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Short Review

Regulation of Amyloid Precursor Protein Cleavage

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Abstract: Multiple lines of evidence suggest that increased production and/or deposition of the β-amyloid peptide, derived from the amyloid precursor protein, contributes to Alzheimer's disease. A growing list of neurotransmitters, growth factors, cytokines, and hormones have been shown to regulate amyloid precursor protein processing. Although traditionally thought to be mediated by activation of protein kinase C, recent data have implicated other signaling mechanisms in the regulation of this process. Moreover, novel mechanisms of regulation involving cholesterol-, apolipoprotein E-, and stress-activated pathways have been identified. As the phenotypic changes associated with Alzheimer's disease encompass many of these signaling systems, it is relevant to determine how altered cell signaling may be contributing to increasing brain amyloid burden. We review the myriad ways in which first messengers regulate amyloid precursor protein catabolism as well as the signal transduction cascades that give rise to these effects. **Key Words:** Amyloid precursor protein—β-Amyloid—Alzheimer's disease—Second messengers.
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Extracellular accumulation of fibrillar β-amyloid (Aβ) in the cerebral and limbic cortices and in the walls of the cerebral microvasculature is a hallmark of Alzheimer's disease (AD) pathology (Selkoe, 1991). Evidence that Aβ deposition plays a pivotal role in the cause of AD comes from several lines of inquiry. Perhaps the most convincing is genetic analysis indicating that three genetic alterations underlying familial AD increase the production and/or the deposition of Aβ in the brain (Roses, 1996; Selkoe, 1997). These include mutations in the genes encoding the amyloid precursor protein (APP, the precursor to Aβ) (Goate et al., 1991; Mullan et al., 1992) and presenilin-1 and -2, (Rogaev et al., 1995; Sherrington et al., 1995). Further support for the amyloid hypothesis is the fact that individuals with trisomy 21 develop AD in their fourth or fifth decade of life (Tanzi et al., 1987). As the APP gene is located on chromosome 21, it has been suggested that this may be due to a gene–dosage effect. Finally, allelic variation of apolipoprotein E is a significant risk factor for sporadic AD (Corder et al., 1993; Rebeck et al., 1993; Brousseau et al., 1994); as apolipoprotein E binds to Aβ (Strittmatter et al., 1993*a*,*b*), it may be involved in the formation of senile plaques (Ma et al., 1994).

Animal and cell culture studies characterizing the effects of familial AD mutations also support the amyloid hypothesis. Expression of these mutants in cell lines results in increased production of Aβ or relative increases in production of the longer, more amyloidogenic form, $A\beta_{1-42}$ (Citron et al., 1992, 1997; Cai et al., 1993; Barelli et al., 1997). Most important, transgenic mice expressing APP genes bearing the familial AD mutations exhibit some characteristics of the classic AD phenotype including neuritic plaques, age-dependent memory deficits, and hyperphosphorylated tau (Games et al., 1995; Hsiao et al., 1996; Masliah et al., 1996; Nalbantoglu et al., 1997; Sturchler-Pierrat et al., 1997).

SECRETORY PROCESSING PATHWAYS

APP is so named because it contains the Aβ peptide (39–43 amino acids in length) within its sequence. APP comprises a family of type 1 membrane-spanning glycoproteins (Kang et al., 1987; Tanzi et al., 1987); alternative splicing generates APP mRNAs giving rise to isoforms ranging from 365–770 amino acid residues (Kosik,

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Abbreviations used: Aβ, β-amyloid; AD, Alzheimer's disease; APLP, amyloid precursor-like protein; APP, amyloid precursor protein; sAPP, soluble ectodomain of APP; sAPPα, sAPP derived from the α-secretase cleavage; sAPPβ, sAPP derived from the β-secretase cleavage; sAPPγ, sAPP derived following γ-secretase cleavage; CHO, Chinese hamster ovary; ERK, extracellular signal-regulated protein kinase; HEK, human embryonic kidney; 5-HT, serotonin; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; p3, truncated β-amyloid fragment; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; SREBPs, sterol-regulating element binding proteins.

1993). The three major APP isoforms expressed in the brain are APP_{695}, APP_{751}, and APP_{770}. Of these, APP_{695} is the only isoform lacking a 57-residue domain homologous to the family of kunitz serine protease inhibitors and is the isoform most highly expressed in neurons (Goedert, 1987; LeBlanc et al., 1991). APP matures while being transported through the secretory pathway, becoming *N*- and *O*-glycosylated and tyrosyl-sulfated while moving through the *trans*-Golgi network (Weidemann et al., 1989). Immature APP (being *N*-glycosylated only) may be cleaved in the endoplasmic reticulum or the *cis*-Golgi but the mature APP is degraded rapidly as it is transported to or from the cell surface via either a biosynthetic or an endocytic pathway (Haass et al., 1992; Sambamurti et al., 1992; Shoji et al., 1992; De Strooper et al., 1993; Kuentzel, 1993; Cook et al., 1997; Hartmann et al., 1997; Marambaud et al., 1997*b*).

Cleavage of APP is complex and can occur via several different routes (Nitsch et al., 1994; Checler, 1995; Selkoe et al., 1996). Traditionally, these pathways have been categorized as either amyloidogenic (generating Aβ, the main constituent of fibrils found in end-stage senile plaques) or nonamyloidogenic (generating sAPP, an N-terminal APP fragment and p3, a truncated Aβ fragment). However, this classification scheme appears to be an oversimplification, as N-terminal truncated Aβ peptides such as p3 have been isolated from early-stage diffuse plaques and form amyloid fibrils even more readily than full-length Aβ (Gowing et al., 1994; Pike et al., 1995). For convenience and clarity, in this review we categorize these pathways as being either Aβ generating (the β-secretase pathway) or not (the α-secretase pathway) (Fig. 1). Cleavage of APP in both pathways generates soluble APP N-terminal fragments that together have been designated sAPP (sAPPα derived from the α-secretase pathway and sAPPβ derived from the β-secretase pathway). The generation of N-terminal truncated Aβ peptides and novel cleavage fragments will be discussed; however, these will not be the focus of the present review, as neither their physiological function nor their regulation has been fully characterized.

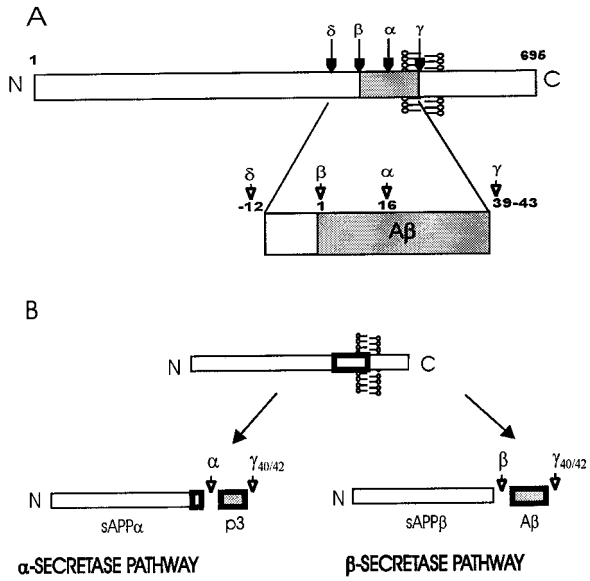

FIG. 1. A: Secretase cleavage sites of APP. APP is cleaved inside the Aβ sequence by α-secretase at Lys^{16} (numbering after Aβ). β- and γ-secretase cleave APP on either side of the Aβ sequence at Met^{1} or in the region of Val^{39}–Thr^{43}, respectively. δ-Secretase cleaves APP 12 residues from the N-terminus of the Aβ domain (Thr^{-12}). **B:** APP is processed via two alternative pathways, resulting in cleavage by α- or β-secretase yielding sAPPα or sAPPβ, respectively. The remaining C-terminal membrane-associated fragments are subsequently processed by γ-secretase to generate either p3 (when following α-secretase cleavage) or Aβ (when following β-secretase cleavage). Cleavage by γ-secretase most commonly generates either soluble p3 and Aβ species (ending at residue 40) or their more insoluble and amyloidogenic counterparts (ending at residue 42). Boldfaced outlines indicate Aβ or Aβ fragments. Stippled boxes indicate fragments that are potentially amyloidogenic. Novel cleavage fragments such as those occurring within the Aβ sequence or the δ-secretase cleavage have not been included, as these fragments have not yet been categorized in detail.

Production of sAPP and p3

Processing of APP by α-secretase was the first pathway to be characterized in detail. This processing route involves cleavage of APP at Lys^{16} within the Aβ sequence by an unidentified enzyme designated α-secretase (Fig. 1) (Esch et al., 1990; Anderson et al., 1991; Wang et al., 1991), which may reside in a late Golgi compartment (Sambamurti et al., 1992; De Strooper et al., 1993; Kuentzel et al., 1993) or at the plasma membrane in microdomains known as caveolae (Ikezu et al., 1998). The principal determinants of cleavage by α-secretase appear to be an α-helical conformation around the cleavage site and the distance of the hydrolyzed peptide bond from the membrane (which occurs most efficiently at a distance of 12 amino acid residues from the plasma membrane on the extracellular side) but is apparently independent of sequence (Sisodia, 1992). As would be expected of an extracellular cleavage event, the presence of the cytoplasmic domain of the holoprotein is not an absolute requirement (Sisodia, 1992; Haass et al., 1993; Efthimiopoulos et al., 1994). Cleavage by α-secretase results in the release of a soluble N-terminal APP fragment designated sAPPα and the retention of its 10-kDa C-terminus at the cell membrane (Weidemann et al., 1989). This 10-kDa C-terminal fragment (also called p10) may undergo additional cleavage by an enzyme known as γ-secretase, which cleaves APP at the C-terminus of Aβ (Haass et al., 1993). The resulting 3-kDa C-terminal fragment of Aβ known as p3 appears to be stoichiometrically coupled to production of sAPPα (Busciglio et al., 1993) and, like sAPPα, is thought to be a product of the α-secretase pathway (Haass et al., 1992, 1993). Both sAPPα and p3 are secreted by a variety of cultured cells and are found in human brain and CSF (Selkoe et al., 1988; Palmert et al., 1989; Schubert et al., 1989*a*,*b*; Weidemann et al., 1989; Oltersdorf et al., 1990). Cleavage of the APP holoprotein by γ-secretase results in a soluble N-terminal APP fragment lengthened

at its C-terminus by the Aβ sequence (sAPPγ). Although sAPPγ appears to occur in cell lines and in the CSF of aged rats (Anderson et al., 1992; Wallace et al., 1995), it has not been detected in the human brain (Pasternack et al., 1992). Although total sAPP does not appear to change in AD (Hock et al., 1998; Moir et al., 1998), alterations in α-secretase cleavage of various APP isoforms have been observed (Moir et al., 1998).

Production of Aβ

An alternative physiological processing pathway for APP results in production of intact Aβ (Fig. 1; Busciglio et al., 1993; Haass et al., 1993). The Aβ segment begins on the extracellular side of APP, 28 amino acids from the membrane, and extends 11–15 residues into the transmembrane domain. Aβ production from the mature APP holoprotein occurs via the sequential action of two enzymes termed β- and γ-secretase. Early studies suggested that these cleavage events occur in an endosomal compartment (Haass et al., 1992; Shoji et al., 1992; Peraus et al., 1997); however, recent evidence suggests that they may also occur in secretory compartments such as the endoplasmic reticulum or Golgi complex (Cook et al., 1997; Hartmann et al., 1997; Tomita et al., 1998). At least in some cases, the β-secretase cleavage appears to occur first (Citron et al., 1995; Paganetti et al., 1996) generating a truncated APP fragment (sAPPβ; 16 residues shorter than the α-secretase product) that ends at the N-terminus of the Aβ domain (Seubert et al., 1993). The remaining 11.5-kDa membrane-associated fragment (Gabuzda et al., 1994) yields intact Aβ following a second cleavage event occurring at the C-terminus of the Aβ domain by γ-secretase (Anderson et al., 1992). This sequence of events does not appear to occur in all cases, as there is evidence for γ-secretase cleavage in the absence of β-secretase cleavage (Anderson et al., 1992; Wallace et al., 1995). The Aβ peptide is heterogeneous at both the amino and the carboxy termini. However, most β-secretase cleavage occurs at the Met–Asp bond preceding the Aβ N-terminus; substitutions at either Met or Asp substantially increase Aβ production (Citron et al., 1995). Soluble Aβ and sAPPβ are secreted by a variety of cells and have been found in human CSF (Sisodia et al., 1990; Haass et al., 1992; Seubert et al., 1992, 1993; Shoji et al., 1992; Busciglio et al., 1993). In the AD brain, soluble Aβ is markedly higher than in the brains of controls (Kuo et al., 1996), whereas CSF levels of soluble Aβ have been to shown decrease with increasing severity of dementia (Hock et al., 1998).

Production of Aβ and N-terminal truncated peptides (N-40 and N-42)

Aβ and N-terminal truncated peptides have heterogeneous C-termini (Cordell, 1994; Selkoe, 1994). Most full-length Aβ peptides fall into one of two populations known as $Aβ_{1–40}$, terminating at amino acid 40 and comprising ~90% of secreted Aβ, or $Aβ_{1–42}$, terminating at amino acid 42 and comprising only 10% of secreted Aβ (Haass et al., 1992; Seubert et al., 1992). C-terminal heterogeneity of Aβ is especially significant to AD pathology, as increased length of the hydrophobic C-terminus has been shown to promote early deposition of fibrillar Aβ in familial AD where there is a relative increase in the production of $Aβ_{1–42}$ (Borchelt et al., 1996; Lemere et al., 1996; Citron et al., 1997). Moreover, $Aβ_{1–42}$ exists in the form of water-soluble dimers that may form the building blocks of insoluble Aβ filaments (Kuo et al., 1996). Another potentially amyloidogenic fragment contains the sequence of residues 17–42 (p3), corresponding to the C-terminal sequence of $Aβ_{1–42}$. These fragments are one of the main constituents of nonfibrillar or diffuse plaques (Gowing et al., 1994).

Although the mechanisms underlying C-terminal heterogeneity of Aβ have not been determined, several hypotheses have been advanced. One is that C-terminal cleavage by γ-secretase occurs in several intracellular compartments. This has been most convincingly demonstrated in neuronal cells where an unusually high percentage of intracellular Aβ is produced from immature APP (De Strooper et al., 1995; Hartmann et al., 1997; Wild-Bode et al., 1997). In neurons, the longer isoform, $Aβ_{1–42}$, has been shown to be generated predominantly in the endoplasmic reticulum and nuclear envelope (Cook et al., 1997; Hartmann et al., 1997), whereas the shorter $Aβ_{1–40}$ is produced in the *trans*-Golgi membrane (Hartmann et al., 1997). Loose sequence specificity for γ-secretase action may be permitted in the endoplasmic reticulum, as this membrane is cholesterol poor, more permeable, and readily deformable relative to that of the *trans*-Golgi membrane (Tischer and Cordell, 1996). A related hypothesis suggests that multiple forms of γ-secretase exist having different preferred cleavage sites (Hartmann et al., 1997). Indeed, differential sensitivity to cleavage at positions 40 and 42 by various protease inhibitors has been observed, suggesting that different γ-secretases are affected (Higaki et al., 1995; Citron et al., 1996; Klafki et al., 1996). A combination of both hypotheses is also possible, such that different ratios of distinct γ-secretases exist in different compartments, thereby resulting in differing $Aβ_{1–40}/Aβ_{1–42}$ ratios.

Novel processing of APP in neurons

Secretory processing of APP may be somewhat more complex in neuronal cells yielding novel fragments. For example, radiosequencing studies of C-terminus APP fragments in rat hippocampal neurons overexpressing human APP_{695} yielded an APP fragment starting at Asn^{585} (12 residues from the N-terminus of the Aβ domain). This cleavage fragment was found in amounts comparable with those of the α- and β-cleaved fragments and was named δ-cleavage (Simons et al., 1996). This cleavage fragment is also thought to exist in vivo (Estus et al., 1992); whether this intermediate serves as a precursor for Aβ production is currently unknown.

Radiosequencing of the 8–8.5-kDa C-terminus fragments traditionally associated with α-secretase cleavage has also displayed N-terminal heterogeneity yielding

three major products in transfected neurons (Simons et al., 1996). One fragment is cleaved at the classic Lys^{16}–Leu^{17} α-secretase site (Esch et al., 1990), whereas the other two fragments are slightly longer. Most noteworthy is the fragment starting at Glu^{11} of the Aβ sequence. $A\beta_{11-42}$ is a major component of AD plaques (Masters et al., 1985; Naslund et al., 1994) and is thought to be produced via activation of the β-secretase pathway (Xu et al., 1998), although direct evidence for this is still forthcoming.

PHYSIOLOGICAL EFFECTS OF sAPP IN NEURONS

Evidence for the physiological importance of sAPPα was examined in vivo by creating mutant mice having complete deficiency of APP. These mice were seen to have reactive gliosis in the hippocampus and cortex (Zheng et al., 1995) as well as behavioral abnormalities (Müller et al., 1994; Zheng et al., 1995). Likewise, mutant mice that were partially defective in α-secretase processing exhibited reactive gliosis, were prone to seizures, and died prematurely (Moechars et al., 1996). Despite these effects, the ability of these mice to maintain normal litter sizes indicated that murine APP is either dispensable for development or that other APP family members (such as the amyloid precursor-like protein, APLP) and their various cleavage products compensated for the loss of APP. In support of the latter hypothesis, 80% of APLP2/APP double-knockout mice were shown to die within the first week after birth. Because soluble N-terminal fragments can be produced from both APP and APLP but Aβ is derived only from APP, these data suggest that sAPP is important in development but Aβ is not (Vonkoch et al., 1997). In adult animals, APP has been shown to be important in neuronal survival, as sAPPα or a peptide fragment protected hippocampal CA1 neurons from ischemic injury or enhanced recovery from ischemic spinal cord injury, respectively (Bowes et al., 1994; Smith-Swintosky et al., 1994). Effects of sAPP on plasticity in the adult brain have also been suggested, as administration of amino acids 319–335 of APP enhances memory retention (Roch et al., 1994), whereas administration of APP antibodies impaired performance on memory tasks (Huber et al., 1993).

In vitro, physiological functions for sAPP in the developing and adult nervous system are well supported. Biological activities of sAPP have been shown to include promotion of neuronal cell survival, adhesive interactions, neurite outgrowth, synaptogenesis, and synaptic plasticity (for review, see Mattson, 1997; Mattson et al., 1997). sAPP has been shown to have effects on ion fluxes and various signaling pathways that may underly its purported physiological roles (Fraser et al., 1997; Mattson, 1997; Mattson et al., 1997). sAPP is a regulator of intraneuronal Ca^{2+} (Mattson et al., 1993; Mattson, 1994; Barger et al., 1995; Furukawa et al., 1996*a*,*b*; Furukawa and Mattson, 1998; Koizumi et al., 1998) and activates high-conductance, charybdotoxin-sensitive K^+ channels (Furukawa et al., 1996*a*). sAPPα has been shown to induce activation of NF-κB (Barger and Mattson, 1996) and to alter various second messengers and effectors including cyclic GMP and protein kinase G (Barger et al., 1995; Furukawa et al., 1996*a*; Furukawa and Mattson, 1998), phospholipase C/protein kinase C (PLC/PKC) (Ishiguro et al., 1998), extracellular signal-regulated protein kinase (ERK) (Greenberg et al., 1994, 1995), and inositol trisphosphate (Ishiguro et al., 1998; Koizumi et al., 1998).

ACTIONS OF Aβ IN NEURONAL CELLS

Studies of Aβ effects in neuronal cells have traditionally emphasized its toxicity, which occurs when Aβ is present at high concentrations and in aggregated form (Mattson, 1997), although recent data indicate that nanomolar concentrations of Aβ oligomers are also neurotoxic (Roher et al., 1996; Lambert et al., 1998). However, soluble Aβ also has biological actions at more physiological concentrations (picomolar to nanomolar). One of the earliest studies to demonstrate such an effect showed that rat hippocampal cultures exposed to picomolar concentrations of Aβ exhibited a trophic response, and that this effect was mediated by a portion of Aβ (amino acids 25–35), which acted as a tachykinin antagonist (Yankner et al., 1990). Subsequent studies have shown that Aβ has effects on neuronal cell signaling. For example, physiological concentrations of Aβ increased tyrosine phosphorylation (Zhang et al., 1994; Luo et al., 1996) and phosphatidylinositol 3-kinase activity in cultured cortical neurons (Luo et al., 1996). $A\beta_{1-42}$ activated hydrolysis of an acidic phospholipid by phospholipase A_2 (PLA_2) (Lehtonen et al., 1996) and $A\beta_{1-40}$ maximally activated NF-κB in primary neurons (Kaltschmidt et al., 1997). Finally, physiological levels of Aβ can induce rapid changes in intracellular Ca^{2+} levels and ecto-PKC (Hogan et al., 1995; Luo et al., 1995; Wolozin et al., 1995).

Soluble Aβ has also been shown to compromise cholinergic neuronal function at concentrations that appear to be physiological. In vitro, long-term exposure to $A\beta_{1-42}$ and $A\beta_{1-28}$ reduced acetylcholine content in a mouse cell line derived from basal forebrain cholinergic neurons and this reduction was accompanied by a proportional decrease in choline acetyltransferase activity (Pedersen et al., 1996). Freshly solubilized oligomeric $A\beta_{1-42}$ suppressed acetylcholine synthesis in cholinergic neurons without affecting neuronal survival (Hoshi et al., 1997). Also, exposure of rodent fetal cortical neurons to nanomolar concentrations of $A\beta_{25-35}$ impaired carbachol stimulation of GTPase activity but had no effect on cell survival (Kelly et al., 1996). Finally, inhibition of K^+-evoked acetylcholine release by Aβ exposure occurred in a region-specific manner, being manifest in the hippocampal formation and frontal cortex, whereas striatal cholinergic neurons were unaffected (Kar et al., 1996).

TABLE 1. *Regulation of α- and β-secretase pathways: lack of mutual exclusivity*

Treatment	Action	In vitro or in vivo system	Effect on APP fragments	References
Phorbol esters	Activators of PKC	Human neuroblastoma	↑ sAPP ↔Aβ	Dyrks et al. (1994) Fuller et al. (1995)
Interleukin-1	Proinflammatory cytokine	Human glioma		Vasilakos et al. (1994) Buxbaum et al. (1994)
Phorbol esters	Activators of PKC	Human glioma Human astrocytes	↔sAPP ↓ Aβ	Gabuzda et al. (1993)
Phorbol esters	Activators of PKC	Human neurons Transgenic mice	↑ sAPP ↑ Aβ	LeBlanc et al. (1998) Savage et al. (1998)
Serum withdrawal	Apoptosis	Human neurons		LeBlanc (1995) LeBlanc et al. (1998)
Caffeine A23187	↑ $[Ca^{2+}]_i$ Ca^{2+} ionophore	HEK 293		Querfurth and Selkoe (1994) Querfurth et al. (1997)

MUTUAL EXCLUSIVITY OF sAPP AND Aβ PRODUCTION

Cleavage of APP is constitutive in most cells. However, the relative amounts of sAPP and Aβ that are produced are subject to regulation by many agents. Traditionally, it has been thought that regulation of sAPPα and Aβ production occurs in a mutually exclusive manner (Nitsch and Growdon, 1994; Checler, 1995). Although this model appears to be correct under many circumstances, it is now clear that reciprocal regulation of these two pathways does not always occur (Table 1). sAPP production increased but Aβ production was unchanged when SY5Y human neuroblastoma cells were exposed to phorbol esters (Dyrks et al., 1994; Fuller et al., 1995) or when human neuroglioma cells were exposed to interleukin-1 (Buxbaum et al., 1994; Vasilakos et al., 1994). In contrast, Aβ levels decreased but sAPP was unchanged when human glioma cells or primary human astrocytes were treated with phorbol 12-myristate 13-acetate (PMA) (Gabuzda et al., 1993). A similar result emerged after application of PMA to transgenic mice harboring the Swedish familial AD mutation and humanized Aβ (Savage et al., 1998). Finally, both α- and β-secretase pathways were stimulated on activation of PKC or serum deprivation in primary human neuronal cultures (LeBlanc, 1995; LeBlanc et al., 1998) and after elevations of intracellular Ca^{2+} in human embryonic kidney (HEK) 293 cells (Querfurth and Selkoe, 1994; Querfurth et al., 1997).

What accounts for these discrepant results? One contributing factor is that APP fragments have often been ill-defined. For example, antibodies directed against the N-terminal of APP are problematic in that they do not discriminate between sAPPα and sAPPβ (an increase in α-secretase activity with a corresponding decrease in β-secretase cleavage would result in no net change). Such antibodies may also detect the production of sAPPγ (Anderson et al., 1992; Wallace et al., 1995) a cleavage fragment that may be part of either the α- or β-secretase pathway. Also, a novel cleavage has recently been identified that occurs within Aβ and may be associated with the β-secretase pathway (Xu et al., 1998). A second confound is that detection methods for APP fragments may yield different results. Pulse-chase studies detect cleavage fragments of APP that have been newly synthesized, whereas other methods such as western blot analysis of secreted proteins do not discriminate new from old. Even given these caveats, under most circumstances when sAPPα increases, Aβ decreases. However, the numerous exceptions to the rule of mutual exclusivity and the widespread availability of antibodies that can detect both sAPPα and Aβ make it imperative that future studies measure both products when examining regulation of APP cleavage.

NEUROTRANSMITTER REGULATION OF APP PROCESSING

Regulation of sAPP secretion by neurotransmitter receptors in cell lines

The first study indicating that APP processing could be regulated by neurotransmitters involved HEK 293 cells overexpressing the human muscarinic acetylcholine receptor (Nitsch et al., 1992). Later studies extended these initial findings in a variety of cell lines overexpressing the muscarinic receptor; cholinergic agonists coincidently increased sAPP release and decreased Aβ production (Hung et al., 1993; Buxbaum et al., 1994; Slack et al., 1995). In a similar manner, cholinergic regulation of sAPP secretion has been shown to occur in cell lines expressing their normal complement of muscarinic receptors (Buxbaum et al., 1992; Wolf et al., 1995). Regulation of sAPP release has been subsequently shown to occur for other neurotransmitters acting at G protein-coupled receptors including the metabotropic glutamate receptor (Lee et al., 1995; Lee and Wurtman, 1997; Nitsch et al., 1997; Jolly-Tornetta et al., 1998) and serotonin (5-HT) receptors (Nitsch et al., 1996).

G protein-coupled receptor stimulation of sAPP release appears to be selective for receptor subtypes that are coupled to phosphatidylinositol hydrolysis. For ex-

ample, stimulation of HEK 293 cells overexpressing the M_1 and M_3 receptor subtypes with the muscarinic agonist carbachol significantly increased sAPP release but stimulation of the M_2 and M_4 receptor subtypes did not. Therefore, it was implied that stimulation of receptors coupled to the PLC pathway increased sAPP release, whereas receptors linked to adenylyl cyclase did not. In a similar manner, application of 5-HT to 3T3 cells stably overexpressing the receptors 5-HT_{2a} or 5-HT_{2c} stimulated phosphatidylinositol turnover and sAPP release in a dose-dependent manner (Nitsch et al., 1996). Finally, glutamate increased both sAPP release and phosphatidylinositol hydrolysis in HEK 293 cells and human N tera 2 neurons expressing metabotropic glutamate receptors (Lee et al., 1995; Jolly-Tornetta et al., 1998), an effect that was antagonized by the metabotropic glutamate receptor antagonist α-methyl-4-carboxyphenylglycine (Nitsch et al., 1997).

Unlike G protein-coupled receptors, few studies have examined regulation of sAPP release by activation of ligand-gated channels. Treatment of PC12 cells with nicotine increased the release of sAPP, an effect that was attenuated by cotreatment with a nicotinic receptor antagonist or EGTA, a calcium chelator (Kim et al., 1997). Likewise, we have observed NMDA receptor stimulation of sAPP release in HEK 293 cells transiently transfected with the NMDA receptor subtypes (J. Mills and P. B. Reiner, unpublished data).

Regulation of sAPP secretion by neurotransmitter receptors in central neurons

Neurotransmitter regulation of APP processing in central neurons has focused predominantly on cholinergic and glutamatergic innervation. Electrical depolarization of hippocampal slices induced a rapid increase in the release of both acetylcholine and sAPP, effects that were inhibited by blocking voltage-sensitive sodium channels with tetrodotoxin (Nitsch et al., 1993). In a similar manner, the muscarinic receptor agonist bethanechol and cholinesterase inhibitors enhanced sAPP release from cortical slices of the rat (Mori et al., 1995). Also, exposure of hippocampal slices to the mixed cholinergic agonist carbachol stimulated sAPP release in the presence of the M_2 antagonist gallamine (Farber et al., 1995). Moreover, a selective M_1 agonist, talsaclidine (WAL 2014) (Ensinger et al., 1993), enhanced sAPP release from cortical or striatal slices (Müller et al., 1997). Finally, reduced cortical cholinergic innervation in rats has been shown to decrease sAPP and increase sAPPγ release (Wallace et al., 1995; Rossner et al., 1997).

In contrast to the above, stimulation of rat cortical cultures or hippocampal slices with the cholinergic agonist carbachol did not increase release of sAPP (Farber et al., 1995; Mills and Reiner, 1996). This appears to be related to the pharmacological complexity of G protein-coupled receptor regulation of sAPP release. Specifically, it has been suggested that M_2 receptor activation may be negatively coupled to sAPP release, thereby explaining both the biphasic nature of the talsaclidine (WAL 2014) response (Müller et al., 1997) and the necessity of gallamine, to observe cholinergic stimulation of sAPP release in hippocampal slices (Farber et al., 1995), although this is not consistent with the observation that bethanechol, a full agonist at muscarinic M_2 receptors, enhanced sAPP release from cortical rat slices (Mori et al., 1995). Nevertheless, these observations have significant implications for the pharmacotherapy of AD. They predict that treatments that aim to alter Aβ production via activation of neurotransmitter receptors must take into account the specificity of receptor subtypes coupling to regulation of APP cleavage.

This view is reinforced by studies of glutamatergic regulation of sAPP release, which has also been shown to be receptor subtype specific, involving metabotropic glutamate receptors coupled to phosphatidylinositol hydrolysis and Ca^{2+} mobilization (Lee et al., 1995; Kirazov et al., 1997; Ulus and Wurtman, 1997). In hippocampal rat neurons, L-glutamate, quisqualic acid, and 1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) stimulated sAPP release, an effect that was antagonized by both the PKC inhibitor GF 109203X and the metabotropic glutamate receptor antagonist L-2-amino-3-phosphonopropionic acid (L-AP3) (Lee et al., 1995). Likewise, in rat brain cortical or hippocampal slices, L-glutamate or a selective metabotropic agonist increased sAPP release (Kirazov et al., 1997; Ulus and Wurtman, 1997) and this effect was blocked by the metabotropic glutamate receptor antagonist α-methyl-4-carboxyphenylglycine and the PKC inhibitor GF 109203X (Ulus and Wurtman, 1997). Ionotropic glutamate agonists had either modest or no effect on sAPP release in all of these preparations. Taken together, these studies suggest that selective activation of subsets of neurotransmitter receptors represents a plausible avenue of regulation of Aβ production.

REGULATION OF APP CLEAVAGE BY INTRACELLULAR SIGNALING PATHWAYS

Regulation of APP processing by PKC-dependent signaling pathways

A strong case has been made for the role of PKC activation in the regulation of APP processing both in vitro (for review, see Nitsch and Growdon, 1994) and in vivo (Caputi et al., 1997; Savage et al., 1998). PKC comprises a large family of serine/threonine kinases, classified as conventional, new, and atypical (Newton, 1995). All are activated, at least partially, by phospholipids, but conventional and new PKC isoenzymes are activated by diacylglycerol or its analogue phorbol ester. Conventional PKCs are the only ones that require Ca^{2+} as a cofactor.

Stimulation of G protein-coupled receptors by neurotransmitters and neuropeptides has been shown to regulate APP processing by PKC-dependent signaling pathways. For example, the PKC inhibitor staurosporine antagonized cholinergic receptor stimulation of sAPP release in HEK 293 cells overexpressing the M_1 or M_3 muscarinic receptors (Nitsch et al., 1992; Slack et al.,

TABLE 2. *APP processing by PKC-independent mechanisms*

Agent	Spectrum of action	Cell system	Effect on APP cleavage	References
Thapsigargin or inositol trisphosphate	↑ $[Ca^{2+}]_i$	HEK 293 CHO Human neuroglioma 3T3	↑ or ↔ sAPP Variable on Aβ	Buxbaum et al. (1994) Querfurth and Selkoe (1994) Nitsch et al. (1996)
A23187	Ca^{2+} ionophore	HEK 293		Nitsch et al. (1992) Querfurth and Selkoe (1994)
Melittin	Activator of PLA_2	CHO-M_1 3T3 HEK 293	↑ sAPP	Emmerling et al. (1993) Nitsch et al. (1996) Nitsch et al. (1997)
Indomethacin	Inhibitor of cyclooxygenase	Human glioblastoma		Kinouchi et al. (1995*a*)
Forskolin	Activator of adenylate cyclase	Rat glioma, PC12 HEK 293	↑ or ↓ sAPP	Efthimiopoulos et al. (1996) Xu et al. (1996) Marambaud et al. (1996)
Dibutyryl cyclic AMP	Cyclic AMP analogue	HEK 293	↔ Aβ	Querfurth and Selkoe (1994)

1995) and bradykinin-dependent increases in sAPP in PC12 cells (Nitsch et al., 1998). In a similar manner, glutamatergic stimulation of the metabotropic glutamate receptor subtype 1α increased sAPP release, an effect that was inhibited with the more specific PKC inhibitor chelerythrine chloride (Nitsch et al., 1997). Down-regulation of PKC by pretreatment with PMA also blocked metabotropic glutamate receptor stimulation of sAPP release in HEK 293 cells (Nitsch et al., 1997). Likewise metabotropic glutamate receptor stimulation of sAPP release from either rat cortical astrocytes or hippocampal cultures was suppressed by the PKC inhibitor GF 109203X (Lee et al., 1995; Lee and Wurtman, 1997).

Direct activation of PKC by phorbol esters has been shown to regulate APP cleavage in several continuous cell lines including PC12 (Buxbaum et al., 1990; Caporaso et al., 1992), Chinese hamster ovary (CHO) (Buxbaum et al., 1993), COS (Gabuzda et al., 1993), neuroblastoma (Dyrks et al., 1994), Swiss 3T3 (Slack et al., 1993), and HEK 293 cells (Nitsch et al., 1992; Jacobsen et al., 1994; Marambaud et al., 1997*a*,*b*) as well as primary neuronal cultures (Lee et al., 1995; Mills and Reiner, 1996; Nitsch et al., 1996, 1997; Mills et al., 1997). Short-term activation of PKC by phorbol esters has been shown to coincidently increase sAPP and decrease Aβ release (Buxbaum et al., 1993; Gabuzda et al., 1993; Hung et al., 1993; Jacobsen et al., 1994). PKC-mediated stimulation of sAPP has been shown to be specific insofar as down-regulation of PKC blocked both phorbol ester stimulation of sAPP release (Buxbaum et al., 1994) and phorbol ester inhibition of Aβ release (Hung et al., 1993; Buxbaum et al., 1994). Moreover, these effects were blocked by the PKC inhibitors 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) (Gabuzda et al., 1993; Slack et al., 1993), staurosporine (Hung et al., 1993), and GF 109203X (Slack et al., 1995). Furthermore, regulation of APP processing was not seen with the inactive phorbol ester analogue 4α-phorbol 12,13-didecanoate (Caporaso et al., 1992; Gabuzda et al., 1993). Also, an analogue of diacylglycerol, the physiological activator of PKC, mimicked the effects of phorbol esters on APP processing (Gabuzda et al., 1993). Finally, further evidence supporting a role for the PLC/PKC signaling pathway in regulating APP processing has come from studies using mastoparan and mastoparan X, activators of PLC that increased formation of sAPP but decreased production of Aβ (Buxbaum et al., 1993).

The role of various PKC isoenzymes in regulating APP processing has not been addressed extensively. The conventional PKC isoenzyme PKCα regulated sAPP release in Swiss 3T3 fibroblast cells; although the total amount of sAPP released was unchanged, the EC_{50} for PMA regulation of sAPP release was lower in cell lines overexpressing PKCα (Slack et al., 1993). A complementary study demonstrated that a specific inhibitor of PKCα (Gö-6976) reduced constitutive and phorbol ester regulation of sAPP in human fibroblasts (Benussi et al., 1998). In a similar manner, in a rat fibroblast cell line, sAPP was increased by PKCα and PKCϵ but not PKCδ after stable overexpression of these isoenzymes (Kinouchi et al., 1995*b*).

Regulation of APP processing by PKC-independent signaling pathways

Although PKC activation can regulate APP processing, neurotransmitter and neuropeptide regulation of APP catabolism has been shown to occur in cells lacking functional PKC (Buxbaum et al., 1994; Slack et al., 1995; Nitsch et al., 1996, 1997, 1998; Racchi et al., 1998). Several intracellular signaling pathways have been suggested to act as intermediaries in PKC-independent neurotransmitter receptor regulation of APP processing, including the second messenger Ca^{2+}, PLA_2, cyclic AMP-dependent protein kinase (PKA), and an unidentified tyrosine kinase (Tables 2 and 3) (Buxbaum et al., 1994; Slack et al., 1995; Nitsch et al., 1996, 1997).

Ca^{2+}. The effects of Ca^{2+} on APP processing are complex and appear somewhat contradictory. For exam-

TABLE 3. *Inhibition of neurotransmitter receptor stimulation of sAPP: PKC-independent pathways*

Neurotransmitter receptor	Receptor subtype	Drug action	Cell system	References
Acetylcholine	M_1	PLA_2 inhibitors	CHO-M_1	Emmerling et al. (1993)
Serotonin	5-HT_{2a} or 5-HT_{2c}		3T3 fibroblasts	Nitsch et al. (1996)
Glutamate	mGluR1α		HEK 293	Nitsch et al. (1997)
Glutamate	Metabotropic	Activator of adenylate cyclase cyclic AMP analogue	Rat cortical astrocytes	Lee and Wurtman (1997)
Acetylcholine	M_1 or M_3	Tyrosine kinase inhibitors	HEK 293	Slack et al. (1995)

mGluR1α, metabotropic glutamate receptor 1α.

ple, Buxbaum et al. (1994) reported that thapsigargin, a compound that raises intracellular Ca^{2+} (Thastrup et al., 1990), increased formation of sAPP from CHO cells overexpressing APP_{751} after down-regulation of PKC. Likewise, in HEK 293 cells overexpressing APP_{751}, Querfurth et al. (1997) reported that the calcium reuptake inhibitors thapsigargin and cyclopiazonic acid potentiated caffeine-stimulated p3 release, a fragment that is presumably stoichiometrically coupled to sAPP and therefore is thought to be a product of the α-secretase pathway. In contrast to these findings, Nitsch et al. (1996) reported that thapsigargin failed to change sAPP release in 3T3 cells using the same concentrations of drug. Finally, in an earlier report using the Ca^{2+} ionophore A23187, Nitsch et al. (1992) demonstrated that Ca^{2+} did not increase sAPP release from HEK 293 cells.

The effects of Ca^{2+} on Aβ release are equally complex. For example, Buxbaum et al. (1994) demonstrated a concentration-dependent effect of thapsigargin on Aβ release, increasing relative Aβ release at 10 n*M* but decreasing release at 20 n*M*. However, direct application of inositol trisphosphate, the second messenger presumed to be responsible for releasing cytoplasmic calcium from intracellular stores, had no effect on Aβ release (Querfurth and Selkoe, 1994). Furthermore, a rise in intracellular Ca^{2+} generated by A23187 or caffeine was shown to increase Aβ release from HEK 293 cells stably expressing APP_{751} (Querfurth and Selkoe, 1994). Finally, thapsigargin and cyclopiazonic acid were both shown to potentiate caffeine-stimulated release of Aβ, presumably by inhibiting reuptake of Ca^{2+} (Querfurth et al., 1997).

Clearly, these contradictions cannot easily be explained by cell-specific differences. However, some of these discrepancies may be explained by effects of these drugs on Ca^{2+} within the acidic luminal environment of the secretory pathway (Querfurth et al., 1997). For example, NH_4Cl decreases Aβ and results in luminal Ca^{2+} depletion. Likewise, an increase in Aβ secretion observed with calcium ionophores and caffeine may be a consequence of increased Ca^{2+} sequestration within the post-Golgi vesicles. That NH_4Cl attenuates caffeine- or A23187-induced stimulation of Aβ secretion supports this hypothesis (Querfurth et al., 1997).

PLA_2. Stimulation of heterotrimeric G protein-coupled receptors activates cytosolic PLA_2 (Farooqui et al., 1997*a*,*b*). Receptor-mediated activation of PLA_2 generates free fatty acids (i.e., arachidonic acid) and lysophosphatidylcholine from membrane phospholipids (Farooqui et al., 1997*a*,*b*). An initial study suggested that PLA_2 can partially mediate muscarinic receptor stimulation of sAPP formation (Emmerling et al., 1993). Subsequent studies have extended these results to include both serotonergic and glutamatergic regulation of APP processing. The PLA_2 inhibitors manoalide, dimethyleicosadienoic acid, or oleyloxyethyl phosphorylcholine inhibited 5-HT stimulation of sAPP secretion in a fibroblast cell line overexpressing either 5-HT_{2a} or 5-HT_{2c} receptors (Nitsch et al., 1996). In a similar manner, these same inhibitors antagonized glutamate receptor stimulation of sAPP release in HEK 293 cells expressing the metabotropic glutamate receptor subtype 1α (Nitsch et al., 1997). Furthermore, melittin, a peptide that stimulates PLA_2, has been shown to augment sAPP release in a variety of cell lines (Emmerling et al., 1993; Nitsch et al., 1996, 1997). Likewise, inhibition of cyclooxygenase, an enzyme that metabolizes arachidonic acid, increases sAPP release in human glioma cells (Kinouchi et al., 1995*a*).

PKA. Recent evidence suggests that like PKC, PKA exerts effects on both constitutive as well as regulated APP processing. PKA-mediated effects on constitutive secretory processing of APP vary between studies. Forskolin, an activator of adenylate cyclase, inhibited constitutive production of sAPP in a glioma cell line, whereas 1,9-dideoxyforskolin, an inactive analogue, had no effect (Efthimiopoulos et al., 1996). Conversely, two independent studies found that either forskolin or 8-bromo cyclic AMP increased constitutive sAPP release from PC12 and HEK 293 cells (Marambaud et al., 1996; Xu et al., 1996). Finally, Querfurth and Selkoe (1994) demonstrated that the cyclic AMP analogue dibutyryl cyclic AMP had no effect on constitutive Aβ release. Resolution of these apparent discrepancies is still a matter of investigation.

In contrast to the variability seen for the effects of PKA on constitutive sAPP release, PKA has been shown to inhibit regulation of APP processing in all studies to date. For example, in a glioma cell line, a rise in intracellular cyclic AMP, levels induced by either stimulation of β-adrenergic receptors, by the cyclic AMP agonist dibutyryl cyclic AMP or by the PKA agonist forskolin all

inhibited PKC stimulation of sAPP release (Efthimiopoulos et al., 1996). In a similar manner, phorbol ester and metabotropic glutamate receptor stimulation of sAPP release was inhibited by either forskolin or dibutyryl cyclic AMP in cortical astrocyte cultures (Lee and Wurtman, 1997).

Tyrosine kinase. Stimulation of a wide range of receptors having intrinsic or associated tyrosine kinase activity has been shown to regulate APP processing. These include receptors for growth factors (Refolo et al., 1989; Schubert et al., 1989*a*; Fukuyama et al., 1993; Clarris et al., 1994; Ringheim et al., 1997), cytokines (Buxbaum et al., 1992, 1994; Vasilakos et al., 1994), thrombin (Davis-Salinas et al., 1994), and neurotransmitters (Slack et al., 1995).

Studies on growth factor receptor stimulation of APP processing were among the first indicating that sAPP release was a regulated event. For example, stimulation of various tyrosine kinase receptors with nerve growth factor, fibroblast growth factor, or epidermal growth factor have all been shown to increase sAPP (Refolo et al., 1989; Schubert et al., 1989*a*; Fukuyama et al., 1993; Clarris et al., 1994; Ringheim et al., 1997). These studies focused on the effects of long-term exposure to growth factors, at which time alterations of APP expression levels have also been reported (Mobley et al., 1988; Gray and Patel, 1993; Lahiri and Nall, 1995; Ringheim et al., 1997). However, recent studies provide evidence that transient stimulation of growth factor receptors increase sAPP release. For example, nerve growth factor stimulates sAPP release from PC12 cells within 15 min of exposure (Mills et al., 1997; Desdouits-Magnen et al., 1998). In a similar manner, exposure of a human epidermoid carcinoma cell line to epidermal growth factor stimulates both sAPP and phosphatidylinositol turnover within 30 min (Slack et al., 1997).

Stimulation of tyrosine kinase receptors and receptors having associated tyrosine kinase activity has been shown to regulate APP cleavage in a PKC-independent manner. Epidermal growth factor receptor regulation of sAPP was found to be predominantly PKC independent, as GF 109203X decreased the effect of epidermal growth factor by 35% at a concentration that completely inhibited the release of sAPP by PKC (Slack et al., 1997). In a similar manner, nerve growth factor receptor stimulation of sAPP in PC12 cells was not blocked by either GF 109203X or PKC down-regulation (Desdouits-Magnen et al., 1998). Also, carbachol and the Ca^{2+} ionophore ionomycin increased sAPP release from HEK 293 cells that were overexpressing the muscarinic receptor M_3; both of these effects were mediated by a tyrosine phosphorylation-dependent mechanism (Slack et al., 1995; Petryniak et al., 1996).

The mitogen-activated protein kinase (MAPK) pathway: a point of convergence for multiple signals

The data cited above predict the existence of an effector system that can be regulated in either a PKC-dependent or independent fashion and may involve activation of tyrosine kinases. The MAPK signaling pathway meets all of these criteria and has recently been implicated in both PKC and tyrosine kinase receptor regulation of APP catabolism (Mills et al., 1997; Desdouits-Magnen et al., 1998). The MAPK pathway refers to a three-level cascade involving the sequential activation of raf, MAPK kinase (MEK), and ERKs (Pelech and Charest, 1996). MAPK was antagonized by inhibiting MEK, its only known physiological activator. The MEK inhibitor PD 98059 antagonized nerve growth factor receptor stimulation of sAPP release and ERK in PC12 cells. Moreover, exposure to PD 98059 or overexpression of a kinase-inactive MEK mutant reduced PKC-mediated effects on APP processing in a variety of cell lines (Mills et al., 1997; Desdouits-Magnen et al., 1998). Indeed, preliminary evidence from our laboratory suggests that the MAPK pathway is critically involved in NMDA receptor stimulation of sAPP secretion (J. Mills and P. B. Reiner, unpublished data). This study suggests that the MAPK cascade may provide a useful target for altering secretory processing of APP.

NOVEL MECHANISMS UNDERLYING REGULATION OF APP CLEAVAGE

Estrogen regulation of APP cleavage

Of potential therapeutic relevance, physiological concentrations of 17β-estradiol have been shown to alter APP cleavage in cell lines and primary cultures of rat, mouse, and human embryonic cerebrocortical neurons (Jaffe et al., 1994; Xu et al., 1998). In an early study, treatment of a breast carcinoma-derived cell line with 17β-estradiol increased sAPPα release (Jaffe et al., 1994). A later study extended these findings, demonstrating that 17β-estradiol decreased both Aβ and a novel 3-kDa protein (Aβ_{11-40}) (Xu et al., 1998). It is noteworthy that maximal increases in sAPP and decreases in Aβ were observed after a 7–10-day treatment period.

The biological mechanism for estrogen regulation of APP cleavage is presently unknown. The estrogen receptor is a ligand-regulated nuclear transcription factor that is widely expressed in the developing forebrain (Tsai and O'Malley, 1994). Because of evidence suggesting that postmenopausal women taking estrogen have a reduced incidence of AD (Wickelgren, 1997), these findings are worthy of further investigation.

Regulation of APP cleavage in response to stress

Recent studies indicate that altered APP catabolism may arise as a result of stressful stimuli associated with oxidant stress, metabolic compromise, or programmed cell death. For example, in COS cells overexpressing APP_{695}, inhibition of oxidative energy metabolism by sodium azide or the mitochondrial uncoupler carbonyl cyanide *m*-chlorophenylhydrazone increased the activity of β-secretase, resulting in an 80-fold increase in the production of an 11.5-kDa C-terminal derivative (Gabuzda et al., 1994). Radiosequencing analysis con-

firmed that this C-terminal fragment of APP resulted from β-secretase cleavage and therefore was a potential processing intermediate in the generation of Aβ (Gabuzda et al., 1994). In a similar manner, both glucose deprivation and sodium azide decreased release of sAPP from COS cells within a 2-h exposure period but had no effect on APP expression levels (Gasparini et al., 1997). Treatment of COS cells with the antioxidant glutathione completely antagonized azide inhibition of sAPP release (Gasparini et al., 1997).

Altered APP catabolism resulting from stressful stimuli in neuronal cell lines or central neurons has also been observed. For example, oxidative stress in neuroblastoma cells increased secretion of Aβ although APP expression levels were also increased (Yan et al., 1995). In a similar manner, serum-free media induced apoptosis in human primary neuronal cultures, resulting in a threefold increase in Aβ release and a corresponding, although somewhat more modest, decrease in sAPP production (LeBlanc, 1995). Also, PC12 cells maintained in serum-free media with or without additional injurious agents released a 60-kDa C-terminal fragment containing the intact Aβ sequence (Baskin et al., 1991).

Although extremely interesting, stress-induced regulation of APP catabolism by these various stimuli remains nothing more than phenomenology at present. Both the signaling cascades and the receptors involved in these regulatory pathways are presently unknown. However, a potential role for PKC has been suggested for altered APP catabolism induced by apoptosis (LeBlanc, 1995).

Sterol-regulated APP processing

A unique mechanism of regulation of APP cleavage was recently reported that correlates with cell membrane cholesterol content. Cellular cholesterol content is controlled either through intracellular synthesis or by uptake of cholesterol through the low-density lipoprotein receptor pathway (Brown and Goldstein, 1986). Dose-dependent inhibition of sAPP release was observed when COS cells were incubated with increasing concentrations of cholesterol (Racchi et al., 1997). In a similar manner, cholesterol, solubilized by methyl-β-cyclodextrin or ethanol, reduced sAPP release but had no effect on cellular expression levels in HEK 293 cells (Bodovitz and Klein, 1996). This inhibition was specific, as cholesterol increased secretion of several other cellular proteins.

Both synthesis and uptake of cholesterol are tightly regulated by sterol-regulating element binding proteins (SREBPs), membrane-bound transcription factors that are proteolytically cleaved and then translocate to the nucleus where they regulate transcription of genes involved in cholesterol biosynthesis and uptake (Brown and Goldstein, 1997). It is noteworthy that several parallels have been drawn between APP and SREBPs. Like APP, membrane-associated proteolytic cleavage of the SREBPs is also highly dependent on sterol membrane content, as cholesterol inhibits proteolytic processing of SREBP-1 and SREBP-2 in cultured cells (Brown and Goldstein, 1997). As SREBPs and APP are the only proteins known to be cleaved within a membrane-spanning segment, it has been suggested that proteases involved in SREBP processing may be similar to γ-secretase (Brown and Goldstein, 1997). That both processes are regulated by membrane cholesterol content suggests that these proteins exhibit multiple functional similarities. However, the recently cloned *S2P* gene (the gene encoding a putative protease-cleaving SREBP) was found to belong to a class of metalloproteases that appear to be different from APP γ-secretase (Rawson et al., 1997).

In neurons, it has been suggested that apolipoprotein E may regulate phospholipid and cholesterol content (Igbavboa et al., 1997); apolipoprotein E–lipoprotein complexes enter neurons by binding to the low-density lipoprotein receptor, thereby increasing cellular cholesterol content. Therefore, cholesterol effects on APP and SREBPs may be mediated by apolipoprotein E and its receptor. This idea is supported by experiments in transgenic mice where circulating cholesterol and apolipoprotein E levels were inversely related to amounts of secreted sAPP and Aβ in the brain (Howland et al., 1998). Likewise, cholesterol depletion in hippocampal neurons decreased the generation of Aβ (Simons et al., 1998). Differential cholesterol and lipid uptake by apolipoproteins ε3 and ε4 (Poirier, 1994; Poirier et al., 1995) may also underlie effects of these proteins on sAPP release. In PC12 cells, nanomolar levels of apolipoprotein ε3 induced a rapid decrease in the secretion of sAPP but apolipoprotein ε4 increased secretion of sAPP (Wolozin et al., 1996). If, as in transgenic mice, sAPP and Aβ levels are regulated in the same manner, apolipoprotein ε4 would be expected to increase secretion of Aβ as well.

The actual mechanisms whereby cholesterol alters cleavage of membrane-bound proteins are not known. However, it has been suggested that increased cholesterol membrane content increases membrane rigidity (Yeagle, 1991), thereby decreasing the interaction of the various secretases with their substrate (Racchi et al., 1997). Altered activities of intrinsic membrane enzymes by changes in membrane lipid and cholesterol content set a precedent for this mechanism of control (Criado et al., 1982; Mitchell et al., 1990).

MECHANISM OF REGULATION

The mechanism by which various kinases regulate the secretory processing of APP is unknown. Although APP is phosphorylated by PKC (Suzuki et al., 1992), direct regulation by PKC through phosphorylation of the APP holoprotein is unlikely, as mutants lacking the phosphate acceptor residues are still cleaved and secreted after PKC activation (da Cruz e Silva et al., 1993; Efthimiopoulos et al., 1994; Hung and Selkoe, 1994). An alternative possibility is that protein kinases may also have a direct effect on the yet-to-be-identified secretases by altering their activity through phosphorylation. Indirect evidence for this has been suggested by studies using an APP construct resistant to proteolysis, which was no longer

susceptible to PKC-dependent regulation (Hung and Selkoe, 1994).

Secretase activity would be reduced, indirectly, if these enzymes and their substrates were compartmentalized and if access of APP to the secretase-containing compartment were somehow altered. Indeed, evidence is now accumulating that α-, β-, and γ-secretases are themselves localized differentially within the cell (Gabuzda et al., 1994; Checler, 1995). Given the short half-life of APP (Oltersdorf et al., 1990; Sisodia et al., 1990), even subtle changes in the rate of APP transport through these various secretase-containing compartments may have large effects on the net steady-state levels of sAPP and Aβ.

Several examples of kinases regulating APP processing indirectly by phosphorylating proteins involved in intracellular trafficking now exist. PKC and PKA have been shown to phosphorylate a tightly associated *trans*-Golgi network protein, thereby altering the formation of constitutive secretory vesicles containing mature APP (Xu et al., 1995, 1996). In a similar manner, PKC and PKA may exert their effects by phosphorylating substrates in the APP secretory pathway, which affects proteasome activity (Marambaud et al., 1996, 1997*a*,*b*). Presenilin-1, a protein that may associate with APP (Weidemann et al., 1997; Xia et al., 1997) and mutations of which alter the $A\beta_{1-40}$/$A\beta_{1-42}$ ratio (Borchelt et al., 1996; Lemere et al., 1996; Ancolio et al., 1997; Citron et al., 1997; Weidemann et al., 1997), has consensus sequences for ERK- and PKC-dependent phosphorylation and has recently been shown to be a substrate for PKC (Seeger et al., 1997; Walter et al., 1997). It is interesting that presenilin-1 has been localized to the nuclear membrane (Li et al., 1997) and the endoplasmic reticulum (Cook et al., 1996; Kovacs et al., 1996; Thinakaran et al., 1996), thus placing presenilins at sites where Aβ formation is thought to occur. At present, it is unknown whether phosphorylation of presenilins alters Aβ formation. Finally, PKC may alter α-secretase-mediated processing by prolonging the amount of time APP spends in cell surface caveolae. The caveolin-1 protein, a principal component of caveolae, is physically associated with APP, and overexpression of caveolin increased secretion of α-secretase processing whereas caveolin depletion prevented this cleavage event (Ikezu et al., 1998). As PKCα is a component of caveolae (Lisanti et al., 1994) and PKC activators antagonize caveolae internalization, PKC activation may prolong the time APP spends in cell surface caveolae, thereby increasing the time spent in contact with α-secretase.

AD AND APP PROCESSING: UNANSWERED QUESTIONS

It is widely hypothesized that the production and deposition of Aβ are early events in AD and may be a central pathological event in the disease process (Hardy, 1997; Selkoe, 1997). However, amyloid as a therapeutic target remains an elusive enemy. Although not necessarily aimed at decreasing the brain amyloid burden per se, many of the current therapeutic strategies for AD would likely decrease Aβ levels. These include (1) replacement of neurotransmitters (Mohr et al., 1994; Schorderet, 1995; Giacobini, 1996) and neurotrophins (Schorderet, 1995; Koliatsos, 1996) and hormones (Wickelgren, 1997), and (2) screening of antioxidants and nonsteroidal antiinflammatory agents (Schorderet, 1995; McGeer and McGeer, 1996; O'Banion and Finch, 1996; Münch et al., 1997). With advances in our understanding of signal transduction mechanisms underlying APP-processing events, the consequences of these treatment strategies on the ability of various cell types to process APP can be predicted. However, given the cell-specific nature of APP processing, the net effect on brain amyloid burden remains unknown.

The idea that altered signal transduction underlies neurodegenerative disorders such as AD has been previously suggested (Morrison and Hof, 1997). For example, it is thought that altered signal transduction may lead to increased brain amyloid burden (Nitsch et al., 1992; Selkoe, 1993; Buxbaum and Greengard, 1996). Moreover, reductions of synaptic inputs have been shown to strongly correlate with the clinical phenotype of AD (Dekosky and Scheff, 1990; Terry et al., 1991). Altered signaling is also apparent by the quantitative differences in either kinase (Jin and Saitoh, 1995) or phosphatase activity (Gong et al., 1993) between the normal aging brain and the AD brain. This is especially interesting given the putative role played by kinases in memory processes (Schwartz, 1993; Kornhauser and Greenberg, 1997). If tyrosine kinase activity is, as has been suggested (Ullrich and Schlessinger, 1990), the primary indicator of signal transduction, these effectors may offer targets for sophisticated drug development in degenerative disease processes (Levitzki and Gazit, 1995).

Perhaps the most intriguing lines of inquiry regarding regulation of APP processing are those addressing the role of signal transduction in regulating trafficking of APP. These studies are particularly interesting given the notion that different pools of APP exist in the cell that are differentially transported and cleaved. Trafficking, and therefore cleavage of APP, may be cell specific. For example, although polarized cells such as neurons do not express significantly more cellular APP, they appear to produce proportionally more intracellular Aβ (Wertkin et al., 1993; De Strooper et al., 1995; Turner et al., 1996; Hartmann et al., 1997; Wild-Bode et al., 1997). As secreted Aβ and intracellular Aβ appear to be generated by different mechanisms (De Strooper et al., 1995; Hartmann et al., 1997; Tienari et al., 1997; Wild-Bode et al., 1997), cell-signaling events controlling the production of these two pools of Aβ may be distinct. Underlying the effects of trafficking on APP regulation may be subcellular localization of secretase isoenzymes. Some precedent for the existence of differential localization of isoenzymes already exists for the γ-secretases. Given

that $A\beta_{1-40}$ and its more amyloidogenic counterpart $A\beta_{1-42}$ are produced in different compartments (Cook et al., 1997; Hartmann et al., 1997), it is conceivable that the γ-secretase isoenzymes that produce them are regulated differentially. Finally, different pools of $A\beta$ may be produced by distinct secretases that cleave either constitutively or in a regulated manner. The idea that $A\beta$ production can be regulated in both a temporal and spatial fashion demands further consideration as a means of reducing the brain amyloid burden through activation of signal transduction cascades.

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