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COMMENTARY

ROLE OF NEUROTRANSMISSION IN THE REGULATION OF AMYLOID β-PROTEIN PRECURSOR PROCESSING

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Because of the central place of amyloid in the histopathology of Alzheimer's disease (AD‡), current research seeks to determine the proteolytic mechanisms involved in the processing of the amyloid β-protein precursor (APP). Cellular APP processing yields several cleavage products some of which appear to be neurotrophic or even neuroprotective, whereas others are potentially amyloidogenic and, in some experimental systems, neurotoxic. Understanding the biochemical mechanisms that regulate cellular APP processing is a requisite for developing treatments that affect APP metabolism and that might slow the rate of amyloid deposition in the brain of AD patients. Recent evidence from cell culture experiments suggests that APP processing pathways are regulated by a variety of first messengers coupled to activation of protein kinase C. These first messengers include the muscarinic acetylcholine receptor m1 and m3 subtypes that increase the secretion of N-terminal APP derivatives lacking the C-terminus (non-amyloidogenic APP^s), and that concomitantly decrease secretion of the potentially amyloidogenic Aβ peptide. Brain tissue slice experiments indicate that similar APP processing mechanisms occur in mammalian brain tissue as in cell culture, and that APP processing in the brain may be a function of neuronal activity. This article reviews recent data on the regulation of APP processing by neurotransmitters, and prompts speculation that activating specific neuronal cell surface receptors will prove a novel pharmacological way to modify APP processing in the intact brain.

ALZHEIMER'S DISEASE AMYLOID

Amyloid deposits in brains of AD patients, Down syndrome subjects and aged individuals consist primarily of aggregates of 39–43 residue peptides, variously termed amyloid β-protein, β-peptide, β-amyloid, A4, βA4, $\beta_{1-39/43}$, or simply Aβ. These peptides are derived, by proteolytic cleavage, from transmembrane glycoproteins known as APP. Aggregates of the 39 residue variant account for the congophilic angiopathy of cerebral blood vessels [1], and the 42/43 amino acid Aβ appears to be the predominant form of extracellular amyloid in senile plaques [2, 3], which are often associated with a neuritic reaction. At high concentrations, Aβ molecules tend to self-aggregate *in vitro* to form insoluble amyloid fibrils [4, 5] that are reminiscent of those deposited in the AD brain, but such additional amyloidotrophic factors as apolipoprotein E [6, 7] or free oxygen radicals [8] may enhance the aggregation of Aβ into amyloid *in vivo*. The biochemical mechanisms that regulate the synthesis of APP, the proteolytic APP degradation pathways that yield Aβ-comprising derivatives, and the factors that promote aggregation of Aβ-containing polypeptides into amyloid are central for understanding brain amyloid formation in health and disease.

STRUCTURE AND POSSIBLE BIOLOGICAL FUNCTIONS OF APP

APP is one of the most abundant proteins in the brain (for recent reviews, see Refs. 9 and 10). Nine APP gene transcripts derived from alternative splicing have been identified to date, encoding corresponding proteins that range from 365 to 770 amino acid residues [11–20]. Some of these (APP695, APP751, APP770) are expressed at high levels in the brain. APP molecules are membrane glycoproteins with a long extracytoplasmic N-terminal domain followed by a single transmembrane segment and a short cytoplasmic C-terminal tail (see Fig. 1). Aβ, the principal amyloidogenic component of AD-type brain amyloid begins 28 residues from the membrane, in the extracytoplasmic region, and extends 11 to 15 amino acids into the transmembrane portion. One set of transcripts that lacks exon 15 was initially identified in white blood cells, and named LAPP [19, 20]. These variants are predominantly expressed by immunocompetent cells and by microglial cells in the brain [19, 20]. Two APP isoforms APP770 and LAPP752, contain,

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‡ Abbreviations: AD, Alzheimer's disease; APP, amyloid β-protein precursor(s); APP^s, secreted forms of APP; KPI, Kunitz serine protease inhibitors; PNII, protease nexin II; FAD, familial AD; mAChR, muscarinic acetylcholine receptor(s); IL-1, interleukin 1; DAG, diacylglycerol; and PKC, protein kinase C.

within the extracytoplasmic N-terminus, a 57-residue domain homologous to the family of Kunitz serine protease inhibitors (KPI) along with a 19-residue segment homologous to the thymocyte OX2 antigen. The APP751 and LAPP733 variants also contain the KPI but not the OX2 segment. Extracytoplasmic portions of mature APP are N- and O-glycosylated and sulfated [21]. Secreted KPI-containing N-terminal APP derivatives are homologous to protease nexin II (PNII) [22, 23], a member of a larger family of transmembrane proteins that bind to, internalize, and inhibit extracellular proteases. This protease inhibitory function of C-terminally truncated, secreted APP/PNII may be involved in the regulation of proteases that control the blood coagulation cascade [22–26]. The membrane-associated forms of APP contain a G protein binding domain that resembles a family of cell surface transmembrane receptors [27], suggesting a possible, yet unidentified, role in cellular signalling. Recently, a metalloprotease inhibitor domain has been described within the extracellular glycosylated portion of APP [28]. This domain reportedly inhibits collagenase type IV, an enzyme involved in the degradation of extracellular matrix proteins. The extracellular N-terminal portion of APP contains segments that specifically bind to collagen, laminin and heparin [29], suggesting a possible biological function in cell adhesion and in cell growth. APP also exists in a chondroitin sulfate proteoglycan form [30], underscoring the possibility of interactions with the extracellular matrix. There is accumulating evidence that the C-terminally truncated, secreted forms of APP (APP^s) protect cultured primary neurons from damage induced by glutamate excitotoxicity and glucose deprivation by limiting the increase in intracellular free Ca^{2+} levels [31]. These observations suggest that APP^s may protect neurons against such toxic insults as excitotoxicity, hypoglycemia and Ca^{2+}-mediated cellular damage. Furthermore, APP and some of its derivatives have trophic effects on a variety of cells in culture by stimulating neurite outgrowth and neurite branching [32] in PC-12 cells, and by enhancing the rate of survival of primary hippocampal neurons in culture [33]. These data on neurotrophic and neuroprotective biological functions of APP imply that its mismetabolism could have two distinct important consequences: the disruption of biologic processes normally influenced by APP and its metabolites, as well as the generation of amyloidogenic derivatives leading to amyloid plaque formation.

APP maps to human chromosome 21 [11, 12]. Additional genes encoding for the APP-like proteins APLP1 and APLP2 with homologous N-terminals but lacking Aβ segments, have identified recently and mapped to human chromosomes 19 and 11, respectively [34, 35]. APLP1 and APLP2 are non-amyloidogenic proteins, but, because of their structural similarity to APP, may interfere with APP processing pathways, and thus may indirectly affect APP function and amyloid formation.

APP PROCESSING PATHWAYS

The full-length, mature forms of APP have a very short half-life (20–30 min in cell culture) as they are degraded proteolytically by at least four alternative processing pathways. Some of these pathways generate derivatives that contain intact (potentially amyloidogenic) Aβ segments, whereas other, non-amyloidogenic, pathways prevent the formation of such fragments by cleaving APP within the Aβ domain (Fig. 1). The specific proteases mediating APP catabolism have not yet been identified and much of the data generated thus far is based upon analyses of APP fragments. The APP processing pathways known to date are referred to as α- and β-secretase cleavage, secretase-II cleavage and endosomal–lysosomal processing (Fig. 1).

The secretory pathways

Soluble APP derivatives that contain a large (110–125 kDa) extracytoplasmic N-terminal portion but not the cytoplasmic C-terminus of APP (APP^s) are secreted by a great variety of cultured cells [21, 37], are also found in superfusates of brain slice preparations [38], in human brain and cerebrospinal fluid [21, 39], as well as in human serum [40]. Human serum APP^s may be largely derived from platelets, which release APP^s in response to activation with heparin [41, 42]. The cellular mechanisms leading to APP^s secretion involve a proteolytic cleavage event that leaves behind a small cell-associated C-terminal fragment [37]. Secretory APP cleavage events of this type are referred to as *secretase* processing pathways (Fig. 1). Three distinct secretase-type cleavage events have been suggested to date. The first is *α-secretase* cleavage which occurs at position Lys16-Leu17 (referring to Aβ1-43) within the Aβ domain and thus generates two non-amyloidogenic metabolites [43–46]. The efficiency of this cleavage event is highest at the precise distance of 12 amino acid residues extracytoplasmic from the membrane surface, i.e. between residues 16 and 17 of Aβ, but appears to be remarkably independent from the specific amino acids flanking the cleavage site [47]. *β-Secretase* cleavage generates a secreted N-terminal derivative that is 16 residues shorter than the α-secretase product and ends at the N-terminus of the Aβ domain [48]. If, indeed, cellular full-length APP is a substrate for this cleavage event (as opposed to the secreted α-secretase product), the remaining cell-associated C-terminus is potentially amyloidogenic and may be a possible progenitor of secreted soluble Aβ [36]. It is, however, also possible that the secreted N-terminal β-secretase product does not reflect a single enzymatic cleavage event but is derived from multi-processing events (e.g. extracellular nibbling) at the C-terminus of the secreted α-secretase cleavage product. A third secretase cleavage reportedly occurs at a yet unidentified site near the C-terminus of Aβ and was named *secretase II* [49] (Fig. 1).

Secretion of soluble Aβ

Soluble 4 kDa Aβ was identified in conditioned cell culture media from transfected and primary cells [50–52] as well as in human cerebrospinal fluid [53] (Fig. 1). Like any Aβ-containing polypeptide, these peptides are potentially amyloidogenic and could theoretically contribute to amyloid formation. On

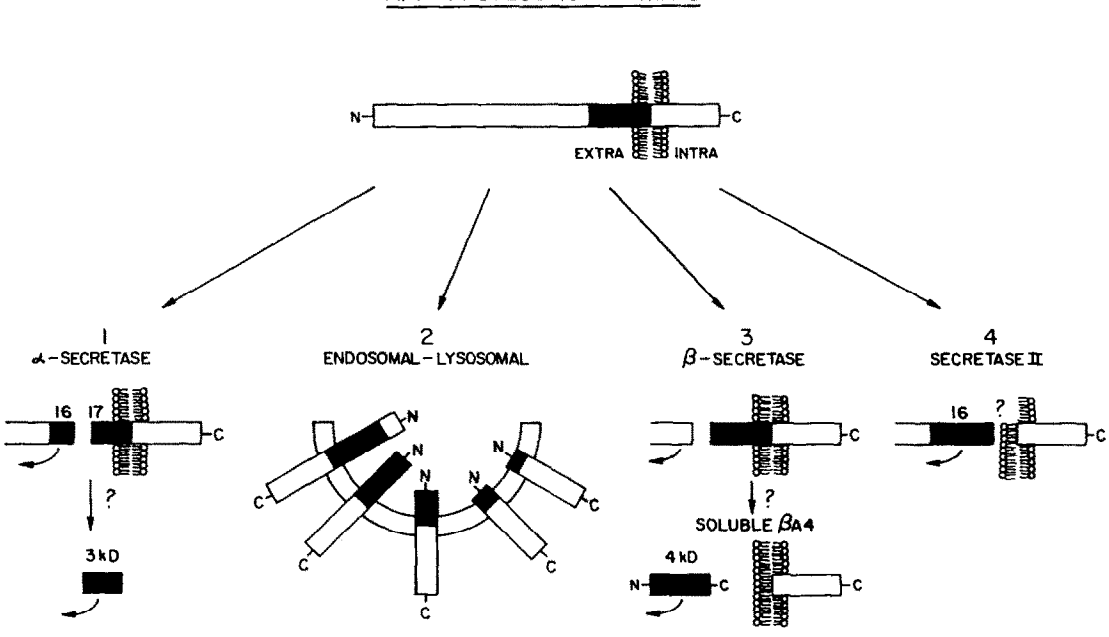

Fig. 1. APP processing pathways (1) α-Secretase cleavage within the Aβ domain prevents the formation of amyloidogenic derivatives and generates secreted APP^s that carries some of the neurotrophic and neuroprotective functions of the molecule. A 3 kDa fragment, which is also secreted, may derive from the remaining C-terminus generated by α-secretase cleavage [36]. (2) Endosomal-lysosomal APP processing generates multiple C-terminal derivatives, some of which contain intact Aβ sequences and, thus, are potentially amyloidogenic. (3) β-Secretase cleavage occurs at the N-terminus of the Aβ domain and generates a large secreted N-terminal derivative. The remaining C-terminus is potentially amyloidogenic and may serve as the progenitor molecule for secreted Aβ. (4) Secretase II cleavage occurs at a yet unidentified site close to the C-terminus of Aβ.

the other hand, the 4 kDa Aβ identified in cell culture media fails to form aggregates as indicated by the absence of Aβ immunoreactivity in high-speed centrifugation pellets of cell culture supernatants. This absence of aggregation could reflect low peptide concentrations (0.3 ng/mL/hr, estimated from [50]), or the absence of residues 41–43 which promote aggregation [5]; or the presence of a cell-derived factor that prevents aggregation; or the lack of amyloidotrophic factors necessary for aggregation [6, 8]. An additional 3 kDa C-terminal fragment of Aβ (p3) is also secreted into cell culture media and cerebrospinal fluid. This fragment could be generated by cleavage of the remaining cell-associated C-terminal APP stump derived from α-secretase cleavage [36]. An initial argument supporting the hypothesis that secreted Aβ may contribute to brain amyloidosis and the development of AD comes from a Swedish familial AD (FAD) kindred that carries a double mutation in the APP gene causing a substitution of two residues close to the N-terminus of the Aβ domain [54]. Overexpression of this mutant in cell culture is associated with a 5- to 6-fold higher Aβ release as compared with normal APP [55, 56].

The endosomal–lysosomal pathways

Alternatively to the secretory pathways, APP holoprotein can be internalized from the plasma membrane and targeted to endosomal–lysosomal compartments [57], possibly via clathrin-coated pit internalization mediated by an Asn-Pro-Thr-Tyr consensus sequence in the C-terminus of APP [58]. In addition to this re-internalization pathway, APP holoprotein may be targeted directly from the post-Golgi to the endosomal–lysosomal compartment. Full-length APP is detectable in endosomal–lysosomal preparations and degraded within this compartment, yielding multiple C-terminal APP fragments [59, 60] (Fig. 1). Some of these endosomal–lysosomal C-terminal APP derivatives appear to contain intact Aβ sequences and thus are potentially amyloidogenic.

CELL SURFACE RECEPTOR REGULATION OF APP PROCESSING

Increase of APP^s secretion by activation of cell surface receptors

The relative activities of individual proteolytic APP processing pathways can be regulated by first messengers via stimulation of neurotransmitter and neuromodulator receptors. Initial evidence for this novel role of cell surface receptors came from stimulation experiments of human embryonic kidney cells transfected with cDNA encoding for human muscarinic acetylcholine receptor (mAChR) subtypes. These experiments demonstrated that

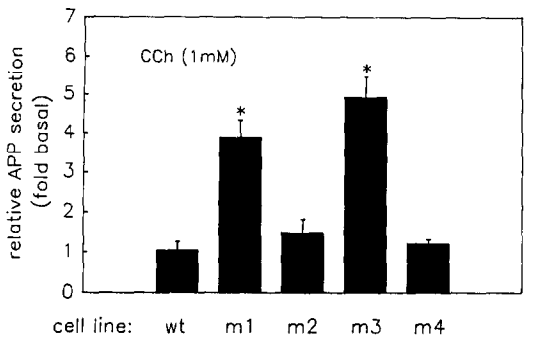

Fig. 2. Increase in APP^s secretion by cell surface receptors. Activation of muscarinic acetylcholine receptor subtypes m1 and m3 with carbachol (CCh) increases the release of APP^s from HEK 293 cells stably transfected with the cDNA encoding for the human receptor subtypes. Wild type HEK cells (wt), as well as cells expressing the cDNA for the m2 and m4 receptor subtypes, did not respond to carbachol with increased APP^s secretion, indicating that APP^s secretion, indicating that APP^s secretion is specifically increased by the m1 and m3 receptor subtypes. Values are means ± SEM, N = 5–7 experiments per group. Key: (*) $P < 0.01$ versus unstimulated control condition in each cell line. Reprinted with permission from Nitsch *et al.*, *Science* **258**: 304–307, 1992. Copyright (1992) AAAS. [Ref. 61].

activation of both the m1 and the m3 AChR with the muscarinic receptor agonist carbachol (a stable acetylcholine analog) significantly increases the release of soluble large N-terminal APP derivatives lacking the C-terminus (APP^s) [61] (Fig. 2). These initial findings were subsequently confirmed in a rat pheochromocytoma PC-12 cell line transfected with the muscarinic m1 receptor gene, and extended by the observation that stimulation of interleukin 1 (IL-1) receptors also increases the release of N-terminal APP derivatives [62].

The increased release of APP derivatives in response to receptor activation is unaffected by the translation inhibitor cycloheximide in the initial 30-min period after stimulation, indicating that preexisting APP molecules are cleaved and released in response to receptor activation. Concomitantly, levels of cell-associated full-length APP decrease to 20–40% of control levels as a result of a 30-min receptor stimulation period. These observations indicate that first messengers can activate, via receptor stimulation, a proteolytic APP processing pathway that cleaves preexisting APP holoprotein, resulting in the secretion of APP^s into the extracellular medium.

Decrease of Aβ secretion by activation of cell surface receptors

Recent evidence from experiments in cell lines overexpressing both human APP and human mAChR indicates that activation of these receptors significantly decreases the release of soluble Aβ by more than 50% as compared with the unstimulated control condition [63]. Concomitantly, the secretion of p3, a 3 kDa fragment of Aβ lacking its N-terminus (see Fig. 1), was increased along with the increase in APP^s secretion, suggesting that the cleavage mechanisms generating p3 and APP^s are stoichiometrically coupled.

Taken together, these data indicate that mAChR affect APP processing in a complex way by enhancing the secretion of both APP^s and p3, and by simultaneously blocking Aβ secretion. Based upon the assumption that APP^s and p3 are derived from proteolytic pathways involving α-secretase cleavage and that Aβ is a product of β-secretase cleavage [36], we propose that receptor activation enhances α-secretase cleavage while decreasing β-secretase processing (Fig. 3). As a consequence, amyloidogenic APP processing may be decreased by receptor activation, while non-amyloidogenic processing may be enhanced.

Previous findings suggest that the double mutation in the APP gene associated with the Swedish FAD kindred [54] causes a 5- to 6-fold increase in Aβ secretion when overexpressed in cell culture [55, 56]. To determine the effect of cell surface receptors on this pathologically high Aβ production, we coexpressed both APP with the Swedish double mutation and m1 AChR in the same cell line and found that increased Aβ secretion was blocked by m1 AChR activation with carbachol [63]. These data suggest that not only normal Aβ secretion but also pathologically high Aβ secretion caused by an APP mutation can be diminished by cell surface receptor activity. This conclusion provides a novel theoretical strategy for the pharmacological modification of Aβ secretion, even in inherited genetic conditions associated with abnormally high rates of Aβ formation.

Receptor subtype specifically

The increase in APP^s release mediated by mAChR is specific to the m1 and m3 receptor subtypes as activation of the m2 and m4 subtypes does not change APP^s release [61]. Because activation of bradykinin [64,*] and IL-1 [62] receptors causes a similar stimulation of the secretory APP processing pathway, it is likely that the second messenger systems coupled to the receptors account for the specificity of the response. Bradykinin, m1 and m3 mAChR, and IL-1 receptors are all coupled to diacylglycerol (DAG) formation via phospholipase activation [65], whereas m2 and m4 receptors are primarily coupled to the cAMP signal transduction pathway. These findings led to the hypothesis that such cell surface receptor types that generate DAG as part of their signal transduction may stimulate APP^s secretion and inhibit Aβ formation.

SIGNAL TRANSDUCTION PATHWAYS IN THE REGULATION OF APP PROCESSING

Mediation of receptor-coupled APP^s secretion by protein phosphorylation

DAG is a central molecule for the regulation of cellular events controlled by protein kinase C (PKC), as DAG is the physiological activator of PKC. DAG is formed by phospholipid hydrolysis as a result of first messenger-induced stimulation of cell surface

* Nitsch *et al.*, unpublished observations.

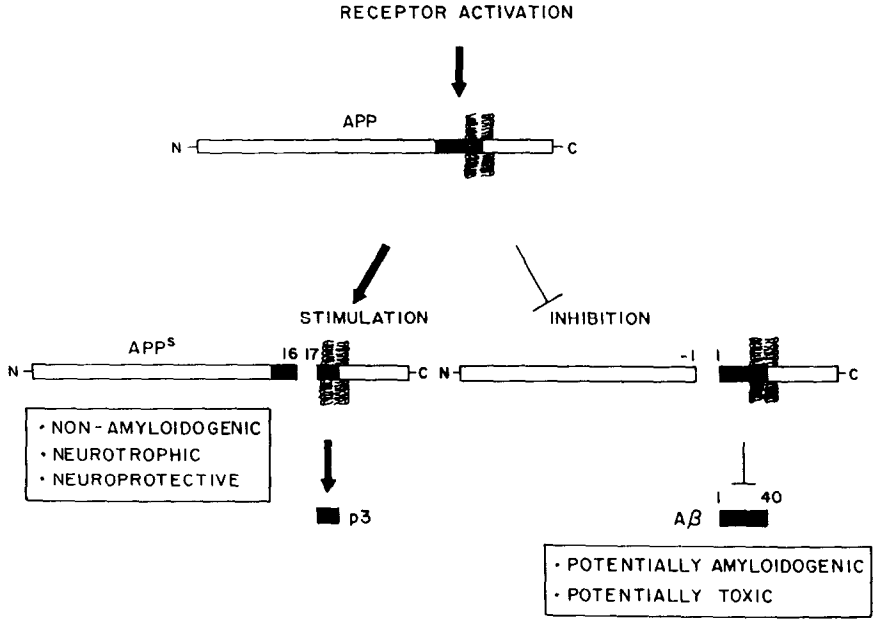

Fig. 3. Regulation of AAP processing by cell surface receptors. Receptor activation increases APP processing into non-amyloidogenic derivatives and prevents processing into amyloidogenic derivatives. Activation of muscarinic m1 and m3 receptors increases secretion of both APP^s and p3, and concomitantly depresses Aβ secretion. Thus, activation of cell surface receptors may promote the formation of neurotrophic and neuroprotective APP derivatives and slow the formation of potentially toxic products.

receptors that are coupled, via G protein, to phospholipase C activation (see Ref. 66 for review). In our experiments, receptor-activated APP^s secretion was blocked by many protein kinase inhibitors including calphostin C, chelerythrine chloride, and staurosporine, indicating that a protein kinase is involved in the signal transduction cascade that links receptor activation to APP processing. Experiments with fibroblasts transfected with the cDNA encoding for the PKCα isoenzyme clearly indicate that this PKC subtype can stimulate APP^s secretion [67]. PKC activation with phorbol esters also increases the secretion of APP^s from cells in culture [67, 68], and raises cellular levels of remaining C-terminal APP cleavage products [69]. Activation of PKC with phorbol esters also decreases the amount of secreted Aβ, thus mimicking the effect of receptor stimulation (see above, [63]). Together, these findings imply a central role for PKC in the signal transduction cascade coupling cell surface receptor activation to both increased APP^s secretion and decreased Aβ formation.

The downstream phosphorylation target of PKC activity with respect to the mechanism of APP^s secretion is yet unclear. The possibility that receptor-activated phosphorylation of the APP molecule itself [70, 71] is necessary for increased secretion is unlikely because mutants lacking the phosphorylation sites are still cleaved and secreted in response to PKC activation [72]. However, it is possible that substrates other than APP, e.g. proteases involved in APP cleavage, are phosphorylated in response to receptor activation.

Possible role of DAG, phospholipases and Ca^{2+}

Activation of PKC in response to stimulation of cell surface receptors can be achieved by at least three independent pathways via receptor-coupled activation of the phospholipases C, D, and A_2, as well as by a combination of any of these. All three enzymes are activated via GTP-binding (G) protein in response to agonist-induced stimulation of the receptor molecules. The large class of receptors coupled to phospholipase activation include the mAChR subtypes m1 and m3, metabotropic glutamate receptor subtypes, serotonin receptors, and neuropeptide receptors [66, 73–76]. Phospholipase C subtypes hydrolyze inositol phospholipids as well as choline phospholipids to yield DAG and inositol triphosphate (IP_3). Membrane-associated (lipophilic) DAG activates PKC by translocating the cytosolic forms to the plasma membrane. Phospholipase D, activated by both m1 and m3 receptor stimulation [75], yields phosphatidic acid which is readily dephosphorylated to form DAG, resulting in PKC activation, as described above. Receptor-mediated activation of phospholipase A_2 yields free fatty acids, in particular arachidonic acid, which can activate PKC directly. The corresponding lysophospholipid, in particular lysophosphatidylcholine, potentiates many effects of DAG, and thus contributes to sustained PKC activation (for a detailed review, see (Ref. 66). In addition to lipase activation, stimulation of m1 and m3 AChR transiently increases levels of intracellular free Ca^{2+} derived from two independent sources.

IP_3, generated by phosphatidylinositol-specific phospholipase C, binds to IP_3 receptors at the endoplasmic reticulum (ER), thereby opening Ca^{2+} channels that release Ca^{2+} from the ER into the cytoplasm. Additionally, m1 and m3 AChR also open receptor-operated calcium channels at the plasma membrane, resulting in influx of extracellular free Ca^{2+} [77]. Receptor stimulation in the absence of extracellular free Ca^{2+}, produced a smaller APP^s secretory response than stimulation in the presence of Ca^{2+}. This observation suggests that the signal transduction cascade that links receptor activation to APP^s secretion is co-activated by DAG and elevation of free intracellular Ca^{2+}. This concept is compatible with the finding that some PKC subtypes are co-activated by DAG and Ca^{2+} [66]. Taken together, these findings suggest that protein kinase isoforms, activated by DAG and Ca^{2+}, are central enzymes in the signal transduction cascade that couples receptor activation to APP^s secretion. Evaluation of the effects on APP processing of the more than ten identified individual PKC isoforms will be necessary to determine potential differences among various isoforms expressed by individual cell types.

REGULATION OF APP PROCESSING BY NEURONAL ACTIVITY

The regulation of biochemical pathways in the intact brain is, in many ways, much more complex than that in cell culture model systems. For example, biochemical reactions in brain cells *in situ* can be affected simultaneously by many neurotransmitters and neuromodulators. This situation is very different from the cell culture condition in which one first messenger is tested at a time. Experiments designed to determine the significance of receptor activation for APP processing in the intact brain are just beginning, and only some initial data are available to date. Our first approach to investigating APP processing in mammalian brain tissue was to develop a hippocampal slice superfusion system for the measurement of APP^s release in response to neuronal depolarization. Electrical stimulation of brain tissue slices in this system depolarizes excitable cells in the preparation and the resultant action potentials trigger influx of sodium and calcium ions into the synaptic terminals, resulting in exocytosis of neurotransmitter-containing vesicles. We found that electrical depolarization of superfused hippocampal slices increased the release of endogenous neurotransmitters including acetylcholine and glutamate 3- to 5-fold. Concomitantly, electrical depolarization enhances APP^s release from the slices 2-fold within 50 min of stimulation [38]. Increased APP^s release correlated with increasing stimulation frequencies in the range of 8 to 18 Hz, which matches the physiological firing frequency of hippocampal pyramidal cells. These data suggest that APP^s release from hippocampal slices *in vitro* may be a function of neuronal activation. Pharmacological blockade of action potential formation with tetrodotoxin (a sodium channel blocker) inhibits depolarization-induced APP^s release. These findings demonstrate that action potentials can regulate APP processing in brain tissue. It is now important to determine the specific neurotransmitter systems involved in APP processing in brain tissue, to determine the cellular signalling mechanisms, and to investigate whether APP processing is modified by neurotransmission in the intact human brain.

POSSIBLE RELEVANCE FOR AD

Are neurotransmission deficits related to amyloid formation in AD?

Synapse loss, along with dysfunction and death of neurons [78, 79], are the elemental lesions in AD that account for dementia. As a result of neuronal damage, levels of many neurotransmitters, including acetylcholine, serotonin, glutamate, somatostatin and norepinephrine, are decreased [80–83]. Furthermore, signal transduction pathways may be impaired as protein kinase alterations occur at an early stage of AD [84, 85]. These observations raise an important question: Does dysfunctional neurotransmission lead to changes in APP metabolism that comprise the normal biological function of APP and promote amyloid formation? Attempts to answer this question must take the following points into account. First, neurotransmission is impaired in many brain diseases including Parkinson's disease, Huntingdon's disease and the system atrophies (e.g. olivopontocerebellar atrophy), yet these diseases are not associated with accelerated amyloid deposition in the brain. Second, in AD, amyloid is deposited throughout the neuropil including brain areas that otherwise are relatively spared from neuronal damage and that presumably have preserved neurotransmission. Third, amyloid formation probably occurs independently from neurotransmitter deficits in specific subgroups of AD patients, e.g. Down syndrome which is associated with an increased APP gene dosage (triplicate chromosomes 21), or the Swedish FAD family with a double mutation in the APP gene that may cause altered APP processing. These points argue that abnormalities in neurotransmission may not be the primary cause of amyloid deposition in all AD cases. However, these arguments do not exclude the possibility that APP mismetabolism as a result of impaired neurotransmission may secondarily contribute to amyloidogenesis.

APP in the pathogenesis of AD

An additional central question regarding the molecular pathology of AD is yet unanswered: What is the etiologic significance of APP in AD? Specifically, do APP and its derivatives cause AD-type dementia because they are neurotoxic and initiate a series of events leading to neuronal death and synaptic failure? Or does amyloid deposition result from the response of the brain to an unknown primary damage causing synapse loss and dysfunctional neurotransmission, and therefore represents a misguided attempt at healing? The latter idea stresses the possibly important biological functions of APP rather than the potential toxicity ascribed to some of its derivatives. These two views both have merits and weaknesses. The strongest evidence for APP having a direct role in causing AD comes from the handful of FAD cases linked to APP

gene mutations [54, 86–89]. Although these mutations are rare and do not account for the vast majority of cases [90, 91], they provide an important lead into the cause of some FAD pedigrees. Second, amyloid deposition in the brain of Down syndrome subjects carrying an extra copy of the APP gene begins early in life, and all become demented by the 4th or 5th decade of life with AD-type histopathology [92]. The third point stems from the observation that Aβ can be neurotoxic in some experimental systems [93, 94]. However, attempts to replicate the initial reports on the toxicity of amyloid revealed the narrow confines in which they were reproducible, and detracted from the neurotoxic hypothesis in causing AD [95]. The fourth argument that initially supported a direct relation of APP expression and AD histopathology came from transgenic mouse experiments which showed that animals carrying the transgene for human APP express increased amounts of human APP, but those papers that reported resultant increased amyloid deposits in brain have been retracted.

In opposition to these arguments, two major observations are poised against a primary role of amyloid in AD etiology: amyloid plaques are found in brains of neurologically normal individuals and, in AD, amyloid plaques do not correlate with clinical estimates of dementia severity, number of neurofibrillary tangles, or with duration of illness [96]. These reservations, however, do not exclude the possibility that amyloid is involved in an early stage of neuronal degeneration that is remote from the final clinical and neuropathologic hallmarks of AD.

An alternative view of the role of amyloid in AD was presented in the section on the biological functions of APP. This line of reasoning is based upon observations that APP derivatives such as APP^s and even Aβ fragments have protective and trophic biological functions [31–33], which may be disrupted by APP mismetabolism, regardless of the additional formation of amyloid.

The concept that neurotransmitters can regulate APP processing pathways does not attempt to discriminate between the neurotrophic and the neurotoxic hypotheses and is relevant to AD regardless of which view will prevail. If Aβ accumulation is toxic and leads to neuronal degeneration, pharmacological activation of neurotransmitter receptors that inhibit Aβ formation may reduce the rate of amyloid deposition, and possibly slow the progression of AD. Alternatively, if APP or some of its non-amyloidogenic fragments are neurotrophic or neuroprotective, stimulation of specific cell surface receptors to increase secretion of such APP derivatives may promote the biological function of APP and thus help restore neuronal function.

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REFERENCES

1. Glenner GG and Wong CW, Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* **120**: 885–890, 1984.
2. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL and Beyreuther K, Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci USA* **82**: 4245–4249, 1985.
3. Selkoe DJ, Abraham CR, Podlisny MB and Duffy LK, Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. *J. Neurochem* **46**: 1820–1834, 1986.
4. Hilbich C, Kisters-Woike B, Reed J, Masters CL and Beyreuther K, Aggregation and secondary structure of synthetic amyloid bA4 peptides of Alzheimer's disease. *J Mol Biol* **218**: 149–163, 1991.
5. Jarrett JT, Berger EP and Lansbury PT Jr, The C-terminus of the beta amyloid protein is critical for the seeding of amyloid formation: Implications of the pathogenesis of Alzheimer's disease. *Biochemistry* **32**: 4693–4697, 1993.,
6. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS and Roses AD, Apolipoprotein E: High-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* **90**: 1977–1981, 1993.
7. Wisniewski T, Golabek A, Matsubara E, Ghiso J and Frangione B, Apolipoprotein E: Binding to soluble Alzheimer's β-amyloid. *Biochem Biophys Res Commun* **192**: 359–365, 1993.
8. Dyrks T, Dyrks E, Hartmann T, Masters C and Beyreuther K, Amyloidogenicity of βA4 and βA4-bearing amyloid protein precursor fragments by metal-catalyzed oxidation. *J Biol Chem* **267**: 18210–18217, 1992.
9. Multhaup G, Masters CL and Beyreuther K, A molecular approach to Alzheimer's diseae. *Biol Chem Hoppe Seyler* **374**: 1–8, 1993.
10. Kosik KS, Alzheimer's disease: A cell biological perspective. *Science* **256**: 780–783, 1992.
11. Kang J, Lemaire H-G, Unterbeck A, Salbaum JM, Masters CL, Grzeschik K-H, Multhaup G, Beyreuther K and Müller-Hill B, The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325**: 733–736, 1987.
12. Tanzi RE, Gusella JF, Watkins PC, Bruns GAP, St. George-Hyslop P, Van Keuren ML, Patterson D, Pagan S, Kurnit DM and Neve RL, Amyloid β protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235**: 880–884, 1987.
13. Tanzi RE, McClachey AI, Lamperti ED, Villa-Komaroff L, Gusella JF and Neve RL, Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature* **331**: 528–530, 1988.
14. Ponte P, Gonzalez-DeWitt P, Schiling J, Miller J, Hsu D Greenberg B, Davis K, Wallace W, Leiberburg I, Fuller F and Cordell B, A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. *Nature* **331**: 525–527, 1988.
15. Kitaguchi N, Takahashi Y, Tokushima YU, Shiojiri S and Ito H, Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. *Nature* **331**: 530–532, 1988.
16. De Sauvage F and Octave J-N, A novel mRNA of the

A4 amyloid precursor gene coding for a possibly secreted protein. *Science* **245**: 651–653, 1989.
17. Golde TE, Estus S, Usiak M, Younkin LH and Younkin SG, Expression of beta amyloid precursor mRNAs: Recognition of a novel alternatively spliced form and quantitation in Alzheimer's disease using PCR. *Neuron* **4**: 253–267, 1990.
18. Jacobsen JS, Muenkel HA, Blume AJ and Vitek MP, A novel species-specific RNA related to alternatively spliced amyloid precursor protein mRNAs. *Neurobiol Aging* **12**: 575–583, 1991.
19. König G, Mönning U, Czech C, Prior R, Banati R, Schreiter-Gasser U, Bauer J, Masters CL and Beyreuther K, Identification and differential expression of a novel alternative splice isoform of the βA4 amyloid precursor protein (APP) mRNA in leukocytes and brain microglial cells. *J Biol Chem* **267**: 10804–10809, 1992.
20. Mönning U, König G, Banati RB, Mechler H, Czech C, Gehrmann J, Schreiter-Gasser U, Masters CL and Beyreuther K, Alzheimer βA4-amyloid protein precursor in immunocompetent cells. *J Biol Chem* **267**: 23950–23956, 1992.
21. Weidemann A, König G, Bunke D, Fischer P, Salbaum JM, Masters CL and Beyreuther K, Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. *Cell* **57**: 115–126, 1989.
22. Van Nostrand WE, Wagner SL, Suzuki M, Choi BH, Farrow JS, Geddes JW, Cotman CW and Cunningham DD, Protease nexin-II, a potent antichymotrypsin, shows identity to amyloid beta-protein precursor. *Nature* **341**: 546–549, 1989.
23. Oltersdorf T, Fritz LC, Schenk DB, Lieberburg I, Johnson-Wood KL, Beattie EC, Ward PJ, Blacher RW, Dovey HF and Sinha S, The secreted form of the Alzheimer's amyloid precursor protein with the Kunitz domain is protease nexin-II. *Nature* **341**: 144–147, 1989.
24. Van Nostrand WE and Cunningham DC, Protease nexin-II (APP): A platelet alpha-granule protein. *Science* **248**: 745–748, 1990.
25. Van Nostrand WE, Wagner SL, Farrow JS and Cunningham DD, Immunopurification and protease inhibitory properties of protease nexin-2/amyloid β-protein precursor. *J Biol Chem* **265**: 9591–9594, 1990.
26. Sinha S, Knops J, Esch F, Moyer ED and Oltersdorf T, Conversion of the Alzheimer's β-amyloid precursor protein (APP) Kunitz domain into a potent human neutrophil elastase inhibitor. *J Biol Chem* **266**: 21011–21013, 1991.
27. Nishimoto I, Okamoto T, Matsuura Y, Takahashi S, Murayama Y and Ogata E, Alzheimer amyloid protein precursor complexes with brain GTP-binding protein G_o. *Nature* **362**: 75–79, 1993.
28. Miyazaki K, Hasegawa M, Funahashi K and Umeda M, A metalloprotease inhibitor domain in Alzheimer amyloid protein precursor. *Nature* **362**: 839–841, 1993.
29. Multhaup G, Bush AI, Pollwein P, Masters CL and Beyreuther K, Specific binding of the Alzheimer βA4 amyloid precursor to collagen, laminin, and heparin. *J Protein Chem* **11**: 398–399, 1992.
30. Shioi J, Anderson JP, Ripellino JA and Robakis NK, Chondroitin sulfate proteoglycan form of the Alzheimer's β-amyloid precursor. *J Biol Chem* **267**: 13819–13822, 1992.
31. Mattson MP, Cheng B, Culwell AR, Esch FS, Lieberburg I and Rydel RE, Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the β-amyloid precursor protein. *Neuron* **10**: 243–254, 1993.
32. Milward EA, Papadopoulos R, Fuller SJ, Moir RD, Small D, Beyreuther K and Masters CL, The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. *Neuron* **9**: 129–137, 1992.
33. Whitson JS, Selkoe DJ and Cotman CW, Amyloid beta protein enhances the survival of hippocampal neurons *in vitro*. *Science* **243**: 1488–1490, 1989.
34. Wasco W, Bupp K, Magendantz M, Gusella JF, Tanzi RE and Solomon F, Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid β protein precursor. *Proc Natl Acad Sci USA* **89**: 10758–10762, 1992.
35. Wasco W, Brook JD and Tanzi RE, The amyloid precursor-like protein (APLP) gene maps to the long arm of human chromosome 19. *Genomics* **15**: 237–239, 1993.
36. Haas C, Hung AY, Schlossmacher MG, Teplow DB and Selkoe DJ, β-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. *J Biol Chem* **268**: 3021–3024, 1993.
37. Oltersdorf T, Ward PJ, Henriksson R, Beattie EC, Neve R, Lieberburg I and Frizt LC, The Alzheimer amyloid precursor protein. Identification of a stable intermediate in the biosynthetic/degradative pathway. *J Biol Chem* **265**: 4492–4497, 1990.
38. Nitsch RM, Farber SA, Growdon JH and Wurtman RJ, Release of amyloid β-protein precursor derivatives by electrical depolarization of rat hippocampal slices. *Proc Natl Acad Sci USA* **90**: 5191–5193, 1993.
39. Palmert MR, Berman Podlinsny M, Witker DS, Oltersdorf T, Younkin LH, Selkoe DJ and Younkin SG, The beta-amyloid protein precursor of Alzheimer's disease has soluble derivatives found in human brain and cerebrospinal fluid. *Proc Natl Acad Sci USA* **86**: 6338–7342, 1989.
40. Bush AI, Whyte S, Thomas LD, Williamson TG, Van Tiggelen CJ, Currie J, Small DH, Moir RD, Li Q-X, Rumble B, Mönning U, Beyreuther K and Masters CL, An abnormality of plasma amyloid protein precursor in Alzheimer's disease. *Am Neurol* **32**: 57–65, 1992.
41. Bush AI, Martins RN, Rumble B, Moir R, Fuller S, Milward E, Currie J, Ames D, Weidemann A, Fischer P, Beyreuther K and Masters CL, The amyloid precursor protein is released by human platelets. *J Biol Chem* **265**: 15977–15983, 1990.
42. Cole GM, Galasko D, Shapiro IP and Saitoh T, Stimulated platelets release amyloid β-protein precursor. *Biochem Biophys Res Commun* **170**: 288–295, 1990.
43. Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, Oltersdorf T, McClure D and Ward P, Cleavage of amyloid β peptide during constitutive processing of its precursor. *Science* **248**: 1122–1124, 1990.
44. Sisodia SS, Koo EH, Beyreuther K, Unterbeck A and Price DL, Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* **248**: 492–495, 1990.
45. Anderson JP, Esch FS, Keim PS, Sambamurti K, Lieberburg I and Robakis NK, Exact cleavage site of Alzheimer myeloid precursor in neuronal PC-12 cells. *Neurosci Lett* **128**: 126–128, 1991.
46. Wang R, Meschia JF, Cotter RJ and Sisodia SS, Secretion of the β/A4 amyloid precursor protein. Identification of a cleavage site in cultured mammalian cells. *J Biol Chem* **266**: 16960–16964, 1991.
47. Sisodia SS, β-Amyloid precursor protein cleavage by a membrane-bound protease. *Proc Natl Acad Sci USA* **89**: 6075–6079, 1992.
48. Seubert P, Oltersdorf T, Lee MG, Barbour R, Blomquist C, Davis DL, Bryant K, Fritz LC, Galasko D, Thal LJ, Lieberburg I and Schenk DB, Secretion of β-amyloid precursor protein cleaved at the amino terminus of the β-amyloid peptide. *Nature* **361**: 260–263, 1993.

49. Anderson JP, Chen Y, Kim KS and Robakis NK, An alternative secretase cleavage produces soluble Alzheimer amyloid precursor protein containing a potentially amyloidogenic sequence. *J Neurochem* **59**: 2328–2331, 1992.
50. Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB and Selkoe DJ, Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature* **359**: 322–325, 1992.
51. Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, Cai X-D, McKay DM, Tintner R, Frangione B and Younkin SG, Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science* **258**: 126–129, 1992.
52. Busciglio J, Gabuzda DH, Matsudaira P and Yankner BA, Generation of β-amyloid in the secretory pathway in neuronal and nonneuronal cells. *Proc Natl Acad Sci USA* **90**: 2092–2096, 1993.
53. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C, McCormack R, Wolfert R, Selkoe D, Lieberburg I and Schenk D, Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids. *Nature* **359**: 325–327, 1992.
54. Mullan M, Crawford F, Axelman K, Houlden H, Lilius L, Winblad B and Lannfelt D, A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nature Genet* **1**: 345–347, 1992.
55. Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, Vigo-Pelfrey C, Lieberburg I and Selkoe DJ, Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. *Nature* **360**: 672–674, 1992.
56. Cai X-D, Golde TE and Younkin SG, Release of excess amyloid β protein from a mutant amyloid β protein precursor. *Science* **259**: 514–516, 1993.
57. Haass C, Koo EH, Mellon A, Hung AY and Selkoe DJ, Targeting of cell surface β-amyloid precursor protein to lysosomes: Alternative processing into amyloid-bearing fragments. *Nature* **357**: 500–5003, 1992.
58. Chen W-J, Goldstein JL and Brown MS, NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of low density lipoprotein receptor. *J Biol Chem* **265**: 3116–3123, 1990.
59. Estus S, Golde TE, Kinishita T, Blades D, Lowery D, Eisen M, Usiak M, Qu X, Tabira T, Greenberg BD and Younkin SG, Potentially amyloidogenic, carboxyl-terminal derivatives of the amyloid protein precursor. *Science* **255**: 726–728, 1992.
60. Golde TE, Estus S, Younkin LH, Selkoe DJ and Younkin SG, Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* **255**: 728–730, 1992.
61. Nitsch RM, Slack BE, Wurtman RJ and Growdon JH, Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* **258**: 304–307, 1992.
62. Buxbaum JD, Oishi M, Chen HI, Pinkas-Kramarski R, Jaffe EA, Gandy SE and Greengard P, Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer β/A4 amyloid protein precursor. *Proc Natl Acad Sci USA* **89**: 10075–10078, 1992.
63. Hung AY, Haass C, Nitsch RM, Qiu WQ, Citron M, Wurtman RJ, Growdon JH and Selkoe DJ, Activation of protein kinase C inhibits cellular production of the amyloid β-protein. *J Biol Chem* 22959–22962, 1993.
64. Francis PT, Webster M-T, Chessell IP, Holmes C, Stratmann GC, Procter AW, Cross AJ, Green AR and Bowen DM, Neurotransmitters and second messengers in aging and Alzheimer's disease. *Ann NY Acad Sci* **695**: 19–26, 1993.
65. Fisher SK, Heakock AM and Agranoff BW, Inositol phospholipids and signal transduction in the nervous system: An update. *J Neurochem* **58**: 18–38, 1992.
66. Nishizuka Y, Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C *Science* **258**: 607–614, 1992.
67. Slack BE, Nitsch RM, Livneh E, Kunz GM Jr, Breu J, Eldar H and Wurtman RJ, Regulation by phorbol esters of amyloid precursor protein release from Swiss 3T3 fibroblasts overexpressing protein kinase Cα. *J Biol Chem* **268**: 21097–21101, 1993.
68. Caporaso GL, Gandy SE, Buxbaum JD, Ramabhadran TV and Greengard P, Protein phosphorylation regulates secretion of Alzheimer β/A4 amyloid precursor protein. *Proc Natl Acad Sci USA* **89**: 3055–3059, 1992.
69. Buxbaum JD, Gandy SE, Cicchetti P, Ehrlich ME, Czernik AJ, Fracasso RP, Ramabhadran TV, Unterbeck AJ and Greengard P, Processing of Alzheimer βA4 amyloid precursor protein: Modulation by agents that regulate protein phosphorylation. *Proc Natl Acad Sci USA* **87**: 6003–6006, 1990.
70. Gandy SE, Czernik AJ and Greengard P, Phosphorylation of Alzheimer disease amyloid precursor peptide by protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II. *Proc Natl Acad Sci USA* **85**: 6218–6221, 1988.
71. Suzuki T, Nairn AC, Gandy SE and Greengard P, Phosphorylation of Alzheimer amyloid precursor protein by protein kinase C. *Neuroscience* **48**: 755–761, 1992.
72. Hung AY and Selko DO, Selective ectodomain phosphorylation and regulated cleavage of β-amyloid precursor protein. *EMBO J*, in press.
73. Nishizuka Y, Membrane phospholipid degradation and protein kinase C for cell signalling. *Neurosci Res* **15**: 3–5, 1992.
74. Peralta EG, Ashkenazi A, Winslow JW, Ramachandran J and Capon DJ, Differential regulation of PI hydrolysis and adenylyl cyclase activity by muscarinic receptor subtypes. *Nature* **334**: 434–437, 1988.
75. Sandmann J, Peralta EG and Wurtman RJ, Coupling of transfected acetylcholine receptor subtypes to phospholipase D. *J Biol Chem* **266**: 6031–6034, 1991.
76. Axelrod J, Burch RM and Jelsema JL, Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins: Arachidonic acid and its metabolites as second messengers. *Trends Neurosci* **11**: 117–123, 1988.
77. Felder CC, Poulter MO and Wess J, Muscarinic receptor-operated Ca^{2+} influx in transfected fibroblast cells is independent of inositol phosphates and release of intracellular Ca^{2+}. *Proc Natl Acad Sci USA* **89**: 509–513, 1992.
78. DeKosky ST and Scheff SW, Synapse loss of frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Ann Neurol* **27**: 457–464, 1990.
79. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA and Katzman R, Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann Neurol* **30**: 572–580, 1991.
80. Francis PT, Sims NR, Procter AW and Bowen DM, Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment of Alzheimer's disease: Investigative and therapeutic perspectives. *J Neurochem* **60**: 1589–1604, 1993.
81. Sparks DL, Hunsaker JC III, Slevin JT, DeKovsky ST, Kryscio RJ and Markesbery WR, Monoaminergic and cholinergic synaptic markers in the nucleus basalis of Meynert (nbM): Normal age-related changes and the

effect of heart disease and Alzheimer's disease. *Ann Neurol* **31**: 611–620, 1992.
82. DeKosky ST, Harbaugh RE, Schmitt FA, Bakay RAE, Chang Chui H, Knopman DS, Reeder TM, Shetter AG, Senter HJ, Markesbery WR and Intraventricular Bethanecol Study Group, Cortical biopsy in Alzheimer's disease: Diagnostic accuracy and neurochemical, neurpathological, and cognitive correlations. *Ann Neurol* **32**: 625–632, 1992.
83. Bowen DM, Najlerahim A, Procter AW, Francis PT and Murphy E, Circumscribed changes of the cerebral cortex in neuropsychiatric disorders of later life. *Proc Natl Acad Sci USA* **86**: 9504–9508, 1989.
84. Masliah E, Cole GM, Hansen LA, Mallory M, Albright T, Terry RD and Saitoh T, Protein kinase C alteration is an early biochemical marker in Alzheimer's disease. *J Neurosci* **11**: 2759–2767, 1991.
85. Masliah E, Mallory M, Ge N and Saitoh T, Protein kinases and growth associated proteins in plaque formation in Alzheimer's disease. *Rev Neurosci* **3**: 99–107, 1992.
86. Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M and Hardy J, Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**: 704–706, 1991.
87. Murrell J, Farlow M, Ghetti B and Benson MD, A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science* **254**: 97–99, 1991.
88. Chartier-Harlin M-C, Crawford F, Houlden H, Warren A, Hughes D, Fidani L, Goate A, Rossor M, Roques P, Hardy J and Mullan M, Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. *Nature* **353**: 844–846, 1991.
89. Hendriks L, Van Duijen CJ, Cras P, Cruts M, Van Hule W, Van Harskamp F, Warren A, McInnes MG, Antonarakis SE, Martin G, Hofman A and Van Broeckhaven C, Presenile dementia and cerebral hemorrhage linked to a mutation at codon 692 at the β-amyloid precursor protein gene. *Nature Genet* **1**: 218–221, 1992.
90. Schellenberg GD, Anderson L, O'dahl S, Wisjman EM, Sadovnick AD, Ball MJ, Larson EB, Kukull WA, Martin GM, Roses AD and Bird TD, APP_{717}, APP_{693}, and PRIP gene mutations are rare in Alzheimer disease. *Am J Hum Genet* **49**: 511–517, 1991.
91. Tanzi RE, Vaula G, Romano DM, Mortilla M, Huang TL, Tupler RG, Wasco W, Hyman BT, Haines JL, Jenkins BJ, Kalaitsidaki M, Warren AC, McInnis MC, Antonarakis SE, Karlinsky H, Percy ME, Connor L, Growdon J, Crapper-Mclachlan DR, Gusella JF and St. George-Hyslop PH, Assessment of amyloid β-protein precursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. *Am J Hum Genet* **51**: 273–282, 1992.
92. Rumble B, Retallack R, Hilbich C, Simms G, Multhaup G, Martins R, Hockey A, Montgomery P, Beyreuther K and Masters C, Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease. *N Engl J Med* **320**: 1446–1452, 1989.
93. Yankner BA, Duffy LK and Kirschner DA, Neurotrophic and neurotoxic effects of amyloid β protein: Reversal by tachikinin neuropeptides. *Science* **250**: 279–282, 1990.
94. Kowall NW, Beal MF, Busciglio J, Duffy LK and Yankner BA, An *in vivo* model for the neurodegenerative effects of β amyloid and protection by substance P. *Proc Natl Acad Sci USA* **88**: 7247–7251, 1991.
95. Kosik K and Coleman P, Is β-amyloid neurotoxic? *Neurobiol Aging* **13**: 535–625, 1992.
96. Arriagada PV, Growdon JH, Hedley-Whyte ET and Hyman BT, Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**: 631–639, 1992.