FREQUENCY-DEPENDENT BLOCKADE OF Na CHANNELS OF ISOLATED RAT CARIOMYOCYTES BY THE ANTIARRHYTHMIC ETHMOZINE

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Ethmozine [2-carbethoxyamine-10-(3-morpholylpropionyl)-phenothiazine hydrochloride] has marked antiarrhythmic properties. In recent years it has proved highly effective in clinical practice for controlling various disturbances of the cardiac rhythm [2, 13]. Experiments on trabeculae of the frog atrium have shown [5] that ethmozine (E) (10^{-5}-10^{-6} g/ml) blocks the Na current (I_{Na}). A similar result has been obtained in experiments on single adult rat cardiomyocytes [4]. It was concluded from these observations that the antiarrhythmic action of E in the late stage of myocardial infarction is due to inhibition of the fast inward Na current [3], due to a decrease in maximal Na conductance. Similar investigations of the action of E on I_{Na} of the frog Ranvier node [1] showed that blockade of Na channels is largely dependent on the membrane depolarization frequency. The problem of the possible frequency dependence of the action of E on Na channels in the heart remains unsolved.

The aim of this investigation was to study the action of E on I_{Na} of isolated heart cells at different depolarization frequencies.

EXPERIMENTAL METHODS

Single heart cells were isolated by the method described previously [15]. Intact, noncontracting cardiomyocytes, selected under the microscope, were transferred to an experimental chamber and drawn up into the pore of a V-shaped polyethylene pipet by the method described in [15]. The experimental chamber contained a standard solution of the following composition (in mM): NaCl 130, KCl 4, $MgCl_2$ 0.5, glucose 10, Tris-buffer 10 (pH 7.4), with the addition of 0.9 mM $CaCl_2$. The control solution in the suction cap was of similar composition but 1 mM $MnCl_2$ and 1 mM 4-aminopyridine were added to it to block Ca and K channels respectively. All experiments were conducted at room temperature (20-22°C). The fast inward Na current was measured by the patch clamp method [15]. The holding potential in all experiments ensured complete abolition of inactivation ($h_\infty \approx 1$). The amplitude of the testing stimuli was chosen so as to achieve complete activation ($m_\infty \approx 1$) of Na channels. The amplitude of the current under these circumstances was 500-800 pA. Cells in which the decrease in amplitude of I_{Na} during stimulation with a frequency of 5 Hz for 30 sec did not exceed 15% were used in the experiments.

RESULTS

Replacement of the control solution in the suction cap by solution containing 10^{-5}, $2 \cdot 10^{-5}$, and $4 \cdot 10^{-5}$ M E caused a decrease in the amplitude of I_{Na} in all 15 experiments. Under these circumstances, according to recommendations given in [14], three phases of the I_{Na} block were distinguished: 1) initial block; 2) cumulative (frequency-dependent) block; 3) tonic block. The initial block developed during the first 10 min after the change of solution without membrane depolarization. Its depth depended on the concentration of E. With E in a concentration of 10^{-5} M the amplitude of I_{Na} decreased by 20.5 ± 12% (n = 5). An increase in the concentration up to $2 \cdot 10^{-5}$ and $4 \cdot 10^{-5}$ M led to a decrease in I_{Na} by 30 and 40% respectively (n = 3). A study of dependence of the cumulative (frequency-dependent) block of I_{Na} in response to E in a concentration of 10^{-5} M on frequency of stimulation showed

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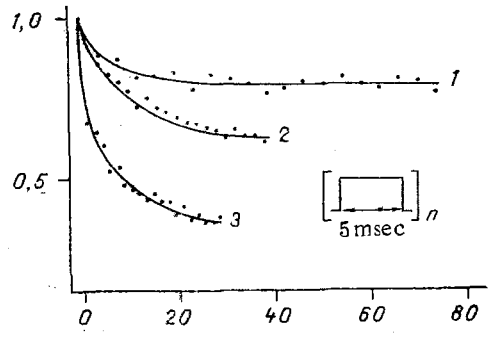

Fig. 1

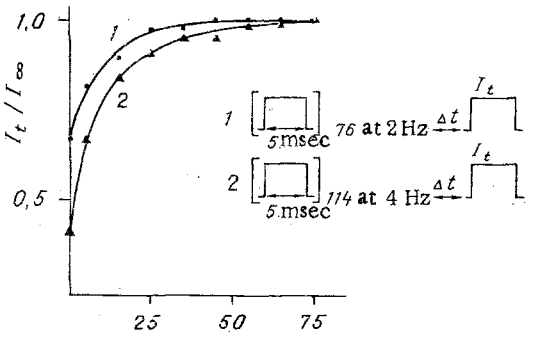

Fig. 2

Fig. 1. Cumulative block of I_{Na} under the influence of 10^{-5} M E depending on frequency of stimulation. Abscissa, time of stimulation (in sec); ordinate, peak values of I_{Na} in response to testing stimuli 5 msec in duration, evoking maximal I_{Na}. 1, 2, 3) 0.5, 2, and 4 Hz respectively. Inset: program of stimuli shown schematically. Amplitude of current at time t = 0 corresponds to 75% of control amplitude without drug.

Fig. 2. Kinetics of recovery of amplitude of I_{Na} from cumulative block in the presence of E in concentration of 10^{-5} M. Abcissa, time from beginning of recovery period (in sec, ΔT); ordinate, amplitude of I_{Na} at each second pulse. Insets: program of stimuli. Amplitude of currents (I_t) normalized for amplitude of I_{Na} at 5th minute after beginning of recovery period (I_∞).

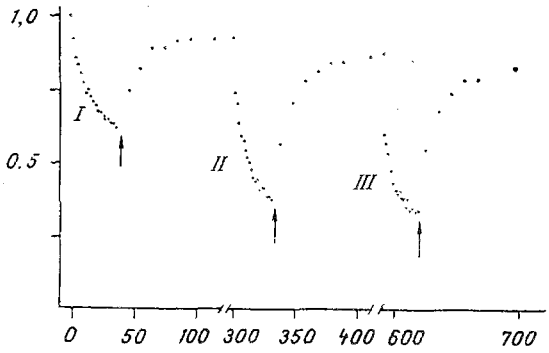

Fig. 3. Effect of frequency of membrane stimulation on development of cumulative and tonic blocks in the presence of 10^{-5} M E. Abscissa, time (in sec); ordinate, amplitude of peak of I_{Na}, normalized relative to amplitudes of I_{Na} to first stimulus in series I. Degree of initial block in this case 25%. Separate points indicate peaks of I_{Na} arising in response to stimulation. I, II, III) fall of I_{Na} peaks during stimulation with frequencies of 2, 3, and 4 Hz, by depolarizing pulses for 40, 33, and 30 sec respectively. Arrows indicate time of changing to frequency of 0.2 Hz.

that if the frequency was increased the block developed faster and the steady-state level of the block at the end of the series of pulses was lower, i.e., the block was stronger (Fig. 1). Repetitive depolarization with frequencies of 0.5, 2, and 4 Hz caused a smooth (approximately exponential) decrease in peak values of I_{Na}. After 25 sec, with a frequency of stimulation of 4 Hz (i.e., after 100 stimuli), the amplitude of I_{Na} fell to 40% of the amplitude of I_{Na} to the first stimulus in the series. After 50 sec, with a frequency of stimulation of 0.5 Hz, I_{Na} fell to 78%, and after 35 sec at 2 Hz, it fell to 62% (Fig. 1). The kinetics of recovery of the amplitude of I_{Na} from cumulative (frequency-dependent) blockade of various depths was studied by means of program of stimuli (Fig. 2). The Na channels were first converted into the E-blocked state by different numbers of pulses in series with the specified frequency. At the end of these series the block reached a steady-state level at different depths. The frequency of stimulation was then abruptly changed by 0.2 Hz. It will be clear

from Fig. 2 that the kinetics of recovery of I_{Na} followed an approximately monoexponential course, with a time constant of about 10 sec. However, under these circumstances the amplitude of I_{Na} did not recover completely.

The difference in amplitude of I_{Na} at the end of the 5-min recovery period from cumulative block by E compared with the initial block is a measure of the tonic block. Amplitudes of I_{Na} to pulses in series with different frequencies, following one after the other, are shown in Fig. 3. In the course of the experiments of series I, II, and III the membrane was depolarized by a frequency of 2, 3, and 4 Hz respectively. Amplitudes of I_{Na} were normalized relative to amplitudes of I_{Na} to the first pulse in series I. After each series, for 75 sec, recovery of I_{Na} from cumulative block at a frequency of 0.2 Hz was observed. The pause before the beginning of the next series was 3 min. It will be clear from Fig. 3 that the magnitude of the tonic block increased gradually.

Investigations into the action of local anesthetics and of certain similarly acting antiarrhythmics on I_{Na} have shown that blockade of Na channels is strongly dependent on stimulation [6-8, 11]. It has been suggested that stimulus-dependent blockade of Na channels in heart cells may play an important role in the mechanism of action of antiarrhythmics [9, 10]. The frequency-dependent action of E on I_{Na} of isolated heart cells is similar to its action on I_{Na} in the Ranvier node [1]. According to the data E interacts mainly with open Na channels. The main result of the present experiments is to demonstrate the frequency-dependence of the action of E on I_{Na} of single adult rat cardiomyocytes. This provides a good explanation of clinical data on the marked therapeutic effect of E in ventricular and atrial forms of tachycardia. The question why E should interact predominantly with open or inactivated Na channels remains unanswered. An investigation of dependence of the depth of the cumulative block on the duration of the testing pulses is required. Quantitative evaluation of steady interactions of E with open Na channels and with inactivated Na channels, in terms of the theoretical model in [9, 10] would enable accurate conclusions to be drawn in this respect.

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