Journal of Medicinal Chemistry

Volume 46, Number 22 October 23, 2003

Miniperspective

Human β-Secretase (BACE) and BACE Inhibitors

Varghese John,*,† James P. Beck,‡ Michael J. Bienkowski,‡ Sukanto Sinha,† and Robert L. Heinrikson‡

Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, California 94080, and Pfizer Corporation, 301 Henrietta Street, Kalamazoo, Michigan 49007

Received May 20, 2003

Introduction

β-Secretase (BACE) is a proteolytic enzyme involved in the processing of an integral membrane protein known as amyloid precursor protein, or APP. The proteolysis of APP by BACE, followed by subsequent C-terminal cleavage(s) by γ-secretase, results in the formation of the amyloid β (Aβ) peptide. Aβ is a neurotoxic and highly aggregatory peptide segment of APP that is the principal component of the neuritic plaque found in the brains of Alzheimer's disease (AD) patients. The amyloid hypothesis holds that the neuronal dysfunction and clinical manifestation of AD are a consequence of the long-term deposition and accumulation of 40−42 amino acid long Aβ peptides and that this process leads to the onset and progression of AD. Because of the apparent causal relationship between Aβ and AD, the so-called "secretases" that produce Aβ have been targeted for development of inhibitors that might serve as therapeutic agents for treatment of this dreaded and ever more prevalent disease. Herein will be discussed our current understanding of BACE, its role in the formation of neuritic plaques, and the known inhibitors of the enzyme.

Background

Following the discovery in the mid-1980s that Aβ is a principal component of the neuritic plaque of AD brain,[1] an intense search was launched to identify the enzymes responsible for liberating this 40−42 residue segment of APP. With no knowledge of their likely identity, these enzymes were called β- and γ-secretases, in reference to their sites of cleavage at the N- and C-terminal sites, respectively, in the Aβ region of APP. Finally, in 1999, several independent laboratories published evidence demonstrating that β-secretase is a new member of the pepsin family of aspartyl proteinases.[2−4] The identification of β-secretase as BACE was carried out by the congruence of several independent approaches. The enzyme activity was purified from human brain tissue,[4] using a combination of a specific biochemical assay and a statine-based inhibitor derived from a short peptide substrate of the enzyme. N-terminal amino acid sequence of the purified enzyme was used to clone and express the enzyme and to verify that it functioned as a β-secretase in cells. In another approach,[3] database mining was utilized to identify potentially novel aspartic proteases from organisms such as *C. elegans*, and this information was then used to search human EST databases for novel aspartic protease sequences. BACE was cloned from the hits obtained and shown to behave as an authentic β-secretase by transfection and antisense experiments in cell culture. Unambiguous proof that the enzyme identified by the three separate groups was indeed β-secretase came about with the generation of BACE knockout (BACE −/−) animals. BACE −/− animals were shown to be devoid of the ability to generate Aβ, whether from endogenous APP[5] or when crossed to transgenic mice expressing FAD mutant APP.[8] Remarkably, the BACE −/− animals were found to be normal in all measures looked at, undistinguished from the BACE +/+ animals except for their inability to generate Aβ.

Like its substrate APP, BACE is a type I transmembrane protein, the first example of a human member of

* To whom correspondence should be addressed. Phone: 650-877-7623. Fax: 650-877-7486. E-mail: varghese.john@elan.com.
† Elan Pharmaceuticals.
‡ Pfizer Corporation.

10.1021/jm030247h CCC: $25.00
Published on Web 09/24/2003

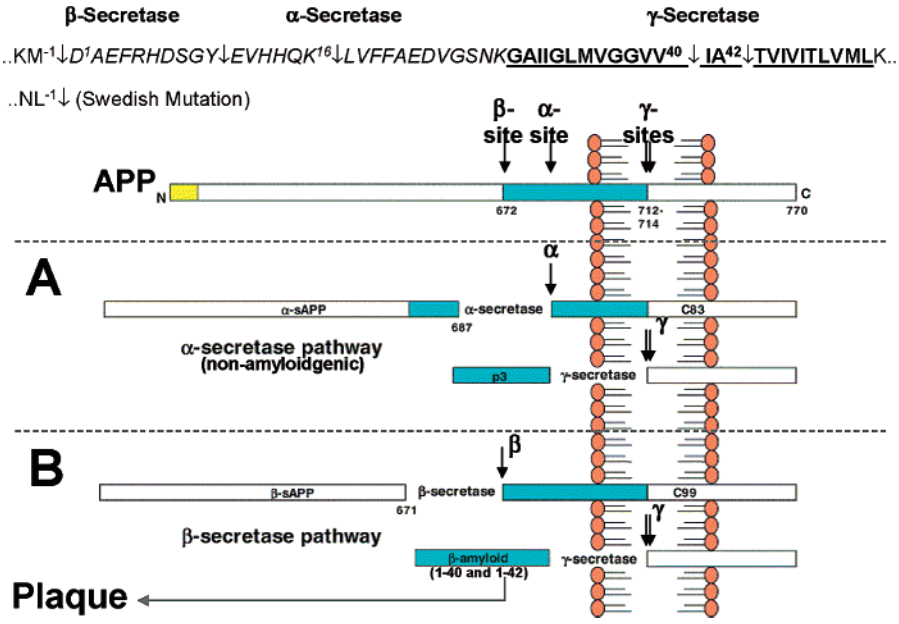

Figure 1. Schematic overview of APP processing by the α-, *β*-, and *γ*-secretases. The top panel shows the amino acid sequence of APP upstream of the transmembrane segment (underlined, bold) and encompassing the sequences of Aβ_{1-40} and Aβ_{1-42} (D^1-V^{40} and D^1- A^{42}, respectively). The *β*-secretase cleaves at D^1 and Y^{10}. The α-secretase cleaves at Lys^{16}, and the *γ*-secretase cleaves at Val^{40} and/or Ala^{42}. Below the sequence is a representation of APP emphasizing its membrane localization and the residue numbers of interest in *β*- and *γ*-secretase processing. Panel A represents the *nonamyloidogenic* α-secretase pathway in which sAPPα and C83 are generated. Subsequent hydrolysis by the *γ*-secretase produces a p3 peptide that does not form amyloid deposits. Panel B represents the *amyloidogenic* pathway in which cleavage of APP by the *β*-secretase to liberate sAPP*β* and C99 is followed by *γ*-secretase processing to release *β*-amyloid peptides (Aβ_{1-40} and Aβ_{1-42}) found in plaque deposits.

the pepsin family of aspartic proteases that exists as an integral membrane protein.[6] The catalysis-competent protease domain is entirely on the N-terminal side of the predicted transmembrane region, suggesting that the enzyme−substrate interaction takes place inside the lumen of the trans Golgi network[6] and in early endosomes, intracellular compartments to which the enzyme has been localized.[9] Like other aspartic proteases, the enzyme is made as a *pre*proenzyme, and its intracellular maturation occurs by a sequential excision of the signal peptide, followed by the removal of a 23 aa "pro"-peptide sequence in a mechanism common to many other aspartyl proteases.[7] The latter occurs in the Golgi and is carried out by the activity of furin or other related proprotein processing enzymes.[10] *β*-Secretase has been referred to by a number of designations in the literature, but the term BACE (*β*-site **A**PP **c**leaving **e**nzyme) has become most widely adopted.[2] With the discovery of BACE, another human homologue with a transmembrane segment was identified, and this has now come to be called BACE2.[11] However, it is not clear that APP is a physiological substrate of this enzyme, and the lack of any detectable A*β* in BACE −/− animals suggests that BACE2 does not contribute to the generation of A*β*.

The assertion that BACE is, in fact, the long-sought *β*-secretase involved in APP processing has considerable experimental support. BACE is highly expressed in the brain, but its message is also found in other tissues,[2] thus explaining the fact that many cell types can process APP to A*β*. Antisense oligonucleotides that block expression of BACE greatly diminish production of A*β*, and conversely, overexpression of BACE in a number of cell lines leads to enhanced A*β* production.[3] BACE −/− mice show no adverse phenotype but have no detectable levels of A*β*.[8] This demonstrates not only that BACE is the true *β*-site APP processing enzyme but that its absence does not pose serious consequences for the animal, which suggests that targeting BACE for inhibition in AD therapy may be chronically tolerable in humans.

A schematic representation of the A*β* region of APP showing the amino acid sequence of A*β* and the major sites of processing by the α-, *β*-, and *γ*-secretases is presented in Figure 1. BACE cleaves on the lumenal or extracellular side of APP at the Met-↓-Asp bond in the so-called *β*-site, located 28 residues upstream of the membrane-spanning region of APP. An arrow between Tyr and Glu in Figure 1 indicates a secondary site of BACE cleavage (*β*′-site). Processing by α-secretase [12] at the Lys-↓-Leu bond gives rise to fragments that are nonamyloidogenic, so this activity is beneficial from the standpoint of the *β*-amyloid hypothesis in AD. Efforts to enhance α-secretase activity represent another approach for diminishing levels of A*β* and plaque formation.[13] In this regard, BACE inhibition may result in shunting of APP into the α-secretase pathway, indirectly resulting in amplification of this pathway (Figure 1). The third important secretase in APP processing is *γ*-secretase, a hetero-oligomeric enzyme complex containing presenilin that carries out its proteolytic function within the membrane (Figure 1).[14a] Processing by *γ*-secretase at positions 40 and 42 gives rise to the major Aβ_{40} and Aβ_{42} species observed in plaque, cerebrospinal fluid, and plasma. Whereas BACE thus far appears to have a single substrate, APP, *γ*-secretase is responsible for processing a variety of transmembrane proteins, including Notch.[14] This broader specificity raises an issue of whether *γ*-secretase inhibitors of APP cleavage might have safety liabilities because of their impact on the processing of other proteins required for normal

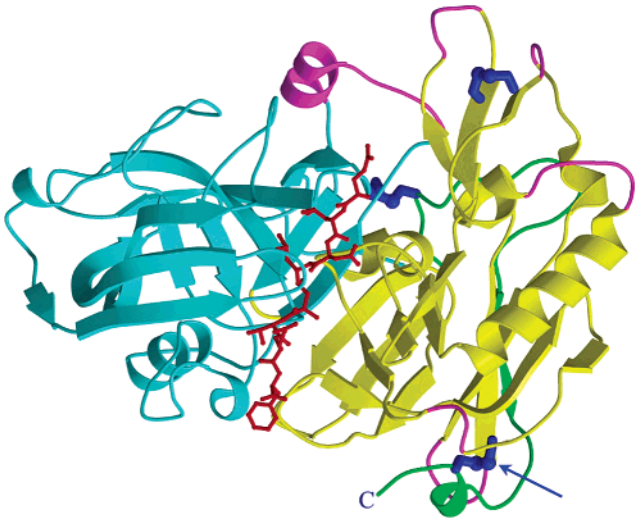

Figure 2. Schematic representation of the three-dimensional structure of the BACE (β-secretase) catalytic unit complexed with the inhibitor OM-99-1 (red), as determined by X-ray crystallography.[15] Arrows and ribbons designate β-strands and α-helices, respectively. The inhibitor is shown bound in the cleft defined by the amino (cyan) and carboxyl (gold) terminal halves of the molecule. The C-terminus of the catalytic unit (green) is marked "C" to indicate the amino acid residue immediately preceding the transmembrane and cytoplasmic domains of BACE. The arrow marks a disulfide bridge that maintains the C-terminus in close structural association with the body of the catalytic unit. The catalytic entity as depicted sits directly on the membrane surface, thereby restricting its motion relative to protein substrates. (Figure kindly provided by Dr. Lin Hong, Oklahoma Medical Research Foundation, Oklahoma City, OK).

function. Since γ-secretase processing cannot take place without prior cleavage by BACE, a BACE inhibitor prevents γ-secretase cleavages in APP without blocking its action on other important transmembrane proteins. Accordingly, BACE might be considered the primary target for lowering Aβ.

BACE 3-D Structure

Great strides in the development of BACE inhibitors have become possible because of the availability of three-dimensional structural information on the BACE target. The X-ray crystal structure of BACE complexed with an inhibitor is represented schematically in Figure 2.[15] Homology with the pepsin-like aspartyl proteases is reflected in the similar folding pattern of BACE, with extensive β-sheet organization, and the proximal location of the two aspartyl residues that comprise the catalytic apparatus for peptide bond cleavage. However, the C-terminal lobe of the molecule is larger than is customarily seen in the aspartyl proteases and contains extra elements of structure with an as yet unexplained impact on function. In fact, before the crystal structure was solved, it was thought that this larger C-terminal region might terminate with a spacer to distance the catalytic unit from the membrane and to provide mobility. This does not appear to be the case. As denoted by the arrow in Figure 2, there is a critical disulfide bridge linking the C-terminal region just upstream of the transmembrane segment to the body of the molecule. Therefore, the globular BACE molecule is proximal to the membrane surface and is not attached via a mobile stalk that would permit much motion. This steric

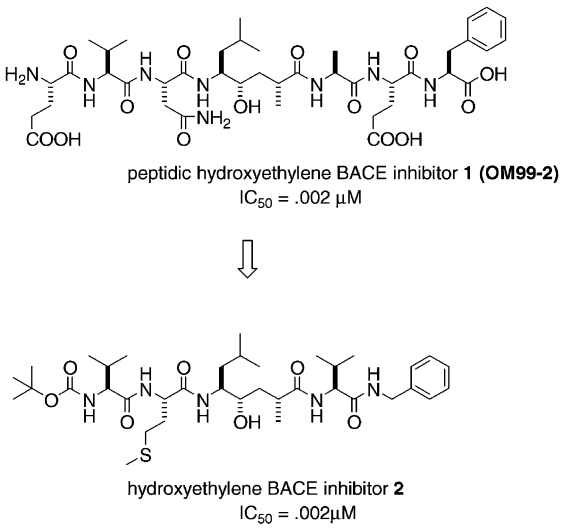

Figure 3. Hydroxyethylene-based inhibitors reported by the Oklahoma group.

localization would be expected to limit the repertoire of protein substrates that are accessible to BACE because it resides in the Golgi network. Crystal structures of BACE/inhibitor complexes have revealed much about the nature of protein−ligand interactions, and information regarding the nature of binding sites obtained by this approach has been of critical importance in the design and development of inhibitors that will be effective drugs in the treatment of AD.

BACE Inhibitors

Peptidomimetic BACE Inhibitors. Substrate-based inhibitors of BACE were designed using the knowledge of the specificity and kinetics of BACE. Tang and co-workers at the University of Oklahoma have reported on the development of peptidic hydroxyethylene-based BACE inhibitor OM99-2, which has a BACE IC_{50} of 0.002 μM.[16] They have further reported on using X-ray structure-based modification of the lead **1** that led to the discovery of a series of potent and considerably low molecular weight peptidomimetic BACE inhibitors such as **2** (Figure 3).

The Elan/Pharmacia team has reported on the development of cell-permeable BACE inhibitors that demonstrate dose-dependent and mechanism-specific reduction of Aβ in human embroyonic kidney (HEK) cells. The evolution of the Elan BACE inhibitors began with the definition of the P_1 and P_1' in a BACE substrate spanning $P_{16}-P_5'$. Replacement of the P_1 residue with a noncleavable statine (sta) residue and replacement of the P_1' Asp with the valine residue resulted in analogue **3** (IC_{50} = 0.03 μM; Figure 4). The Elan team further used **3** in the affinity purification of the crude human brain preparation to yield purified β-secretase that was subsequently sequenced and cloned.[4] Further truncation of the N-terminus and C-terminus resulted in the identification of the smaller peptidic inhibitor **4** (IC_{50} = 0.3 μM; Figure 4).[17] The conversion of the peptide inhibitor **4** into a cell-permeable peptidomimetic BACE inhibitor was done by conceptually subdividing the peptide into three regions: an N-terminal portion, a central statine-containing core, and a C-terminus. These sections were individually targeted for modification to

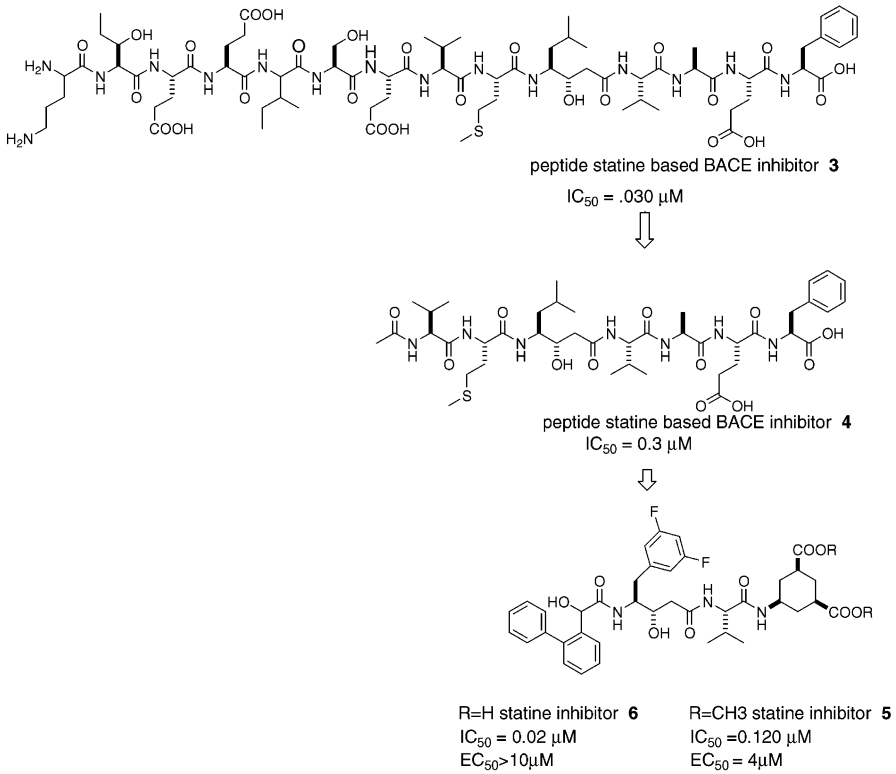

Figure 4. The evolution of the Elan/Pharmacia cell-permeable BACE inhibitors.

replace the amino acid residues with functionalities exhibiting less peptidic character, with retention of BACE enzyme activity. Inhibition of the enzyme was determined using the MBP-C125 (maltose-binding protein C-125) substrate assay as previously described.[4,18]

Using a resin-based strategy and coupling of commercially available carboxylic acids to NH_2[sta]VAEF-resin-bound peptide followed by standard peptide workup quickly led to replacement of the an N-terminal Ac-VM moiety.[18] A variety of substituted phenylacetyl analogues were investigated. The most potent N-terminal replacements were α-hydroxyarylacetic acids. Concurrently the transformation of the C-terminal "AEF" region into a mimetic was pursued, resulting in the identification of a cyclohexyl dicarboxylate held in a geometrically constrained format as an effective surrogate. Further modification of the central core region and combination of the optimal N- and C-terminal mimetics resulted in a potent but cell-permeable BACE inhibitor **5** ($IC_{50} = 0.12\ \mu M$; $EC_{50} = 4\ \mu M$; Figure 4).[18] The corresponding diacid **6** was a potent inhibitor of the enzyme ($IC_{50} = 0.02\ \mu M$) but had no inhibition of Aβ in HEK cells presumably because of poor cellular permeability.

It is notable that the sizes of the modified inhibitors from the Oklahoma and the Elan groups, such as **2** and **5** (molecular weight of ~723), are comparable to a number of approved peptidomimetic HIV protease inhibitor drugs. Recently several peptidomimetic patent publications from Elan/Pharmacia[19] and the Oklahama group[20] have appeared.

Nonpeptidomimetic BACE Inhibitors. The identification of nonpeptidomimetic BACE inhibitors has proven to be a challenging undertaking. To augment the disclosure of peptide-derived BACE inhibitors, a few companies have published nonpeptidomimetic BACE inhibitors, albeit the disclosures to date are few and limited to published patent applications. In 2001, Takeda disclosed the first such purported BACE inhibitors, specifically aminoethyl-substituted tetralins represented by **7** (Figure 5).[21] Among the nine exemplified, but not specifically claimed, analogues, **7** was identified as the most potent inhibitor of BACE with an IC_{50} of 0.35 μM as determined by a fluorescence assay; other compounds possessed IC_{50} values in the nanomolar to low micromolar range. To date, crystallographic and in vivo data have not been disclosed for these non-amide-containing low molecular weight BACE inhibitors. More recently, Neurologic has disclosed piperazine-substituted phosphinyl- and phosphorylmethyl succinic acid derivatives **8**−**10** (LQ β-1−3) as inhibitors of BACE (Figure 5).[22] Inhibition of BACE by these heterocyclic compounds has been indirectly associated through measurements of the compound's ability to increase α-sAPP (see Figure 1), presumably by shifting APP processing toward α-secretase, consequently increasing the pool of non-amyloidogenic derivatives. The analogue **8** (LQ β-3) demonstrated a dose proportional response on α-sAPP secretion with effects starting at 0.001 μM. The phosphorus-containing tetrapeptides **11** and **12** (LQ β-4 and -5) were also disclosed.[23]

The most detailed account of nonpeptidomimetic BACE inhibitors to date comes from Vertex, again via a published patent application. In late 2002, Vertex disclosed[22] several hundred compounds along with associated K_i ranges of BACE inhibition; these compounds spanned multiple classes of heterocyclic templates. Among the more potent classes reported (BACE K_i < 3 μM) were the halogen-substituted biarylnaphthalenes represented by **13** (Figure 6). From these results, Vertex proposed[23] the first 3-D pharmacophore map of BACE to guide the design and optimization of inhibitors,

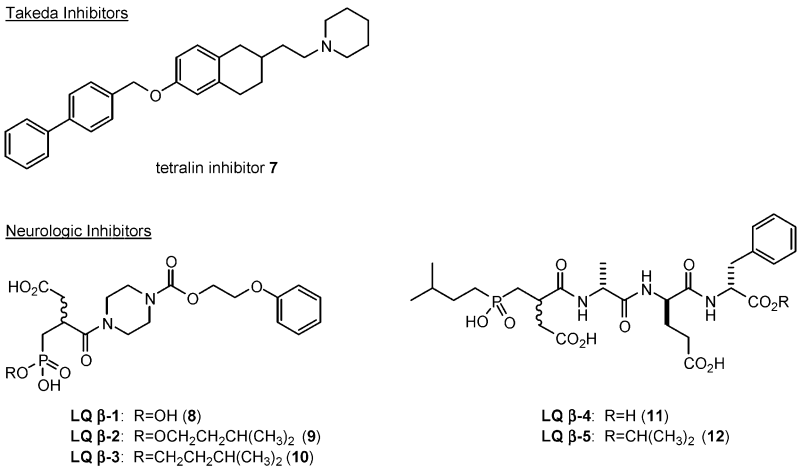

Figure 5. Takeda and Neurologic BACE inhibitors.

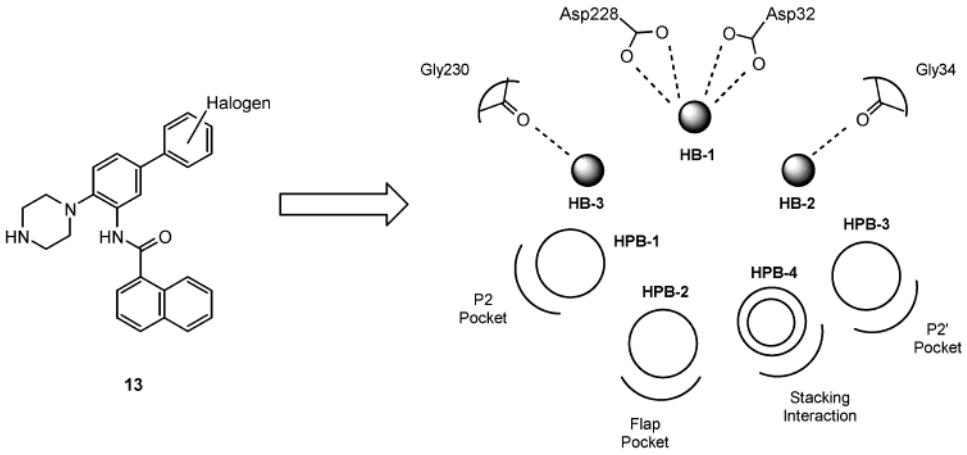

Figure 6. Vertex inhibitor and 3-D BACE pharmacophore map.

wherein HB represents hydrogen-bonding moiety interactions with the active site and other key residues of BACE and HPB represents hydrophobic moiety interactions with BACE subsites (Figure 6). Crystallographic data to substantiate the 3-D model were not provided.

Conclusion

The availability of three-dimensional structural information for BACE in complexation with a variety of inhibitors has enabled great strides in development of new classes of inhibitors, and a number of potent inhibitors of the enzyme have been reported. BACE has been shown to be the rate-limiting enzyme in the processing of APP into Aβ, and given its central role in the liberation of the Aβ peptide and data suggesting that it is a target that can be safely blocked, BACE continues to be an attractive target for inhibition in the treatment of Alzheimer's disease.

Acknowledgment. The authors dedicate this review to the BACE collaboration between Elan Pharmaceuticals and the legacy Pharmacia site at Kalamazoo, an outstanding example of shared drug discovery effort toward a major target.

Biographies

Varghese John is Senior Scientist at Elan Pharmaceuticals in South San Francisco working on development of therapies for Alzheimer's disease. Elan is a biopharmaceutical company focused on three core areas: neurology, pain management, and autoimmune diseases. Since 1987 he was with Athena Neurosciences, prior to its acquisition by Elan, where he was involved in research primarily in the central nervous system area, focusing on developing drugs for brain tumors and neurological disorders. He has several scientific journal articles and patents in his field. He received his Ph.D. in medicinal chemistry at the University of Minnesota and completed postdoctoral fellowships with Prof. Josef Fried at the University of Chicago and with Prof. Carl Djerassi at Stanford University.

James P. Beck was a Research Advisor and Associate Chemistry Fellow at Pfizer in Kalamazoo, working on the development of therapies for Alzheimer's disease. He has previously worked with Pharmacia (2000−2003) and DuPont Pharmaceuticals (1994−2000) where his research focus has been on neurobehavioral and neurodegenerative receptor and enzyme targets involved in anxiety, depression, attentional disorders, and AD. He received his Ph.D. in organic chemistry at Indiana University with Prof. Paul A. Grieco. He has recently moved to Eli Lilly in Indianapolis.

Michael J. Bienkowski is a Senior Scientist at Pfizer in Kalamazoo Michigan, working on the discovery of disease-modifying therapies for the treatment of Alzheimer's disease. Prior to the acquisition of Pharmacia by Pfizer, he has been with the legacy companies Upjohn, Pharamacia & Upjohn, and Pharmacia since 1985, working primarily on the discovery of central nervous system drugs for the treatment of psychiatric diseases and neurodegeneration. He has multiple peer-reviewed scientific articles and issued patents in his field. He received his Ph.D. in biochemistry from the University of Illinois and completed a postdoctoral fellowship with John Shively at the Beckman Research Institute of the City of Hope in Duarte, CA.

Sukanto Sinha is a Research Fellow at Elan Pharmaceuticals, South San Francisco, CA, where he is project leader for the BACE program, a joint collaboration between Elan and Pfizer (since 2000). He has been with Elan (previously Athena Neurosciences) since 1988 and headed the effort that led to the discovery of BACE. Prior to coming to Athena, he was a Research Assistant Professor at SUNY Stony Brook (1985−1988). He received his Ph.D. in biochemistry from the University of Georgia in 1983 and followed that with a postdoctoral fellowship with Prof. James Travis at the University of Georgia (1983−1985).

Robert L. Heinrikson is Distinguished Fellow at Pfizer, Inc., Kalamazoo, MI. Following a career in academia in which Dr. Heinrikson rose to the level of Full Professor of Biochemistry at the University of Chicago, he joined the Upjohn Company in Kalamazoo in 1985 to head a group in protein chemistry. During his 19-year tenure in the pharmaceutical industry, Dr. Heinrikson has led teams in adhesion biology and in targeting proteolytic enzymes such as renin, the HIV-protease, and caspases. He was codiscoverer of BACE and served as project head of that team as well.

References

(1) Glenner, G. G.; Wong, C. W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 885−890.

(2) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J.-C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **1999**, *286*, 735−741.

(3) Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashier, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* **1999**, *402*, 533−537.

(4) Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* **1999**, *402*, 537−540.

(5) Cai, H.; Wang, Y.; McCarthy, D.; Wen, H.; Borchelt, D. R.; Price, D. L.; Wong, P. C. BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nat. Neurosci.* **2001**, *4*, 233−234.

(6) Yan, R.; Han, P.; Miao, H.; Greengard, P.; Xu, H. The transmembrane domain of the Alzheimer's β-secretase (BACE1) determines its late Golgi localization and access to β-amyloid precursor protein (APP) substrate. *J. Biol. Chem.* **2001**, *276*, 36788−36796.

(7) Shi, X.-P.; Chen, E.; Yin, K.-C.; Na, S.; Garsky, V. M.; Lai, M.-T.; Li, Y.-M.; Platchek, M.; Register, R. B.; Sardana, M. K.; Tang, M.-J.; Thiebeau, J.; Wood, T.; Shafer, J.; Gardell, S. J. The pro domain of β-secretase does not confer strict zymogen-like properties but assists proper refolding of the protease domain. *J. Biol. Chem.* **2001**, *267*, 10366−10373.

(8) Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sihna, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. BACE knockout mice are healthy despite lacking the primary β-secretase activity in the brain: implications for Alzheimer's disease therapeutics. *Hum. Mol. Genet.* **2001**, *10*, 1317−1324.

(9) Huse, J. T.; Pijak, D. S.; Leslie, G. J.; Lee, V. M.; Doms, R. W. Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. *J. Biol. Chem.* **2000**, *275*, 33729−33737.

(10) Bennett, B. D.; Denis, P.; Haniu, M.; Teplow, D. B.; Kahn, S.; Louis, J. C.; Citron, M.; Vassar, R. A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's beta-secretase. *J. Biol. Chem.* **2000**, *275*, 37712−37717.

(11) Acquati, F.; Accarino, M.; Nucci, C.; Fumagalli, P.; Jovine, L.; Ottolenghi, S.; Taramelli, R. The gene encoding DRAP (BACE2), a glycosylated transmembrane protein of the aspartic protease family, maps to the down critical region. *FEBS Lett.* **2000**, *468*, 59−64.

(12) Sisodia, S. S.; Koo, E. H.; Beyreuther, K.; Unterbeck, A.; Price, D. Evidence that the β-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* **1990**, *248*, 492−495.

(13) Esler, W. P.; Wolfe, M. S. A portrait of Alzheimer secretases—New features and familiar faces. *Science* **2001**, *293*, 1449−1454.

(14) (a) Sisodia, S. S.; St. George-Hyslop, P. H. γ-Secretase, Notch, Aβ and Alzheimer's disease: where do the presenilins fit in? *Nat. Rev. Neurosci.* **2002**, *3*, 281−289. (b) Schmidt B. Aspartic proteases involved in Alzheimer's disease. *ChemBioChem* **2003**, *4*, 366−378.

(15) Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. Structure of the protease domain of memapsin 2 (β-secretase) complexed with inhibitor. *Science* **2000**, *290*, 150−153.

(16) Ghosh, A. K.; Bilcer, G.; Harwood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. Structure-Based Design: Potent Inhibitors of Human Brain Memapsin 2 (β-secretase). *J. Med. Chem.* **2001**, *44*, 2865−2868.

(17) Tung, J. S.; Davis, D. L.; Anderson, J. P.; Walker, D. E.; Mamo, S.; Jewett, N. E.; Hom, R. K.; Sinha, S.; Thorsett, E. D.; John, V. Design of substrate-based inhibitors of human β-secretase. *J. Med. Chem.* **2002**, *45*, 259−262.

(18) Hom, R. K.; Fang, L. Y.; Mamo, S.; Tung, J. S.; Guinn, A. C.; Walker, D. E.; Davis, D. L.; Gailunas, A. F.; Thorsett, E. D.; Sinha, S.; Knops, J. E.; Jewett, N. E.; Anderson, J. P.; John, V. Design and synthesis of statine-based cell-permeable peptidomimetic inhibitors of human β-secretase. *J. Med. Chem.* **2003**, *46*, 1799−1802.

(19) (a) John, V.; Tung, J.; Fang, L.; Mamo, S. S. Patent WO 00/077030, 2000. (b) Hom, R.; Mamo, S.; Tung, J.; Gailunas, A.; John, V.; Fang, L. Patent WO 01/070672, 2001. (c) Fang, L. Y.; Hom, R.; John, V.; Maillaird, M. Patent WO 02/002505, 2002. (d) Fang, L. Y.; John, V. Patent WO 02/002506, 2002. (e) Maillaird, M.; Hom, R.; Gailunas, A.; Jagodzinska, B.; Fang, L. Y.; John, V.; Freskos, J. N.; Pulley, S. R.; Beck, J. P.; TenBrink, R. E. Patent WO 02/002512, 2002. (f) Beck, J. P.; Gailunas, A.; Hom, R.; Jagodzinska, B.; John, V.; Maillaird, M. Patent WO 02/002520, 2002. (g) Schostarez, H.; Chrusciel, R. A.; Centko, R. S. Patent WO 02/094768, 2002. (h) Freskos, J. N.; Aquino, J.; Brown, D. L.; Fang, L.; Fobian, Y. M.; Gailunas, A.; Guinn, A.; John, V.; Romero, A. G.; Tucker, J.; Tung, J.; Walker, D. Patent WO 02/098849, 2002. (i) Pulley, S. R.; Beck, J. P.; TenBrink, R. E.; Jacobs, J. S. Patent WO 02/100399, 2002. (j) Schostarez, H. J.; Chrusciel, R. A. Patent WO 02/100818, 2002. (k) Maillaird, M.; Tucker, J. A. Patent WO 02/100820, 2002. (l) Pulley, S. R.; Beck, J. P.; TenBrink, R. E. Patent WO 02/100856, 2002. (m) John, V.; Hom, R.; Tucker, J. Patent WO 03/002122, 2003. (n) Schostarez, H. J.; Chrusciel, R. A. Patent WO 03/006013, 2003. (o) Schostarez, H. J.; Chrusciel, R. A. Patent WO 03/006021, 2003. (p) Gailunas, A.; Hom, R.; John, V.; Maillard, M.; Chrusciel, R. A.; Fisher, J.; Jacobs, J.; Freskos, J. N.; Brown, D. L.; Fobian, Y. M. Patent WO 03/006423, 2003. (q) Schostarez, J. H.; Chrusciel, R. A. Patent WO 03/006453, 2003. (r) Gailunas, A.; Tucker, J. A.; John, V. Patent WO 03/027068, 2003. (s) Fisher, J. F.; Jacobs, J. S.; Sherer, B. A. Patent WO 03/029169, 2003.

(20) (a) Tang, J. N.; Koelsch, G.; Ghosh, A. K. Patent WO 02/53594, 2002. (b) Tang, J. J. N.; Hong, L.; Ghosh, A. K. Patent WO 01/00665, 2001.

(21) Miyamoto, M.; Matsui, J.; Fukumoto, H.; Tarui, N. Patent WO 01/187293, 2001.

(22) Qiao, L.; Etcheberrigaray, R. Patent WO 02/96897, 2002.

(23) Bhisetti, G. R.; Saunders, J. O.; Murcko, M. A.; Lepre, C. A.; Britt, S. D.; Come, J. H.; Deninger, D. D.; Wang, T. Patent WO 02/88101, 2002.

JM030247H