

Hypothesis

Alzheimer's α -secretase may be a calcium-dependent protease

Ming Chen^{a,b,*}

^aDepartment of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, FL 33612, USA

^bMedical Research Service (151), Bay Pines VA Medical Center, 10,000 Bay Pines Blvd., St. Petersburg, FL 33744, USA

Received 22 July 1997

Abstract Proteolytic processing of β -amyloid precursor protein (APP) is believed to be fundamental to the understanding of Alzheimer's disease. The identities and the regulatory elements of the proteases involved in the process, known as $\alpha/\beta/\gamma$ secretases, are unclear. In this study, by examining reported data, we found some indications suggesting that the putative α -secretase may be a calcium-dependent protease, and that this enzyme may play a primary role in the regulation of APP processing. Based on this, we proposed a model for the membrane orientations of the secretases for further discussions.

© 1997 Federation of European Biochemical Societies.

Key words: Alzheimer; β -Amyloid; Secretase; Calcium; Calpain

1. Introduction

Alzheimer's disease (AD) is characterized by the presence of amyloid plaques and neurofibrillary tangles in the brain. Amyloid plaques are mainly comprised of β -amyloid peptide (A β), which is a proteolytic product of β -amyloid precursor protein (APP). In normal individuals, processing of APP involves predominantly cleavage within the A β domain at the Lys¹⁶ site by a putative α -secretase, thereby precluding the formation of intact A β . In patients with AD, APP is excessively cleaved at both ends of A β by β - and γ -secretases [1,2]. The reasons for this are unclear. A number of proteases has been proposed to be candidates for these secretases thus far, but a consensus has not been reached over their enzyme classifications or regulatory elements [3,4]. This issue is important to the disease origin since the overproduction of A β precedes its damaging effects to the cells. Attempts to resolve this issue have been complicated primarily by: difficulties in tracing enzyme action *in situ*; complexity of protease specificity; and continuing discrepancies over the putative regulatory elements involved. In this study, we present evidence to suggest some characteristics of the secretases.

2. α -Secretase may be a calcium-dependent protease

We examined a number of reports regarding the regulation of APP processing in various cells. These studies have shown that secretion of soluble APP (APP_s) from cells can be affected by a wide variety of agents. By sorting through the reported data, we noticed that agents that increase APP_s secretion include the following: phorbol esters [5], thapsigargin

[5], calcium ionophores [6], carbachol [7], thrombin [8], interleukin-1 (IL-1) [9], acetylcholine (ACh) [9], basic fibroblast growth factor (bFGF) [10], lipopolysaccharide (LPS) [11], indomethacin [12], serotonin [13], estrogen [14], bradykinin [15], melittin [16], glutamate [17], electrical depolarization [18] and others. These agents have a wide diversity of biochemical and physiological effects. For example, glutamate and serotonin are neurotransmitters, and indomethacin is a cyclooxygenase inhibitor. Authors of these reports have attributed the observed effects on APP_s secretion to the best known actions of the respective agents, such as activation of neurotransmitter receptor, arachidonate metabolism, and so on. A common pathway has not been identified.

By examining the known actions of each agent individually, it came to our attention that the above 16 agents manifest at least one unifying effect. Each of them is able to elevate intracellular calcium levels, either through transmembrane influx or through release from intracellular stores [5–7,17–28]. This effect is well-known for some of these agents, i.e. calcium ionophore [6], phorbol esters [5], thapsigargin [5], carbachol [7], thrombin [19], ACh [21], bFGF [22], estrogen [26], bradykinin [27], glutamate [17] and electric depolarization [18]. The cellular effects of these agents are known to be mediated by calcium mobilization. For others, the calcium-elevating effect may not be as well-known, but has been experimentally confirmed (LPS [23], indomethacin [25], IL-1 [20], melittin [28], serotonin [24]). Whereas most of these studies have not considered the calcium effect to be the primary factor affecting APP_s secretion, Buxbaum et al. [5] have pointed out that the effect of the agents (phorbol esters, IL-1, thapsigargin and carbachol) on APP_s secretion is due to calcium changes and can be independent of protein kinase C activation.

Calcium ionophore has also been reported to increase A β secretion [29]. Since a corresponding decrease of APP_s has not been observed in this report, the finding may not necessarily conflict with the other reports (e.g. [6]). It is possible that this effect on A β could be due to the highly sensitive detection by radioimmunoprecipitation of minor A β changes induced by a slight activation of cellular exocytosis under the conditions used. Indeed, the increase of A β is only associated with low concentrations of the ionophore (0.1–0.5 μ M) and higher concentrations of the ionophore (1–5 μ M) have resulted in a decline of A β [29]. Presumably, a simultaneous determination of both APP_s and A β released from the same cells will resolve this discrepancy.

In concert with the effect of the above 16 calcium-elevating agents, another group of agents that can offset or oppose the calcium effects under certain circumstances, such as cAMP, forskolin, cholera toxin [30], nordihydroguaiaretic acid (NDGA) [31], brefeldin A [32] and atropine [21], have been

*Fax: (1) (813) 398-9467. (b, mailing address.)
E-mail: michen@com1.med.usf.edu

observed to decrease APP_s secretion (cAMP, forskolin [33,34], cholera toxin [33], NDGA [12], brefeldin A [35], atropine [9]). The effects of these six agents on calcium are also known. NDGA, for example, suppresses calcium elevation induced by glutamate [31]. Brefeldin A exerts its effect by sequestering calcium to intracellular stores [32]. We and others have previously shown that the cAMP-related signal pathway is able to oppose the effects of the phospholipase C/Ca²⁺ pathway in some cell types (but not universally) [33,34,36,37]. It has been noted that the effects of some of the agents are concentration-dependent and sometimes cell-type specific. cAMP has also been observed to increase APP_s secretion [38], probably with a different time frame and under different conditions from those used in other reports [33,34]. Lee et al. [34] have shown that cAMP can also increase APP gene expression.

Based on the bulk of the experimental data, it can be inferred that there is a previously unknown (or not generalized) and intimate correlation between the free intracellular calcium levels and APP_s secretion. As calcium is an important factor regulating many life processes, this correlation may suggest some interesting features of APP processing.

Elevation of the intracellular calcium levels can elicit several responses [39]. Among those, neuronal excitability and activation of protein kinases are unable to uniformly and directly explain the observed effects in different cells. Also, cellular exocytosis can be activated by calcium and contribute to the increased release of intracellular proteins. Exocytosis itself, however, would not account for the increased secretion of APP_s that has been proteolytically cleaved from its precursor, the full-length APP.

It seems that the correlation between calcium levels and APP_s secretion would not be reasonably explained without the implication of a protease, which cleaves APP within the Aβ domain to produce APP_s. By definition this protease is the putative α-secretase, and the observed APP_s changes are a measure of the activity of that protease. Although most of these studies have not determined the actual cleavage site of APP_s, it is likely that its C-terminus predominantly ends at

Lys¹⁶ of Aβ. Direct biochemical sequencings of APP_s from a variety of cells including yeast have found only this form of APP_s as the predominant species [40–45]; thus other shorter or longer APP_s derivatives, being detected only by radio-sequencings, should be minor [46]. Sisodia and others have shown that α-secretase is a highly specific protease which acts only at the APP sites having certain distances from the membrane [47–49].

Thus, a reasonable explanation for the observed correlation between calcium levels and APP_s would be that the elevation of calcium activates, among other things, the putative α-secretase. Synergistic cooperation of this protease and cellular exocytosis would account for the observed increase of APP_s secretion. As such, the regulatory mechanism of α-secretase should be calcium-dependent. It is noteworthy that the regulatory mechanism of α-secretase by calcium is characterized by high sensitivity (some agents elicit a moderate Ca²⁺ transient), reliability (repeatedly observed under a diversity of experimental conditions: temperature, action duration and measurement methods) and ubiquity (found in a wide variety of cell types: peripheral and neuronal, natural and recombinant).

3. A model for membrane orientations of α-, β- and γ-secretases

3.1. α-Secretase

If α-secretase is a calcium-dependent protease, then membrane orientation of APP would suggest that this enzyme would have access to cytosol and meanwhile be able to reach the Lys¹⁶ site (α-secretase site) located at the outside surface of the plasma membrane (or in the lumen of Golgi apparatus where most secretory proteins are processed [50], including α-cleavage of APP [3,51] (Fig. 1). As such, it should be a membrane-associated (or transmembrane) protease, in line with a known feature of α-secretase [47]. Interestingly, such an unusual double membrane anchorage of both an enzyme and its substrate would impose a rigid steric restraint at the cleavage site relative to the membrane, due to hindrance of free move-

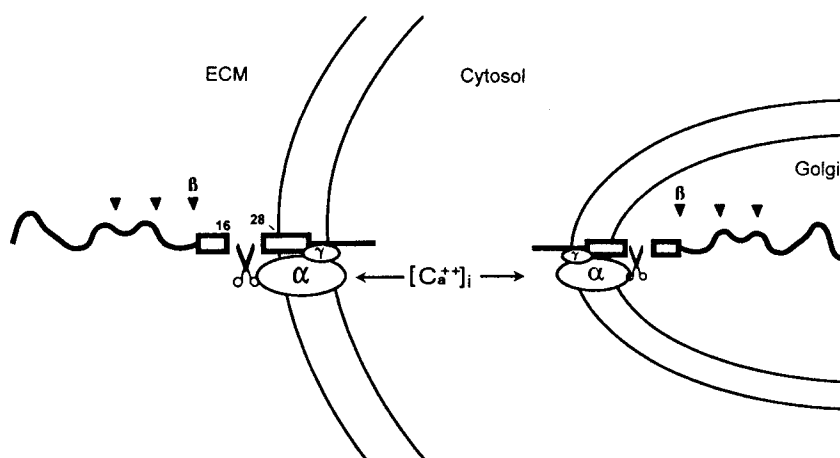


Fig. 1. A proposed model for membrane orientations and subcellular localizations of α-, β- (and β-like) and γ-secretases. The schematic representation is intended to show that: (1) APP, α- and γ-secretases are located in the membranes of both plasma and intracellular organelles (Golgi, endoplasmic reticulum or endosome/lysosome; only Golgi is shown), and α-secretase is a transmembrane protease regulated by calcium in the cytosol; (2) double membrane anchorage of both α-secretase and APP may impose a 'distance-over-sequence' preference during the cleavage of APP by α-secretase; (3) β-like secretases (β-secretase and other proteases that produce Aβ-containing fragments) (arrowheads) should be found in ECM or in the lumen of the intracellular organelles; (4) membrane association of γ-secretase and its likely contact by α-secretase may give it freedom of mobility and sensitivity to steric distortions (e.g. APP717 mutations) that may somehow cause γ-secretase 'swinging' along the hydrophobic residues of APP to produce longer Aβ (see Fig. 2).

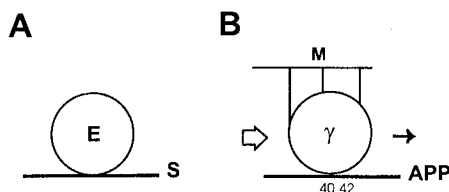


Fig. 2. Flexibility of γ -secretase. Compared with a normal pair of enzyme (E)/substrate (S)(A), the recognition of γ -secretase (γ) to APP (B) is additionally influenced by the binding of the enzyme to the membrane (M). Such a binding may render the enzyme more freedom of mobility relative to APP. It is possible that APP717 mutations (at A β 45 or A β 46) might alter the conformation of APP that normally holds γ -secretase at the A β 40 position, thereby allowing the enzyme to move.

ment. This can explain the elegant observations that α -secretase has a 'distance-over-sequence' preference during its catalytic action, in which the enzyme only acts at sites 12–13 amino acids from the membrane [47–49].

However, calcium-dependent proteases, to our knowledge, have not been considered thus far to be candidates for α -secretase, though such proteases (e.g. calpain) have been suggested as possible β -like secretases [4,52,53]. Whereas the discrepancy awaits clarification, here we consider some known features of β -secretase.

3.2. β -Like secretases

The membrane orientation of APP (Fig. 1) also suggests that the β -like secretases (including β -secretase and those cleaving at upstream sites of APP) should be located in the extracellular milieu (ECM) or in the lumen of intracellular organelles. Cytosolic enzymes cannot have access to the β -secretase site, or to the upstream sites of APP to generate the A β -containing fragments (8, 12 kDa or larger). Such fragments are physiologically produced [3,54] and would not be explained by postulating that a membrane damage leaks the cytosolic enzymes to ECM.

As extracellular enzymes, β -like secretases will not be sensitively regulated by calcium, because calcium concentration in ECM is 10 000-fold higher than in cytosol [50]. Sensitive regulation of an enzyme by a cellular element requires that the element and the enzyme should both be located in a compartment in which the element concentration is low (cytosol), and that there is a large gradient outside tending to drive the element across the membrane into that compartment. The high calcium levels in ECM cannot be significantly changed by any stimuli and thus β -like secretases would not explain the sensitive response of APP processing to the various agents.

Alternatively, β -like secretases can function in the lumen of endoplasmic reticulum (not shown) or Golgi apparatus (Fig. 1) where APP processing by β -secretase has been observed [51]. Calcium levels in these organelles are also high, since they are actually the intracellular calcium stores [32,50]. Also, β -processing of APP may occur in endosome/lysosome (not shown; but the same orientations as in Golgi) [55]. Calcium levels in these compartments are high as well, because they are formed by endocytosis of ECM (along with the internalized APP on the plasma membrane) or derived from Golgi, thus they are equivalent to ECM or Golgi in the ingredients [50].

Therefore, it appears that the β -like secretases in any of these locations are unlikely to be sensitively regulated by cal-

cium. Indeed, sensitive regulation of calpain, the best known calcium-dependent protease, has only been found to function physiologically in the cytosol or in the membrane-associated sites in touch with cytosol [56].

Can β -secretase be a transmembrane protease (similar to the model of α -secretase as we proposed)? Clearly, such a protease would first reach the Lys¹⁶ site (12 amino acids from the membrane) before it can reach the β -secretase site (28 amino acids from the membrane), if it can do so at all (Fig. 1). In other words, this protease would only be able to act as α -secretase rather than β -secretase, because the membrane-bound secretase has an out-reaching limit of only 12–13 amino acids from the membrane and no cleavage at the upstream sites has been found [47–49].

As calpain is membrane-associated when activated *in vivo*, an interesting question is: can calpain act as α -secretase? We have recently observed that non-amyloidogenic cleavage of APP at or near Lys¹⁶ in a cell-free system is mediated by a calcium-dependent protease (unpublished data). However, it is unclear whether calpain, as a cytosolic enzyme only known to be associated with the inner surface of the membrane, can 'protrude' to the cell surface. If calpain is uncertain even to act as α -secretase, then the possibility of it acting as β -secretase, or as other enzymes that have to reach the sites far upstream to produce the amyloidogenic fragments, could perhaps be ruled out.

3.3. γ -Secretase

γ -Secretase exhibits a peculiar flexibility in its substrate specificity. Since it can cleave the APP sites within the membrane under physiological conditions, it is reasonable to assume that it is also a membrane-associated protease (Fig. 1). In this model, the membrane-binding of γ -secretase would render the enzyme more freedom of mobility relative to APP (Fig. 2). Also, the membrane-associated α - and γ -secretases, acting on the same substrate, would be in a close proximity (likely contacted) within a short distance on APP between the two enzymes' acting sites (24 residues between Lys¹⁶ and Val⁴⁰) relative to the much larger masses of the proteases themselves (Fig. 1). Such a 'cluster' of the membrane proteins would be expected to be sensitive to any distortions (e.g., APP717 mutations [57]), which might exert their effects by somehow 'dislocating' γ -secretase (probably through a changed APP conformation which normally hold γ -secretase in place) from Val⁴⁰ to Ala⁴² [3,58]. Alternatively, a second γ -like secretase may be responsible for the genesis of A β 42; but the limited space of only two amino acids on APP may make it difficult to accommodate two separate enzymes (or several enzymes since A β 39,41,43 are also observed).

If α -secretase is calcium-dependent, then neither β - nor γ -secretase would be regulated by the same signal. Studies have shown that an increased APP_s level *in vivo* is accompanied by a decreased A β [59] and vice versa [60], suggesting that α -secretase and β/γ -secretases act reciprocally competing for the same pool of the full-length APP. This implies that neither β - nor γ -secretase could be a calcium-dependent protease.

4. α -Secretase may be the primary determinant in APP processing

If α -secretase is a calcium-dependent protease, then this would imply that α -secretase is regulated *in vivo* more sensi-

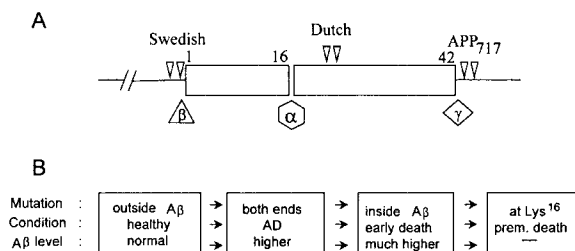


Fig. 3. APP mutations and their consequences. A: Reported APP missense mutations. The three secretase sites are denoted as α , β and γ , respectively. B: The relationships between the distances of the mutations relative to the α -secretase site, their clinical conditions and A β level changes in their hosts. Note that the distances of the mutations to the Lys¹⁶ site correspond to an increasing severity of the conditions and roughly to increasing A β levels. Mutations 'outside A β ' are inferred from the absence of such clinical cases; 'both end', the Swedish and APP717 types; 'inside A β ', the Dutch type. The A β levels are deduced from the reports (particularly in the brain vessels). Prem., premature.

tively than β/γ -secretases since Ca^{2+} is perhaps the most sensitive cellular signal that can directly regulate proteases (most proteases are unregulated though they can be affected by less sensitive factors such as gene expression and pH changes) [61]. It seems likely that α -secretase is primarily responsible for the effects of the various agents that affect APP processing [3,4] (see also above), and thus plays a dominant role in the regulation of APP processing *in vivo*.

This possibility appears to be consistent with the genetic studies. Familial AD phenotypes have been segregated with APP gene missense mutations. If α -secretase plays a primary role in APP processing, then mutations occurring at different 'distances' relative to the α -secretase site would be expected to result in different A β levels and distinct pathological consequences in their hosts (Fig. 3A). There may be such a phenomenon.

First, mutations are typically located at or near the A β domain (the Swedish and APP717 types at both ends of A β [57,62]) since this is where they can affect the outcome of the secretase cleavages. Logically, mutations occurring far outside the A β domain would not affect the cleavage as much and may not be pathological. Second, mutations that occur in closer proximity to Lys¹⁶ than the Swedish and APP717 types cause more severe conditions than the latter two types. Dutch-type mutations at Glu²² and Ala²¹ cause hemorrhage with a 'severe amyloidosis' in the blood vessels and 'early death' of their hosts in the fifth or sixth decade (typical AD patients have a normal life expectancy) [58,63,64]. Third, this trend would suggest that if mutations occur directly at the Lys¹⁶ site, they are probably fatal (lethal). In fact, such natural mutations have not been reported, but Moechars et al. [65] have shown that artificial mutations at this site have resulted in 'premature death' in transgenic animals, suggesting that such mutations are not tolerated in humans. The relationships between these mutations and their phenotypes are summarized in Fig. 3B. Notably, an increasing severity of the phenotypes also seems to correspond to the A β level (the A β level in the Lys¹⁶ mutant animals might be high as well and might have been deposited had the animals lived longer, because the mutant APP_s secretion is reduced by ~50% [65]).

Such a 'proximity rule' relative to α -secretase site, but not to the β/γ -secretase sites, suggests that: (1) AD phenotypes caused by known APP mutations are primarily due to the

perturbation of α -secretase function; (2) β/γ -secretase functions may not be as important in causing the phenotypes (if they were important, then Dutch types and Lys¹⁶ mutations, which have moved away from the β/γ -secretase sites, would cause lower levels of A β and less severe conditions than the Swedish and APP717 types); (3) the functional integrity of α -secretase on APP can have far-reaching consequences in the body.

Whereas the mechanisms and implications behind these phenomena can be profound, the leading role of α -secretase in the phenotype development, together with its regulation by calcium, appears to support the possibility that this enzyme is the primary determinant in the competition with its β/γ -counterparts for the outcome of APP processing.

If our interpretations of the reported data are correct, then they would imply that α -secretase should receive more attention in the attempts to understand the abnormal processing of APP in AD. The regulatory mechanism of this enzyme may have important implications in the understanding of AD pathogenesis [66]. As the issues are controversial, it is hoped that the views presented here, many of which are from a theoretical perspective, will induce further discussions about these issues.

Acknowledgements: This work is supported by the Medical Research Service of the Department of Veterans Affairs. I am grateful to Dr. Hugo L. Fernandez for his generous support in this work. I also wish to thank Drs. Michael Mullan, Jacques Durr, Katherine Siegler, Paul Gottschall, David Morgan, Michael Lombardo and Max Polo for helpful discussion, and Rob Giles for artwork.

References

- [1] Glenner, G.G. and Wong, C.W. (1984) *Biochem. Biophys. Res. Commun.* 120, 885–890.
- [2] Kang, J., Lemaire, H., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K., Multhaup, G., Beyreuther, K. and Muller-Hill, B. (1987) *Nature* 325, 733–736.
- [3] Selkoe, D.J. (1996) *J. Biol. Chem.* 271, 18295–18298.
- [4] Cordell, B. (1994) *Annu. Rev. Pharmacol. Toxicol.* 34, 69–89.
- [5] Buxbaum, J.D., Ruefli, A.A., Parker, C.A., Cypess, A.M. and Greengard, P. (1994) *Proc. Natl. Acad. Sci. USA* 91, 4489–4493.
- [6] Löffler, J. and Huber, G. (1993) *Biochem. Biophys. Res. Commun.* 195, 97–103.
- [7] Wolf, B.A., Wertkin, A.M., Jolly, Y.C., Yasuda, R.P., Wolfe, B.B., Konrad, R.J., Manning, D., Ravi, S., Williamson, J.R. and Lee, V.M. (1995) *J. Biol. Chem.* 270, 4916–4922.
- [8] Davis-Salinas, J., Saporito-Irwin, S.M., Donovan, F.M., Cunningham, D.D. and Van Nostrand, W.E. (1994) *J. Biol. Chem.* 269, 22623–22627.
- [9] Buxbaum, J.D., Oishi, M., Chen, H.I., Pinkas-Kramarski, R., Jaffe, E.A., Gandy, S.E. and Greengard, P. (1992) *Proc. Natl. Acad. Sci. USA* 89, 10075–10078.
- [10] Schubert, D., Jin, L.W., Saitoh, T. and Cole, G. (1989) *Neuron* 3, 689–694.
- [11] Monning, U., Sandbrink, R., Banati, R.B., Masters, C.L. and Beyreuther, K. (1994) *FEBS Lett.* 342, 267–272.
- [12] Kinouchi, T., Ono, Y., Sorimachi, H., Ishiura, S. and Suzuki, K. (1995) *Biochem. Biophys. Res. Commun.* 209, 841–849.
- [13] Nitsch, R.M., Deng, M., Growdon, J.H. and Wurtman, R.J. (1996) *J. Biol. Chem.* 271, 4188–4194.
- [14] Jaffe, A.B., Toran-Allerand, C.D., Greengard, P. and Gandy, S.E. (1994) *J. Biol. Chem.* 269, 13065–13068.
- [15] Nitsch, R.M., Slack, B.E., Farber, S.A., Borghesani, P.R., Schulz, J.G., Kim, C., Felder, C.C., Growdon, J.H. and Wurtman, R.J. (1993) *Ann. NY Acad. Sci.* 695, 122–127.
- [16] Emmerling, M.R., Moore, C.J., Doyle, P.D., Carroll, R.T. and Davis, R.E. (1993) *Biochem. Biophys. Res. Commun.* 197, 292–297.

- [17] Lee, R.K.K., Wurtman, R.J., Cox, A.J. and Nitsch, R.M. (1995) *Proc. Natl. Acad. Sci. USA* 92, 8083–8087.
- [18] Nitsch, R.M., Farber, S.A., Growdon, J.H. and Wurtman, R.J. (1993) *Proc. Natl. Acad. Sci. USA* 90, 5191–5193.
- [19] Rittenhouse, S.E., Sorisky, A., Kucera, G.L. and King, W.G. (1990) *Adv. Second Messenger Phospholipid Metab.* 24, 370–380.
- [20] Inokuchi, N., Zeki, K., Morimoto, I., Nakano, Y., Fujihira, T., Yamashita, U., Yanagihara, N., Izumi, F. and Eto, S. (1995) *Jap. J. Cancer Res.* 86, 670–676.
- [21] Heikkilä, J., Jansson, C. and Akerman, K.E. (1991) *Eur. J. Pharmacol.* 208, 9–15.
- [22] Chandrasekar, B. and Korc, M. (1991) *Biochem. Biophys. Res. Commun.* 177, 166–170.
- [23] Letari, O., Nicosia, S., Chiavaroli, C., Vacher, P. and Schlegel, W. (1991) *J. Immunol.* 147, 980–983.
- [24] Tani, A., Yoshihar, Y. and Mori, K. (1992) *Neuroscience* 49, 193–199.
- [25] Fiorucci, S., Santucci, L., Gresele, P., Luinetti, O. and Morelli, A. (1995) *Am. J. Physiol.* 268, G968–978.
- [26] Morley, P., Whitfield, J.F., Vanderhyden, B.C., Tsang, B.K. and Schwartz, J.L. (1992) *Endocrinology* 131, 1305–1312.
- [27] Watanabe, H., Takahashi, R., Zhang, X.X., Kakizawa, H., Hayashi, H. and Ohno, R. (1996) *Biochem. Biophys. Res. Commun.* 225, 777–784.
- [28] Reiter, Y., Ciobotariu, A., Jones, J., Morgan, B.P. and Fishelson, Z. (1995) *J. Immunol.* 155, 2203–2210.
- [29] Querfurth, H.W. and Selkoe, D.J. (1994) *Biochemistry* 33, 4450–4561.
- [30] Yang, C.M., Hsu, M.C., Tsao, H.L., Chiu, C.T., Ong, R., Hsieh, J.T. and Fan, L.W. (1996) *Cell Calcium* 19, 243–254.
- [31] Goodman, Y., Steiner, M.R., Steiner, S.M. and Mattson, M.P. (1994) *Brain Res.* 654, 171–176.
- [32] Ivessa, N.E., De Lemos-Chiarandini, C., Gravotta, D., Sabatini, D.D. and Kreibich, G. (1995) *J. Biol. Chem.* 270, 25960–25967.
- [33] Efthimiopoulos, S., Punj, S., Manolopoulos, V., Pangalos, M., Wang, G.P., Refolo, L.M. and Robakis, N.K. (1996) *J. Neurochem.* 67, 872–875.
- [34] Lee, K.K.R., Araki, W. and Wurtman, R.J. (1997) *Proc. Natl. Acad. Sci. USA* 94, 5422–5426.
- [35] Gabuzda, D., Busciglio, J., Chen, L.B., Matsudaira, P. and Yankner, B.A. (1994) *J. Biol. Chem.* 269, 13623–13628.
- [36] Cooper, D.M., Mons, N. and Karpen, J.W. (1995) *Nature* 374, 421–424.
- [37] Chen, M. and Stracher, A. (1989) *J. Biol. Chem.* 264, 14282–14289.
- [38] Xu, H., Sweeney, D., Greengard, P. and Gandy, S. (1996) *Proc. Natl. Acad. Sci. USA* 93, 4081–4084.
- [39] Müller, W.E., Hartmann, H., Eckert, A., Velbinger, K. and Forstl, H. (1996) *Ann. NY Acad. Sci.* 786, 305–320.
- [40] Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Culwell, A.R., Oltersdorf, T., McClure, D. and Ward, P.J. (1990) *Science* 248, 1122–1124.
- [41] Wang, R., Meschia, J.F., Cotter, R.J. and Sisodia, S.S. (1991) *J. Biol. Chem.* 266, 16960–16964.
- [42] Lowery, D.E., Pasternack, J.M., Gonzalez-DeWhitt, P.A., Zurcher-Neely, H., Tomich, C.C., Altman, R.A., Fairbanks, M.B., Heinrikson, R.L., Younkin, S.G. and Greenberg, B.D. (1991) *J. Biol. Chem.* 266, 19842–19850.
- [43] Ramabhadran, T.V., Gandy, S.E., Ghiso, J., Czernik, A.J., Ferris, D., Bhasin, R., Goldgaber, D., Frangione, B. and Greengard, P. (1993) *J. Biol. Chem.* 268, 2009–2012.
- [44] Zhang, H., Komano, H., Fuller, R.S., Gandy, S.E. and Frail, D.E. (1994) *J. Biol. Chem.* 269, 27799–27802.
- [45] Anderson, J.P., Esch, F.S., Keim, P.S., Sambamurti, K., Lieberburg, I. and Robakis, N.K. (1991) *Neurosci. Lett.* 128, 126–128.
- [46] Zhong, Z., Higaki, J., Murakami, K., Wang, Y., Catalano, R., Quon, D. and Cordell, B. (1994) *J. Biol. Chem.* 269, 627–632.
- [47] Sisodia, S.S. (1992) *Proc. Natl. Acad. Sci. USA* 89, 6075–6079.
- [48] Vitek, M.P., Blume, A.J. and Sonnenberg, J.L. (1993) *J. Biol. Chem.* 268, 16699–16705.
- [49] Maruyama, K., Kametani, F., Usami, M., Yamao-Harigaya, W. and Tanaka, K. (1991) *Biochem. Biophys. Res. Commun.* 179, 1670–1676.
- [50] Albert, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D. (1994) *Molecular Biology of the Cell*, 3rd edn., Garland, New York.
- [51] Haass, C., Lemere, C.A., Capell, A., Citron, M., Seubert, P., Schenk, D., Lannfelt, L. and Selkoe, D.J. (1995) *Nature Med.* 1, 1291–1296.
- [52] Siman, R., Card, J.P. and Davis, L.G. (1990) *J. Neurosci.* 10, 2400–2411.
- [53] Klafki, H., Abramowski, D., Swoboda, R., Paganetti, P.A. and Staufenbiel, M. (1996) *J. Biol. Chem.* 271, 28655–28659.
- [54] Estus, S., Golde, T.E., Kunishita, T., Blades, D., Lowery, D., Eisen, M., Usiak, M., Qu, X.M., Tabira, T., Greenberg, B.D. and Younkin, S.G. (1992) *Science* 255, 726–728.
- [55] Golde, T.E., Estus, S., Younkin, L.H., Selkoe, D.J. and Younkin, S.G. (1992) *Science* 255, 728–730.
- [56] Suzuki, K., Sorimachi, H., Yoshizawa, T., Kinbara, K. and Ishiura, S. (1995) *Biol. Chem. Hoppe-Seyler* 376, 523–529.
- [57] Chartier-Harlin, M.C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J. and Mullan, M. (1991) *Nature* 353, 844–846.
- [58] Hendriks, L. and Van Broeckhoven, C. (1996) *Eur. J. Biochem.* 237, 6–15.
- [59] Hung, A.Y., Haass, C., Nitsch, R.M., Qiu, W.Q., Citron, M., Wurtman, R.J., Growdon, J.H. and Selkoe, D.J. (1993) *J. Biol. Chem.* 268, 22959–22962.
- [60] Van Nostrand, W.E., Wagner, S.L., Shankle, W.R., Farrow, J.S., Dick, M., Rozemuller, J.M., Kuiper, M.A., Wolters, E.C., Zimmerman, J. and Cotman, C.W. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89, 2551–2555.
- [61] Bond, J.S. and Butler, P.E. (1987) *Annu. Rev. Biochem.* 56, 333–364.
- [62] Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B. and Lannfelt, L. (1992) *Nature Genet.* 1, 345–347.
- [63] Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., van Duinen, S.G., Bots, G.T., Luyendijk, W. and Frangione, B. (1990) *Science* 248, 1124–1126.
- [64] Hendriks, L., van Duijn, C.M., Cras, P., Cruts, M., Van Hul, W., van Harskamp, F., Warren, A., McInnis, M.G., Antonarakis, S.E. and Martin, J.J. et al. (1992) *Nature Genet.* 1, 218–221.
- [65] Moechars, D., Lorent, K., De Strooper, B., Dewachter, I. and Van Leuven, F. (1996) *EMBO J.* 15, 1265–1274.
- [66] Chen, M. (1997) *Med. Hypotheses* (in press).