CHANGES IN THE SODIUM CURRENT DUE TO THE ACTION OF ETHMOZINE AND LIGNOCAINE FROM THE OUTER AND INNER SIDES OF THE MEMBRANE OF SINGLE RAT MYOCARDIAL CELLS

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Ethmozine, a new antiarrhythmic drug of the phenothiazine series, reduces the rate of rise of the front ($\dot{V}_{max}$) of the action potential in isolated Purkinje fibers [1, 6] and in fibers of the frog's atria [2], and it also slows the conduction of excitation in the working myocardium of the dog's heart in situ [1]. These observations show that ethmozine reduces the fast inward sodium current (I_{Na}). Direct measurements of I_{Na} of a membrane under voltage clamp conditions have shown that ethmozine reduces the maximal sodium conductance of frog atrial fibers [2]. However, it is not yet clear whether ethmozine acts on I_{Na} from the outer side of the cell membrane or whether, like lignocaine [7], it affects the sodium channels from the inner side of the cell membrane.

In the present investigation, by means of the intracellular perfusion method, the extracellular and intracellular action of ethmozine and lignocaine on the fast inward I_{Na} of single rat heart cells was compared.

EXPERIMENTAL METHOD

Experiments were carried out on single myocytes isolated from the heart of adult male rats weighing 200-400 g [10]. The heart was isolated under ether anesthesia. A cannula was introduced into the aorta and the heart was perfused (37°C) at the rate of 15 ml/min for 15 min with normal perfusion solution of the following composition (in mM): NaCl 118, KCl 4.8, $CaCl_2$ 0.9, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25; mg/ml bovine serum (from Serva, West Germany), pH 7.4. The solution was saturated with carbogen (95 O_2 + 5% CO_2). To soften the heart tissue the heart was perfused for 30 min with calcium-free solution containing 0.8 mg/ml of the enzyme collagenase (type I, from Sigma, USA). After this the remainder of the enzyme was washed out with calcium-free solution for 5 min. The heart was then disconnected from the cannula and placed in a Petri dish; the atria and ventricles were cut up into small pieces with scissors and placed in 50 ml calcium-free perfusion solution. The suspension was filtered through nylon tissue and allowed to stand for 5 min for sedimentation of the cells, after which the supernatant was carefully decanted. The cells were resuspended in 5-10 ml of perfusion solution with the addition of Ca^{++} (0.9 mM) and were kept at 20°C, the surface being ventilated with carbogen. The solution with which the isolated heart was perfused was used in the subsequent experiments as the "extracellular" solution.

The method of intracellular dialysis, which has been used on neuroblastoma cells [8], was used for voltage clamping. The scheme of the experimental arrangements is shown in Fig. 1. For intracellular perfusion of heart cells a V-shaped polyethylene tube with conical pore at the end was made. The external and internal diameters of the pore were 20-30 and 10-15 μ respectively. The walls of the pore were covered with special glue of the following composition: 50% parafilm (USA) and 50% mineral oil. The V-shaped tube was perfused with "intracellular" solution (Tris-aspartic acid 140 mM, EGTA 2 mM, pH 7.3). Electrodes (Ag-AgCl/3M KCl) to measure the membrane potential (V_m) and to apply the current, were placed in the branch of the tube from which the "intracellular" solution flowed out. The electrodes were connected to a preamplifier and the output of the operational amplifier. As a first step, a few heart cells were transferred to the perfusion chamber with

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TABLE 1. Action of Ethmozine (8×10^{-5} g/ml) from Outside the Cell on Reactivation of I_{Na} (M ± m; n = 4)

Experimental conditions	Reactivation constant, msec
Control	12,5±1,24
Ethmozine:	
5 min	15,8±1,8 $P>0,05$
7 min	20,17±4,02 $P>0,05$

Legend. Constant was measured by means of two depolarizing stimuli with variable interval between stimuli.

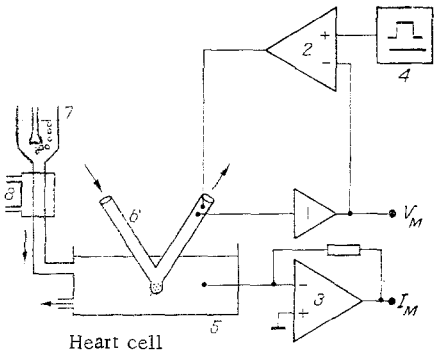

Fig. 1. Scheme of arrangements for clamping membrane potential on single heart cells. 1) Preamplifier; 2) operational comparison amplifier; 3) current–voltage converter; 4) stimulator; 5) perfusion chamber; 6) V-shaped polyethylene tube with conical pore at its end; 7) vessel with perfusion solution, aerated with carbogen; 8) thermostat. Arrows indicate direction of motion of "extracellular" and "intracellular" solution.

the aid of a pipet and the part of the tube with the pore was immersed in it. Under visual control the necessary cell was selected and, by creation of a negative hydrostatic pressure, it was drawn up into the pore. As soon as the cell was firmly fixed in the pore, by a sudden thrust of negative pressure part of the cell membrane was ruptured and the cell became accessible for intracellular perfusion.

To clamp V_m a special electronic device was used. The technical characteristics of the device were: The zero drift applied to the input was less than 1 mV/h; band 10 kHz (with feedback disconnected); amplification factor of operational amplifier 150; signal 10 mV at the output of the current amplifier corresponds to a current of 5×10^{-9} A. Ionic currents and membrane potential were recorded on a dual-beam storage oscilloscope (5103N, D-13, Tektronix, USA), equipped with polaroid camera (C-5A, Tektronix, USA). The command and holding potentials were applied from a two-channel stimulator (302T, WPI, USA). The frequency of stimulation of the cells was 0,5 Hz. Experiments were carried out at room temperature (20 ± 2°C).

EXPERIMENTAL RESULTS

Ethmozine (8×10^{-5} g/ml), applied from within the cell, depressed the maximal fast inward I_{Na} very slightly (Fig. 2A). For instance, by the 5th minute of action of ethmozine from within the cell the amplitude of

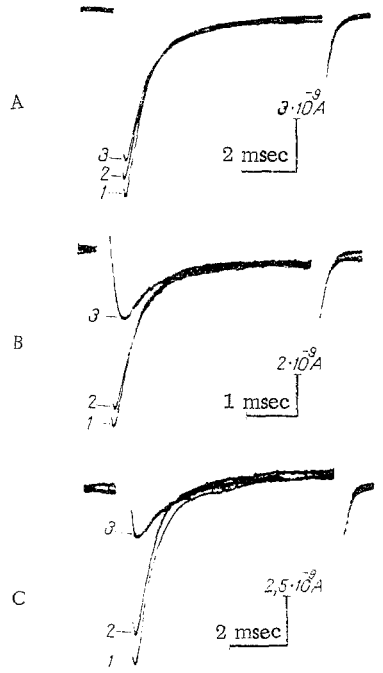

Fig. 2

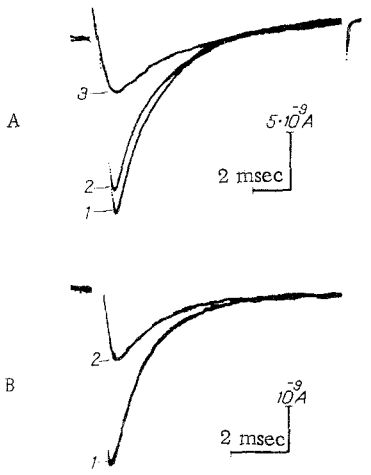

Fig. 3

Fig. 2. Changes in fast inward I_{Na} of a single rat heart cell during intracellular and extracellular action of ethmozine (8×10^{-5} g/ml). A: 1) maximal I_{Na} recorded during depolarization of membrane to −35 mV under normal conditions; 2, 3) after action of ethmozine for 3, 5, and 7 min respectively from inside; B: 1) maximal I_{Na} recorded during depolarization of membrane to −34 mV under normal conditions; 2, 3) after action of ethmozine from outside for 2 and 5 min respectively; C: 1) maximal I_{Na} recorded during depolarization of membrane to −31 mV under normal conditions; 2) after action of ethmozine from inside for 5 min; 3) after action of ethmozine from outside for 5 min (normal intracellular solution inside the cell). Holding potential on cell membrane in all experiments −100 mV.

Fig. 3. Reduction in fast inward I_{Na} of a single rat heart cell during intracellular (A) and extracellular (B) action of lignocaine (8×10^{-5} g/ml). A: 1) maximal I_{Na} recorded during depolarization of membrane to −38 mV under normal conditions; 2, 3) after action of lignocaine from inside for 2 and 5 min respectively; B: 1) maximal I_{Na} recorded during depolarization of membrane to −32 mV under normal conditions; 2) after action of lignocaine externally for 10 min. A and B) Data obtained on different cells. Holding potential on cell membrane in these experiments −100 mV.

I_{Na} was 88 ± 2% (M ± m, n=6) of its initial value. Addition of ethmozine to the "extracellular" solution in the same concentration effectively inhibited I_{Na} (Fig. 2B), so that after the action of the drug for 5 min its mean amplitude was 43 ± 5.9% (n=6) of the initial value. The reactivation constant of I_{Na} during extracellular application of ethmozine showed no significant change (Table 1). The action of ethmozine on I_{Na} from inside and outside in an experiment on the same cell is illustrated in Fig. 2C. The degree of reduction of I_{Na} during the action of ethmozine from inside and outside the cell differed significantly ($P < 0.001$).

Similar experiments were carried out with the other antiarrhythmic drug lignocaine. Addition of lignocaine (8×10^{-5} g/ml) to the intracellular solution reduced the amplitude of I_{Na} (Fig. 3A), which fell to 35 ± 5.4% (n=7) of its initial value 5 min after the beginning of its action. To achieve the same degree of depression of I_{Na} by extracellular application of lignocaine, a longer time was required. A fall in I_{Na} to 36 ± 7.4% (n=7) was observed after the action of lignocaine externally for 8 min. The results of an experiment to study the extracellular action of lignocaine are given in Fig. 3B.

Experiments on the frog atrium showed that ethmozine inhibits I_{Na} on account of a decrease in the maximal conductance of Na^+ [2]. The kinetic properties of the sodium channels – activation, inactivation, and re-

activation – were virtually unchanged under the influence of ethmozine. It was suggested on the basis of these findings that ethmozine, like tetrodotoxin, acts from outside the cell membrane, reducing the number of channels capable of conducting Na^+. In the present investigation, when intracellular and extracellular perfusion of single heart cells was used, direct proof was obtained that ethmozine acts predominantly from the outside of the sarcolemma.

Lignocaine has a more complex action on electrical activity of heart tissue. For instance, besides the reduction in $\dot{V}_{max}$ and, consequently, of I_{Na} also, under the influence of lignocaine the action potential of the Purkinje fibers was significantly shortened [3, 4], evidence that lignocaine affects not only I_{Na}, but also other ionic currents. The action of lignocaine on I_{Na} cannot be reduced purely to depression of maximal conductance, but it is accompanied by a significant increase in the reactivation constant of I_{Na} [5, 11]. It has been shown on internally perfused squid giant axons [9] and Purkinje cardiac cells [7] by iontophoretic application of lignocaine that inhibition of I_{Na} by lignocaine is connected mainly with the intracellular action of this drug. Comparison of the action of lignocaine and ethmozine on I_{Na} from inside and outside the heart cell in the present investigation showed that the sites of action of these two antiarrhythmics on the cell membrane are different.

LITERATURE CITED

1. L. V. Rozenshtraukh, R. Raffi, V. Élkharar, et al., Kardiologiya, No. 5, 63 (1979).
2. I. A. Yuryavichyus, L. V. Rozenshtraukh, A. I. Undrovinas, et al., Kardiologiya, No. 9, 118 (1978).
3. M. F. Arnsdorf and J. T. Bigger, Jr., J. Clin. Invest., 51, 2252 (1972).
4. J. T. Bigger, Jr. and W. J. Mandel, J. Clin. Invest., 49, 63 (1970).
5. C. M. Chen, L. S. Gettes, and B. C. Katzung, Circulat. Res., 37, 20 (1975).
6. P. Danilo, W. B. Langan, M. R. Posen, et al., Eur. J. Pharmacol., 45, 127 (1977).
7. J. I. Gliklich and B. F. Hoffman, Circulat. Res., 43, 638 (1978).
8. P. G. Kostiuk, O. A. Krishtal, V. I. Pidoplichko, et al., Neuroscience, 3, 327 (1978).
9. T. Narahashi and D. T. Frazur, J. Pharmacol. Exp. Ther., 171, 32 (1970).
10. Y. Rajs, M. Sundberg, N. Danell, et al., Exp. Cell Res., 115, 183 (1978).
11. F. M. Weld and J. T. Bigger, Jr., Circulat. Res., 37, 630 (1975).

EFFECT OF ETHIMIZOLE AND PROPYLNORANTIPHEIN ON ACTIVITY OF RESPIRATORY ENZYMES OF THE MYOCARDIUM AFTER NEUROGENIC INJURY

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One of the main causes of development of neurogenic trophic disorders of the internal organs is insufficiency of energy metabolism in the damaged tissues [5, 11]. Ethimizole,* an imidazole dicarboxylic acid derivative, accelerates the healing of gastric ulcers and has a preventive and therapeutic action in neurogenic lesions of heart muscle [6, 7]. The writer showed previously that ethimizole considerably accelerates energy forming processes in the CNS of healthy animals [4].

The object of the present investigation was accordingly to study the effect of ethimizole and of another imidazole-dicarboxylic acid derivative, propylnorantiphein, on some indices of metabolism of the myocardium

*1-ethylimidazole-4,5-dicarboxylic acid-bis-methylamide.

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