affecting mRNA levels. We analyzed the effect of several deletion mutants in order to determine regions within the 5′-UTR which influence the translation efficiency of ADAM10. Successive deletion of the first part of the ADAM10 5′-UTR resulted in a significant increase in ADAM10 protein expression, arguing that this part of the 5′-UTR contains inhibitory elements. Using CD-spectroscopy, we identified a G-quadruplex motif, which forms a very stable secondary structure within the first half of the ADAM10 5′-UTR. Mutation of this G-quadruplex motif results in enhanced ADAM10 expression. Conclusions: We provide evidence that a 30 nucleotide long G-rich region within the first portion of the ADAM10 5′UTR is able to form an extremely stable intramolecular G-quadruplex secondary structure which contributes to the inhibitory effect of the 5′-UTR on the translation of ADAM10.

**P2-308 DEVELOPMENTAL REGULATION OF PROTEIN O-GlcNAcylation, O-GlcNac Transferase, and O-GlcNAcase in Mammalian Brain**

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**Background:** O-GlcNAcylation is a dynamic, regulatory posttranslational modification of protein by β-N-acetylglucosamine (GlcNAc), which is transferred enzymatically from UDP-GlcNAc donor to the hydroxyl group of serine or threonine residues of proteins. O-GlcNAcylation is catalyzed by O-GlcNAc transferase (OGT), and the O-GlcNAc groups attached to proteins can be removed with the catalysis of O-GlcNAcase (OGA). Numerous neuronal proteins, including transcription factors, synaptic and cytoskeletal proteins, are modified by O-GlcNAc, suggesting that it regulates many brain functions. Both OGT and OGA are highly expressed in the brain. Methods: Here, we investigated immunohistochemically the regional distributions of global O-GlcNAcylation, OGT and OGA in rat brains at ages of embryonic 19 days (E19d), postnatal 5 days, 6 months and 12 months. Results: We found wide distributions of O-GlcNA cylated proteins, OGT and OGA at all ages examined, but they are regulated during development. At E19d, O-GlcNAcylated proteins, OGT and OGA had similar distributions with the highest staining in the cortical plate and sub plate. More brain region-specific distributions were seen in rat brains after birth, but the distributions of O-GlcNAcylated proteins, OGT and OGA were similar at all ages examined. Higher immuno-staining was seen in the cerebral cortex and the pyramidal neurons of some sectors of the cornu ammonis of the hippocampus. Conclusions: These observations provide fundamental knowledge for understanding the regional regulation of brain functions by O-GlcNAcylation during development.

**P2-309 METABOLOME CHANGES INDUCED BY ANAESTHETIC IN AN IN VITRO ALZHEIMER’S MODEL.**

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**Background:** A link between AD and anaesthesia has been suggested. In prelude to a full behavioural study examining anaesthesia, surgery and hippocampal pathology we are investigating early, sub cellular responses to anaesthetic exposure in vitro. Combining a novel Nuclear Magnetic Resonance (NMR) profiling technique with Western Blotting, we examined changes to the metabolome and expression of AD associated peptides in a cell culture model of AD susceptibility. Methods: Neuroglioma cells stably transfected to over express Amyloid Precursor Protein (H4-APP) were exposed for 6 hours to (1) no treatment, (2) 2% Isoflurane, (3) 75% Nitrous Oxide and (4) 70% Xenon, with 21%O2 and 5% CO2 balanced in Nitrogen. For NMR, metabolism was halted by quenching in ice cold 100% methanol, before scraping and analysis using 800 MHz Proton Nuclear Magnetic Resonance Spectroscopy. Western Blotting was performed on cell lysate isolated immediately postexposure, analysing for 4 AD associated proteins of interest: Caspase-3 fragment (functional executor of Apoptosis), APP, gamma and beta-secretase. Densitometric analysis was normalised by internal control of alpha-tubulin and presented as mean ± SEM (n = 3). Results: Preliminary NMR analysis shows a small molecule metabolome rich in lactate, folate, phosphorylcholine (PC) and Glycero-phosphorylcholine (GPC). Western Blotting shows Caspase-3 fragment was increased by Isoflurane, but xenon significantly reduced both Caspase-3 fragment and gamma-secretase as compared to Isoflurane exposure (0.46 ± 0.35 and 0.44 ± 0.35 fold reduction respectively, p < 0.05). beta-secretase expression was reduced in the Xenon group (0.29 ± 0.11, p < 0.05). Nitrous Oxide had no statistically significant effects. Conclusions: We have successfully profiled the metabolome of the H4 neuroglioma cell line model of Alzheimer’s susceptibility. Preliminary findings reveal a choline precursor molecule rich metabolome. Elevated levels of these precursors (GPC/PC) within the CNS have previously been associated with. Western results indicate that anaesthetic agents influence cell death and modulate Alzheimer’s associated enzyme systems. NMR may prove useful in further characterising this. Kuehn B. JAMA 2007;297:1760.

**Figure 1. 800MHz Nuclear Magnetic Resonance spectrum of H4-APP metabolome post-anaesthesia.**

**P2-310 CANDIDATE-BASED SEARCH FOR AMYLOID PRECURSOR PROTEIN LIGANDS THAT MODULATE z-SECRETASE CLEAVAGE**

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**Background:** Recently, a series of candidate APP ligands, including F- Spondin, Contactins, and Reelin, have been reported to bind the APP ectodomain and modulate cleavage of APP by γ-secretase. Also, in an unbiased screen, our lab has identified Pancortin as a novel APP binding partner. However, these candidate APP ligands have not yet been widely validated and systematically compared. Methods: To rigorously evaluate the ability of published candidate APP ligands to modulate cleavage by α-secretase, we developed a co-culture APPα shedding assay using primary cortical neurons (as the reporter cell) and stable HEK293 cell lines (as the ligand source). We used a rodent-specific APPα shedding assay using primary cortical neurons (as the reporter cell) and stable HEK293 cell lines (as the ligand source). We used a rodent-specific APPα ELISA (IBL) to detect endogenous APPα produced by the rat neurons (but not the HEK cells). The co-culture APPα shedding assay has advantages over prior systems in that 1) APP is endogenously shedding, with a statistically significant 3). Results: In our co-culture assay, robust changes in APPα shedding were not stimulated by F-Spondin, Integrin β1, or CNTN2-Fc. Reelin produced the clearest change in APPα shedding, with a statistically significant 20% decrease in APPα shedding. However, this is in contrast to published data that Reelin increases APPα levels. We observed a biochemical
interaction of Pancoratin and APP and found that the M domain of Pancoratin interacts with the E1 region of the APP ectodomain. Pancoratin did not significantly modulate APPs accumulation nor did it functionally interact with APP in neurite outgrowth. However, in utero electroporation experiments suggest that Pancoratin and APP do interact functionally during neuronal cortical migration. **Conclusions:** Our APPs accumulation assays underscore the inconsistency of some published candidate APP ligands to affect APP processing. Although some candidate ligands did not modulate APP cleavage in our assays, these proteins still may have important functional interactions with APP. This is exemplified by our studies suggesting that Pancoratin does not affect APPs shedding but has functional interactions with APP in neuronal migration during cortical development.

**P2-311 ACETYLCHOLINESTERASE EXPRESSION LEVELS MODULATES PRESENILIN-1**

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**Background:** In the intensive ongoing study to elucidate the relationship between the hallmarks of Alzheimer’s disease (AD), the reciprocal interaction between amyloid metabolism and the cholinergic enzyme acetylcholinesterase (AChE) has been addressed and various levels of interactions have been suggested. We have previously identified presenilin-1 (PS1), the active component of the γ-secretase complex, as an interacting protein of AChE. In this study, we have explored some of the consequences of AChE-PS1 interactions. **Methods:** The expression of the cholinergic AChE (also called Tor “tailed”) and the “read thought” (R) splice variant was modulated in SH-SYSY neuroblastoma cells by transgenic over-expression and by knock-down with siRNA. We also tested whether the classical agonist of acetylcholine receptors, carbachol may affect PS1 levels. Finally, we evaluated PS1 levels in amyloid Aβ42-treated SH-SYSY cells with and without AChE knock-down. **Results:** We showed an AChE influence on PS1 levels by showing that AChE knock-down with siRNA in SH-SYSY neuroblastoma cells decreased PS1 levels. We found that AChE over-expression exerts opposing effects on PS1 levels, leading to increased level of PS1 protein in transfected cells. Interestingly, over-expression of the AChE-R variant, which is normally present at low levels in the mammalian brain, was more effective influencing PS1 than the over-expression of the major cholinergic AChE-T variant. The cholinergic agonist carbachol failed to exert an effect on PS1. Finally we expressed neuroblastoma cells to Aβ42 which triggered elevation of both AChE and PS1 levels. The Aβ42-induced PS1 increase was abolished by pre-treatment of SH-SYSY with siRNA AChE, suggesting that AChE may participate in the pathological feed-back loop between PS1 and Aβ. **Conclusions:** Our results provide insight into AChE-amyloid interrelationships and identify a new molecular interaction that may contribute to AD pathology and have therapeutic implications.

**P2-312 TRANSGENIC MOUSE MODELS WITH INCREASED PYROGLUTAMATE-ABETA FORMATION SHOW EARLY PHENOTYPIC CHANGES**

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**Background:** Pyroglutamate (pGlu)-modified amyloid peptides are found in deposits in sporadic and inherited Alzheimer’s disease as well as in Familial British and Familial Danish dementia. Formation of pGlu at the N-Terminus confers resistance against cleavage by proteases and peptidases, increases the cytotoxicity of the peptides and speeds up Ab aggregate formation. Thus, the accumulation of modified Ab might represent one driving force of the neurodegenerative amyloidoses. In order to further characterize theophysiological potential of N-truncated and pGlu-modified Ab peptides, we aimed at an increase of pGlu-Ab formation in mouse models. **Methods:** We cross-bred APPsw/1 with mice, which express human Glutaminyl Cyclase (QC) neuron-specifically. Phenotypic changes were assessed in Morris Water Maze and Contextual fear conditioning paradigms. The Amyloid pathology was characterized by immunohistochemistry and ELISA techniques detecting total Aβ and pGlu-modified amyloid. **Results:** Pyroglutamate-modified peptides were detected at an age of 7 months in the brain homogenates of double transgenic mice. At 9 months of age, the pGlu-Ab load was 2-4fold higher in double transgensics compared to APPsw/1. Behavioral changes of TASD41/hQC double transgensics started to develop at 6 months of age, as assessed in Morris water maze and contextual fear conditioning paradigms. Pharmacologic treatment of these mice with two QC inhibitors attenuated the amyloid pathology, suggesting involvement of QC activity rather than expression of the protein. **Conclusions:** Expression of the enzyme responsible for pGlu-Ab production led to significantly increased pGlu-Ab pathology. Although the pGlu-Ab load appears to be moderate compared to human AD, the formation of these species promotes early behavioral changes in the mice. The results support that N-terminal heterogeneity of Aβ influences the pathophysiological potential of amyloid peptides, supporting a causal involvement rather than a bystander-role.

**P2-313 QUANTITATIVE MODELING OF AMYLOIDOGENIC PROCESSING AND ITS INFLUENCE BY SORL1/SORL1 IN ALZHEIMER’S DISEASE**

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**Background:** Trafficking of amyloid precursor protein (APP) through the intracellular compartments of neurons is a major regulatory process in the breakdown of this precursor, leading to Abeta production and senile plaque formation. However, the cellular mechanisms that control APP transport and that, when altered, cause Alzheimer’s disease (AD) remain incompletely understood. Since quantitative aspects of APP processing are considered critical both to normal neuronal function and to neurodegeneration, a better understand of the tightly regulated kinetics of transport and proteolytic turn-over of the precursor protein in neurons is warranted. Recently, we demonstrated that SORLA (aka SORL1 or LR11), a neuronal receptor that controls intracellular transport and processing of APP, acts as retention factor for the precursor protein in the trans-Golg network, preventing release of the precursor into regular processing pathways. SORLA-mediated APP retention leads to decreased Abeta levels in neuronal cell lines, whereas ablation of receptor expression in knockout mice results in increased levels of Abeta in the brain similar to the situation in patients with late-onset AD who exhibit poor receptor expression. **Methods:** To test there levance of subtle alterations in SORLA levels for risk of amyloidogenic processing, we developed a novel cell system in which the amount of APP and SORLA that are being produced can be varied. These cell lines have been applied to produce quantitative data to estimate reaction constants of the proteolytic processing of APP into sAPPalpha, sAPPbeta, and Abeta in the presence or absence of SORLA. Based on the experimental data, we developed nonlinear ordinary differential equation models faithfully describing the cleavage of APP by alpha- and beta- secretases and the effect of SORLA on these processes. **Results:** Taken together, our quantitative biochemical data demonstrate that alpha- and beta-secretases are allosteric enzymes that depend on formation of APP oligomers for efficient processing. We also demonstrate that SORLA-mediated APP retention leads to decreased Abeta levels in neuronal cell lines, whereas ablation of receptor expression in knockout mice results in increased levels of Abeta in the brain similar to the situation in patients with late-onset AD who exhibit poor receptor expression. **Conclusions:** Rather it acts as competitive inhibitor that prevents APP oligomerization, thereby eliminating the preferred form of the secretase substrate and reducing amyloidogenic processing.

**P2-314 MOLECULAR STUDIES OF NICA STRIN IN THE γ-SECRETASE COMPLEX USING AN ARTIFICIAL NICA STRIN INDEPENDENT PRESENILIN 1 DOUBLE MUTANT**

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