Neuroscience Letters 561 (2014) 58–63

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Glycine receptor in hippocampal neurons as a target for action of extracellular cyclic nucleotides

Julia V. Bukanova, Elena I. Solntseva*, Rodion V. Kondratenko, Vladimir G. Skrebitsky

Research Center of Neurology, Russian Academy of Medical Sciences, Moscow, Russia

HIGHLIGHTS

- Isolated rat hippocampal neurons, patch-clamp method, glycine-evoked chloride current.
- Acceleration of desensitization of glycine-evoked current by extracellular cAMP and cGMP.
- A novel mode of action of cyclic nucleotides.

ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form
10 December 2013
Accepted 17 December 2013

Keywords:
Glycine receptor
Desensitization
Cyclic AMP
Cyclic GMP
Hippocampus

ABSTRACT

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are well known intracellular second messengers. At present study, we describe the effects of extracellularly applied cAMP and cGMP on glycine-induced chloride currents (I_{Gly}) in isolated rat hippocampal pyramidal neurons. 50 or 500 μM glycine was applied for 600 ms with 1 min intervals. cAMP and cGMP were co-applied with glycine. We found that both cAMP and cGMP rapidly, reversibly and in a dose-dependent manner accelerated the I_{Gly} desensitization. The effect was more prominent on I_{Gly} induced by 500 μM than by 50 μM glycine. Dose–response curves were constructed in the 0.1–100,000 nM range of cAMP and cGMP concentrations. They demonstrate that threshold concentration of both compounds was about 1 nM and maximal effect was manifested at 100 nM. When cAMP and cGMP were added to the recording pipette, their extracellular application caused the effects similar to those obtained with normal intracellular medium. The effects of cyclic nucleotides remained unchanged in the presence of the antagonist of adenosine receptors in extracellular solution, and the agonist of adenosine receptors did not mimic the effect of cyclic nucleotides. The changes in the decay kinetics were equally pronounced at negative and positive membrane potentials. When co-administered 1 nM cAMP and 1 nM cGMP caused a weaker effect than either of the compounds alone which suggests a negative interaction between binding sites for cAMP and cGMP. This work describes a novel mode of action of cyclic nucleotides, namely, the modulation of GlyRs functions from extracellular side.

1. Introduction

Glycine is a crucial inhibitory neurotransmitter acting on specific glycine receptors (GlyRs), which are plentifully expressed in retina, spinal cord, brain stem and cerebellum [for a review, 1,2]. Their localization can be both synaptic and extra-synaptic allowing glycine to influence cellular excitability through both fast synaptic transmission and tonic extra-synaptic inhibition [3,4]. Although no glycinergic synaptic currents have been found in the hippocampus, accumulating evidence indicates the presence of functional GlyR in hippocampal CA1, CA3 and dentate gyrus regions [5–7]. It has been demonstrated that in hippocampal neurons GlyRs can be activated by a number of endogenous agonists including taurine, β-alanine, and glycine, leading to the opening of strychnine-sensitive chloride channels [6–8]. Extra-synaptic GlyRs in hippocampus provide a tonic inhibition [9], which is very important for information processing within a neuronal network and can make a contribution to many pathophysiological processes.

Numerous structurally diverse molecules have been shown to modulate GlyRs, including anesthetics, alcohols, synthetic neurosteroids, antagonists of 5-HT_3 receptor, cannabinoids, ginkgolide B, cyclothiazide and quercetin [for a review, see 9,10]. GlyRs were shown to be a subject to phosphorylation and regulation by different protein kinases, such as protein kinase A (PKA), protein kinase C (PKC) and protein tyrosine kinase (PTK) [9]. However, up to date, there have been no reports on the investigations

* Corresponding author. Tel.: +7 9096427758; fax: +7 4959172382.
E-mail addresses: soln@front.ru, synaptology@mail.ru (E.I. Solntseva).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.12.037

of possible direct effects of activator of PKA, cyclic adenosine monophosphate (cAMP), and of activator of protein kinase G, cyclic guanosine monophosphate (cGMP), on GlyRs currents. cAMP and cGMP are well known intracellular second messengers [for a review, see 11]. Their well established mechanism of action is activation of corresponding protein kinases with subsequent protein phosphorylation. At the same time, efflux of cAMP and cGMP into extracellular medium [12–15] and their ability to affect cellular functions from extracellular side have been described for many different cell types, including neurons [16–18]. However, extracellular targets for either cAMP or cGMP are not known yet. The results of present study suggest that GlyRs may be considered as possible extracellular targets for cAMP and cGMP.

2. Materials 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 [19]. Briefly, the slices (200–500 μm) of Wistar rats (11–14 days of age) were incubated at room temperature for at least 2 h in a solution containing the following components (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 25 $NaHCO_3$, 1.3 NaH2PO_4, 10 D-glucose, pH 7.4. The saline was continuously stirred and bubbled with carbogen (95% O_2 + 5% CO_2). Single pyramidal neurons from CA1 and CA3 were isolated from the stratum pyramidale by a vibrating fused glass pipette with a spherical tip [19].

2.2. Current recordings

Glycine-activated currents in isolated neurons were induced by a step application of agonist for 600 ms with 1 min intervals. The application was made through glass capillary, 0.1 mm in diameter, which could be rapidly displaced laterally under control of home-made software [20]. 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): 40 CsF, 100 CsCl, 0.1 $CaCl_2$, 1 EGTA, 3 $MgCl_2$, 4 NaATP, 5 HEPES, pH 7.3. The composition of 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). 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. Reagents

Glycine, adenosine 3′,5′-cyclic monophosphate sodium salt monohydrate (cAMP), guanosine 3′,5′-cyclic monophosphate sodium salt (cGMP), 3-isobutyl-1-methylxanthine (IBMX), γ-aminobutyric acid (GABA), adenosine 5′-triphosphate disodium salt hydrate (ATP), as well as all of the chemicals for intracellular and extracellular solutions were purchased from “Sigma”. The tested substances were dissolved in distilled water to make 0.1–1 mM stock solution, which was divided into daily aliquots and kept frozen at −20 °C. The substances were dissolved in external saline to their final concentration immediately before the experiments.

2.4. Data analysis

All statistical analysis was performed with the help of *Prism Graphpad* software. All comparisons were made with paired two-tailed Student’s *t*-test and nonparametric Mann–Whitney test at a significance level of $P = 0.05$. In result descriptions, mean and standard error of mean (SEM) are specified. In figures, error bars represent SEM.

3. Results

3.1. Glycine-activated chloride currents in rat hippocampal pyramidal neurons

Experiments were performed in CA1 and CA3 pyramidal neurons isolated from the hippocampus of rats (11–14 d of age). Application of glycine evoked chloride currents (I_{Gly}) which amplitude and kinetics were dependent on glycine concentration (Fig. 1A). The EC_{50} value of glycine was 90 ± 7 μM ($n = 6$) which corresponds well with the value reported for acutely isolated hippocampal neurons by other authors [21]. An 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). The I_{Gly} had low sensitivity to the $GABA_AR$ antagonist bicuculline (10 μM), but was completely and reversibly blocked by specific GlyR blocker strychnine (3 μM) (Fig. 1B). No differences between peak amplitude of I_{Gly} recorded in neurons isolated from CA1 and CA3 were noticed (3.3 ± 0.9 nA *vs* 3.8 ± 1.1 nA). Since there was also no difference in cyclic nucleotides effects, the results obtained from cells of CA1 and CA3 regions were grouped together.

3.2. Modulation of glycine-activated currents by extracellular cyclic nucleotides

The I_{Gly} was activated by 50 μM or 500 μM glycine applied for 600 ms with 1 min intervals. Different concentrations (0.1–100,000 nM) of cAMP and cGMP were co-applied with glycine. Application of cAMP or cGMP alone did not evoke any direct membrane response. When co-applied with glycine, both cAMP and cGMP barely affected the I_{Gly} peak amplitude, but rapidly, reversibly and in a dose-dependent manner accelerated the I_{Gly} desensitization (Fig. 1C and E). The effect was well pronounced in the 1–100,000 nM range of cAMP and cGMP concentrations. To quantitatively assess the alteration of the current kinetics, we measured the halftime of a decay (τ) of I_{Gly} in the absence or presence of cAMP or cGMP. The I_{Gly} desensitization kinetics was fitted with mono-exponential function (*Prism Graphpad* software). However, in some experiments, especially with 50 μM glycine, a decay of the control current was not prominent. In such cases, the amplitude of I_{Gly} at the end of glycine application (I_{Gly} at 600 ms) was measured, and the effect was estimated as a ratio of I_{Gly} at 600 ms in the presence and absence of the cyclic nucleotide tested. It was found that cAMP and cGMP affected I_{Gly} in a similar manner (Fig. 1C–G). The effect was more prominent for I_{Gly} induced by 500 μM glycine than for I_{Gly} induced by 50 μM. Fig. 1D and F shows dose–effect relationship for a reduction of normalized I_{Gly} at 600 ms evoked by 50 and 500 μM glycine. One can see that the threshold concentration of cyclic nucleotides is close to 1 nM and maximal effect is manifested at 100 nM. The maximal decrease of I_{Gly} evoked by 50 and 500 μM glycine averaged 47 ± 6% ($P < 0.0001$, $n = 8$) and 55 ± 3% ($P < 0.0001$, $n = 10$), accordingly, in the presence of 100 nM cAMP, and 43 ± 6% ($P < 0.0001$, $n = 9$) and 53 ± 5% ($P < 0.0001$, $n = 10$), accordingly, in the presence of 100 nM cGMP. The concentrations of cyclic nucleotides above 100 nM caused weaker effects with large dispersion. It seems that high