

GAMMA-SECRETASE MODULATORS SHOW SELECTIVITY FOR GAMMA-SECRETASE MEDIATED A β PRODUCTION

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Background and objectives

The process of A β amyloidosis plays a pivotal role in the onset of Alzheimer's disease (AD) and starts decades prior to symptomatic disease. It is thus conceivable that an A β -targeting therapy would be most beneficial as a preventive therapy initiated during preclinical AD. The major constituent of A β amyloid is the post-proteolytical A β peptide, which stems from γ -secretase mediated processing of amyloid precursor protein (APP). γ -Secretase has a large number of substrates and is involved in many contexts of cell signaling. γ -Secretase modulators (GSMs) represent a promising class of orally available A β modulatory agents for the treatment of early AD. In this study we have asked what impact GSMs may have on other γ -secretase reactions, with emphasis on EphA4, EphB2 and E-cadherin signaling.

Methods

Expression constructs encoding N-terminally truncated and FLAG-epitope tagged EphA4, EphB2 and E-cadherin were expressed in murine cells lacking presenilin (PS) expression (BD8 cells) and in human HEK293 cells. Cells expressing APP_{swe} and C99-GVP (direct substrate of A β) had been generated before. γ -Secretase mediated intracellular domain (ICD) formation and secretion of A β and A β -like peptides were analyzed in cell-based assays using a reporter gene system and by a combined immunoprecipitation/mass spectrometric analysis, respectively. Ectopic expression of PS1 in BD8 cells and the γ -secretase inhibitor L685,458 were used in experiments to determine the γ -secretase dependency of different reactions. Three structurally distinct GSMs were used to explore the impact of GSMs on the intramembrane processing of EphA4, EphB2 and E-cadherin.

Results

Cells overexpressing EphA4 and EphB2 resulted in a PS and γ -secretase dependent generation of ICDs and A β -like peptides (Fig 1). In contrast, E-cadherin overexpression resulted in γ -secretase independent formation of ICD and A β -like peptides (Fig 1A and data not shown). The amount of ICD generated from EphA4, EphB2, E-cadherin and C99 expressing cells were unaffected by all GSMs tested (Fig 2A). None of the secreted A β -like peptides generated from EphB2 or E-cadherin expressing cells were modulated by the GSMs (Fig 2 and data not shown). Only the GSM AZ4126 modulated EphA4 derived A β -like peptide formation (Fig 2D), but with an order of magnitude lower potency as compared to its A β modulatory effect (Fig 3).

Conclusion

Our studies confirm that γ -secretase mediates intramembrane processing of EphA4 and EphB2 and show that it results in multiple secreted A β -like peptides. Our data also suggest that E-cadherin could be subject to intramembrane processing by an enzymatic activity that is distinct from γ -secretase. Importantly, our data demonstrate that GSMs, developed for targeting APP processing and A β production, are selective for A β modulation. The molecular basis for this selectivity remains unknown, but strongly support the development of a GSM therapy for the targeted lowering of amyloidogenic A β production in the preclinical, pre-symptomatic phase of AD.

Figure 1. γ -Secretase dependent EphA4 and EphB2 ICD (A) and A β -like peptide (B and C) generation. PS deficient cells in A. and HEK293 cells in B and C. PS1 (=PS1 overexpression), L685,458 (= γ -secretase inhibitor treatment)

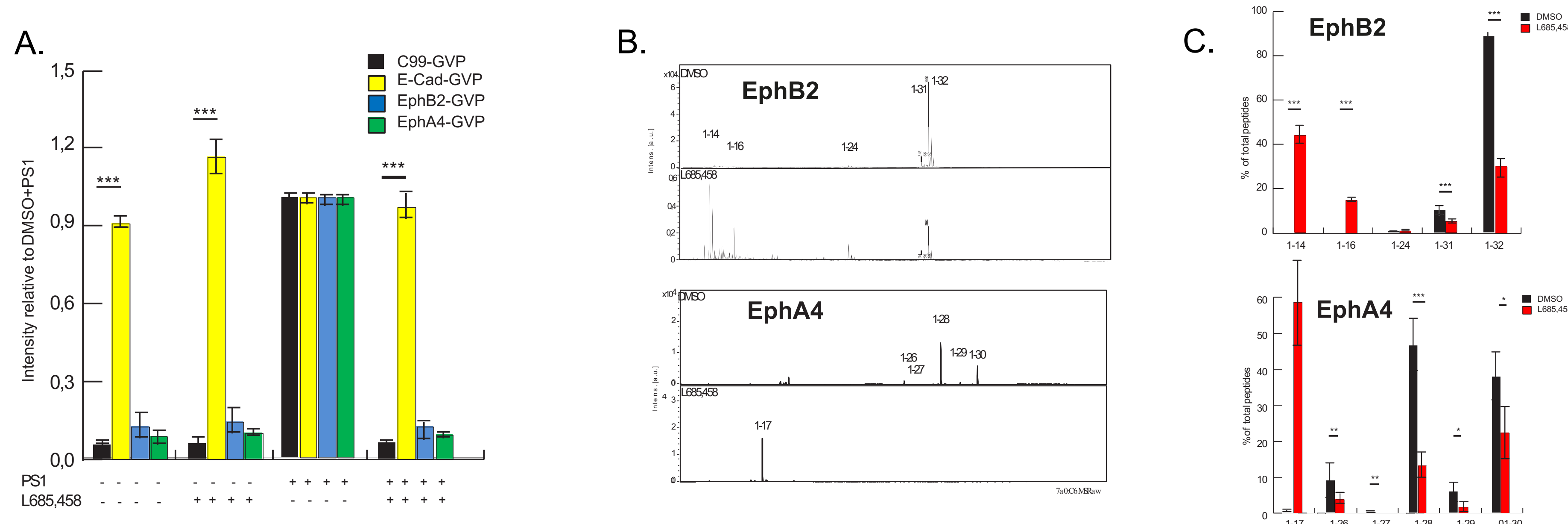


Figure 2. The impact of GSMs on the γ -secretase mediated ICD (A), and A β -like peptide formation in cells overexpressing N-terminally truncated and epitope tagged EphB2 (B) and EphA4 (C).

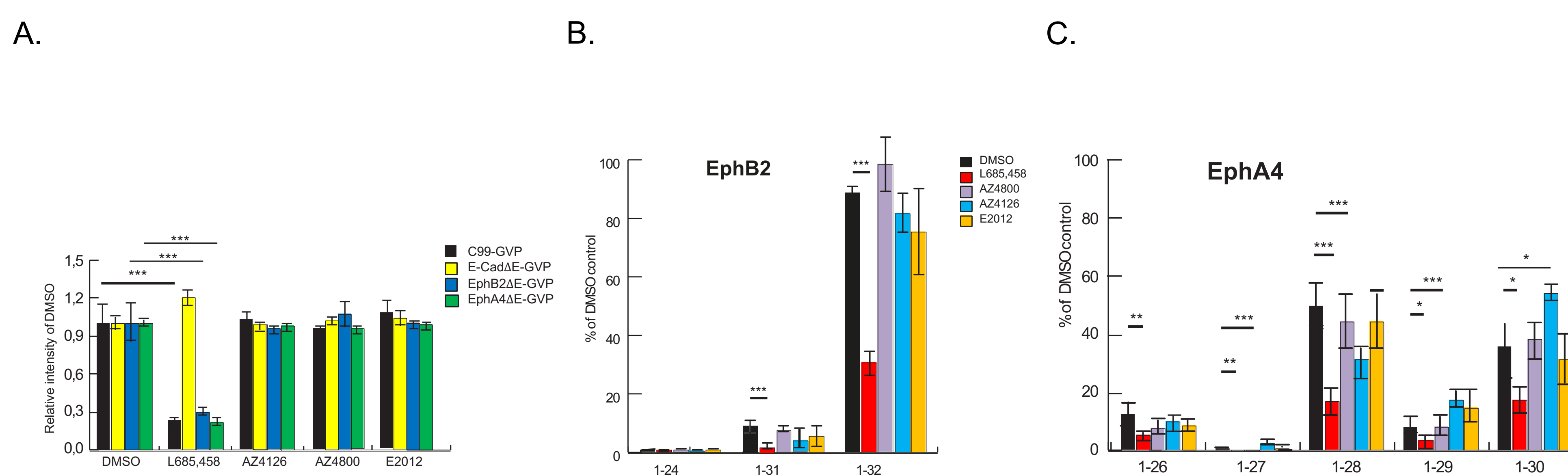
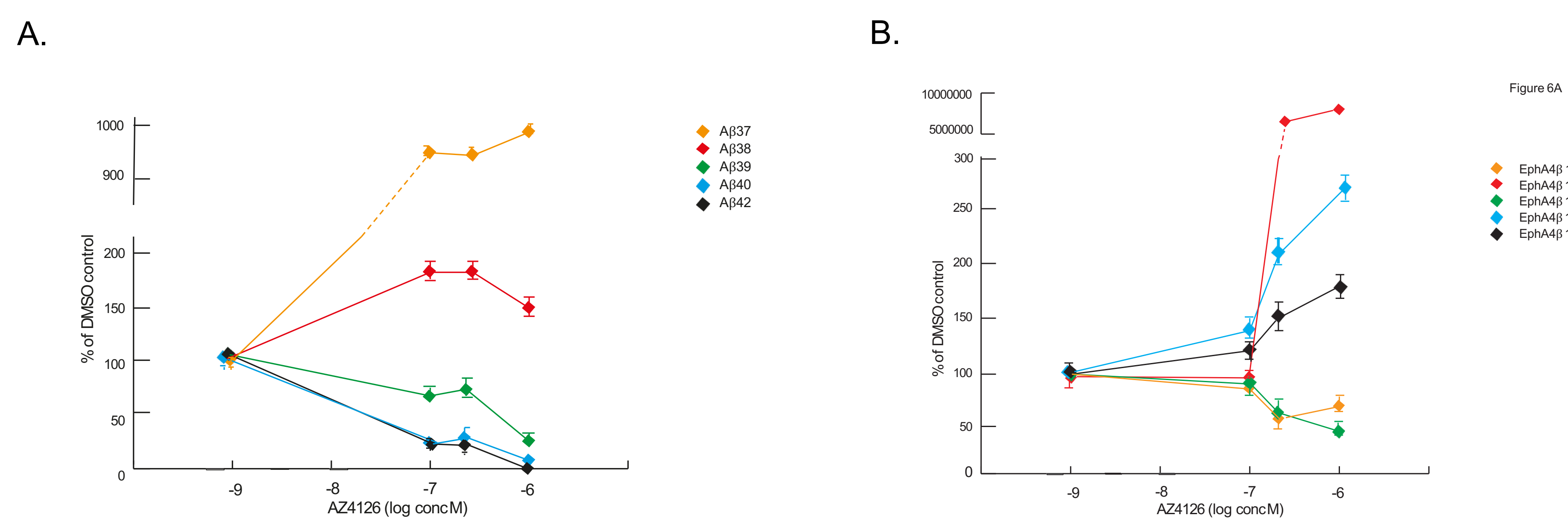


Figure 3. The GSM 4126 shows a more potent modulation of A β (A) as compared to EphA4 A β -like peptide formation (B)



Key findings:

1. γ -Secretase processing of EphB2 and EphA4 results in the release of multiple A β -like peptides
2. GSMs developed for A β modulation appear not as effective on modulating γ -secretase mediated EphB2 and EphA4 processing
3. The GSM AZ4126 modulates the pattern of EphA4-derived A β -like peptides differently as compared to its effect on A β