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Mechanisms Underlying the Antiarrhythmic Action of Compound ALM-802

I. B. Tsorin[1], I. Yu. Teplov[2], V. P. Zinchenko[2], V. V. Barchukov[1], M. B. Vititnova[1], G. V. Mokrov[1], and S. A. Kryzhanovskii[1]

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The mechanisms underlying the antiarrhythmic action of compound trihydrochloride N^1-(2,3,4-trimethoxy)-N^2-{2-[(2,3,4-trimethoxybenzyl)amino]ethyl}-1,2-ethane-diamine (code ALM-802) were studied *in vitro*. The experiments were performed on a culture of rat hippocampal neurons. The electrical activity of neurons was recorded by the patch-clamp method in the whole cell configuration. It is shown that the compound ALM-802 effectively blocks potential-dependent Na^+ and K^+ channels and does not affect the activity of potential-dependent Ca^{2+} channels. The inhibition of currents through these channels is dose-dependent; the IC_{50} of Na^+ and K^+ channels were 94±4 and 67±3 μM, respectively. These findings indicate that compound ALM-802 combines the properties of class I and class III antiarrhythmic agents according to the Vaughan–Williams classification.

Key Words: *compound ALM-802; patch-clamp; Na^+ channels; K^+ channels; Ca^{2+} channels*

Cardiovascular diseases (CVD), mainly coronary artery disease and stroke, are the main cause of both global mortality and disability in the population. The largest epidemiological study that included 204 countries and territories showed that mortality from CVD continued to grow steadily and increased from 12.1 million cases in 1990 to 18.6 million in 2019 despite significant advances in the development of cardiac care [1]. Disability from CVD in 1990-2019 almost doubled, from 17.7 to 34.4 million cases [1]. Apparently, this is due to the significant spread of CVD during this period (from 271 to 523 million cases of the disease).

A similar trend is observed in Russia. So, according to the data of the Central Research Institute of Organization and Informatization of Healthcare of the Ministry of Health of Russia, for the period from 2007 to 2019 the primary incidence of CVD increased by 42%, and the overall incidence by 24% [2]. The COVID-19 pandemic aggravates the problem [3,4]. There is every reason to believe that in the near future, against the backdrop of a pandemic, morbidity, disability, and mortality from CVD will only increase. This is due not only to the cardiovascular complications of COVID-19 itself, but also to a significant decrease in the availability of specialized medical care. According to the operational data of the Ministry of Health, in the period from January 1, 2020 to November 19, 2020, 818,422 people died from CVD, which is 9.4% more than in the same period in 2019 [5]. In Russia over the first half of 2020 (January–June), the death rate from COVID-19 was 7317 people and from other causes in patients with COVID-19 – 5825 people. During the same period, 39,985 people died from acute coronary syndrome, 220,719 people died from coronary artery disease [6]. In 2022, these figures are likely to be even higher.

Heart rhythm disturbances are one of the most frequent complications in hospitalized patients diagnosed with COVID-19 [7,8]. In particular, in patients with COVID-19 requiring intensive care, arrhythmia was observed in 44% of cases [9]. Thus, the search for

[1]V. V. Zakusov Research Institute of Pharmacology, Moscow, Russia; [2]Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, Russia. ***Address for correspondence:*** tsorinib@yandex.ru. I. B. Tsorin

innovative cardiotropic drugs that have, among other things, antiarrhythmic activity seems to be extremely relevant.

Screening performed at the V. V. Zakusov Research Institute of Pharmacology in the series of α,ω-diarylmethyl derivatives of bis-(ω-aminoalkyl) amines allowed identifying compound trihydrochloride N^1-(2,3,4-trimethoxy)-N^2-{2-[(2,3,4-trimethoxybenzyl)amino]ethyl}-1,2-ethanediamine (code ALM-802) that exhibits pronounced cardioprotective activity [10], including antiarrhythmic and antifibrillatory effects [10,11].

In the present study, we analyzed the effect of the antiarrhythmic ALM-802 compound on the activity of voltage-dependent Na^+, K^+, and Ca^{2+} channels of excitatory neurons in the rat brain in experiments *in vitro* using electrophysiological methods.

MATERIALS AND METHODS

We used a mixed neuroglial culture of hippocampal cells isolated from the brain of newborn (P1-3) Wistar rats [12]. The study was approved by the Commission on Biomedical Ethics of the V. V. Zakusov Research Institute of Pharmacology (Protocol No. 01, January 29, 2021).

Changes in the membrane potential and ionic currents were studied by the electrophysiological method (patch-clamp) in the whole-cell configuration. The studies were carried out on an AxioObserver Z1 Fluorescent Station, a unique scientific station with a built-in microincubator, a patch-clamp electrophysiological setup equipped with a Hamamatsu ORCA-Flash high-speed camera.

A round cover glass with cell culture was mounted in a special measuring chamber containing 1 ml medium. The reagents were applied in a continuous flow of Hanks' solution using a special perfusion system that allows quick replacement of the solution in the chamber. The addition of reagents and washings were performed by replacing the medium in a 10-fold volume using a system that provides perfusion at a rate of 15 ml/min.

To measure the neuronal membrane potential, a cell with a cover glass filled with 1 ml of Hanks' extracellular solution (HBSS) was placed on the grounded stage of an AxioImager Z1 microscope (Carl Zeiss) equipped with a micromanipulator for applying patch pipettes.

Changes in the membrane potential were recorded in the current clamp mode, and ion currents were recorded in the potential clamp mode, for which an Axopatch 200B amplifier (Molecular Devices), a Digidata 1440A ADC, and pClamp 10.2 software (Molecular Devices) were used.

Experiments were performed using a patch pipette solution containing (in mM): 5 KCl, 130 potassium gluconate, 1 $MgCl_2 \times 6H_2O$, 0.25 EGTA, 4 HEPES, 2 Na_2-ATP, 0.3 Mg-ATP, 0.3 Na-GTP, 10 Na_2-phosphocreatine (305-310 mOsm, pH 7.2). The HBSS solution used for all recordings contained (in mM): 156 NaCl, 3 KCl, 2 $MgSO_4$, 1.25 K_2HPO_4, 1.4 $CaCl_2$, 10 glucose, and 10 HEPES (pH 7.35 at 25-28°C).

To study the effect of ALM-802 on evoked action potentials (AP) and the intervals between them, 12-14-day cultures were used. Recordings were made in the current clamp mode. Current stimuli from 10 to 200 pA (increments 5 pA) were applied to the cells, the duration of stimulation was 1000 msec, and the intervals between stimuli were 5 sec.

To study the effect of the drug on K^+ and Na^+ currents, experiments were carried out on young 2-3-day-old neuroglial cultures. To record Na^+ currents, an intrapipette solution was used, in which all potassium salts were replaced by cesium salts to inhibit potassium currents (a CsOH solution was used to adjust pH). To record K^+ currents, 100 nM tetrodotoxin was added to the extracellular solution to block Na^+ current. Recordings were made in the potential fixation mode. After the addition of the substance, voltage steps of 30 mV were applied to the cell (the current reached its maximum values in most cells under this stimulus) with a duration of 50 msec and with an interval of 5 sec for 5 min. To standardize the current values, the results of recording from each cell were divided by the capacity of this cell.

Experiments with each concentration of the ALM-802 compound were repeated on at least three cultures. Solutions of the ALM-802 compound were prepared each time immediately before the start of the experiment.

Half-maximal inhibition concentrations (IC_{50}) were calculated using OriginPro 8.5 software. Statistical processing of the results was carried out using the BioStat 2009 program. The normality of the distribution of the obtained data was checked using the Shapiro–Wilk test. Since the distribution of the samples practically did not differ from the normal one, the significance of differences in the IC_{50} of the ALM-802 compound for Na^+ and K^+ channels was determined using Student's *t* test for independent samples. The results obtained were presented as $M \pm SEM$. The differences were considered significant at $p \leqslant 0.05$.

RESULTS

To study the effect of the ALM-802 compound on mature 12-14 day neuroglial cultures, control recordings of evoked AP were made (the scheme of the stimulation protocol is shown in Figure 1, *a*), after

which Hanks' solution containing 69.8 μM ALM-802 was added through the application system into the experimental chamber (this concentration is equivalent to the effective dose of the ALM-802 compound determined in *in vivo* experiments (2 mg/kg intravenously) [10,11]), and repeated recordings were made. At these excitation parameters, AP generation was observed (Fig. 1, *b*), the amplitude of which gradually decreased due to inactivation of a part of the voltage-dependent Na^+ channels [13]. ALM-802 compound caused a decrease in the amplitude and frequency of AP, which may indicate inhibition of voltage-gated Na^+ channels and inactivation of K^+ channels.

In the next experiment on young 2-3-day neuroglial cultures, we studied the effect of ALM-802 compound directly on Na^+ and K^+ currents. At this stage of development, neurons do not yet express a large number of Na^+ and K^+ channels, and the Na^+ and K^+ currents generated in response to stimulation are small, which facilitates their analysis. The stimulation protocol was used to measure Na^+ and K^+ currents (Fig. 2, *a*). The retained potential of the membrane varied from -70 to -100 mV for 150 msec, which made it possible to remove all voltage-dependent Na^+ and K^+ channels from the inactivation state. Then, to activate the voltage-dependent Na^+ and K^+ channels, the potential was increased to -30 mV, after which it was reduced to -70 mV. The protocol was repeated every 5 sec for 5 min. The Na^+ and K^+ currents generated in response to depolarization of -30 mV decreased

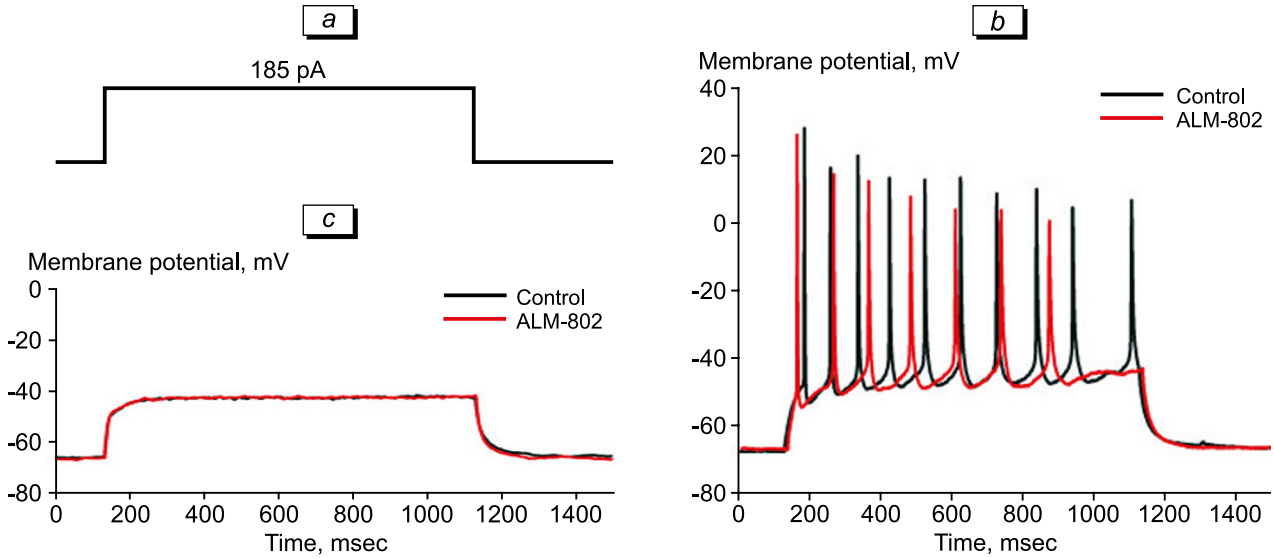

Fig. 1. Effect of ALM-802 on induced AP in mature 12-14-day neuroglial cultures. *a*) Current stimulation protocol; *b*) generation of evoked AP in the control and in the presence of ALM-802 (69.8 μM); *c*) a step of membrane potential (without AP generation, Na^+ channels are inhibited) arising due to activation of voltage-gated Ca^{2+} channels in the control and in the presence of ALM-802 (69.8 μM).

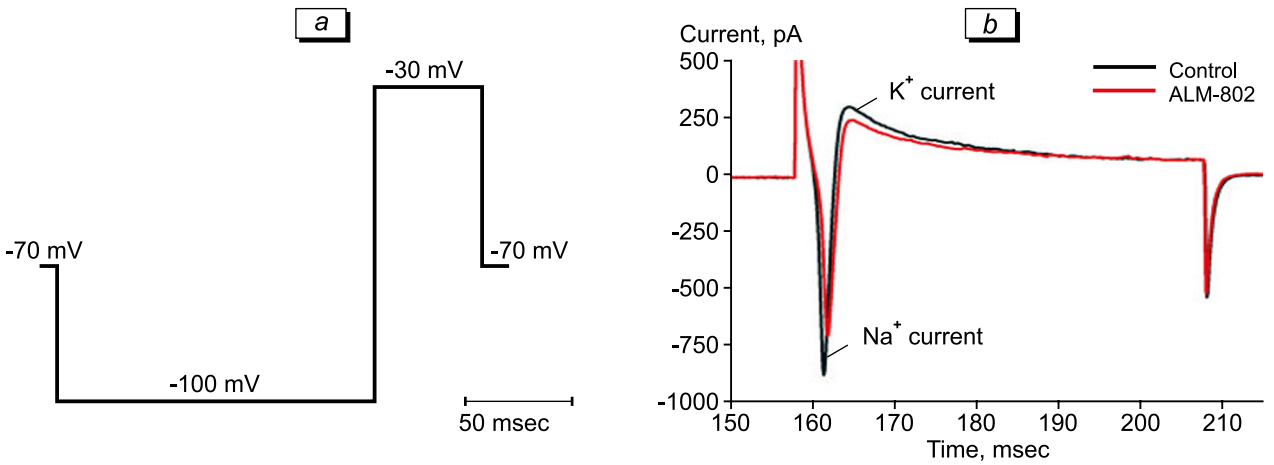

Fig. 2. Effect of ALM-802 compound on induced AP in young (2-3 day) neuroglial cultures. *a*) Stimulation protocol; *b*) generation in response to stimulation of Na^+ and K^+ currents in the control and in the presence of ALM-802 compound (69.8 μM).

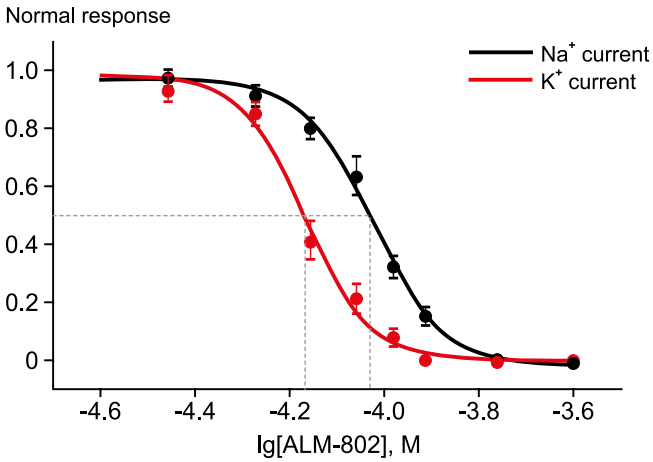

Fig. 3. Dependence of the amplitude of Na^+ and K^+ currents in response to a depolarization pulse up to -30 mV on the concentration of the ALM-802 compound. The amplitude of the response in the control is taken as 1. Each point is the mean of 3 experiments. For Na^+ channels, IC_{50}=94±4 μM, for K^+ channels, IC_{50}=67±3 μM (p<0.01).

relative to the control in the presence of ALM-802 in a concentration of 69.8 μM (Fig. 2, *b*).

A series of experiments was also carried out to quantify the dependence of Na^+ and K^+ currents on the concentration of the ALM-802 compound. The experiments were carried out separately for each channel in order to exclude distortions of the values of the current responses due to interaction of Na^+ and K^+ currents. To record Na^+ currents, potassium salts in the intrapipette solution were replaced by cesium salts to inhibit K^+ currents. To record K^+ currents, tetrodotoxin (100 nM) was added to the extracellular solution to inhibit Na^+ currents. To standardize the current values, the results of recording from each cell were divided by the capacity of this cell. The experiment with each concentration of ALM-802 compound was repeated at least 3 times. As a result, dose–response curves were constructed (Fig. 3). ALM-802 compound blocked voltage-gated Na^+ and K^+ channels. Since the IC_{50} for Na^+ channels is significantly higher than for K^+ channels (94±4 and 67±3 μM, respectively, p<0.01), it can be assumed that ALM-802 compound has higher affinity for voltage-gated K^+ channels.

To evaluate the effect of ALM-802 compound on voltage-gated Ca^{2+} channels, similar experiments were carried out in the presence of tetrodotoxin (100 nM) in an extracellular solution. When AP generation is blocked, the step value of the membrane potential caused by neuron current stimulation, among other things, depends on the activity of voltage-gated Ca^{2+} channels, and if the ALM-802 compound affects their activity, the value of the membrane potential during stimulation should change. These changes were not detected either under the conditions of adding the ALM-802 compound to the solution at a concentration of 69.8 μM, or at a 2- and 3-fold increase in concentration (Fig. 1, *c*). Therefore, the ALM-802 compound does not affect the activity of voltage-gated Ca^{2+} channels.

Thus, the antiarrhythmic activity of the ALM-802 compound is largely related to its ability to block voltage-dependent Na^+ channels and, to a greater extent, K^+ channels, which suggests a combination of properties of antiarrhythmic drugs of classes I and III according to the Vaughan–Williams classification.

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