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Research report

Functional modulation of strychnine-sensitive glycine receptors in rat hippocampal pyramidal neurons by amyloid-β protein (1-42)

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ABSTRACT

Amyloid-β peptide (Aβ) is considered a key protein in the pathogenesis of Alzheimer's disease because of its neurotoxicity, resulting in impaired synaptic function and memory. On the other hand, it was demonstrated that low (picomolar) concentrations of Aβ enhance synaptic plasticity and memory, suggesting that in the healthy brain, physiological Aβ concentrations are necessary for normal cognitive functions. In the present study, we found that Aβ (1 – 42) in concentrations of 10 pM – 100 nM enhanced desensitization of the glycine-activated current in isolated CA3 pyramidal neurons and also reversibly suppressed its peak amplitude during short (600 ms) co-application with agonist. The effect was most prominent at low glycine concentrations. When glycine receptors were activated by other receptor agonists – taurine and β-alanine, the changes of current kinetics and amplitudes induced by Aβ had a similar character. When Aβ (100 pM) was added to the bath solution, it caused, besides acceleration of desensitization, more pronounced reduction of peak current amplitude. This effect developed slowly, during a few minutes, and was more prominent at saturating concentrations of agonists. The results suggest that Aβ interacts with glycine receptors through three different mechanisms - by enhancing receptor desensitization, by rapid inhibition of the receptor, and also by means of a slowly developing inhibition of the amplitude of the current, possibly through intracellular mechanisms. The observed changes in the activity of glycine receptors induced by Aβ can lead to suppression of the tonic inhibition of hippocampal neurons mediated by extrasynaptic glycine receptors.

1. Introduction

Extensive studies demonstrate that the overproduction of soluble β-amyloid (Aβ) peptides in elderly brain is a key pathogenic factor at the onset of Alzheimer's disease (AD) (Selkoe, 2001, 2008; Haass and Selkoe, 2007). Aβ is considered as a trigger in the pathogenesis of this disease because of its neurotoxicity, resulting in impaired synaptic function and memory. However, the presence of Aβ in the cerebrospinal fluid (CSF) of non-demented individuals and in media from neuronal cell cultures (Tamaoka et al., 1997; Haass et al., 1992; Shoji, 2002) indicates that, as well as having a potential pathological role in Alzheimer's disease, Aβ has a role in the normal physiology of the central nervous system (for review, see Nehls, 2016). It was demonstrated that normal levels (picomolar range) of Aβ peptides regulate synaptic function by augmenting presynaptic release at hippocampal synapses and facilitating learning and LTP in CA1 (Abramov et al., 2009; Puzzo et al., 2008; Palop and Mucke, 2010). Moreover, normal levels of Aβ may play a neurotrophic role, because prevention of Aβ production by adding β- or γ-secretase inhibitors in cultured neurons causes cell death, which can be rescued by application of synthetic Aβ peptides to culture medium (Plant et al., 2003). It was also demonstrated that picomolar Aβ concentrations enhanced synaptic plasticity and memory (Puzzo et al., 2008, 2011, 2012; Morley et al., 2010), suggesting that in the healthy brain, physiological Aβ concentrations are necessary for normal cognitive functions.

Several putative molecular targets for soluble Aβ have been identified (Patel and Jhamandas, 2012). Two possible targets at the synapse include the nicotinic acetylcholine receptor (nAChR) (Wang et al., 2000; Liu et al., 2001; Pettit et al., 2001) and certain metabotropic glutamate receptors (Chin et al., 2007), both of which have been shown to be functionally regulated by Aβ. Soluble Aβ (1 – 40) modulates nicotinic and muscarinic receptor subtypes which stimulate in vitro and in vivo the release of glycine in the rat hippocampus (Zappettini et al., 2012) and inhibits both nicotinic and muscarinic cholinergic modulation of DA release from rat nerve endings (Olivero et al., 2014). Recently, we have demonstrated that low picomolar amounts of exogenously applied Aβ (25 – 35) can interact with strychnine-sensitive glycine receptors (GlyRs) in isolated hippocampal pyramidal cells (Bukanova

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et al., 2014). We have determined that Aβ (25 – 35) in picomolar concentrations reversibly augments the desensitization of glycine current, probably by binding to external sites on glycine receptors. We also observed that Aβ (25 – 35) can suppress the peak amplitude of glycine current, but this effect develops slowly and may be mediated through some intracellular machinery.

Aβ (25 – 35) peptide represents the neurotoxic domain of the native, full-length peptides Aβ (1 – 40) or Aβ (1 – 42), and is implicated in the progressive neurodegeneration in AD (Gruden et al., 2007). Therefore, Aβ (25–35) peptide has been widely used in vivo and in vitro to investigate the neurotoxic mechanisms of AD (Misiti et al., 2005). However, there is some difference in the effects of full-length amyloid-β peptide and Aβ (25–35) fragment. For example, similar behavioral but different neurophysiological effects for Aβ (25 – 35) and Aβ (1 – 42) were found in the feeding circuitry of the snail *Lymnaea stagnalis* (Ford et al., 2015). Acute exposure of hippocampal CA1 cells to nanomolar doses of Aβ peptides (25–35) or (1–40) cause an increase in the activity of different calcium channel types (Rovira et al., 2002). The toxic fragment, Aβ (25–35), increases the L-type calcium channel activity while Aβ (1–40) potentiates the non-L-type calcium channels. These data suggest that the mechanism of the destabilization of calcium homeostasis may be different for the two peptides, and could be mediated through different types of receptors (Rovira et al., 2002).

Here we demonstrate that the endogenous peptide, Aβ (1–42), like its 25-25 fragment (Bukanova et al., 2014), increases the speed of decay of glycine current, but also supresses the peak current amplitude both during short co-application with agonists and after long cell exposure to the peptide. Our results suggest that Aβ (1 – 42) in the picomolar concentrations may plays a physiological role in synaptic processes through modulation of glycine receptors.

2. Results

2.1. Modulation of GlyR-mediated currents by Aβ (1-42)

The experiments were performed on the pyramidal neurons isolated from CA3 region of rat hippocampus. According to our previous study (Bukanova et al., 2014), glycine applied to hippocampal pyramidal neurons elicited a transient inward current at −70 mV (as shown in Fig. 1A). The peak amplitudes and desensitization rates of GlyR currents both increased in a concentration-dependent manner. The peak amplitude induced by 500 μM Gly was close to the maximum value, and the desensitization rate at 600 ms remained relatively slow.

The effects of Aβ (1 – 42) on Gly-currents were examined under two experimental conditions, which differed with respect to drug exposure time. In the first set of experiments, Aβ was co-applied with glycine for 600 ms (Fig. 1A), whereas in the second set of experiments, the cells were continuously perfused with Aβ for 1–2 min, prior to co-application of glycine plus peptide for 600 ms.

When co-applied with glycine, Aβ (1 – 42), like Aβ (25 – 35), accelerated the decay of Gly-current (Fig. 1A,C). Glycine currents were measured at the beginning and at the end of agonist application. To estimate the extend of desensitization, we calculated the ratio of the current amplitude at the end of 600 ms pulse ($I_{600\ ms}$), to the peak current amplitude (I_{peak}) in control conditions and during glycine co-application with Aβ. To evaluate the effect of Aβ we then calculated the ratio of degree of current desensitization

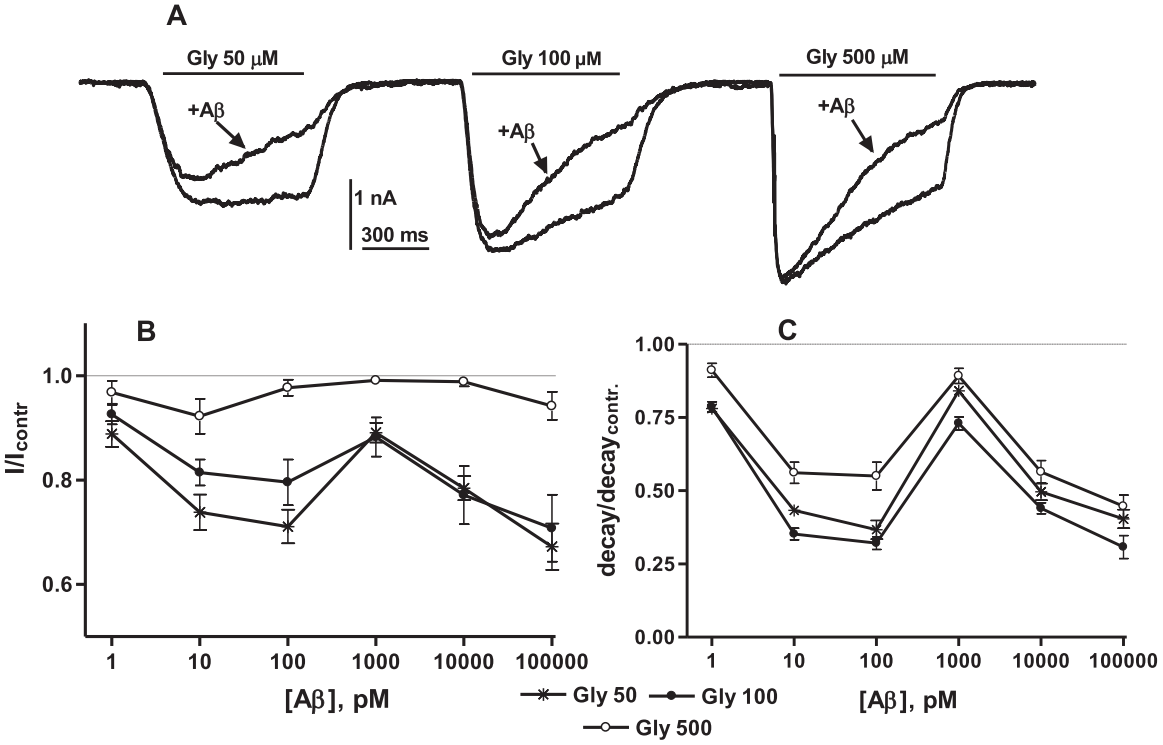

Fig. 1. Amyloid-β peptide (Aβ) 1–42 at concentrations 1pM-100 nM suppresses glycine-induced whole-cell Cl- currents in acutely isolated rat hippocampal CA3 pyramidal neurons. (A) Aβ co-application enhances desensitization of glycine-activated currents and suppresses its peak amplitude at glycine concentrations 50 and 100 μM. Representative current traces elicited by different concentrations of glycine applied for 600 ms in control and during co-application with 100 nM Aβ. Neurons were voltage-clamped at −70 mV. (B) Averaged effects of Aβ at various concentrations on the peak currents amplitude evoked by 50 μM glycine (asterisks), 100 μM glycine (filed circles) and 500 μM glycine (open circles). Data points represent the relative peak current amplitude for each glycine concentration tested (mean ± SEM of 9–11 separate experiments for each glycine concentration). (C) Averaged effects of different concentrations of Aβ on the extent of Gly-current desensitization. Each point represents the ratio of degree of current desensitization (I_{600ms}/I_{peak}) in control to the degree of desensitization in the presence of Aβ for different glycine concentrations. Data are shown as mean ± SEM, n=9–11.