhage with amyloid angiopathy and paradoxically is not associated with dementia.

Neurons containing the 670/671 mutations do not produce sAPPα indicating reduced alpha-secretase activity. Neuronal nicotinic receptors are reduced in the brains of patients with the Swedish double mutation again providing further evidence for a relationship between APP and cholinergic neurotransmission.
volved in the deposition of Aβ and the neuropathology of 
AD. Mutations in presenilin genes located on chromo-
some 14 (presenilin 1) and chromosome 1 (presenilin 2) 
are also associated with familial AD and their contribu-
tion to the pathophysiology of AD must be considered 
[312,313]. Over 60 missense and deletion mutations in 
PS1 have been observed in families worldwide and PS1 
mutations amount to over 60% of familial AD [314]. 
Other genes causing familial AD will probably be iden-
tified in the future. Analysis of the APP promoter in fibro-
blasts from patients with chromosome 14-related familial 
AD showed hyperactivity but normal gene structure and 
revealed that fibroblasts from these patients produced 
two times the amount of APP mRNA, APP, and Aβ in 
comparison to controls when grown in low (0.5%) serum 
[315]. 

Cell lines expressing PS1 mutations have an increased 
ratio of Aβ (1-43) to Aβ (1-40) and the brains of double 
transgenic animals possessing both APP and PS1 muta-
tions contain elevated levels Aβ (1-43) to Aβ (1-40) con-
firming that PS1 is involved in the metabolism of APP as 
part of the γ-secretase complex [316,317]. PS1 and the γ-
secretase complex have other important functions in the 
processing of other membrane bound proteins such as the 
products of Notch genes [318]. Cell lines and knock-out 
mice not containing PS1 do not produce Aβ [319]. 

The long forms of Aβ (1-42, 1-43) are initially depos-
ited in sporadic and familial AD, but not in APP muta-
tions associated with autosomal dominant cerebral haem-
morrhages without Alzheimer pathology [320-322]. APP 
mutations have been shown to activate the G protein Go 
(composed of beta and gamma subunits) which mediate 
apoptosis; observations which may be inhibited by a 
β-adrenergic receptor kinase [323]. 

The tissue factor pathway inhibitor-1 (TFPI-1), a KPI 
inhibitor, is localized to microglia and neuritic plaques 
and may play a role in the protease regulation of human 
APP [324]. 

The CSF concentration of sAPPα is low and correlates 
with impaired neuropsychological scores in AD and 
ageing; findings which suggest impaired APP metabo-
ism is related to cognitive deficits [325]. 

There are conflicting reports on changes in APP gene 
expression in normal aging brains and AD. Ncase protec-
tion assay showed that the ratio of APP KPI+ to KPI−
increased 1.5 times with age and AD [326,327]. An ele-
vated KPI+/KPI− ratio correlated directly with plaque 
density [328]. These findings were confirmed using immu-
nocytochemistry and in situ hybridisation [329]. Nuc-
lease protection assays showed APP 695 was strongly 
expressed in fetal brain. Adults, the aged, and patients 
with AD had similar splicing patterns [330]. An increase 
in the APP transcript lacking the Aβ sequence has been 
reported [281]. 

Lymphoid cells from patients with familial AD have 
reduced levels of APP KPI+ which might influence the 
balance of function of the secretases favouring Aβ depo-
sition [331]. Northern analysis suggests APP mRNA 
might decrease with age [332]. 

A combined immunoreactivity and Western analysis 
detected no difference in APP isoforms between AD and 
controls [333]. Nonisotopic in situ hybridization also 
found no trends in APP mRNA isoform expression when 
correlated with neurofibrillary tangles or senile plaques, 
even when neurons up to 100 µm from a senile plaque 
were examined [334,335]. 

In situ hybridization of AD frontal cortex revealed re-
ductions in both KPI− and KPI+ isoforms, not found in 
other neurodegenerative diseases [336]. Other studies 
have suggested differences in neuronal subpopulation 
expression of APP mRNA [190]. An effect of age and anato-
mal region is supported by a study of cynomolgus 
monkeys in which APP 695 and 751 were increased 2 - 7 
times in the thalamus in 17-year-old monkeys when com-
pared with 3-year olds [337]. Solution hybridization 
Studies have not demonstrated changes in APP in ageing 
and AD [338]. 

An immunocytochemical study showed that APP 
KPI+ correlates with neuritic plaque density [339]. There 
was a relative increase in the purified soluble fraction of 
KPI+ protein in AD using Western blots implying that 
APP may be involved in the regulation of its own me-
tabolism [149]. 

These variable findings in APP isoforms in AD might 
relate to the interval of time from death to brain sampling, 
agalonal state, and techniques employed (e.g. nuclease 
protection assays versus in situ hybridization). A relatively 
recent study using radioactive in situ hybridization ana-
lysis of APP isoforms and larger numbers of AD patients 
and neurodegenerative controls has revealed no differ-
ences in differential mRNA expression of APP isoforms 
[340]. An investigation using PT-PCR in AD 
post-mortem brains showed an increase in the KPI+ iso-
forms controlling for age at death, brain pH and gender 
[341]. Caution must be exercised in investigating changes 
in gene expression at death in a slowly evolving neuro-
logical pro- cess, since critical changes in gene expression 
may have occurred some time before. 

Aβ deposition is not unique to AD but is also found in 
ageing, head injury, and other neurodegenerative disorders 
[342]. Twenty-four hours after head injury enhanced Aβ 
immunoreactivity has been observed throughout the cor-
tical mantle, not just in the area adjacent to the impact 
[343]. Even after concussion, the mildest form of head
injury not associated with macroscopic or microscopic evidence of brain damage, increased APP immunoreactivity has been found in axons within the fornix and may be a marker of diffuse axonal injury [344]. The immediate early gene c-jun may be associated with amyloid deposition in blood vessels and glial cells [345,346]. The APOEε4 isoform has been associated with increased Aβ deposition after head injury [34]. Microglia with IL-1α immuno-reactivity were found after head injury adjacent to neurons expressing APP, suggesting a role in its production [347].

Interleukin 1 expression has been demonstrated in the vicinity of senile plaques, possibly in microglial cells, in AD and DS [348]. Microglial IL-1 was associated with increased neuronal APP in temporal lobectomy specimens from patients with refractory epilepsy [349]. APP KPI+ isoforms were increased in comparison to controls, without any change in APP KPI−. An IL-1 antagonist

### Table 1. The Disease Associations of APP and Aβ.

<table>
<thead>
<tr>
<th></th>
<th>Alzheimer’s disease</th>
<th>Cerebral amyloid angiopathy</th>
<th>Primary angitis of the central nervous system</th>
<th>Inclusion body myopathy</th>
<th>Downs syndrome and dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Aβ</td>
<td>+</td>
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Interleukin 1 expression has been demonstrated in the vicinity of senile plaques, possibly in microglial cells, in AD and DS [348]. Microglial IL-1 was associated with increased neuronal APP in temporal lobectomy specimens from patients with refractory epilepsy [349]. APP KPI+ isoforms were increased in comparison to controls, without any change in APP KPI−. An IL-1 antagonist

### Figure 1. The Functions of APP and Aβ.
rhIL-1ra prevented excitotoxicity induced changes in APP [102]. These observations provide evidence for an interaction between neuronal injury and loss, microglial activation, cytokines—in particular IL-1—and changes in APP isoform expression. The synthesis of APP mRNA in cultured human endothelial cells has been shown to be influenced by IL-1 [350].

Human platelets may have differential expression of APP isoforms in AD and APP might function to inhibit platelet activation post-granulation [351,352].

Aβ deposition is associated with some forms of primary angiitis of the CNS and is found in the presence of cerebral amyloid angiopathy [353]. In the muscle disease amyotrophic lateral spinocerebellar atrophy (ALS) [354] (Table 1). The functions of APP and Aβ are summarised in the figure (Figure 1).

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