VIROLOGY

Antiviral Drug Ivermectin at Nanomolar Concentrations Inhibits Glycine-Induced Chloride Current in Rat Hippocampal Neurons

J. V. Bukanova, E. I. Solntseva, R. V. Kondratenko, and V. G. Skrebitsky

Ivermectin (IVM) belongs to the class of macrocyclic lactones, which is used as an antiparasitic agent. At present, the researchers focus on possibility to use IVM in treatment of certain forms of cancer and viral diseases such as COVID-19. The mechanisms of IVM action are not clear. It is assumed that IVM affects chloride channels and increases cytoplasmic concentration of chloride. This study examines the effect of IVM on chloride currents induced by glycine (I_{Gly}). Experiments were carried out on isolated pyramidal neurons of the rat hippocampus with whole-cell patch clamp. A short-term (600 msec) application of IVM in a concentration of 10 μM induced a slow inward current, which persisted after washing the neurons. The low concentrations (0.1-1000 nM) of IVM did not induce any novel current, but it rapidly and reversibly reduced the peak amplitude and accelerated desensitization of I_{Gly} in a dose-dependent manner. The threshold concentrations of IVM sufficient to reduce peak amplitude of I_{Gly} and to accelerate desensitization of I_{Gly} were 100 nM and 0.1 nM, respectively. The study revealed a high sensitivity of neuronal glycine receptors to IVM.

Key Words: *glycine receptor; ivermectin; hippocampus; patch clamp*

Ivermectin (IVM) belongs to the class of macrocyclic lactones. It is a product of secretion of Streptomyces bacteria, which live in the soil. IVM is known for pronounced antiparasitic action, so it is widely used in veterinary and therapeutics against helminths and some insects [6]. In addition, IVM exerts many other physiological effects such as anticonvulsant action [4], alleviation of neuropathic pain [13], destruction of the malignant leukemic cells [5,6,9]. A present, a promising vista to use IVM as chemotherapeutic medication in treatment of certain types of cancer [5,7] and as antiviral agent against COVID-19 [3] is widely discussed. A large number of molecular targets have been identified for IVM to exert its physiological effects [5]. Of them, an important role is given to ligand-operated chloride channels in plasmalemma, which can increase the intracellular concentration of chloride and hyperpolarize the cell membrane as a result of binding with IVM. In neurons of invertebrate animals, IVM in nanomolar concentrations enhances the chloride current through glutamate-activated chloride channels (GluClC) [14]. In mammalian cells, it activates GABA-dependent chloride channels, although at higher concentrations [4]. However, the data on IVM-induced modification of glycine-activated chloride current (I_{Gly}) are contradictory. Actually, IVM stimulates I_{Gly} due to interaction with recombinant glycine receptors (GlyR) [8], but inhibits the cortical neurons in rats [4].

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This work was designed to study the effects of IVM on I_{Gly} in pyramidal neurons of rat hippocampus.

MATERIALS AND METHODS

Experiments were carried out on pyramidal neurons isolated from hippocampal CA3 region of 11-14-day-old Wistar rat. Glycine was applied to neuron at concentration of 100 or 500 µM via a 0.1-mm glass pipette during its rapid lateral displacement. The application time and interapplication interval were 600 msec and 30-40 sec, respectively. The ionic currents were recorded with patch clamp in whole cell configuration [1]. The holding potential was -70 mV. The recording pipette was filed with solution containing (in mM): 40 CsF, 100 CsCl, 5 EGTA, 0.5 $CaCl_2$, 4 $MgCl_2$, 6 NaATP, and 5 HEPES (pH 7.3). The extracellular perfusion solution contained (in mM): 140 NaCl, 3 KCl, 3 $CaCl_2$, 3 $_{MgCl2}$, 10 D-glucose, and 10 HEPES hemisodium (pH 7.4). The perfusion rate was 0.6 ml/min. IVM was purchased from Tocris. The reagents of physiological saline were purchased from Sigma.

The results were analyzed statistically using GraphPad Prism software and unpaired Student's *t* test. The data were averaged from the measurements in several neurons (*n*=7-8) and presented as *m±SEM*.

RESULTS

A short-term application (600 msec) of glycine to hippocampal pyramidal neurons induced I_{Gly} [2]. Dependence of its amplitude on concentration of glycine was
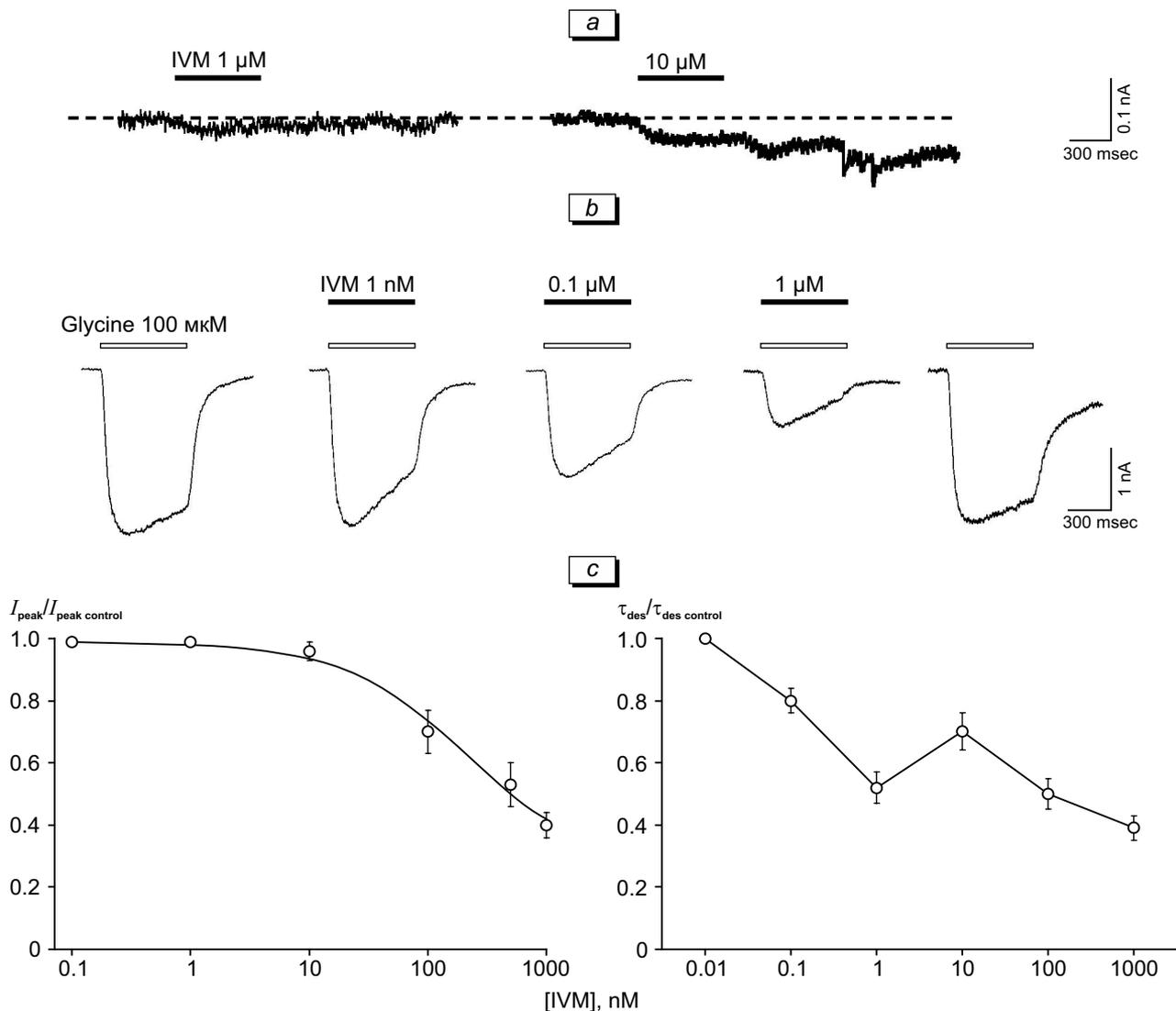

Fig. 1. Effect of IVM on holding current and I_{Gly} induced by 100 µM glycine. *a*) Effect of 1 and 10 µM IVM on holding current at holding potential of -70 mV; *b*) recordings of I_{Gly} before adding IVM to patch-clamp pipette, in the presence of various concentrations of IVM, and after removing IVM from the pipette; *c*) normalized plots of dose-dependent effect of IVM on peak amplitude (I_{peak}) and desensitization time constant (τ_{des}) of I_{Gly}.

characterized with EC50=90±7 μM. I_{Gly} was induced with glycine at concentrations of 100 or 500 μM, which were close to EC_{50} or to concentration evoking maximal I_{Gly}, respectively.

In preliminary experiments, the effect of IVM on holding current was examined. At low concentrations (≤1 μM), a short-term (600 msec) application of IVM produced no effect on this current (Fig. 1, a). Application of IVM in higher concentration (10 μM) induced a slow inward current, that was did not return to initial level throughout the experiment (Fig. 1, a). According to [9], IVM-induced current results in accumulation of cytoplasmic chloride and shifts the chloride equilibrium potential. Evidently, it is problematic to examine I_{Gly}, which is carried out by chloride ions. Logically, further experiments focusing on the effect of IVM on I_{Gly} were carried out at low IVM concentrations (0.1-1000 nM), which did not evoke ionic current *per se*.

In further experiments, we studied the effect of IVM on I_{Gly} caused by application of 100 μM glycine. To this end, IVM was supplemented to glycine-containing pipette and applied to neurons with gly-

cine for 600 msec. In all examined neurons (n=12), application of IVM resulted in rapid, reversible, and dose-dependent inhibition of I_{Gly} (Fig. 1, b). IVM produced two effects manifested by a decrease in peak amplitude of I_{Gly} and acceleration of its desensitization. The normalized dose-dependence plots for peak amplitude (I_{peak}) and τ_{des} of I_{Gly} show that the threshold concentrations of IVM affecting τ_{des} and I_{peak} are 0.1 and 100 nM, respectively. The parameters of dose-dependence plots for both IVM effects also differed (Fig. 1, c). For I_{peak}, this plot had the form of a smooth regression curve with maximum effect at 1000 nM IVM, while the dose-dependence plot for τ_{des} was N-shaped with two maxima at 1 and 1000 nM IVM. In relation to control levels, I_{peak} at 1000 nM IVM was 0.41±0.04 (p<0.0001, n=8), while the values of τ_{des} at 1 nM and 1000 nM IVM were 0.52±0.05 (p<0.0001) and 0.39±0.04 (p<0.0001), respectively.

The next experiments were carried out with I_{Gly} induced by 500 μM glycine (Fig. 2). They showed that elevation of glycine concentration weakened the effect of IVM on I_{peak}, but did not change the effect of
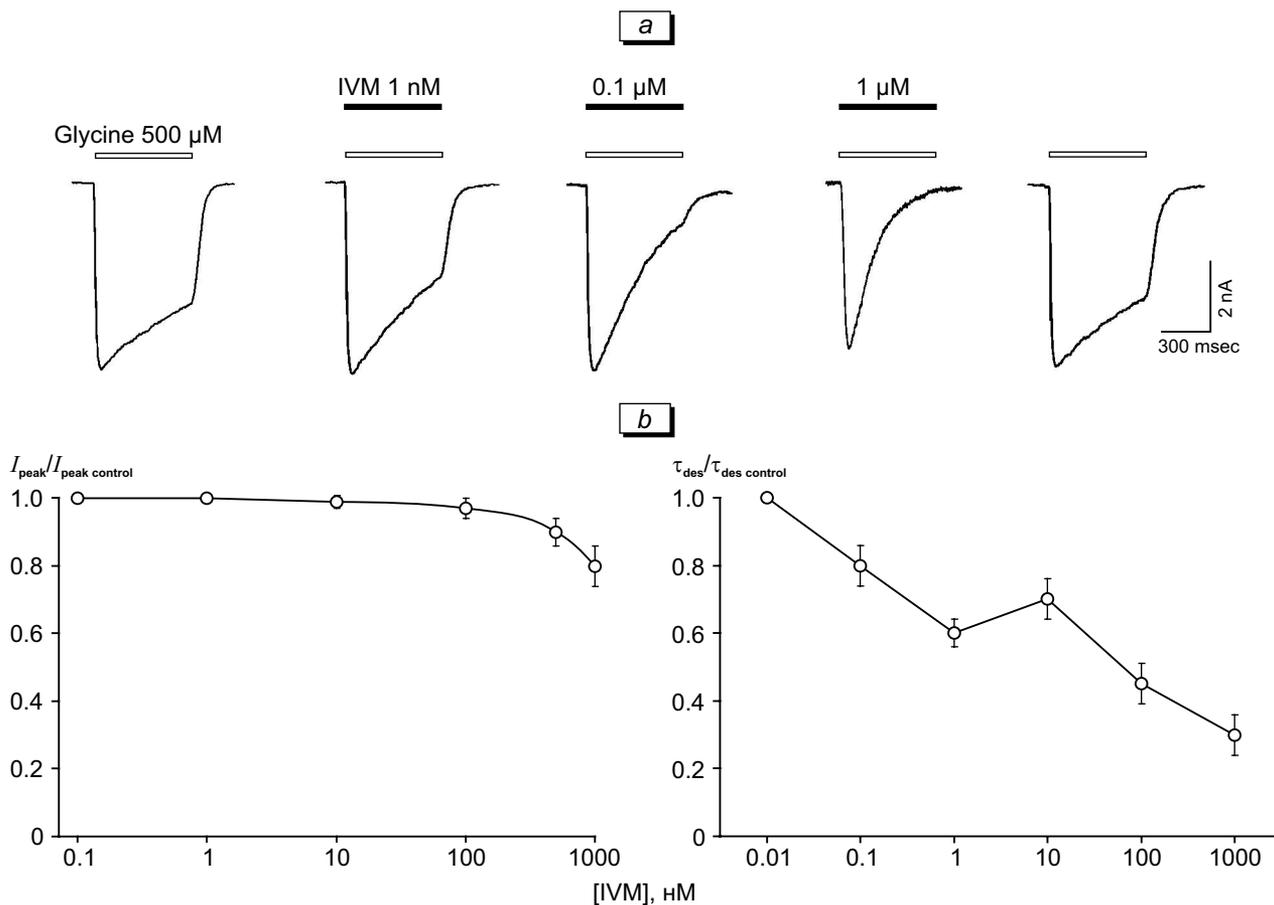

Fig. 2. Effect of IVM on I_{Gly} induced by 500 μM glycine. *a*) Recordings of I_{Gly} before adding IVM to patch clamp pipette, in the presence of various concentrations of IVM, and after removing IVM from the pipette; *b*) normalized plots of dose-dependent effect of IVM on peak amplitude (I_{peak}) and the time constant of desensitization (τdes) of I_{Gly}.

IVM on τ_{des}. At a glycine concentration of 500 µM, the threshold IVM concentration affecting I_{peak} was 500 nM, while I_{peak} at 1000 nM IVM was 0.81±0.06 relatively to the control level ($p<0.05$, $n=7$), which was significantly higher than the corresponding value obtained with 100 µM glycine ($p<0.01$, $n=7$). In this case, the values of τ_{des} at 1 and 1000 nM IVM were 0.60±0.04 ($p<0.0001$) and 0.29±0.06 ($p<0.0001$), respectively, relatively to control value, and they did not significantly differ from the corresponding τ_{des} values obtained at 100 µM glycine.

It was established that IVM activates chloride channels and elevates cytoplasmic chloride [9], and both effects are viewed as important mechanisms of physiological action of this drug. In neurons and muscle cells of invertebrates, IVM enhances the chloride current flowing through glutamate-activated chloride channels (GluClC) [14], which explains its antiparasitic effect. No GluClC were found on vertebrate animal cells although at least five other types of chloride channels were described that are regulated by various factors, including neurotransmitters (GABA, glycine), cAMP-dependent phosphorylation, membrane potential, Ca^{2+} ions, and intracellular turgor [12]. In various cells (frog oocytes, HEK293, rat cortical neurons, and fibroblasts) IVM at high concentrations induces a slow and irreversible chloride current, which cannot be blocked by picrotoxin and, therefore, cannot be explained by potentiation of GABA- or glycine-activated receptors [8]. Here, IVM (10 µM) induced a slow and irreversible current in native neurons, which cannot be eliminated by washing. However, the type of chloride channels or pores responsible for this current is presently unclear.

The effect of IVM on GABA- and glycine-activated chloride current (I_{GABA} and I_{Gly}) was studied in electrophysiological experiments both on recombinant receptors expressed in oocytes or HEK293 cells, and on native neurons and fibroblasts [4,8]. It was found that the effects of IVM on I_{GABA} and I_{Gly} in recombinant receptors and native cells were different. IVM augmented IGABA, recorded in recombinant receptors and native neurons of rat cortex, but diminished this current in fibroblasts [4]. In recombinant GlyRs, IVM exerted the stimulating effect on I_{Gly} [8], whereas it inhibited this current in rat cortical neurons [4]. Therefore, the recombinant receptors in oocytes or kidney cells (HEK293) have different pharmacological properties in comparison with those of native neurons. Evidently, further studies of IVM effects exerted on ligand-operated chloride channels should be carried out on native neurons.

In pyramidal neurons of rat hippocampus, IVM at low concentration did not induce its own chloride current but decreased I_{Gly}. IVM exerted dual effect on I_{Gly}:

it decreased the peak amplitude of I_{Gly} and τ_{des}. The threshold concentration of IVM for affecting τ_{des} was smaller by 3 orders of magnitude than that for acting on the peak amplitude of I_{Gly}. The dose-dependence plot for the effect of IVM on τ_{des} was N-shaped. The effect of IVM on peak amplitude of I_{Gly} decreased when concentration of glycine increased, while its effect on τ_{des} did not change, which attests to independent nature of both effects. Probably, attenuation of I_{Gly} peak amplitude is underlain by a competitive mechanism, in contrast to IVM effect on desensitization of I_{Gly}.

Enhanced sensitivity of τ_{des} to IVM, N-shape of its dose-dependence plot, and stability of this effect despite increasing glycine concentration are similar to the effects of other drugs (cyclic nucleotides, beta-amyloid peptide, and lithium ions) exerted on I_{Gly}, which we described previously in isolated pyramidal neurons of rat hippocampus [2,10,11]. Overall, these results suggest that GlyR in these neurons has a non-specific site located near the desensitization gate of chloride channel, which is able to bind various drugs with different affinities.

Our study showed that antiviral drug IVM is an effective antagonist of neuronal GlyR. The present data not only widen our views on pharmacological properties of GlyR, but they can also be useful in analyzing the mechanisms of side effects, which can emerge when using IVM as antiparasitic, antiviral, or anticancer agent.

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