Aβ Imaging Ligand Pittsburgh Compound B Binding in AD Frontal Cortical Synaptosomes is in Glutamatergic Synapses

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Background: Introduction of the PET imaging agent 11C-Pittsburgh Compound B (PIB) revolutionized AD diagnosis by enabling detection of early stage fibrillar β-amyloid brain pathology in living subjects before clinical symptoms manifest. PIB binding in humans correlates with both early stage clinical diagnosis and levels of Aβ pathology. Both PIB binding and AD are only found in humans and not in animal Aβ pathology models ranging from nonhuman primates to transgenic mice. Our previous work showed that plaque-associated 3H-PIB binding is in a specific, detergent- resistant, insoluble Aβ protein-lipid AD PIB-binding complex (ADPBC) that contains ~95% of the PIB binding present in AD frontal cortex.

Purpose/Hypothesis: To determine the neuronal types whose synapses bind the human-specific Aβ imaging ligand PIB.

Methods: Synaptosomes were prepared by homogenization in 0.32M sucrose followed by differential centrifugation. The P2 fraction in 0.32M sucrose was further purified over a two-step sucrose gradient collecting the synaptosomes from the 0.8 M/1.2M interface. Under these conditions the ADPBC (plaque) pellets away from the synaptosomes. The highly fluorescent analog of PIB, CN-PIB, was used to identify PIB-binding synaptosomes and for colocalization with pre-synaptic markers (VGlut1, SNAP-25) and the postsynaptic marker, PSD-95. Aβ content was assessed with monoclonal antibodies to Aβ (6E10 and 4G8 - Covance) and MOAB-2 (AβO- selective and non-APP reactive – Novusbio NBP2-13075). We used a Sony SY3200 High Performance Cell Sorter (UK Flow Cytometry Core Facility) to analyze synaptosomes for co- localization of CN-PIB binding with multiple other ligands and proteins of interest. The same instrument was also used to sort and isolate VGlut1+ synaptosomes that were either PIB+ or PIB- for further biochemical analysis.

Results & Conclusions: Non-plaque high-affinity PIB binding (Kd = 2-5 nM) is disrupted by low concentrations of SDS, in contrast to the highly resistant plaque-like ADPBC. This labile binding is in synaptosomes, a subcellular brain fraction containing the pre- and post-synaptic compartments of neurons. PIB binding is present in a small fraction of total synaptosomes from AD frontal cortex, and is restricted to glutamatergic (VGlut1+) endings. A larger proportion of 0.5-1.5 µm diameter synaptosomes from AD brain bound high levels of CN-PIB compared with those from non-cognitively impaired cases. We found a nearly identical structure-activity-relationship for a series of PIB analogues binding to the synaptosomes and ADPBC, suggesting that the PIB binding pocket is highly conserved. Antibody reactivity for Aβ was detected only in VGlut1+ synaptosomes that also bound CN -PIB. This suggests that the accumulation of synaptic Aβ Frontal cortex neurons are primarily glutamatergic, and glutamate excitotoxicity is a major contributor to neuronal death in AD. One trigger for neocortical synaptic dysfunction is thought to be at the level of glutamate receptor regulation, followed by dendritic and spine alterations and axonal transport defects that culminate in loss of glutamatergic synapses. Flow cytometry allows us to analyze, select, and enrich for specific, matched populations of synaptosomes to probe events occurring at the synapses of neurons with the same or different neurotransmitter types within the same brain region of the same individual.