sets covering the whole transcribed rat genome. For the genes detected by the microarray analysis, we surveyed the binding sites of transcription factors, using the database produced by UCSC Genome Bioinformatics. Results: The increase in the DNA-binding activity of NF-kappa B and AP-1 after exposure to Abeta fibrils was suppressed by PARP-1 inhibitor, 1,5-dihydroxyisoquinoline (DHIQ), and also PARP-1 siRNA. The microarray analysis demonstrated that among 31,042 probe sets used, 345 and 224 probe sets showed up-regulation and down-regulation, respectively, in the astrocytes after exposure to Abeta fibrils. Furthermore, 87 probe sets showed down-regulation, while only three probe sets showed up-regulation, after addition of DHIQ. Upstream and downstream of the genes detected by these probe sets, the DNA-binding sites of other transcription factors than NF-kappa B and AP-1 were identified. Conclusions: PARP-1 plays an important role in the change of gene expression profile of astrocytes after exposure to Abeta fibrils through the activation of a variety of transcription factors. By regulation of these factors, PARP-1 inhibitors could be new therapeutic and/or neuroprotective agents for Alzheimer’s disease.

P2-275 COMPUTATIONAL AND EXPERIMENTAL ELUCIDATION OF AMYLOID-BETA OLIGOMERIZATION AND ITS BIOLOGICAL EFFECTS

David Teplow, UCLA, Los Angeles, California, United States.

Background: AD is one of the most difficult medical problems to understand and treat. Over 200 clinical trials now have been completed. All have failed. A significant fraction of these studies targeted AII or tau. This approach is reasonable strategically. However, the tactics used in developing AII- or tau-centric therapies appear to have been unsuccessful. We believe that the underlying reason for these tactical failures has been an incomplete elucidation of the “structural biology” of these two important proteins, i.e., the formal determination of their structure-activity relationship. Methods: We discuss here current computational and experimental approaches to determine the structural dynamics of AII oligomerization. Beginning with monomer dynamics, we proceed to the consideration of differential oligomerization pathways for AII40 and AII42, and then to the differential effects of specific assemblies on neuronal function and viability. Results: Prior computational work suggested that AII exists as four relatively rigid segments that are connected by three flexible turns. What may be the most important of these rigid regions is the C-terminus. Our simulations revealed that the C-terminus of AII42 can fold into a hairpin-like conformation. To validate this finding, and to explore the potential disease implications of such a structure, we designed changes into the AII40 and AII42 peptides that were predicted either to stabilize or destabilize this turn. We then chemically synthesized the corresponding peptides and studied their oligomerization experimentally. We found that changes stabilizing the turn resulted in the production of very stable hexamer (paramacous) and di-hexamer units. In contrast, destabilizing changes blocked formation of paramacous. In parallel, we executed a scanning Tyr mutagenesis approach to allow us to use Tyr-based chemical cross-linking to study oligomer formation. We correlated Tyr position with oligomer stability and found substantial stabilization of AII42 oligomers with C-terminal Tyr. Conclusions: The mechanism by which the C-terminal Ile and Ala residues of AII produce the distinct monomer folding and oligomerization behavior of AII42 versus AII40 may involve the stabilization of a C-terminal turn. This turn produces a hydrophobic center for solvophobic intramolecular interactions that stabilize the overall monomer fold.

P2-276 INVESTIGATION OF ALTERNATIVE BINDING EVENTS BETWEEN THE GOLGI-LOCALIZED, GAMMA EAR-CONTAINING, ARF-BINDING (GGA) PROTEIN FAMILY AND BACE1 AND ITS INFLUENCE ON THE PROCESSING OF AMYLOID PRECURSOR PROTEIN (APP)

Bjoern von Einem1, Anke Hellring1, Daniel Schwanzar1, Cathrin Schnack1, Cornelia Steinmetz1, Frank Dolp2, Angelika Rueck2, Christian Proepper1, Tobias Boeckers1, Christine von Arnim1, Ulm University, Ulm, Germany; 2Institute for Laser Technologies in Medicine and Metrology, Ulm, Germany.

Background: Essential for the proteolytic processing of APP by BACE1 - and therewith for the generation of Abeta - is the transport and sorting of both proteins through endosomal and Golgi compartments. The family of Golgi-localized γ ear-containing ARF-binding (GGA) proteins was shown to have striking functions in cargo sorting in these pathways. Recently, it was shown that GGAs and GGA3 can interact with BACE1, that they are expressed in neurons and GGA3 is reduced in AD brain tissues. The GGA VHS-domain was found to be the major binding motif necessary for interaction with BACE1. Methods: We applied Immunoprecipitation and Fluorescence Lifetime imaging Microscopy (FLIM) with different GGA-domain deletions to analyze VHS-domain independent BACE1-GGA interaction and the binding capacity of the different deletion mutants. We further extended our approach by ELISA assays and Western Blot analysis to measure the influence on APP processing. Results: Co-immunoprecipitation experiments showed that all GGA VHS deletion-mutants can still be precipitated by BACE1, though to a lower extent than their wild type forms. Additionally, in FLIM experiments the lifetime of GGA-VHS deletion-mutants was only slightly increased compared to wild-type GGAs. Furthermore, cells co-transfected with APP, BACE and either wild-type GGAs or VHS deletion-mutants showed no significant differences in APP processing in Mesoscale Elisa assays and Western Blot analysis. Therefore, we extended our approach to identify other domains responsible for GGA-BACE1 interaction using several GGA domain-deletions and point-mutations to identify the major binding motif necessary for GGA-BACE interaction. Conclusions: In conclusion, our data suggest that the GGA VHS-domain has only limited influence upon the processing of APP and upon interaction with and trafficking of BACE1. As all other known GGA cargo proteins seem to be dependent on the VHS-domain differing BACE1 interactions are of high interest in the attempt to find BACE1 specific transport and trafficking modifiers.

P2-277 LACTIC ACID INDUCES ABERRANT AMYLOID PRECURSOR PROTEIN PROCESSING BY PROMOTING ITS INTERACTION WITH ENDOPLASMIC RETICULUM CHAPERONE PROTEINS

Yiwen Xiang, University of Florida, Gainesville, Florida, United States.

Background: Lactic acid, a natural by-product of glycolysis, is produced at excess levels in response to impaired mitochondrial function, high-energy demand, and low oxygen availability. The enzyme involved in the production of b amyloid peptide (Ab) of Alzheimer’s disease, BACE1, functions optimally at lower pH, which led us to investigate a potential role of lactic acid in the processing of amyloid precursor protein (APP). Methods: ELISA, Western blot, fluorescence microscopy studies and coimmunoprecipitation were implied on SHSY5y cell model. Results: Lactic acid increased levels of Ab40 and 42, as measured by ELISA, in culture medium of human neuroblastoma cells (SH-SY5Y), whereas it decreased APP metabolites, such as sAPPa. In cell lysates, APP levels were increased and APP was found to interact with ER-chaperones in a perinuclear region, as determined by coimmunoprecipitation and fluorescence microscopy studies. Lactic acid had only a modest effect on cellular pH, did increase the levels of ER chaperones Grp78 and Grp94 and led to APP aggregate formation reminiscent of aggresomes. Conclusions: These findings suggest that sustained elevations in lactic acid levels could be a risk factor in amyloidogenesis related to Alzheimers’s disease through enhanced APP interaction with ER chaperone proteins and aberrant APP processing leading to increased generation of amyloid peptides and APP aggregates.