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The influence of acidic media on the effect of beta-amyloid peptide on the function of glycine receptor in hippocampal neurons

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ABSTRACT

We have previously shown that application of beta-amyloid peptide 1–42 (Aβ) at picomolar/nanomolar concentrations caused a decrease in the peak amplitude and acceleration of desensitization of the glycine-activated chloride current (I_{Gly}) in hippocampal pyramidal neurons (Bukanova et al., 2016). The aim of this work was to study the effect of Aβ on I_{Gly} in an acidified medium. The relevance of this work is determined by the fact that the pathogenic effects of Aβ in Alzheimer's disease are usually accompanied by inflammatory processes and acidosis. The I_{Gly} was induced by 600 ms application of 100 μM (nearly EC_{50}) or 500 μM (nearly saturating) glycine on isolated rat hippocampal neurons. The solution of glycine was neutral (pH 7.4) or acidic over a pH range of 5.0–7.0. It was found that 600 ms application of protons rapidly, reversibly and in dose-dependent manner decreased the peak amplitude and accelerated the desensitization of I_{Gly}. The effect of H^+ on I_{Gly} desensitization did not depend on glycine concentration and may be considered noncompetitive, while the effect on I_{Gly} peak disappeared at saturating glycine concentration and can be regarded as a competitive. These characteristics of the proton effects on I_{Gly} coincide with the characteristics of the Aβ effects on I_{Gly}. Experiments with joint application of Aβ and H^+ showed interdependence of their effects. Addition of Aβ to perfusing solution reduced H^+ effects on I_{Gly} while long pretreatment of Aβ with acid solution prevented the effects of the peptide on I_{Gly}. Our results suggest the existence of common sites for Aβ and H^+ on the GlyR and indicate a mutual weakening of the inhibitory action of these molecules on I_{Gly}.

1. Introduction

Transient changes in extracellular pH occur in both physiological (Krishtal et al., 1987) and pathophysiological conditions such as inflammation, ischemia and nociception (Chesler, 1990; Reeh and Steen, 1996). Altered neuronal H^+ homeostasis may also accompany head injury and Alzheimer's disease (AD) (Yates et al., 1990). Increase in H^+ concentration has been clearly shown to influence functional properties of various proteins/peptides due to global conformational changes of the molecules. H^+ has been reported to act as a modulator of several ion channel receptors, including the P2X2 purinoreceptor (Clyne et al., 2002), the GABAA receptor (Zhou et al., 2007; Chen and Huang, 2014), the AMPA receptor (Ihle and Patneau, 2000), the NMDA receptor (Cummings and Popescu, 2016) and the Gly receptor (Li et al., 2003; Chen et al., 2004; Chen and Huang, 2007; Song et al., 2010).

In the literature, much attention is paid to the action of protons on the structural and functional properties of the beta-amyloid peptide (Aβ). Aβ has important influence on neuronal functions and can cause both positive and negative effects on synaptic transmission depending upon the concentration (Puzzo and Arancio, 2013; Parihar and Brewer, 2010). A large body of data suggests that, at least in the early stages of Alzheimer's disease, synaptic disorders underlying memory impairment could be due to the elevated Aβ levels (Small et al., 2001; Tanzi and Bertram, 2005). The action of Aβ on nerve tissue was shown to be complicated by cerebral acidosis, which may be related to the inflammatory response seen in AD-affected brain tissue (Yates et al., 1990). With this background, the study of interaction of Aβ and protons is of undoubted interest. In the literature, multiple influences of acidosis on the properties of the Aβ is described: under these conditions the charge, conformation, binding of metals and the ability of the peptide molecules to aggregate change significantly (Ghalebani et al., 2012; Guo et al., 2005; Atwood et al., 1998; Matsunaga et al., 2002).

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In our previous work, we showed that Aβ1-42 in picomolar-nanomolar concentrations modulated the functional properties of the GlyR in hippocampal neurons, causing a change in the glycine-activated chloride current (I_{Gly}) (Bukanova et al., 2016). In this study, we examined the effects of acidosis on the I_{Gly} in the absence and presence of Aβ. With this work, we pursued two goals. The first goal was to study the effect of acidosis on GlyR of hippocampal pyramidal neurons. In a number of studies, the effect of acidosis on I_{Gly} was described in isolated spinal neurons of rat (Li et al., 2003) and human embryonic kidney (HEK) 293 cells (Chen et al., 2004; Chen and Huang, 2007; Song et al., 2010). We have shown for the first time that GlyRs of hippocampal pyramidal neurons contain two functionally different sites for H^+. Secondly, we wanted to find out whether effects of Aβ and proton on the I_{Gly} are interdependent. Our results showed that addition of Aβ to the perfusion medium neutralized the changes in the I_{Gly} caused by 600 ms proton application while long pretreatment of Aβ with acid solution prevented the effects of the peptide on I_{Gly}.

2. Material and methods

2.1. Cell preparation

All procedures were performed in accordance with the institutional guidelines on the care and use of experimental animals set by the Russian Academy of Sciences. The cells were isolated from transverse hippocampal slices as described previously (Bukanova et al., 2016). Briefly, the slices (200–500 μm) of Wistar rats (11–14 days of age) hippocampus were incubated at room temperature for at least 2 h in a solution containing the following components (in mM): 124 NaCl, 3 KC1, 2 $CaCl_2$, 2 $MgSO_4$, 25 $NaHCO_3$, 1.3 NaH_2PO_4, 10 D-glucose, pH 7.4. The saline was continuously stirred and bubbled with carbogen (95% 0_2 + 5% CO_2). Single pyramidal neurons from CA3 region were isolated from the stratum pyramidale by a vibrating fused glass pipette with a spherical tip.

2.2. Current recordings

Glycine-activated chloride currents (I_{Gly}) in isolated neurons were induced by a step application of agonist for 600 ms with 30–40 s intervals. Transmembrane currents were recorded using conventional patch-clamp technique in the whole-cell configuration. Patch-clamp electrodes had tip resistance of ~2 MΩ. The solution in the recording pipette contained the following components (in mM): 40 CsF, 100 CsCl, 0.5 $CaCl_2$, 5 EGTA, 3 $MgCl_2$, 4 NaATP, 5 HEPES, 4 ATP, pH 7.3. The composition of the extracellular solution was as follows (in mM): 140 NaCl, 3 KCl, 3 $CaCl_2$, 3 $MgCl_2$, 10 D-glucose, 10 HEPES hemisodium, pH 7.4. The speed of perfusion was 0.6 ml/min. Recording of the currents was performed using EPC7 patch-clamp amplifier (HEKA Electronik, Germany). The holding potential was maintained at −70 mV. Transmembrane currents were filtered at 3 kHz, stored and analyzed with IBM-PC computer, using homemade software.

2.3. Drug application

Glycine was applied through glass capillary, 0.1 mm in diameter, which could be rapidly displaced laterally under control of home-made software. The system allows a complete exchange of external solution surrounding the neuron within 20 ms. The solution of glycine was neutral (pH 7.4) or acidic over a pH range of 5.0–7.0. Aβ was applied to the cell in two ways. In the first set of experiments 100 nM Aβ was co-applied with glycine through micropipette during 600 ms, and in the second series of experiments, 100 nM Aβ was added into an extracellular perfusate for 10 min using two reservoirs system. To avoid the reduction in the concentration of Aβ during the application of glycine, we added Aβ also to the glycine-containing pipette.

2.4. Reagents

All the drugs were purchased from “Sigma”. Aβ was dissolved in DMSO at a concentration of 100 μM, aliquoted and stored at −20 °C. Final concentration of DMSO was 0.1%, and DMSO itself at this concentration had no effect on the I_{Gly}. Stocks of drugs were diluted shortly before the experiment in the bath solution to reach the final concentration.

2.5. Data analysis

All statistical analysis was performed with the help of *Prism Graphpad* software. In results descriptions, mean and standard error of mean (SEM) are specified. Significance of the effect was tested by one-way ANOVA (Newman-Keuls's post hoc analysis with repeated measures test) and two-way ANOVA analysis with the P-value of 0.05. The meanings of asterisks (probability levels) in figures is the following: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Glycine activates chloride currents (I_{Gly}) in rat hippocampal neurons

Short application of glycine for 600 ms on pyramidal neurons evoked chloride currents (I_{Gly}) described in detail in our previous work (Bukanova et al., 2016). Shortly, the amplitude of I_{Gly} depended on glycine concentration with EC_{50} value of 90 ± 7 μM. The average value of the reversal potential of I_{Gly} −9.8 ± 0.9 mV matched well the chloride reversal potential calculated for the chloride concentrations used (−9.5 mV). In this paper, the effects of H^+ on the I_{Gly} were studied with two glycine concentrations: 100 μM (nearly EC_{50}) and 500 μM (nearly saturating). The mean peak amplitude of the I_{Gly} at glycine concentration of 100 μM was 2.6 ± 5 nA, and at glycine concentration of 500 μM - 5.0 ± 0.7 nA.

3.2. Acid-sensing ion channels (ASICs) in rat hippocampal neurons

When studying the effect of protons on the I_{Gly}, we were faced with a problem of the I_{Gly} contamination by current passing through acid-sensing ion channels (ASICs). ASICs are H^+-gated Na^+ channels, which are present in most neurons, including hippocampal neurons. The typical ASIC current is transient and is elicited by a rapid drop in the extracellular pH (Gründer and Chen, 2010). In our experiments, short application (600 ms) of highly acidic solution (pH of 5) on hippocampal neurons caused no effect or an inward current with amplitude ranging from 0.1 to 2 nA. We used cells with no or negligeable (0.15 nA or less) ASIC current. Fig. 1A shows weak inward current with peak amplitude of 0.1 nA evoked by proton application in one of the cells tested.

3.3. The effect of protons on the I_{Gly} evoked by 100 μM glycine

In order to study the effect of protons on the I_{Gly} of rat hippocampal neurons, we used a short (600 ms) application of a solution containing 100 μM glycine, the pH of which varied between 7.4 (control), 7.0, 6.0, and 5.0. It was found that a rapid drop in the extracellular pH rapidly, reversibly and in a dose-dependent manner changed the I_{Gly} in all cells tested ($n = 9$). Namely, H^+ reduced the I_{Gly} peak amplitude and accelerated the current decay. Fig. 1B (left) shows representative traces of I_{Gly}, induced by 600 ms application of