The affects of amyloid precursor protein (APP) trafficking, processing and interaction with p75 in the presence of amyloid-β

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Alzheimer's disease (AD) is one of the most well known and most debilitating neurodegenerative diseases. Neurons die in AD due to toxic levels of amyloid beta (Aβ) proteins and tangled clusters of tau proteins within the cell. The Aβ originates from cleavage of APP (amyloid precursor protein). APP has been shown to interact with p75NTR (the neurotrophin receptor), leading to cell death (Fombonne *et al.*, 2009). However, how APP interacts with p75NTR and whether the receptor ligands affect their interactions are not known. We propose that p75NTR ligands may affect APP-p75TR interactions and the interaction may participate in the development of AD.

In the present study we attempted to use FRET (fluorescence resonance energy transfer) analysis to characterize the nature of the APP-p75NTR interaction in HEK293 cells which were transfected with p75NTR-CFP and APP-YFP plasmids and controls. These cells were subjected to a range of neurotrophins, and Aβ, at differing concentrations. The FRET efficiency of APP-p75NTR was significantly higher than the negative control and comparable to the positive control, suggesting these two molecules are close to each other. All neurotrophins at least in a lower concentration triggered an increase in the FRET efficiency between p75NTR and APP, suggesting neurotrophins can enhance the interaction between p75NTR and APP. Interestingly, Aβ at all concentrations triggered a dramatic increase in FRET efficiency, reaching over 3-fold of the positive control, suggesting that Aβ can cause p75NTR-APP clumping together and trigger an aggressive interaction between these molecules. As Aβ is the pathogenic agent for AD, the subsequent experiments had focused on the nature of the Aβ induced interactions.

Aβ time course of Aβ's effect on p75NTR-APP interaction was undertaken. Results showed that the interaction increased dramatically within the first 1-3 minutes of the addition of Aβ to the cells. Aβ not only had a profound effect on p75NTR-APP interaction, but also its effect was almost immediate, suggesting a quick biochemical reaction. It is known that both p75NTR and APP have phosphorylation sites within their intracellular domains. We propose that a phosphorylation event may play a role in the interaction of p75NTR-APP upon addition of the ligand Aβ. When a phosphorylation inhibitor KT5720 was added to transfected cells with added Aβ, FRET efficiency dropped. Reciprocally, when a phosphorylation activator 8-Br-cAMP was added, FRET efficiency increased significantly. These data seem to support the idea that p75NTR-APP interaction is under the influence of a phosphorylation event. Furthermore we proposed that endogenous Aβ may constitutively activate the interactions of p75NTR and APP. To test the idea, FRET efficiency dropped further by blocking endogenous Aβ with an antibody 6E10, indicating endogenous Aβ may increase the interaction of APP and p75NTR. Also, the preliminary data support that Aβ increases APP transcription possibly *via* activation of the p75NTR-APP pathway. This could suggest that Aβ may have a positive feed forward mechanism that increases APP transcription, which may in turn increase an output of Aβ. We conclude that p75NTR ligands, Aβ and neurotrophins enhance the interaction of p75NTR-APP in a phosphorylation dependent manner, and the increased interaction of p75NTR-APP may participate in the pathogenesis of AD.

Fombonne J, Rabizadeh S, Banwait S, Mehlen P, Bredesen DE (2009) Selective vulnerability in Alzheimer's disease: amyloid precursor protein and p75(NTR) interaction. *Annals of Neurology* **65:** 294-303.