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Research Report

Amyloid β peptide (25–35) in picomolar concentrations modulates the function of glycine receptors in rat hippocampal pyramidal neurons through interaction with extracellular site(s)

*J.V. Bukanova**, *I.N. Sharonova*, *V.G. Skrebitsky*

Brain Research Department, Research Center of Neurology, Russian Academy of Medical Sciences, Per. Obukha 5, Moscow 105064, Russia

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ABSTRACT

β-Amyloid peptide (Aβ) plays a central role in the pathogenesis of Alzheimer's disease, but in lower amounts it is found in normal brains where it participates in physiological processes and probably regulates synaptic plasticity. This study investigated the effects of physiologically relevant concentrations of Aβ (1 pM–100 nM), fragment 25–35, on glycine-mediated membrane current in acutely isolated rat hippocampal pyramidal neurons using whole-cell patch-clamp technique. We have found that short (600 ms) co-application of glycine with Aβ caused reversible dose-dependent and voltage-independent acceleration of desensitization of glycine current. The peak amplitude of the current remained unchanged. The effect of picomolar Aβ concentrations persisted in the presence of 1 μM Aβ in the pipette solution, implying that Aβ bounds to extracellular site(s). Concentration-dependence curve was N-shaped with maximums at 100 pM and 100 nM, suggesting the existence of two binding sites, which may interact with each other. Glycine current resistant to 100 μM picrotoxin, was insensitive to Aβ, which suggests that Aβ affected mainly homomeric glycine receptors. When Aβ was added to bath solution, besides acceleration of desensitization, it caused reversible dose-dependent reduction of glycine current peak amplitude. These results demonstrate that physiological (picomolar) concentrations of Aβ reversibly augment the desensitization of glycine current, probably by binding to external sites on homomeric glycine receptors. Furthermore, Aβ can suppress the peak amplitude of glycine current, but this effect develops slowly and may be mediated through some intracellular machinery.

*Corresponding author. Fax: +7 495 917 9664.
E-mail addresses: bukanovaj@mail.ru (J.V. Bukanova), sharonova.irina@gmail.com (I.N. Sharonova), skrebitsky@yahoo.com (V.G. Skrebitsky).

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1. Introduction

Amyloid-β protein (Aβ) is thought to play a major role in pathogenesis of Alzheimer's disease (Tanzi and Bertram, 2005). Aβ is a derivate of amyloid precursor protein (APP), which is an integral membrane protein abundantly expressed in neurons and glia. Proteolytic processing of APP results in the production of different fragments depending on the secretase involved. Processing of APP by α-secretase leads to the release of secreted APP and prevents the formation of Aβ, cleavage of APP by β- and ɤ-secretases generates amyloid-β peptides (Thinakaran and Koo, 2008). A variety of studies have indicated that elevation of Aβ level and its accumulation result in cognitive dysfunction, including memory deficits (Selkoe, 2008, Small et al, 2001, Tanzi and Bertram, 2005).

But in contrast to the pathological accumulation, in normal brain Aβ is produced at lower, picomolar concentration (Cirrito et al., 2003; Garcia-Osta and Alberini, 2009; Selkoe, 2008), and there is evidence that it plays various physiologically important roles (for review, see Parihar and Brewer, 2010).

There is also evidence that Aβ production increases with synaptic activity (Cirrito et al., 2008) and this increased level of Aβ depress excitatory synapses and reduces neuronal activity, the mechanism which may participate in a negative feedback that could control neuronal excitability under physiological conditions (Kamenetz et al., 2003; Parihar and Brewer, 2010). Puzzo et al. reported that in low picomolar concentration Aβ markedly increased hippocampal long-term potentiation, while in high nanomolar concentrations caused well established reduction of potentiation. Moreover, picomolar concentration of Aβ enhances both reference and contextual fear memory (Puzzo et al., 2008). Garcia-Osta and Alberini have shown that hippocampal injection of picomolar concentrations of Aβ enhances memory consolidation (Garcia-Osta and Alberini, 2009).

Aβ can act through various targets (Small et al., 2001), including L-type voltage-dependent Ca^{2+}-channels (Pearson and Peers, 2006; Mezler et al., 2012) and chemo-sensitive receptors, such as ACh receptors (Dougherty et al., 2003) and NMDA receptors (Snyder et al., 2005; Texidó et al., 2011; Wu et al., 1995). There is evidence that Aβ can suppress GABA-dependent chloride current in molluscan neurons (Sawada and Ichinose, 1996). But no data are available so far on the possible effect of Aβ on glycine receptors. In the present study, therefore, we explored the effect of Aβ on glycine receptors in acutely dissociated rat hippocampal pyramidal neurons.

Glycine, along with GABA, is a major inhibitory neurotransmitter in the nervous system. Glycine mediates fast inhibitory transmission via ionotropic glycine receptors (GlyR), which predominate in the spinal cord and brainstem. Although no glycinergic synaptic current has been found in mature hippocampus, there is evidence that in hippocampal CA1, CA3 and dentate gyrus regions functional GlyRs are widely expressed (Chattipakorn and McMahon, 2002, 2003). In hippocampus GlyRs are believed to be located mainly at extrasynaptic sites (Aroeira et al., 2011; Keck and White, 2009). According to literature data, in hippocampal pyramidal neurons and interneurons GlyR can be activated by a number of endogenous agonists including taurine, β-alanine and glycine, leading to the opening of strychnine-sensitive chloride channels (Chattipakorn and McMahon, 2002, 2003; Mori et al., 2002). The extracellular concentrations of these amino acids in the hippocampus are relatively high, in the micromolar range (Shibanoki et al., 1993), and they can tonically activate glycine receptors. So, GlyRs may provide tonic inhibition, which is important for information processing and for maintaining healthy level of activity (Song et al, 2006). Adequate level of inhibition is particularly important in hippocampus to prevent epileptiform activity (Keck and White, 2009; Lozovaya et al., 2005; Chattipakorn and McMahon, 2003). The level of tonic activation of GlyRs can be regulated in two ways. Firstly, there is a system of specific transporters which control extracellular concentration of GlyR agonists (Chattipakorn and McMahon, 2002; Jursky and Nelson, 1995; Keck and White, 2009). Secondly, the function of GlyR can be modulated by a wide range of endogenous and exogenous compounds such as divalent cations Zn^{2+} and Cu^{2+}, steroids, cannabinoids, general anesthetics and others (Betz and Laube, 2006; Chattipakorn and McMahon, 2002; Webb and Lynch, 2007).

In this work we show that Aβ (25–35) in low picomolar concentration can modulate function of hippocampal GlyR, probably, by interaction with some extracellular site(s).

2. Results

2.1. *Glycine-activated currents in isolated hippocampal pyramidal neurons*

Experiments were performed on isolated pyramidal neurons from CA1 and CA3 fields of rat hippocampus. In the whole-cell configuration of the patch-clamp technique and in the voltage-clamp mode at holding potential of −70 mV application of glycine evoked slow-desensitizing inward current with an EC_{50} of 102.8±5.7 μM (n=5) (Fig. 1A). The reversal potential was around −5 mV, which is consistent with current mediated by Cl^- ions. The current had low sensitivity to 10 μM bicuculline, but was strongly suppressed by 3 μM strychnine (Fig. 1B). All these features corresponded to the properties of glycine current (Gly-current) described by other authors (Chattipakorn and McMahon, 2002, 2003; Lynch, 2004; Lozovaya et al., 2005).

2.2. *A β effects on Gly-current*

In the first series of experiments (14 neurons), we studied the influence of different concentrations of Aβ (25–35) on Gly-current evoked by 50 μM and 500 μM glycine. Aβ was used in the concentration range of 1 pM – 100 nM, because we wanted to focus on its normal physiological functions. The results obtained on neurons from CA1 and CA3 fields had no notable differences, so they were combined.

Short (600 ms) co-application of Aβ with glycine induced reversible acceleration of Gly-current decay. The peak amplitude of the current remained unchanged. The effect was observed on currents elicited by both 50 μM and 500 μM glycine, but was more pronounced with 500 μM glycine (Fig. 2). When the current was induced by 50 μM glycine, 100 pM Aβ reduced the amplitude of Gly-current, measured at 600 ms from the beginning of application (Gly-current at