EVIDENCE FOR “BACE-INHIBITOR RESISTANT” PRODUCTION OF SPECIFIC Aβ VARIANTS FROM WILD TYPE BUT NOT FROM SWEDISH MUTANT APP

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Introduction: Based on the “tripartite structure” concept, β-secretase inhibitors can be targeted to the lipid rafts by linking them via an appropriate spacer to a membrane anchor (T. Braxmeier et al., International Patent, WO 2005/097199). These compounds accumulate in endosomes and efficiently reduce Aβ-peptide secretion (L. Rajendran et al., Science 2008, 320, 520-523).

Aims: To analyze in detail the generation of different Aβ-peptide variants from wildtype APP (APPwt) and Swedish mutant APP (APPsw) in the presence and absence of selected tripartite BACE inhibitors.

Methods: Primary chicken neurons and transfected SH-SY5Y cell lines, expressing APPwt or APPsw, were treated with selected test compounds. Aβ-peptides and secreted sAPPα and sAPPβ were quantified with electrochemiluminescence immunoassays. C- and N-terminal variants of the Aβ-peptide were studied by 1- and 2-dimensional urea SDS-PAGE/Western blot and immunoprecipitation / mass spectrometry.

Results: “Tripartite structures” carrying two different β-secretase inhibitors efficiently reduced Aβ-secretion in all three cellular models. At the same time, sAPPβ and CTFβ was reduced and sAPPα increased, confirming the inhibition of β-site cleavage. A tripartite transition state mimic inhibitor was less potent on the generation of Aβ-peptides from APPsw than from APPwt. One- and 2-dimensional SDS-PAGE / Western blot analysis revealed specific Aβ-peptide variants generated from APPwt but not from APPsw which were not reduced by tripartite BACE inhibitors.

Conclusions: Tripartite inhibitors are an attractive tool for the analysis of APP processing. Our data support the theory of the existence of more than one enzyme displaying “β-secretase like activity” for APPwt.