EFFECT OF ALLAPININE ON SODIUM CURRENTS IN SINGLE TRIGEMINAL NEURONS AND CARDIOMYOCYTES OF RATS

A. E. Valeev, A. N. Verkhratskii, and F. N. Dzhakhangirov

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Allapinine is the hydrobromide of the alkaloid lappaconitine, found in *Aconitum leucostomum* and *Aconitum orientale* plants. Experimental and clinical studies have shown that allapinine is a new antiarrhythmic agent which is highly effective in the treatment of supraventricular and ventricular cardiac arrhythmias of varied etiology [4]. The results of clinical investigations suggest that the mechanisms whereby allapinine realizes its antiarrhythmic effects differs from those of the familiar antiarrhythmic agents currently used in clinical practice. It was accordingly necessary to study the action of allapinine on components of transmembrane conduction of the membranes of excitable cells.

EXPERIMENTAL METHOD

Experiments were carried out on single neurons from rat trigeminal ganglia and rat cardiomyocytes in culture, using the membrane voltage clamp method and intracellular perfusion [1, 6]. The methods of isolating and culturing the cells were described [1, 3]. The compositions of the (in mM) in the experiments on nerve cells were: 1) extracellular solution: NaCl – 130, KCl – 5, $CaCl_2$ – 2, $MgCl_2$ – 0.5, HEPES/NaOH – 10, glucose – 10, pH 7.4; 2) intracellular solution: KF – 100, TrisF – 40, pH – 7.4; in the experiments on isolated cardiomyocytes: 1) extracellular solution: NaCl – 120, KCl – 5.4, $CaCl_2$ – 5, $MgCl_2$ – 1.1, HEPES/NaOH – 10, glucose – 10, pH 7.4; 2) intracellular solution: CsF – 110, NaF – 30, HEPES/NaOH – 10, pH 7.3.

EXPERIMENTAL RESULTS

In both types of cells tested on the addition of allapinine to the external physiological saline in concentrations of 1-10 μM the tetrodotoxin sensitive inward sodium current was reduced (Figs. 1 and 2). In both neurons and cardiomyocytes allapinine did not change the voltage-dependent properties of the sodium channels. The action of allapinine on the outward potassium and inward calcium currents and on chemically controlled currents activated by different neurotransmitters, such as glycine, taurine, γ-aminobutyric acid, glutamate, and ATP, in neurons of the trigeminal ganglia also was tested. All these types of transmembrane ionic conductance were unaffected by allapinine in the concentration range which we used.

Under laboratory conditions, in order to induce experimental cardiac arrhythmias, another alkaloid of the diterpene class, namely aconitine, is frequently used [7]. The arrhythmogenic action of aconitine is based on an increase in the inflow of sodium ions into the cell through voltage-dependent sodium channels; under these circumstances the excitability of the cardiac cell is increased, and on the other hand, the cytoplasmic sodium ion concentration is increased, and this is one of the key factors causing the development of triggering activity in the myocardium [9]. Studies of the action of aconitine on the sodium current of the heart cell membrane showed that aconitine shifts the threshold of activation of Na^+-channels toward hyperpolarization, alters their selectivity, and delays inactivation of the sodium currents in the region of voltages to –40 mV. These changes are based on direct interaction of aconitine with sodium channels, leading to a change in the gating properties of the channel, which is expressed at the elementary current level as a significant increase in the proportion of bursts of open channels [8].

A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. Institute of Chemistry of Plant Compounds, Academy of Sciences of the Uzbek SSR, Tashkent. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Romodanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 4, pp. 388-390, April, 1991. Original article submitted August 8, 1989.

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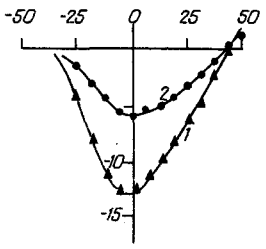

Fig. 1

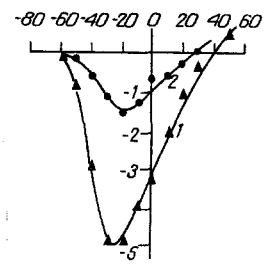

Fig. 2

Fig. 1. Current—voltage characteristic curve of inward sodium current recorded on single rat trigeminal neuron before (1) and after (2) action of allapinine (10 μM).

Fig. 2. Current—voltage characteristic curve of inward sodium current recorded on rat cardiomyocytes in culture before (1) and after (2) action of allapinine (10 μM).

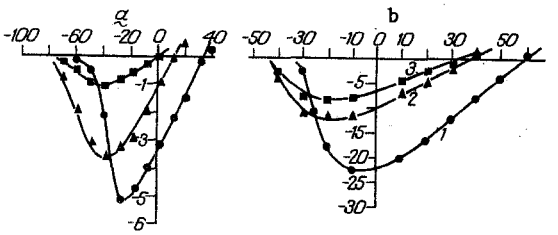

Fig. 3. Current—voltage characteristic curves of inward sodium current on cardiomyocytes (a) and isolated trigeminal ganglionic neurons (b). 1) Current—voltage characteristic curve plotted from measurements of currents in normal extracellular solution; 2) in solution containing aconitine, 10 μM; 3) in solution containing 10 μM aconotine and 10 μM allapinine.

Changes in the current—voltage characteristic curve of sodium currents in the membrane of single neurons and cardiomyocytes under the influence of aconotine are shown in Fig. 3. These changes consist of displacement of the region of the activation threshold of the sodium current and the maximum of the current—voltage characteristic curve toward negative values of membrane potential. Simultaneously with this, the reversal potential of the sodium current is also shifted toward hyperpolarization, due to changes in selectivity of the Na^+-channels [8]. The addition of allapinine after aconitine did not change the voltage-dependent parameters of the sodium current, but caused significant depression of its amplitude.

Most antiarrhythmic agents are characterized by a so-called stimulus dependent block, manifested as an increase in the degree of blocking of the current during a successive series of depolarizations [2, 5].

To test the possible stimulus-dependence of the allapinine-induced block of the sodium current the following experiments were carried out. Cardiomyocytes and neurons were kept in external solutions containing allapinine, and the amplitudes of the sodium currents induced by depolarizations separated by different intertrial intervals, were compared. The degree of blocking of the sodium channels did not depend on the duration of the intertrial intervals. In addition, changes in the degree of blocking of the sodium current depending on the frequency of application of the testing shifts of membrane potential also were studied. The degree of depression of the sodium current was found not to change during variation of the frequency of stimulation within the 0.5-5 Hz range. On the basis of these data it can be postulated that allapinine is not characterized by a stimulus dependent block of the sodium channels; in turn, this suggests that allapinine binds with sodium channels that are in a resting state.

The results are thus evidence that the basic action of allapinine is inhibition of sodium conductance of membranes of excitable objects. Allapinine has an inhibitory action on the sodium current, without changing the threshold of its activation, in experiments both on cardiomyocytes and on single trigeminal ganglionic neurons.

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SERUM CREATINE KINASE ISOZYME SPECTRUM OF RATS DURING AGING AND ACUTE ALCOHOL INTOXICATION

I. V. Chuvaev

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Ethanol has a many-sided action on all systems of the body [1-3]. Many workers have shown that ethanol penetrates freely through the blood brain barrier, and thus disturbs its protective function [1]. The direct action of alcohol and acetaldehyde on cells may lead to increased "flowability" of the cell membranes [2], thereby changing their permeability. An early response of brain tissue to ethanol poisoning is disturbance of oxidative processes and acidification of the intracellular medium, and disturbance of tissue respiration [1]. Similar changes in energy metabolism may also lead to increased cellular permeability for several metabolites and proteins, one of which is creatine kinase.

Creatine kinase (CK) is a cytoplasmic enzyme involved in energy metabolism. A significant increase in CK activity in the blood of rats has been demonstrated after a single injection of a large dose of ethanol [7]. There is also information on the increase in CK activity in the blood serum of alcoholics [4].

We know that CK exists in three molecular forms, each characterized by marked organ-specificity. Skeletal muscle contains MM-CK, MB-CK is a cardiospecific isozyme, and BB-CK is found mainly in the brain, smooth muscle, and gonads [5].

The question accordingly arises: on account of which isozymes does the increase in total CK activity in the blood serum take place in acute alcohol intoxication, and from which tissues does the enzyme leak into the blood stream. Other interesting questions are age changes in the activity of this enzyme. The investigation described below was carried out to study these problems.

Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.

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