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Lithium ions in nanomolar concentration modulate glycine-activated chloride current in rat hippocampal neurons

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ABSTRACT

Lithium salts are successfully used to treat bipolar disorder. At the same time, according to recent data lithium may be considered as a candidate medication for the treatment of neurodegenerative disorders. The mechanisms of therapeutic action of lithium have not been fully elucidated. In particular, in the literature there are no data on the effect of lithium on the glycine receptors. In the present study we investigated the effect of Li^+ on glycine-activated chloride current (I_{Gly}) in rat isolated pyramidal hippocampal neurons using patch-clamp technique. The effects of Li^+ were studied with two glycine concentrations: 100 μM (EC_{50}) and 500 μM (nearly saturating). Li^+ was applied to the cell in two ways: first, by 600 ms co-application with glycine through micropipette (short application), and, second, by addition to an extracellular perfusate for 10 min (longer application). Li^+ was used in the range of concentrations of 1 nM–1 mM. Short application of Li^+ caused two effects: (1) an acceleration of desensitization (a decrease in the time of half-decay, or "τ") of I_{Gly} induced by both 100 μM and 500 μM glycine, and (2) a reduction of the peak amplitude of the I_{Gly}, induced by 100 μM, but not by 500 μM glycine. Both effects were not voltage-dependent. Dose-response curves for both effects were N-shaped with two maximums at 100 nM and 1 mM of Li^+ and a minimum at 1 μM of Li^+. This complex form of dose-response may indicate that the process activated by high concentrations of lithium inhibits the process that is sensitive to low concentrations of lithium.

Longer application of Li^+caused similar effects, but in this case 1 μM lithium was effective and the dose-effect curves were not N-shaped. The inhibitory effect of lithium ions on glycine-activated current suggests that lithium in low concentrations is able to modulate tonic inhibition in the hippocampus. This important property of lithium should be considered when using this drug as a therapeutic agent.

1. Introduction

For many decades lithium salts have been successfully used to treat bipolar disorder (BPD) (for a review: Curran and Ravindran, 2014). At the same time, over recent years evidence of the positive effect of lithium ions in treatment of other diseases, including neuro-, cardio- and nephroprotection has been accumulated (for a review: Malhi et al., 2013; Plotnikov et al., 2014; Vo et al., 2015). The possibility of neuroprotective effects of lithium is particularly relevant. According to recent data lithium may be considered as a candidate medication for the treatment of the most significant neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (for a review: Vo et al., 2015). In in vitro experiments lithium has been shown to facilitate neural plasticity (Gray and McEwen, 2013) and regulate intracellular calcium levels as well as calcium turnover (Sourial-Bassillious et al., 2009). However, to date no definitive mechanism for lithium effects has been established. It has been proposed that lithium exerts its therapeutic effects by interfering with signal transduction through G-protein-coupled receptor (GPCR) pathways or by direct inhibition of specific targets in signaling systems, including inositol monophosphatase and glycogen synthase kinase-3 (GSK-3) (for a review: Beaulieu et al., 2009; Plotnikov et al., 2014).

Ionic channels of neuronal membrane are also considered as potential targets for lithium ions. The experimental results show that lithium can modulate both ligand-activated and voltage-gated channels. However, in some cases the effective lithium concentrations exceeded therapeutic dose of 0.6–1.2 mM in neurons, with the concentrations >2 mM considered toxic (Plotnikov et al., 2014).

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Lithium at 10 mM was found to facilitate AMPAR currents in hippocampal CA1 cells by selectively increasing the probability of channel opening (Gebhardt and Cull-Candy, 2010). Na^+-dependent K^+ current responsible for after-hyperpolarization was shown to be reduced in the presence of 1–10 mM lithium, which resulted in increased neuronal excitability (Safronov and Vogel, 1996; Butler-Munro et al., 2010). In adrenal chromaffin cells, lithium inhibited voltage-dependent sodium channels in a concentration-dependent manner (IC_{50} = 23.4 mM) (Yanagita et al., 2007). Finally, dual regulation of G-protein-activated K^+-channels (GIRK) was described in mouse hippocampal neurons where 1–2 mM Li^+ increased GIRK basal current but attenuated neurotransmitter-evoked GIRK-current (Tselnicker et al., 2014). In our work, we demonstrate for the first time, that Li^+ at nanomolar concentrations can modulate glycine-activated chloride current in rat hippocampus neurons.

Glycine is a crucial inhibitory neurotransmitter acting on specific glycine receptors (GlyRs), whose localization can be both synaptic and extra-synaptic (Lynch, 2009; Song et al., 2006). Extrasynaptic GlyRs in hippocampus provide a tonic inhibition (Xu and Gong, 2010), which is very important for information processing within a neuronal network and disturbance of which can contribute to many pathophysiological processes. Numerous structurally diverse compounds have been shown to modulate GlyRs, including neurosteroids, alcohols, anesthetics, cannabinoids, antagonists of 5-HT_3 receptor, ginkgolide B, cyclothiazide and quercetin (for a review: Xu and Gong, 2010; Yevenes and Zeilhofer, 2011). Recently we demonstrated the ability of cyclic nucleotides (cAMP and cGMP) and beta-amyloid peptide to exert extracellular modulation of GlyRs (Bukanova et al., 2014a, 2014b). A lot of attention was given to the study of GlyRs modulation by cations (for a review: Lynch, 2004; Xu and Gong, 2010). Divalent cation Zn^{2+} was shown to modulate GlyRs extracellularly in a bidirectional manner depending on its concentration. There is no convincing evidence of the ability of other metal ions to mimic the biphasic action of zinc. However, the potentiating site is recognized by several metal ions with the following potency sequence: zinc > lanthanide > lead > cobalt, whereas the inhibitory site exhibits a different potency sequence: zinc > copper > nickel (for a review: Lynch, 2004). 100 μM of Ba^{2+}, Sr^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} and Al^{3+} were shown to have no effect on GlyR currents in rat septal neurons (Kumamoto and Murata, 1996). We have shown in our previous study conducted in rat hippocampus neurons that Fe^{2+} and Fe^{3+} can modulate glycine-evoked current (I_{Gly}) in a similar manner. These ions in micromolar concentrations caused an acceleration of desensitization and a decrease of peak amplitude of I_{Gly} (Solntseva et al., 2015). We could not find any data on Li^+ influence on I_{Gly} in the available literature. Thus, our data on the effect of lithium on the I_{Gly} can help to elucidate the mechanisms of therapeutic action of lithium, and to understand the functional properties of glycine receptors.

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 in detail elsewhere (Vorobjev, 1991). 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): 124NaCl, 3KC1, 2$CaCl_2$, 2$MgSO_4$, 25$NaHCO_3$, 1.3NaH_2PO_4, 10D-glucose, pH 7.4. The saline was continuously stirred and bubbled with carbogen (95% 0_2 + 5% CO_2). Single pyramidal neurons from CA3 were isolated from the stratum pyramidale by a vibrating fused glass pipette with a spherical tip (Vorobjev, 1991).

2.2. Current recordings

Glycine-activated currents in isolated neurons were induced by a step application of agonist for 600 ms with 30–40 s intervals. Transmembrane currents were recorded using a conventional patch-clamp technique in the whole-cell configuration. Patch-clamp electrodes had a tip resistance of ~2 MΩ. The solution in the recording pipette contained the following (in mM): 40CsF, 100CsCl, 0.5$CaCl_2$, 5EGTA, 3$MgCl_2$, 4NaATP, 5HEPES, 4ATP, pH 7.3. The composition of extracellular solution was as follows (in mM): 140NaCl, 3KCl, 3$CaCl_2$, 3$MgCl_2$, 10D-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). Unless noted otherwise, 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 homemade software (Vorobjev et al., 1996). The system allows a complete exchange of external solution surrounding the neuron within 20 ms. Li^+ was applied to the cell in two ways. In the first set of experiments Li^+ was co-applied with glycine through micropipette during 600 ms (short application), and in the second series of experiments, Li^+ was added to an extracellular perfusate for 10 min (longer application) using two reservoirs system. The speed of perfusion was 0.6 ml/min. To avoid the reduction in the concentration of lithium during the application of glycine, we added lithium in corresponding concentration also to the glycine-containing pipette.

2.4. Reagents

All the drugs were purchased from “Sigma”. LiCl was used as the source of Li^+. The tested substances were dissolved in distilled water to make 0.1–1 mM stock solution, which was dissolved in external saline to their final concentration immediately before the experiments.

2.5. Data analysis

All statistical analysis was performed with the help of *Prism Graphpad* software. All comparisons were made with one-way repeated measures ANOVA at a significance level of $p = 0.05$. In results descriptions, mean and standard error of mean (SEM) are specified. The meanings of asterisks (probability levels) in figures are the following: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Glycine activates chloride currents in rat hippocampal neurons

Short application of glycine for 600 ms on pyramidal neurons evoked chloride currents (I_{Gly}) which characteristics were described in details in our previous works (Bukanova et al., 2014a, 2014b). Shortly, the amplitude of I_{Gly} depended on glycine concentration with EC_{50} value of 90 ± 7 μM. The average value of the