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Research Report

Altered distribution of mGlu2 receptors in β-amyloid-affected brain regions of Alzheimer cases and aged PS2APP mice

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ABSTRACT

Altered glutamatergic synaptic transmission is among the key events defining the course of Alzheimer's disease (AD). mGlu2 receptors, a subtype of group II metabotropic glutamate receptors, regulate (as autoreceptors) fast synaptic transmission in the CNS via the controlled release of the excitatory amino acid glutamate. Since their pharmacological manipulation in rodents has been reported to affect cognition, they are potential drug targets for AD therapy. We examined the fate of these receptors in cases of AD as well as in aging PS2APP mice—a proposed model of the disease. *In vitro* binding of [^{3}H]LY354740, a selective group II agonist (with selective affinity for mGlu2 receptors, under the assay conditions used) and quantitative radioautography revealed a partial, but highly significant, loss of receptors in amyloid-affected discrete brain regions of AD cases and PS2APP mice. Among the mouse brain regions affected were, above all, the subiculum but also frontolateral cortex, dentate gyrus, lacunosum moleculare and caudate putamen. In AD, significant receptor losses were registered in entorhinal cortex and lacunosum moleculare (40% and 35%, respectively). These findings have implications for the development of selective ligands for symptomatic therapy in AD and for its diagnosis.

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Abbreviations: AID, agranular insular cortex, dorsal part; AIV, agranular insular cortex, ventral part; CA1, cornu ammonis region 1; CA3, cornu ammonis region 3; CPu, caudate putamen; DG, dentate gyrus; EC, entorhinal cortex; LMol, lacunosum moleculare; Mol, molecular layer dentate gyrus; M1, primary motor cortex; M2, secondary motor cortex; PrS, presubiculum; S, subiculum; SI, substantia innominata; S1, primary somatosensory cortex; SIDZ, primary somatosensory cortex, dysgranular region; SIBF, primary somatosensory cortex, barrel field; V2L, secondary visual cortex, lateral area

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1. Introduction

Cognitive impairment associated with Alzheimer's Disease (AD) is accompanied by progressive degeneration in the mediotemporal lobe (Celone et al., 2006; Devanand et al. 2007) which is strongly affected by both β-amyloid (Aβ) deposition and τ-phosphorylation (Braak and Braak, 1991; Braak et al., 2006; Lazarov et al., 2002; Thal et al., 2000; von Gunten et al., 2006). Glutamatergic neurons in these regions, particularly the entorhinal cortex, are among the most damaged in early AD (Gomez-Isla et al. 1996; Carter et al., 2004; Kirvell et al., 2006) leading to altered processing of amyloid precursor protein (APP), reduced synaptic connectivity and formation of neurofibrillary tangles (NFT; Adalbert et al., 2007; Bell et al., 2003, 2006; Buxbaum et al., 1998; Conforti et al., 2007; Jacobsen et al., 2006; Yoshiyama et al., 2007).

Glutamate excitatory synaptic neurotransmission is mediated by receptors classified into two functional groups: ionotropic and metabotropic (Kew and Kemp, 2005; Foster and Kemp, 2006). The group II metabotropic glutamate (mGlu) receptors (mGlu2 and mGlu3) are broadly distributed in rodent brain (see Richards et al., 2005; Ferraguti and Shigemoto, 2006) where they mainly exert a presynaptic control on the release of glutamate (Schoepp, 2001). mGlu2 receptors, in particular, are highly concentrated in the perforant path regulating the level of excitation in the hippocampal formation (Kew et al., 2001, 2002). It was recently shown that pharmacological manipulation of mGlu2 receptors in rodents affects cognition (Higgins et al., 2004) with mGlu2 receptor agonists having a negative effect on cognitive functions. Since the hippocampus is known to play a crucial role in emotions, learning and memory that are severely affected in Alzheimer's disease (AD), hypothetically the blocking action of mGlu2 antagonists (Woltering et al., 2008) on presynaptic receptors of surviving glutamatergic neurons in the corticohippocampal circuitry in AD should facilitate excitatory neurotransmission and thereby restore cognitive functions.

Previous studies of glutamate receptors (Albasanz et al., 2005; Cha et al., 2001; Dewar et al., 1991; Greenamyre and Young, 1989; Hsia et al., 1999; Hyman et al., 1987; Jansen et al., 1990; Lee et al., 2004; Penney et al., 1990; Taylor et al., 2002; Thorns et al., 1997; Young and Penney, 1994) have used the non-selective ligand [^{3}H] glutamate. However, the subsequent development of more selective mGlu receptor ligands (see Schoepp et al., 1999; Kew and Kemp, 2005) has provided the opportunity of creating receptor subtype-selective radioligands (e.g. Schaffhauser et al., 1998; Johnson et al., 1999; Mutel et al., 1998; Wright et al., 2001). Therefore, we examined the binding of the selective group II agonist LY354740 ((+)-2-aminobicyclo[3,1,0]-hexane-2,6-dicarboxylic acid), in its tritiated form, in the hippocampal formation and entorhinal cortex in AD postmortem and during aging in the PS2APP mouse model of AD (Richards et al., 2003). The overexpression, in mice, of mutant forms of human presenilins (PS1 or PS2) and human APP transgenes causes cognitive deficits, amyloidosis and axonopathies in an age-dependent brain region-specific manner indicating a causal relationship between the altered expression of the transgenes and some key aspects of AD neuropathology (Almeida et al., 2005; Dong et al., 2007; Gotz et al., 2006; Lazarov et al., 2006; Priller et al., 2006, 2007; Richards et al., 2003). A longitudinal *in vivo* multiphoton microscopy study of young APPswe/-PS1d9xYFP (B6C3-YFP) transgenic mice (Meyer-Luehmann et al., 2008) has recently demonstrated the extremely rapid formation of plaques and established them as a critical mediator of neuritic pathology.

2. Results

2.1. *Age-related [^{3}H]LY354740 binding to brain regions of control and PS2APP mice*

The distribution and abundance of [^{3}H]LY354740 binding sites in control mouse brain at 8 months (Fig. 1) were similar to that previously reported (Schaffhauser et al., 1998; Richards et al., 2005; Malherbe et al., 2005). The highest level of specific binding was found in the lacunosum moleculare of the hippocampus (6314 ± 878 fmol/mg prot.) with little or no binding in white matter. Non-specific binding (not competed for by 10 μM DCG IV) was approximately 10% of total. Binding to control mouse and Fischer rat brain regions did not differ significantly with age (Supplementary Figures 1 and 2 online).

The affinity of LY354740 for the long form of dopamine D2 receptor (high affinity binding site) was recently reported (Seeman et al., 2008, 2009) although this is disputed (Fell et al., 2009). In house data could not confirm the results of Seeman et al. (unpublished affinity studies with [^{3}H]domperidone and functional studies using the GTPγS assay). Moreover, the distribution of binding sites for [^{3}H]LY354740 in mouse and human brain, as described in this study, is generally well correlated with that of primary transcripts and gene products for mGlu2 and in contrast to earlier studies on D2 receptors revealing their abundance in mesolimbic and nigrostriatal brain regions. Our results are consistent with previously published pharmacologic and genetic studies of mGlu2 receptors (Richards et al. 2005; Higgins et al. 2004; Malherbe et al., 2005).

At 5 months (Fig. 2; Supplementary Table 1 online), in most brain regions of PS2APP mice there was a trend (albeit a statistically non-significant one) to bind [^{3}H]LY354740 to a slightly lower degree than controls. However, at 9 months binding was significantly reduced in the dentate gyrus, lacunosum moleculare and subiculum. Marginally significant reductions also occurred in the cerebellum, caudate putamen, frontolateral cortex and parietal cortices at this age. At 13 months, binding to the caudate putamen, frontolateral cortex and subiculum was reduced significantly compared to controls. Of the remaining regions, only the dentate gyrus was marginally reduced. Finally, at 17 months, only the subiculum showed a statistically significant change. Highly significant reduced binding was also measured consistently in the brains of a cohort of 21-month-old PS2APP mice. In contrast, all brain regions of 17.5-month-old single transgenic $hAPP_{swe}$ mice bound the radioligand to a degree similar to controls. The observed variability in binding to brain regions of mice at the same age is probably due to not only the use of a radiolabeled agonist (possibly reflecting variable amounts of the endogenous transmitter bound to the receptor competing with the radioligand) but also to the reported variability within the PS2APP strain (Richards et al., 2003).