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NEUROPROTECTIVE AMYLOID B N-TERMINAL PEPTIDES DIFFERENTIALLY ALTER HUMAN A7- AND A7B2-NICOTINIC ACETYLCHOLINE (NACH) RECEPTOR SINGLE-CHANNEL PROPERTIES

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Abstract :

Background and Purpose

Oligomeric amyloid β $_{1-42}$ (oAβ$_{1-42}$) exhibits agonist-like action at human α7- and α7β2-containing nicotinic receptors. The N-terminal amyloid β$_{1-15}$ fragment (N-Aβ fragment) modulates presynaptic calcium and enhances hippocampal-based synaptic plasticity via α7-containing nicotinic receptors. Further, the N-Aβ fragment and its core sequence, the N-amyloid-beta core hexapeptide (N-Aβcore), protect against oAβ$_{1-42}$-associated synapto- and neurotoxicity. Here, we investigated how oAβ$_{1-42}$, the N-Aβ fragment, and the N-Aβcore regulate the single-channel properties of α7- and α7β2-nicotinic receptors.

Experimental Approach

Single-channel recordings measured the impact of acetylcholine, oAβ$_{1-42}$, the N-Aβ fragment, and the N-Aβcore on the unitary properties of human α7- and α7β2-containing nicotinic receptors expressed in nicotinic-null SH-EP1 cells. Molecular dynamics simulations identified potential sites of interaction between the N-Aβ fragment and orthosteric α7+/α7- and α7+/β2- nicotinic receptor binding interfaces.

Key Results

The N-Aβ fragment and N-Aβcore induced α7- and α7β2-nicotinic receptor single-channel openings. Relative to acetylcholine, oAβ$_{1-42}$ preferentially enhanced α7β2-nicotinic receptor single-channel open probability and open-dwell times. Co-application with the N-Aβcore neutralized these effects. Further, administration of the N-Aβ fragment alone, or in combination with acetylcholine or oAβ$_{1-42}$, selectively enhanced α7-nicotinic receptor open probability and open-dwell times (compared to acetylcholine or oAβ$_{1-42}$).

Conclusions and Implications

Amyloid-beta peptides demonstrate functional diversity in regulating α7- and α7β2-nicotinic receptor function, with implications for a wide range of nicotinic receptor-mediated functions in Alzheimer's disease. The effects of these peptides on α7- and/or α7β2-nicotinic receptors revealed complex interactions with these subtypes, providing novel insights into the neuroprotective actions of amyloid β-derived fragments against the toxic effects of oAβ$_{1-42}$.

Keywords : iso-form , receptors , amyloid beta peptides

Introduction :

Alzheimer's disease (AD) is a neurodegenerative disorder that robs individuals of their language, reasoning, and memory. An estimated 6.7 million Americans ≥65 years of age and 20–30 million individuals worldwide are thought to suffer from Alzheimer's disease. Alzheimer's disease is typically confirmed via *in vivo* imaging or post-mortem assessment of distinct neuropathological biomarkers:- amyloid plaques primarily composed of amyloid β_{1-42}, neurofibrillary tangles (filaments of hyperphosphorylated tau) and neurodegeneration in select areas of the brain (Jack et al., 2018).

Evidence now suggests that accumulation of amyloid plaques and neurofibrillary tangles contribute marginally to the development of cognitive decline in Alzheimer's disease patients (Castellani et al., 2007; Marchesi, 2012). Instead, there is increased appreciation of the role(s) played by the elevation of soluble, oligomeric forms of amyloid β_{1-42} ($oA\beta_{1-42}$) in Alzheimer's disease etiopathogenesis during a long prodromal phase, up to 15 years prior to diagnosis (Cline et al., 2018; Inayathullah & Teplow, 2011), particularly with respect to the development and progression of synapse dysfunction and loss (Chiantia et al., 2023; Gavello et al., 2018). Specifically, $oA\beta_{1-42}$ is released at the synapse in response to neuronal activity (Cirrito et al., 2005) and physiological levels of the neuropeptide increase presynaptic Ca^{2+} and positively modulate hippocampus-based synaptic plasticity and contextual fear conditioning (Lawrence et al., 2014; Puzzo et al., 2008), indicating that amyloid β normally functions as a synaptic regulator.

Among the classes of molecular targets for amyloid β and derivatives in their specific forms are nicotinic acetylcholine (nACh) receptors, including those that contain α7 and perhaps other subunits (α7*-nACh receptor; * indicates the possible incorporation of other nACh receptor subunits) (Lukas et al., 1999; Whiteaker & George, 2023). Whereas α7*-nACh receptors primarily exist as homopentamers (α7-nACh receptors) in the majority of brain regions, a minority also contain β2 subunits (e.g. α7β2-nACh receptors). These heteromeric α7β2-nACh receptors exhibit several features distinct from those of homomeric α7-nACh receptors (Khiroug et al., 2002; Moretti et al., 2014; Murray et al., 2012). α7β2-nACh receptors are enriched in specific populations of cholinergic and non-cholinergic neurons of the basal forebrain (Azam et al., 2003; George et al., 2021; Thinschmidt et al., 2005; Whiteaker & George, 2023). α7β2-nACh receptors are particularly sensitive to functional modulation by various amyloid β forms (Liu et al., 2009, 2012). At the synapse, $oA\beta_{1-42}$ at physiological concentrations (pM-nM) has been shown to activate presynaptic α7*-nACh receptors (Lawrence et al., 2014), including α7β2-nACh receptors (Mehta et al., 2009), via direct agonistic interaction with the ligand binding domain (Tong et al., 2011), this signaling pathway largely accounting for the Aβ-induced regulation of presynaptic Ca^{2+}.

Material and method :

Fully pentameric α7- and α7β2-nACh receptor concatemers were constructed as previously described (George et al., 2021). We have previously shown these α7*-nACh receptor concatenated constructs to be functionally and pharmacologically indistinguishable when compared with unconcatenated α7*-nACh receptors (George et al., 2021). Briefly, human α7- and α7β2-nACh receptor cDNA constructs were subcloned into high-expression mammalian vectors (pCEP4 and pCDNA 3.1+, respectively) that harboured the Kozak consensus sequence and native α7- and α7β2-nACh receptor signal peptide sequences (Figure 1). All α7*-nACh receptor constructs were engineered to express the fluorescent protein mCherry, facilitating the identification of α7*-nACh receptors that successfully transfected SH-EP1 cells (RRID:CVCL_0524). Genes that encoded nACh receptor subunits were arranged in the order 5′-α7-α7-α7-α7-α7-3′ for homomeric α7-nACh receptors and 5′-α7-β2-α7-β2-α7-3′ for heteromeric α7β2-nACh receptors. Sequences of all subunits, together with their associated partial linkers, were confirmed by DNA sequencing (Thermo Fisher Scientific, Waltham, MA, USA), and the assembly of each translated pentamer was verified by restriction digest. For α7-nACh receptors, orthosteric acetylcholine (ACh) binding sites

are formed at all α7/α7 subunit interfaces and involve the principal (+) face containing subunit loop C of one subunit and the adjacent subunit's complementary (−) face containing loop D. As previously demonstrated for α7β2-containing nACh receptors (George et al., 2021), α7/β2 subunit pairs of the α7β2-nACh receptor construct may form ACh binding pockets between the principal (+) face of the α7 subunit and the complementary (−) face of the β2 subunit. Whether there is ligand interaction at β2(+)/α7(−) interfaces is not clear. To enhance α7*-nACh receptor cell-surface expression, SH-EP1 cells were co-transfected with the human nACh receptor chaperone protein NACHO (Gu et al., 2016). Human NACHO was engineered in the mammalian expression vector pIRES (Addgene, Watertown, MA) to facilitate the expression of ZsGreen1 fluorophore and to identify successfully transfected cells. Fluorescence microscopy imaging was used both to assess the efficacy of transfections and to identify cells exhibiting both mCherry and ZsGreen1 fluorescence as optimal candidates for patch clamp recording

Single-channel bursts corresponding to these precise amplitudes were segregated from isolated openings and only bursts were used for single-channel analysis. Bursts of single-channel activity were defined as series of openings separated by closures shorter than the minimum inter-burst closed duration or T_{crit} (Grosman & Auerbach, 2000) and from other such episodes by prolonged channel desensitization (Chakrapani et al., 2004). For all groups tested, T_{crit} was calculated by using QuB software. Bursts that contained overlapping currents, which indicate two simultaneously active channels, were rare and were discarded from the analysis. The advantages of using burst analysis have been described (Ciuraszkiewicz et al., 2013), as it increases the probability that adjacent openings arise from the same receptor.

Results

We began by investigating the functional interaction between α7*-nACh receptors and the N-Aβ fragment or the N-Aβcore by determining whether the N-Aβ fragment or N-Aβcore altered α7- or α7β2-nACh receptor single-channel amplitudes (Figure 2; Table 2). No significant α7*-nACh receptor by treatment interaction was observed for single-channel amplitude ($F_{(7,139)} = 0.9$; $P > 0.05$). Furthermore, single-channel amplitudes across all treatment groups were similar between homomeric α7-nACh receptors and heteromeric α7β2-nACh receptors (Figure 2c,d; Figure S3A). These data discount the possibilities that single-channel responses induced by oA$\beta_{1\text{-}42}$, the N-Aβ fragment, or the N-Aβcore occur via nicotinic receptor subtypes other than α7*-nACh receptors and are consistent with earlier work showing blockade of oA$\beta_{1\text{-}42}$ effects by nicotinic antagonists (Liu et al., 2012).

Measurements of single-channel burst rates (bursts per second) were also not significantly different between α7*-nACh receptor subtypes (Figure 2e,f) or across treatments. Cross-group comparisons revealed that single-channel burst rates were similar between homomeric α7-nACh receptors and heteromeric α7β2-nACh receptors (Figure 2c,d; Figure S3B). These data show that when compared with ACh or oA$\beta_{1\text{-}42}$, the application of N-Aβ fragment or N-Aβcore exhibits similar single-channel burst rates when applied alone or in combination with ACh or oA$\beta_{1\text{-}42}$. Taken together, these findings demonstrate that the unitary amplitude and frequency of single-channel activity are not altered in the exclusive presence of oA$\beta_{1\text{-}42}$, the N-Aβ fragment, or the N-Aβcore or when these Aβ fragments are co-administered with the endogenous ligand, ACh.

Discussion

In this study, we identified for the first time the activation of human homomeric α7− and heteromeric α7β2-nACh receptor single-channel openings by the human N-Aβ fragment (N-terminal 15 amino acid Aβ peptide fragment) and by the N-Aβcore (essential core sequence hexapeptide within the N-Aβ fragment), as we have done for oligomeric A$\beta_{1\text{–}42}$ (George et al., 2021). Specifically, we demonstrated that N-Aβ fragment and N-Aβcore differentially alter the biophysical properties of α7*-nACh receptor subtypes in comparison to activation by ACh and oA$\beta_{1\text{-}42}$

Here, we provide evidence that N-Aβ fragment exposure alone increases α7-nACh receptor component 2 (C2) open-dwell time within bursts and burst duration relative to the effects of ACh alone. Co-administration of the N-Aβ fragment plus ACh increased α7-nACh receptor C2, burst duration and P_{open} relative to effects of ACh alone, while the α7-nACh receptor burst duration was higher in the presence of N-Aβ fragment alone or co-administered with $oA\beta_{1-42}$ than upon exposure to $oA\beta_{1-42}$ alone. However, N-Aβ fragment plus $oA\beta_{1-42}$ exposure reduces α7β2-nACh receptor open-dwell times within bursts (component 1; C1) relative to effects of $oA\beta_{1-42}$ alone. Importantly, co-application of the N-Aβcore with $oA\beta_{1-42}$ reduces α7β2-nACh receptor C1 open-dwell time, C2 open-dwell time, burst duration, and P_{open} compared to $oA\beta_{1-42}$ treatment alone and to those seen upon exposure to ACh, N-Aβ fragment, or N-Aβ core alone. The current observations, therefore, substantially expand our understanding of the functional interactions of α7*-nACh receptors with amyloid β (Grassi et al., 2003; Liu et al., 2009) and the N-terminal domain amyloid β peptides at the single-channel level.

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