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Cellular and molecular basis of ß-amyloid precursor protein metabolism

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TABLE OF CONTENTS

1. ABSTRACT

In molecular neurobiology, perhaps no molecule has been as thoroughly examined as Alzheimer's ß-amyloid precursor protein (ßAPP). In the ten years since the cDNA encoding ßAPP was cloned, the protein has been the subject of unparalleled scrutiny on all levels. From molecular genetics and cellular biology to neuroanatomy and epidemiology, no scientific discipline has been left unexplored - and with good reason. ß-amyloid (Aß) is the main constituent of the amyloidogenic plaques which are a primary pathological hallmark of Alzheimer's disease, and ßAPP is the protein from which Aß is cleaved and released. Unraveling the molecular events underlying Aß generation has been, and remains, of paramount importance to scientists in our field. In this review we will trace the progress that has been made in understanding the molecular and cellular basis of ßAPP trafficking and processing, or alternatively stated, the molecular basis for Aß generation. Imperative to a complete understanding of Aß generation is the delineation of its subcellular localization and the identification of proteins which play either direct or accessory roles in Aß generation. We will focus on the regulation of ßAPP cleavage through diverse signal transduction mechanisms and discuss possible points of therapeutic intercession in what has been postulated to be a seminal molecular step in the cascade of events terminating in the onset of dementia, a loss of neurons, and tragically, eventual death from Alzheimer's disease.

2. INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly, accounting for approximately 50% of the typical, late-onset cases of dementia. AD is characterized clinically by the insidious onset and inexorable progression of dementia and pathologically by the abnormal accumulation of neuritic plaques and neurofibrillary tangles in vulnerable brain regions. Plaques consist of deposits of 40-43 amino acid peptides called ß-amyloid (Aß) (1,2) which is derived through proteolytic processing of the ß-amyloid precursor protein (ßAPP). The neurofibrillary tangles are composed largely of hyperphosphorylated twisted fillaments of a cytoskeletal protein, tau (3). Evidence causally linking ßAPP to AD was provided by the discovery of mutations within the ßAPP coding sequence that segregated with disease phenotypes in autosomally dominant familial AD (FAD) (4-6). Although documented FAD is rare (<10% of all AD), the characteristic clinicopathological features, amyloid plaques, neurofibrillary tangles, synaptic and neuronal loss, neurotransmitter deficits and dementia are apparently indistinguishable when FAD is compared with typical, common, "non-familial," or sporadic AD.

In addition to mutations within ßAPP, there are now many reported pedigrees in which early onset FAD segregates with two other genetic loci. It is now accepted that mutations in the presenilin 1 gene on chromosome 14 (7) and the presenilin 2 gene on chromosome 1 (8) also cause FAD. PS mutations cause AD by altering ßAPP metabolism: specifically they cause a selective presenilin mutations and cause a selective increase in the production of the 42 amino acid form of Aß (Aß42) (9-10). This increase of Aß42 occurs in the plasma and in media from cultured skin fibroblasts derived from patients carrying these mutations, and this rise can be detected presymptomatically (10). This elevation is significant because Aß42 is more highly amyloidogenic (11,12) and is believed to form the core of the amyloidogenic plaques (13,14), despite being produced far less abundantly than Aß40, the major amyloid species generated by all cells. Evidence from recent studies strongly support this hypothesis. For instance, cells transfected with mutant PS1 cDNA secrete higher levels of Aß42 (9,15); similarly, transgenic mice expressing mutant PS1 show increased Aß42 levels in their brains (16). This data from humans,

transgenic mice and cells expressing mutated ßAPP and PS1 has only further strengthened an integrated amyloid cascade hypothesis; diverse molecular anomalies all lead to the enhanced production and aggregation of Aß peptides. Furthermore, it was already appreciated that patients with Down's syndrome or trisomy 21 (ßAPP is localized to chromosome 21) invariably develop AD pathology by age 50 and show Aß42 immunoreactive plaques by age 12 (17). It is the goal of this review to introduce the various hypothesis regarding the metabolism of ßAPP and the generation of Aß - and to suggest how interrupting or delaying this early and invariable event in AD may be therapeutically feasible.

3. DISCUSSION

3.1. ßAPP Structure Gives Clues to its Function

The partial purification of amyloid peptides from the microvasculature of AD brains by Glenner and Wong began the modern era of AD research; within three years, the sequence of this small peptide was successfully used to clone the full length cDNA of ßAPP (18). The deduced amino acid sequence of ßAPP predicts a type 1 transmembrane protein encoded by alternatively spliced mRNA resulting in isoform diversity. Isoforms of 751 and 770 amino acids include a protease inhibitor domain in the extracellular region of the ßAPP molecule (19,20), however, an isoform of 695 amino acids missing this domain is the main isoform found in neurons. Physiological roles of ßAPP have been suggested to include transmembrane signal transduction (21) and calcium metabolism (22). However, a definitive cell biological role for ßAPP and its metabolites has yet to be ascribed. Potential functional motifs in ßAPP have been identified by the presence of consensus sequences. Some of these motifs suggest a role in metal ion binding (23), heparin binding (24), cell adhesion (25) and/or as a receptor for a currently unrecognized ligand. Some have even suggested that ßAPP plays a role in regulating cell growth (26). Despite these varied reports, ßAPP knockout mice do not have an obvious disease phenotype (27) further confounding the search for a normal physiological role for ßAPP. A family of novel ßAPP-like proteins (APLPs) were discovered (28, 29) suggesting that ßAPP may be a member of a larger family of related molecules, however, APLPs lack the Aß domain and therefore cannot serve as precursors to Aß.

3.1.1 ßAPP is Trafficked and Processed Through the Secretory Pathway

ßAPP is initially synthesized and cotranslationally inserted into membranes in the endoplasmic reticulum (ER). Although it has been recently suggested that Aß42 can be generated within a compartment early in secretory pathway (30), such as the ER or early Golgi, a majority of ßAPP molecules exit the ER uncleaved. Once exiting the ER, ßAPP molecules are transported to the Golgi apparatus where the majority of ßAPP molecules are found under steady state conditions. Within the Golgi apparatus, ßAPP is significantly modified by N- and O-glycosylation, tyrosyl sulfation, and sialylation (31, 32). ßAPP is also phosphorylated in both the extracellular and intracellular domains (2, 33). In addition, some ßAPP molecules are chondroitin-sulfated in their ectodomains (34).

The proteolytic processing events underlying ßAPP metabolism have been the subject of intense scrutiny. The first proteolytic cleavage product of ßAPP processing to be definitively identified by purification and sequencing was a fragment which results primarily from a cleavage event within the Aß domain. The entire amino-terminal fragment of the ßAPP extracellular domain, termed s-ßAPP or ßAPPs, is released into the medium of cultured cells (25, 32, 35, 36) and into the cerebrospinal fluid, leaving a nonamyloidogenic carboxyl-terminal fragment associated with the cell (37) . This pathway was designated the α-secretory pathway after the still unidentified enzymatic activity termed the α-secretase which cleaves the protein in this manner. Thus an important processing event in the biology of ßAPP acts to preclude Aß formation by cleaving ßAPP within the Aß domain.

3.1.2. α-Secretase Cleaves ßAPP at the Cell Surface

An increasing number of secreted proteins are derived from integral plasma membrane proteins in which the secretory event is actually post-translational hydrolysis from the cell surface. This shedding or solubilization involves either an endoprotease or a phospholipase. ßAPP appears to belong to this large family of proteins including membrane receptors, receptor ligands, ectoenzymes, leukocyte antigens, and cell adhesion molecules in which an enzymatic event at the cell surface may be physiologically relevant (38). Indeed, it was demonstrated that α-secretase is highly active at the plasma membrane (39). The α-cleaved ßAPP molecule may have physiological relevance that extends beyond merely preventing Aß formation: both *in vitro* and *in vivo* effects of ßAPPs on cytoprotection and cell neurotrophy have been documented (26). Nevertheless, ßAPP knockout mice failed to exhibit any striking neuronal phenotype (27).

Amino acid substitutions around the cleavage site do not alter ßAPP cleavage suggesting that α-secretase cleavage relies more upon the distance from the membrane than on the primary amino acids (39). Progressive deletion of the extracellular juxtamembrane amino acids demonstrated that only 11 amino acids of the natural sequence were required to sustain ßAPP cleavage at the cell surface (39). The cell regulation of the α-secretase pathway will be examined later in this review; simply, however, the activation of Protein Kinase C (PKC) through diverse routes, strongly stimulates α-secretase cleavage, an event which can be ascribed either to indirect effects of PKC on the cellular localization of ßAPP and the α-secretase or direct effects on the enzymes themselves through altering their state of phosphorylation (40-42).

3.1.3. Alternative ßAPP Metabolism Gives Rise to the Amyloid-ß Peptide

Until 1992, Aß was by general consensus, an abnormal metabolite derived from ßAPP. Soluble Aß was not initially detected in brain, cerebrospinal fluid or from cell culture systems due to the limitations imposed by antibody reagent sensitivity, and difficulties due to the conformation, aggregation and low endogenous levels of Aß produced by cells. Most early information regarding ßAPP metabolism was instead derived from experiments

studying the carboxy-terminal fragments of ßAPP. High-level overexpression of human ßAPP using recombinant vaccinia viruses (43), baculoviruses (44), or stable transfections in association with supraphysiological levels of protein phosphorylation (40) resulted in the accumulation of heterogeneous C-terminal ßAPP fragments. These studies provided abundant evidence in support of the hypothesis that an alternative cleavage of ßAPP molecules could occur in which C-terminal fragments not cleaved by the α-secretase, but rather containing the complete Aß sequence, remained as potential precursors from which Aß could be further derived. This cleavage activity was designated the ß-secretase. Sequencing of these putative ß-cleaved C-terminal species did confirm their definitive identification as candidate intermediaries in the pathway to Aß deposition (45).

This non α-secretase mediated cleavage immediately prompted the search for alternative intracellular routes through which ßAPP could be trafficked and metabolized into Aß-generating precursors. The existence of an alternative secretory pathway for ßAPP was difficult to prove. Several groups began searching for the secretory compartments, within which ßAPP was metabolized and Aß generated. A favored hypothesis was that Aß was generated within an acidic intracellular compartment in which the pH would permit maximal activity of the secretases. Vesicle neutralizing agents such as chloroquine and ammonium chloride were applied to cultured cells, facilitating the recovery of full length ßAPP and an array of C-terminal fragments (41, 46, 47) - a population of fragments similar to that recovered from purified lysosomes (48).

The possibility that an endosomal-mediated metabolism of ßAPP was important in the generation of Aß peptides was strengthened by the discovery of a clatherin-coated vesicle (CCV) targeting motif in the LDL receptor. This motif, NPXY, was required for proper internalization of the LDL receptor and was also present on the cytoplasmic tail of ßAPP (49) . The co-purification of ßAPP with CCVs (50) suggested the possibility that ßAPP may be a receptor for a yet undiscovered or recognized ligand. These experiments defined a second normal processing pathway for ßAPP that does not involve a cleavage event within the Aß domain of ßAPP. This work supported the hypothesis that ßAPP can be reinternalized from the cell surface and targeted to the endodome/lysosome pathway where it may contribute, in part, to the generation of ßAPP-derived fragments containing Aß (51).

3.2 Intracellular Generation of Aß

Hypotheses regarding the abundance of an actual 4 kD Aß species were based upon the belief that Aß was an abnormal and toxic species - a product restricted to the brains of aged or demented humans. It was believed that a cell would need to be significantly injured for a protease to gain access to the interior of the membrane bilayer to cleave ßAPP and generate Aß. This concept was soundly refuted when several groups discovered that a soluble Aß species was detectable in body fluids from various species (52) and in the conditioned medium of cultured cells (53) but was not detectable in the lysates of cultured cells. Cultured cells were shown to release the 4 kD Aß peptide into medium in high picomolar to low nanomolar concentrations (53, 54). In addition to cultures of neuronal-like cell lines, cortical cultures derived from the brains of fetal mice, rats and humans all secrete Aß peptides in quantifiable levels (52, 55) and Aß can be similarly detected from the cerebrospinal fluid of humans and rats (52).

The subcellular processing pathway involved in Aß generation was also uncertain, but as the subject of innumerable studies over the last six years, the generation of Aß through either endocytic or exocytic pathways, can each be rigorously defended. One shared feature of the two pathways is the unquestioned reliance of ßAPP passage through an acidic intracellular compartment. In support of this, treatment of ßAPP-expressing cells with Bafilomycin A (Baf A), a vacuolar-type H^+-ATPase inhibitor, abolished Aß generation (56). In an attempt to detect Aß in a distinct compartment, Haass *et al.* purified late endosomes/lysosomes from ßAPP-transfected cells that released substantial amounts of Aß, yet they found no Aß within those structures. They interpreted those results to mean that Aß production requires maturation of ßAPP through the Golgi and processing in an acidic compartment other than lysosomes.

Xu *et. al.,* confirmed this hypothesis by demonstrating using a cell-free system, that Aß can be generated from the trans-Golgi network (TGN) in the absence of vesicle formation (57) . This result is consistent with previous findings because the TGN, like the endosome, provides the ideal acidic environment for secretase activity and Aß generation. Thinakaran *et al.*, also demonstrated ß-secretase activity within the late Golgi using cells expressing ßAPP harboring pathogenic point mutations (58) . The late Golgi has many known cellular functions which support it as a likely site for Aß generation. As we previously mentioned, under steady state conditions, ßAPP is principally localized within the TGN. It has been appreciated that physiologically relevant processing occurs here as well: prohormone processing in the TGN is a well-characterized phenomenon (59). Finally, the TGN is a known sorting station for many lysosomal enzymes and plasma membrane proteins. This fact supports the idea that if ßAPP and relevant secretases are co-localized with the TGN, this late secretory compartment could be a likely site of Aß generation. Interestingly, the implication of the TGN as a major location for Aß production raises the possibility that the excess production of Aß seen in AD may be due to a defect in the general secretory/processing apparatus of neurons which could occur after many decades of unfaltering function.

3.2.1. Presenilin Mutations Support the Aß Hypothesis

The discovery of mutations within ßAPP lent support to those who believed the accumulation of Aß was a crucial event in AD pathogenesis because it linked, for the first time, mutations in ßAPP, with early onset forms of AD. All of the ßAPP mutations which have been studied in transfected or in primary cells lead to an increase of Aß secretion, particularly of Aß42 (60, 61) . Strikingly, Aß levels within the plasma of those families harboring these

mutations is significantly raised even well before symptoms of AD begin to manifest (10) . For this reason, there is little doubt that these mutations cause AD, in these patients, by providing an enhanced cleavage site upon which the proteolytic secretases can act more quickly or efficiently. Of all the known genetic causes of FAD, however, ßAPP missense mutation at or near the sites of endoproteolysis are a relatively rare cause of familial AD.

Two other genetic loci known to be important in the etiology of early onset Alzheimer's disease cause the majority of FAD: the presenilin 1 (PS1) gene on chromosome 14 (7) and the presenilin 2 (PS2) gene on chromosome 1 (8). These genes encode polytopic membrane proteins with high homology to one another. The functions of these proteins are not understood very well, but several clues have been provided. Homologues of the PS proteins have been discovered in Caenorhabditis elegans: mutations in spe-4 lead to deficits in spermatogenesis through a disruption of protein trafficking in the Golgi (62) and sel-12 mutations can disrupt a crucial intracellular signalling mechanism called Notch signalling (63). PS1 can rescue sel-12 mutants (64), further underscoring their functional similarities. Finally, PS1 knockout mice exhibit developmental deficits similar to those observed in mice in which other components of the Notch signalling pathway have been mutated or knocked out (65). Extraordinary time and emphasis has been placed on studying the presenilins because of the belief that by understanding their functions, we will gain insight into the role that ßAPP plays, both as a normally functioning protein and as a crucial protein within the unsolved cascade of AD pathogenesis.

While the presenilin molecules unarguably influence ßAPP metabolism, a direct molecular interaction between the two molecules, while proposed (66) , has been difficult to convincingly prove (67), keeping the answer to how that influence is achieved on a molecular level, elusive. Yet, the downstream effects of presenilin mutations on ßAPP metabolism have been extensively documented. Plasma from individuals with PS1 mutations provided the first evidence of this link when it was shown that their Aß42 levels were significantly elevated (10). Similarly, brains, and fibroblasts from these patients all demonstrate this same significant elevation (10, 68). When transgenic animals or cultured cells bearing mutated PS genes were analyzed and compared with animals or cells bearing wild type PS genes, the mutant counterparts all produced a consistent reinforcing result: expression of mutant PS1 effects ßAPP metabolism which results in significantly elevated amounts of the highly amyloidogenic peptide, Aß42 relative to Aß40 (9, 16) .

These results provided powerful support for placement of the presenilin proteins within the framework of the amyloid cascade hypothesis. Mutations in the presenilin proteins could now join Down's syndrome and ßAPP-linked familial AD as directly causing AD pathogenesis through an increase in Aß42 generation.

3.3. Cellular Biology of APP Trafficking

The hypothesis that PS1 may influence Aß42 production via an alteration of ßAPP-trafficking has generated increasing enthusiasm among AD researchers. The subcellular localization of PS1 to the endoplasmic reticulum, and to a lesser extent, the Golgi, together with the evidence that PS1 mutations may regulate, or at least strongly influence Aß42 generation, led to the search in recent years for Aß peptides within the cell - specifically within an early compartment of the secretory pathway, consistent with PS1's localization. In a series of reports, several groups were able to positively identify Aß42 in the ER (69) , the first such novel report of an intracellular Aß species since Aß40 was identified within the late Golgi. This led to the immediate speculation that Aß40 and Aß42 were generated from ßAPP in distinct intracellular compartments: Aß42 first in the ER and Aß40 later in the Golgi - either de novo from full length ßAPP, or from a sequential proteolysis of the two C-terminal amino acids from Aß42. Either scenario provides a satificatory explanation for why cells produce greater quantities of Aß40 versus Aß42 since ßAPP, when not on the cell surface, resides primarily within the Golgi apparatus. However, secretion of ER-generated Aß42 remains to be documented.

The hypothesis that PS1 can regulate ßAPP metabolism received perhaps its most validating experimental evidence from a recent study examining Aß generation in cells cultured from the hippocampus of PS1 knockout mice and infected with a recombinant Semliki Forest virus encoding human ßAPP (70). These cells produced 80% less Aß (both Aß40 and Aß42) than did control cells which had been infected with the same virus. The authors speculate that PS1 may actually be activating the enzyme (γ-secretase) which gives rise to the C-terminal cleavage of the Aß peptide. This model has precedence: the SREBP-(Sterol-regulatory element binding protein) cleavage activating protein (SCAP) was demonstrated to facilitate what is suspected to be intramembranous cleavage of SREBP (71) - an event which may closely parallel γ-secretases intramembranous cleavage of ßAPP. These proteins also share a residence in the ER and a predicted structure of 6-8 transmembrane domains. If this model proves correct, it would assume that mutations in PS1 would act to disrupt PS1's normal function and lead somehow to increased cleavage after residue 42 of Aß, within full length ßAPP, although preliminary evidence suggests this is not mechanistically accurate.

While that possibility remains intriguing, an alternative hypothesis which we favor, bestows a crucial role upon PS1 in the sorting or trafficking of ßAPP, not its proteolysis. In this hypothesis, mutations in PS1 would retard the normal sorting or trafficking of ßAPP at an early stage within the secretory pathway. If normal PS1 is a sorting or trafficking molecule responsible for ensuring ßAPP's timely and properly directed exit from the ER towards the Golgi, by knocking out PS1 entirely, one might expect ßAPP to languish in the ER, perhaps resulting in extremely elevated levels of Aß42. Alternatively, this may cause ßAPP to enter the traditionally viewed anterograde bulk protein trafficking pathway through the Golgi to the cell surface, resulting in extremely reduced levels of both Aß42 and Aß40 due to the limited exposure of ßAPP to the secretases in the ER/Golgi. A trafficking hypothesis is exciting because it may not only account for

the effects of PS1 mutations or knocking out PS1 entirely, on ßAPP metabolism, but it may also account for the lethality of PS1 knockouts, and aid in explaining the phenotype of mutations in PS1 homologues in C. elegans, that is, by retarding the proper sorting and trafficking of membrane proteins to cell surface during development.

3.3.1. Signal Transduction Regulates ßAPP Trafficking

Although the evidence that the mutated presenilin proteins may cause AD by altering ßAPP metabolism is new, the idea that ßAPP metabolism is a highly regulatable phenomenon, is not novel. The addition of phorbol esters to cultured cells leads to a stimulation of the α-secretase pathway, that is, an increase in ßAPPs secretion and membrane-bound C-terminal fragments cleaved within the Aß domain, and a decrease in secreted Aß. Studies showing this (40-42) provided evidence that the α-secretase pathway was enhanced by protein phosphorylation through activation of protein kinase C (PKC). It was demonstrated that PKC can phosphorylate serine 655 of ßAPP, both on synthetic peptides containing this phospho-domain (33), and utilizing in vitro assays using permeabilized PC12 cells (72) .

These results were compelling because they suggested that PKC may act to directly phosphorylate ßAPP, perhaps in response to direct depolarization of the neuron itself. In support of this hypothesis, it was demonstrated that when muscarinic acetylcholine receptors were transfected into PC12 cells, receptor agonists led to the same increase in ßAPPs, but could not elicit that response when staurosporine was present simultaneously (73). Alternatively, it was possible that PKC, although able to directly phosphorylate ßAPP, actually exerted its effects of ßAPP metabolism through phosphorylation of an entirely different target such as the α-secretase or other phospho-proteins. This latter hypothesis revealed itself to be true following the demonstration that PKC can stimulate ßAPPs secretion even when the phosphorylation sites on ßAPP are themselves mutated, or when the entire cytoplasmic domain has been deleted (74) . So although it was clear that PKC played a crucial role in ßAPP metabolism, alternative hypotheses were needed to explain PKC's potent anti-amyloidogenic effects.

We favor a mechanistic explanation in which PKC phosphorylates a TGN phosphoprotein resulting in the redistribution of ßAPP from theTGN to the cell surface. The subcellular localization of ßAPP within the TGN and α-secretase in the plasma membrane support this hypothesis, which we confirmed in studies in which we reconstituted the formation of ßAPP-containing vesicles from the trans-Golgi network (TGN) in a cell-free system (72, 75) . In these studies we demonstrated that PKC does, in fact, redistribute ßAPP from the TGN towards post-TGN compartments where it can undergo α-secretase processing. We also provide evidence that protein kinase A (PKA) can similarly reduce Aß formation and stimulate the release of constitutive secretory proteins from the TGN via a mechanism involving enhanced formation of post-TGN transport vesicles (75) . Although PKC and PKA converge on the level of formation from the TGN of ßAPP-containing vesicles, additional evidence indicates that the regulatory mechanisms involved are distinct; the effects of PKC and PKA are additive, suggestive of independant substrate sites.

An alternative hypothesis which may help explain PKC's effect on α-secretase cleavage involves the multicatalytic complex of the proteasome. It was demonstrated that proteasome inhibitors can drastically decrease PDBu-stimulated ßAPPα secretion and may play a role in the basal secretion levels of ßAPPs (76) . Unfortunately from a therapeutic standpoint, PKC activation can stimulate ßAPP transcription, indirectly increasing Aß formation; therefore, it remains an important goal to definitively identify phosphorylation targets for the kinases if we are to have a realisitc hope of therapeutically intervening in AD pathogenesis via stimulation of this anti-amyloidogenic pathway.

3.3.2 Estrogen Regulates ßAPP Trafficking

Increasing epidemiological evidence has suggested that post-menopausal women receiving estrogen replacement therapy have both a delayed onset and reduced risk for developing AD (77, 78) . The central role of Aß in AD pathogenesis together with the indication that estrogen replacement therapy may prevent AD, suggested to us that one mechanism of action of estrogen in the brain might be to modulate ßAPP metabolism and Aß generation. We recently reported that physiological concentrations of estrogen reduce neuronal Aß generation (79) which is in agreement with prior data from our laboratory demonstrating that estrogen increased secretion of ßAPPs (80) . Importantly, these changes were not due to changes in ßAPP transcription, nor to alteration of PS1 levels. These data suggest one attractive mechanism through which estrogen may protect against AD.

The anti-degenerative effects of estrogen have received increased attention in recent years. In addition to its anti-amyloidogenic properties, estrogen may also modulate (i) basal forebrain cholinergic activity and integrity (81), (ii) dendritic plasticity (82) , (iii) NMDA receptor density (83) , and (iv) neurotrophin signalling (84) , as well as play a role in the prevention of oxidative toxicity due to glutamate, free radicals and Aß (85). Although it plays many roles in the brain, the Aß-reducing effect of estrogen strongly supports the notion that one relevant neuropharmacological activity of estrogen is to reduce Aß generation, and that such an activity contributes to the ability of estrogen replacement therapy to protect against AD.

The cell biological basis for estrogen's regulation of Aß formation remains to be eludcidated. It has been demonstrated that estrogen can affect the morphology of the trans-Golgi network by enhancing the biogenesis and trafficking of post-TGN constitutive secretory vesicles and granules (86, 87) . Since formation of Aß in the TGN and its export from that organelle are regulated by signal transduction pathways, we hypothesize that estrogen promotes egress of ßAPP from the TGN and thereby reduces the local concentration of ßAPP available as a substrate for Aß production.

4. PERSPECTIVE

The last ten years have provided tremendous insight into the molecular mechanism which are

responsible for ßAPP metabolism. In particular several signal transduction pathways have been identified which can regulate ßAPP metabolism to reduce Aß generation, but a major goal remains the elucidation of compounds which while effective at reducing Aß generation are therapeutically safe. One such anti-amyloidogenic candidate which has received attention is estrogen, although the molecular basis through which it exerts these amyloid reducing effects has yet to be defined. Reducing the amyloid burden in the brain, and therefore the progression of AD may eventually prove analagous to lowering one's cholesterol to prevent heart disease. As scientists, we are perched tantalizingly close to fully understanding the molecular basis for ßAPP metabolism and Aß generation, however, that will fulfill only half our mission; as healers we have much work to do before we can offer AD patients realistic hope that their disease will become anything other than an inexorable decline into irreversible dementia.

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