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BRAIN RESEARCH

Research Report

Dopamine release in prefrontal cortex in response to β-amyloid activation of α7* nicotinic receptors

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ABSTRACT

The levels of soluble beta-amyloid (Aβ) are correlated with symptom severity in Alzheimer's disease. Soluble Aβ has been shown to disrupt synaptic function and it has been proposed that accumulation of soluble Aβ triggers synapse loss over the course of the disease. Numerous studies indicate that soluble Aβ has multiple targets, one of which appears to be the nicotinic acetylcholine receptor, particularly for Aβ concentrations of pM to nM. Moreover, pM to nM soluble Aβ was found to increase presynaptic Ca^{2+} levels, suggesting that it may have an impact on neurotransmitter release. In the present study, soluble Aβ was perfused into mouse prefrontal cortex and the effect on the release of dopamine outflow via microdialysis was assessed. In the presence of tetrodotoxin, $Aβ_{1–42}$ at 100 nM evoked the release of dopamine to ~170% of basal levels. The $Aβ_{1–42}$-evoked dopamine release was sensitive to antagonists of α7 nicotinic receptors and was absent in mice harboring a null mutation for the α7 nicotinic subunit, but was intact in mice harboring a null mutation for the β2 nicotinic subunit. The control peptide $Aβ_{40–1}$ was without effect. In contrast, $Aβ_{1–42}$ at 1–10 pM caused a profound but slowly developing decrease in dopamine outflow. These results suggest that Aβ alters dopamine release in mouse prefrontal cortex, perhaps involving distinct targets as it accumulates during Alzheimer's disease and leading to disruption of synaptic signaling.

1. Introduction

One of the major pathological entities in Alzheimer's disease (AD) is beta amyloid (Aβ). Aβ refers to a collection of peptides of 38–43 amino acids in length, which are derived from the amyloid precursor protein (APP) by sequential proteolytic cleavage (reviewed in Walsh and Selkoe, 2007). The predominant forms of Aβ found in AD are $Aβ_{1–40}$ and $Aβ_{1–42}$, with their levels progressively increasing over the course of the disease (Ingelsson et al., 2004). Though Aβ notably accumulates in plaques, a significant portion exists in soluble forms, mainly as oligomers (or ADDLs; Lambert et al., 1998), and it has been proposed that increased levels of soluble Aβ oligomers in the early stages of AD cause synaptic dysfunction (Hardy and Selkoe, 2002; Selkoe and Schenk, 2003) and, later, synapse loss (Lue et al., 1999). The $Aβ_{1–42}$ form, though less abundant than the $Aβ_{1–40}$ form, has the highest propensity to form toxic oligomers (Walsh and Selkoe, 2007) and correlates with disease onset (Kumar-Singh et al., 2006). In addition, aggregated and fibrillar forms of Aβ also accumulate and hence

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Abbreviations: Aβ, β-amyloid; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; APP, amyloid precursor protein; BgTx, α-bungarotoxin; DA, dopamine; DHBE, dihydro-β-erythroidine; HBS, HEPES-buffered saline; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; 3×Tg-AD, triple-transgenic AD mice; TTX, tetrodotoxin

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different forms of Aβ may have different effects in the brain, which, together with neurofibrillary pathology, likely includes synaptic dysfunction, synapse loss, oxidative damage, inflammation and neuron loss (Selkoe, 2002; Mattson, 2004).

The accumulation of Aβ in AD is selective, with particularly high concentrations found in frontal cortex, entorhinal cortex, hippocampus, amygdala and parietal association cortex (Braak and Braak, 1991). Moreover, the accumulation of Aβ is largely linked to its release from synaptic nerve endings, as evidenced by its loss from terminal innervation sites of lesioned pathways in APP transgenic mice (Lazarov et al., 2002; Sheng et al., 2002). In addition, soluble Aβ oligomers, particularly $A\beta_{1-42}$, has been shown to disrupt synaptic plasticity, specifically LTP in the hippocampus (Walsh et al., 2005). On the other hand, it appears that oligomerization of Aβ starts intraneuronally (Oddo et al., 2006). The extent to which Aβ increases or decreases synaptic activity, and its site(s) of action, remains to be fully elucidated.

Previous work has demonstrated a potent action of soluble Aβ on nicotinic acetylcholine receptors (nAChRs), including both antagonist (Pettit et al., 2001; Liu et al., 2001; Grassi et al., 2003; Wu et al., 2004) and agonist (Dineley et al., 2001; Dougherty et al., 2003; Fu and Jhamandas, 2003) effects. We have shown that pM to nM $A\beta_{1-42}$ induces increased $[Ca^{2+}]_i$ in isolated presynaptic terminals from rat cortex and hippocampus in a manner susceptible to partial antagonism by classical nAChR antagonists, such as α-bungarotoxin and dihydro-β-erythroidine. Moreover, prior activation of presynaptic nAChRs attenuated subsequent responses to $A\beta_{1-42}$. However, there remains some reservation regarding the actual target for soluble Aβ, particularly the role of nAChRs, and whether activation of presynaptic nAChRs leads to altered synaptic transmission. Here, we employed preparations from mice harboring null mutations for either the α7 subunit or the β2 subunit of the two major nAChR subtypes present in brain, namely the α-bungarotoxin-sensitive and high-affinity subtypes, respectively (Role and Berg, 1996; Zoli et al., 1998), to examine the effect of Aβ on the release of dopamine in the prefrontal cortex of the intact brain. Dopamine (DA) is a prominent player in the functioning of the prefrontal cortex (Arnsten and Li, 2005) and alterations in its release by Aβ could lead to altered prefrontal cortical function.

2. Results

2.1. *Nicotine and β-amyloid evoke the release of dopamine in mouse prefrontal cortex*

The release of dopamine in prefrontal cortex was measured in freely moving mice via *in vivo* microdialysis (Fig. S1) in the presence of TTX in order to isolate presynaptic regulation from cellular effects. Nicotine (1 μM) in the presence of TTX evoked an increase in DA outflow (~300% over basal) and this increase was blocked by prior treatment with a nAChR antagonist (Fig. 2A). These results are generally consistent with previous reports of nicotine-evoked DA release in rodent prefrontal cortex slices (Rao et al., 2003; Cao et al., 2005). Perfusion with soluble 100 nM of $A\beta_{1-42}$ (Fig. 1) also evoked the release of DA (~170% over basal; $p<0.05$) in a manner sensitive to nAChR antagonists (Fig. 2B). (Perfusion of Aβ using microdialysis has been previously characterized (Parks et al., 2001; Trabace et al., 2007).) Perfusion with 100 nM of the soluble core amyloid fragment $A\beta_{12-28}$ (Wang et al., 2000a; Dougherty et al., 2003) also evoked the release of DA (Fig. 2C), but the response was rather variable. Curiously, perfusion with 1–10 pM $A\beta_{1-42}$ did not evoke an increase in DA outflow, but, rather, caused a slowly developing, profound decrease in DA outflow (Fig. 2D),

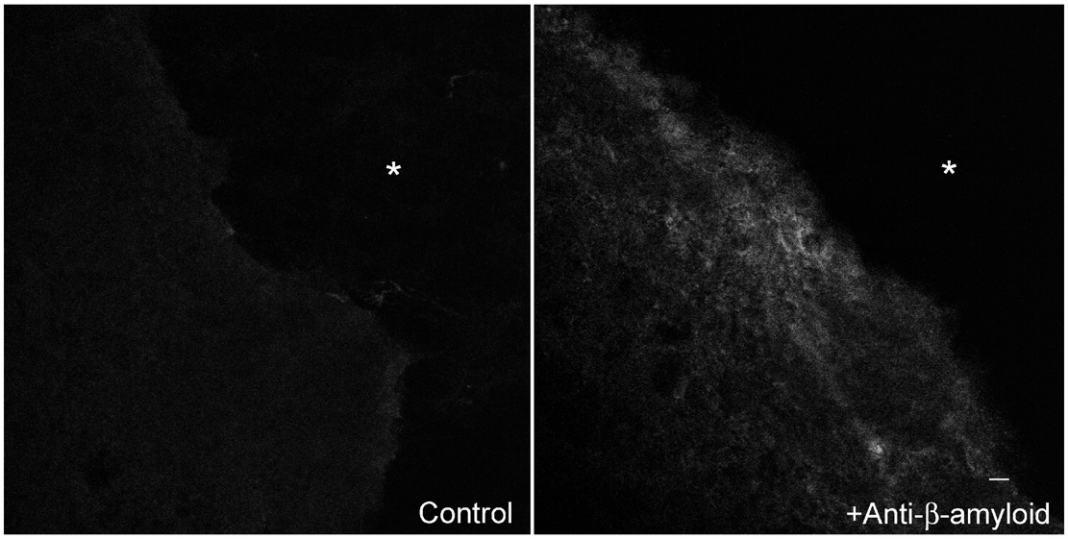

Fig. 1 – Microdialysis perfusion of β-amyloid. β-Amyloid perfused into surrounding tissue via the microdialysis probe (*blank areas along right side of micrographs) placed in the prefrontal cortex for 30 min was detected via immunocytochemistry, as described in Experimental procedures, using an anti-Aβ monoclonal antibody (right) in comparison to a control section incubated with fluorescein-conjugated secondary antibody alone (left). Images of 40-μm sections around the microdialysis probe (*) were taken using confocal microscopy. Scale bar = 12 μm. Post-hoc staining with Hoescht did not reveal any gross alteration in the tissue following Aβ perfusion (not shown).