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Research Report

[^{3}H]-L-685,458 as a radiotracer that maps γ-secretase complex in the rat brain: Relevance to Aβ genesis and presence of active presenilin-1 components

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ABSTRACT

γ-Secretase is a multimeric enzyme important for normal cell/neuronal proliferation, differentiation and plasticity. Determining *in vivo* γ-secretase expression and activity remains a challenge because its subunit proteins can exist in immature and preassembled forms, but may execute cellular roles irrelevant to γ-site cleavage. In this study, we characterized [^{3}H]-L-685,458 as a radiotracer for the detection of active γ-secretase in adult rat brain. *In vitro* autoradiography indicated that [^{3}H]-L-685,458 binding was saturatable, displaceable by peptidomimetic and small molecule γ-secretase inhibitors, and exhibited rapid association and dissociation kinetics. In cultured hippocampal slices, [^{3}H]-L-685,458 binding density correlated with Aβ reduction following in-dish dosing of this radioligand or a non-radioactive γ-secretase inhibitor. [^{3}H]-L-685,458 binding sites in the adult brain were differentially distributed across regions and laminas, with heavy binding localized to the olfactory glomeruli, hippocampal CA3 and cerebellar molecular layer, and moderate binding in the cerebral cortex, amygdala and selected subcortical regions. All of these regions showed labeling for presenilin-1 N-terminal fragments (PS1-NTFs). A distinct correlation of dense binding sites with abundant presence of PS1-NTFs was verified in hippocampal mossy fiber terminals and olfactory bulb glomeruli, suggestive of a rich expression of γ-secretase in the synapses at these locations that are characteristic of dynamic plasticity. Together, [^{3}H]-L-685,458 is an excellent radiotracer for mapping active γ-secretase complex, and may serve as a useful tool for studying the enzyme *in vivo* and *in vitro*.

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1. Introduction

γ-Secretase is an aspartyl protease that cleaves its substrates along their transmembrane regions. This enzyme produces beta-amyloid peptides (Aβ) by catalyzing gamma-site cleavage of beta-amyloid precursor protein (APP). Accumulation of Aβ, especially longer species, is considered to play a pathogenic role in Alzheimer's disease (AD) (Hardy and Allsop, 1991; Selkoe, 1994; Kaether et al., 2006). γ-Secretase also cleaves a growing list of other type I membrane proteins by mediating

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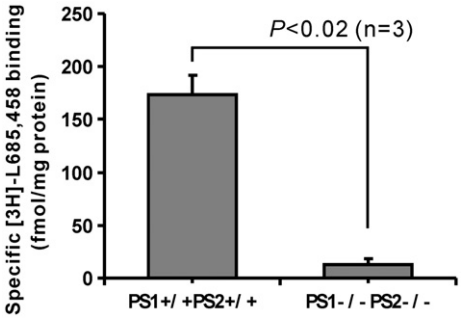

Fig. 1 – Reduced membrane binding of [^{3}H]-L-685,458 in presenilin (PS1 and PS2)-deficient cells. Specific [^{3}H]-L-685,458 binding in PS1$^{-/-}$PS2$^{-/-}$ blastocyst-derived membranes was greatly decreased (up to 90%) relative to wild-type (PS1$^{+/+}$PS2$^{+/+}$) counterparts ($p < 0.02$, 3 assays, Student's *t* test). The remaining radioactivity in PS1$^{-/-}$PS2$^{-/-}$ membranes is not differentiable from the levels of nonspecific reactivity.

the so-called regulated intramembrane proteolysis (Iwatsubo, 2004; Raemaekers et al., 2005). This process liberates active protein fragments that further modulate many basic cellular processes such as receptor activation and signal transduction, which are essential for normal cell/neuronal proliferation, differentiation and plasticity (Hartmann et al., 1997; Figueroa et al., 2002; Parent et al., 2005). Disruption of regulated intramembrane proteolysis may relate to certain disease conditions including tumorigenesis in addition to AD (van Es et al., 2005).

Several proteins have been identified to participate in the formation and function of γ-secretase (Kaether et al., 2006). The N- and C-terminal fragments of presenilins (PS1 and PS2), proteolytic products from the inactive holoproteins, contribute to the enzyme's catalytic core (Kimberly et al., 2003; Laudon et al., 2004). Nicastrin is found to serve as the enzyme receptor, whereas Aph-1 and Pen-2 appear to be involved in the assembly, trafficking and maturation of the enzyme complex (Gu et al., 2003; Luo et al., 2003; Niimura et al., 2005). Lately, a protein called TMP21 is identified as a part of the enzyme complex (Chen et al., 2006).

Conventional detections of γ-secretase subunit proteins or their mRNAs by immunohistochemistry or in situ hybridization may not necessarily or precisely reflect the functional status of the enzyme for several potential reasons: (1) γ-secretase subunit proteins can exist as immature and/or preassembled forms (Kaether et al., 2006); (2) enzyme cofactor proteins may execute independent cellular functions irrelevant to γ-site cleavage (Doglio et al., 2006); (3) active PS fragments constitute a fairly small fraction of the total cellular PS pool (Beher et al., 2003; Lai et al., 2003); (4) changes in the cofactor proteins can alter γ-secretase activity (Shiraishi et al., 2004; Chen et al., 2006).

Specific enzyme inhibitors have been used as molecular probes for *in vitro* characterization of γ-secretase (Li et al., 2000; Tian et al., 2002; Beher et al., 2003). A small molecule inhibitor, compound D, was identified as an ideal radioligand for *in vitro* detection of putative active sites of γ-secretase in mammalian brains (Yan et al., 2004; Patel et al., 2006). L-685,458 is a well-defined and commercially available peptidomimetic inhibitor that directly targets at the catalytic core of γ-secretase (Li et al., 2000). In the present study we extended the utility of this compound to a radioligand for mapping active γ-secretase enzyme sites in the brain. We have defined that [^{3}H]-L-685,458 binding sites coexist with active PS-1 components but likely reflect local Aβ genesis in neuronal or synaptic structures.

2. Results

2.1. *Characterization of [^{3}H]-L-685,458 binding in presenilin-deficient cell membranes*

Association of [^{3}H]-L-685,458 binding with presenilins (PS1, PS2) was verified by *in vitro* binding on membranes from PS1$^{+/+}$ PS2$^{+/+}$ and PS1$^{-/-}$PS2$^{-/-}$ blastocyst-derived cells (Lai et al., 2003). Specific radioactivity of membrane-bound [^{3}H]-L-685,458 was reduced to less than 10% (mean ± SEM, 8 ± 2.8%) in double knockouts (PS1$^{-/-}$PS2$^{-/-}$) relative to wild type (PS1$^{+/+}$ PS2$^{+/+}$) (100% ± 10.8) (Fig. 1). Statistical analyses indicated a significant difference in specific binding density between the two types of cells ($n = 3$, $p < 0.02$, Student's *t* test). However, no difference in radioactivity (standardized to background) existed between double knockouts and nonspecific signals from membranes assayed in the presence of excessive cold ligand ($n = 3$, $p = 0.31$, Student's *t* test). Therefore, membrane binding activity of [^{3}H]-L-685,458 appeared to be dependent on the presence of PS1 and PS2.

2.2. *Characterization of [^{3}H]-L-685,458 binding in brain sections*

In order to determine the utility of [^{3}H]-L-685,458 in autoradiography, we first tested this radioligand on brain sections

Fig. 2 – Autoradiographic characterizations of [^{3}H]-L-685,458 binding on forebrain sections. Panels A–D are representative autoradiographs of binding sites yielded with 5 nM [^{3}H]-L-685,458 in the absence (A) and presence of excessive (0.5 μM) cold ligands, including L-685,458 (B), DAPT (C) and compound-E (D). Panels E–F are fitting curves showing *in vitro* radioligand binding profiles. The binding is saturatable with a specific to total binding ratio over 90% (E). [^{3}H]-L-685,458 binding is concentration dependently inhibited by non-radiolabeled peptidomimetic (L-685,458 and L-852,631) or small molecule (DAPT and compound-E) γ-secretase inhibitors (F). [^{3}H]-L-685,458 binds to the enzyme sites considerably fast, with saturation equilibrium occurring within 1 h (G). Ligand and site dissociation is also fast in fresh assay buffer, with a decay half time ~11 min. Specific binding densities are calculated by subtracting nonspecific binding from total binding measured over the gray matter of the frontal cortices from 4 rats. Binding densities of all groups are normalized to either the mean of densities from longest incubation group (G), or from the group not subjected to further incubation in the cold assay buffer (H). DLU/mm^2: digital light units/mm^2. Scale bar = 0.5 mm in panel D applying to A–C.