ACTION OF THE DIETHYLAMINO ANALOG OF ETHMOZINE ON PARAMETERS OF THE SODIUM CURRENT OF SINGLE RAT CARDIOMYOCYTES

A. I. Undrovinas, L. V. Rozenshtraukh, and A. V. Yushmanova

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New preparations of the phenothiazine series have recently been introduced into clinical practice for the treatment of arrhythmias: ethmozine* and its diethylamino analog (DAAE) [1]. Investigation of the antiarrhythmic activity of these compounds has shown that DAAE has a stronger and longer action than ethmozine on arrhythmias arising in the late stage of myocardial infarction in dogs [2]. On the basis of experimental data in the course of the study of the action of these compounds on ionic currents it has been suggested that the antiarrhythmic activity of ethmozine and its analog is connected with their influence on the fast inward sodium current (I_{Na}) [4]. DAAE thus depresses I_{Na} by a greater degree than ethmozine and for a longer time.

The aim of the present investigation was to study the effect of DAAE on I_{Na}. Since some antiarrhythmic agents, lidocaine for example, affect sodium conductance by their action from the inner side of the cell membrane [3, 5, 7], whereas other preparations, notably ethmozine, affect I_{Na} from the outer side of the cell membrane [3, 4], it was interesting to study the time course of changes in I_{Na} under the influence of DAAE when added to the extra- and intracellular medium of the cardiomyocytes.

EXPERIMENTAL METHOD

Experiments were carried out on single myocytes isolated from the adult rat heart. The method of isolation of the myocytes was as follows [8]. Under ether anesthesia the heart was removed from adult Wistar rats weighing 180-250 g, placed in cold normal perfusion solution, and a cannula was introduced into the aorta. To wash the heart free from blood it was perfused for 5 min by Langendorff's method (37°C) at the rate of 8-10 ml/min with normal perfusion solution with 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, bovine serum 1 mg/ml (from Serva, West Germany), pH 7.4. The solution was saturated with carbogen (95% O_2 + 5% CO_2). The perfusion pressure in the aorta was 60-80 mm Hg. After the heart had been rinsed free from blood the normal perfusion solution was replaced by calcium-free solution containing 0.8 mg/ml of the enzyme collagenase (from Sigma, USA). After perfusion for 20-30 min with solution containing collagenase, the pressure in the aorta fell to 30-40 mm Hg; perfusion with the enzymes ceased at this period and for 5 min the preparation was rinsed with calcium-free solution. The heart was then removed from the cannula and cut into pieces with scissors, with vigorous shaking in 50 ml of the same solution. The resulting suspension was filtered through nylon tissue and allowed to stand for 5 min to sediment the cells. The supernatant was then carefully poured off and the cells resuspended in 10-15 ml of calcium-free solution. The cell suspension was kept at room temperature. Calcium-free perfusion solution was used in the experiments as the extracellular solution.

Voltage clamping was carried out by intracellular dialysis, a technique originally developed for nerve cells [6] and adapted by the writers for heart cells [10]. The method is essentially as follows: a single heart cell was drawn up into a conical pore (the outer and inner diameters of which were 20-30 and 10-15 μ respectively) at the end of a V-shaped polyethylene tube, under visual control (Diavert inverted microscope, from Leitz, West Germany). One cell was fixed in the pore, part of the cell membrane was ruptured by means of a

*Ethmozine is 2-carbethoxyamino-10-(3-morpholylpropionyl)-phenothiazine hydrochloride.

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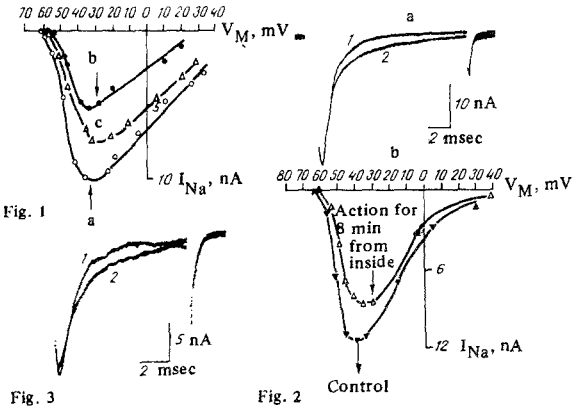

Fig. 1. Action of DAAE (8×10^{-6} g/ml) on current–voltage characteristic curve of I_{Na} of a single heart cell (substance added from outer side of membrane); a) control, b) 3 min of action of DAAE, c) 15 min of rinsing with normal perfusion solution. Experimental conditions: holding potential −100 mV; intracellular-medium: Tris-F 140 mM, EGTA 2 mM, pH 7.3, t = 22°C.

Fig. 2. Action of DAAE (8×10^{-6} g/ml) inside membrane on I_{Na} of single heart cell. I: 1) Trace of $I_{Na_{max}}$ recorded in control during membrane depolarization to −37 mV, 2) action of DAAE inside cell for 6 min. II: current–voltage characteristic curve of I_{Na} of single heart cell in control (a) and after action of DAAE for 8 min (b). Experimental conditions: holding potential −100 mV; intracellular fluid: Tris-F 140 mM, EGTA 2 mM, pH 7.3; t = 21°C.

Fig. 3. Action of pronase (0.8 mg/ml) on I_{Na} of cardiomyocytes from inside membrane: 1) control, 2) action of enzyme for 10 min. Experimental conditions: holding potential −90 mV; membrane potential at which current was recorded −35 mV; intracellular fluid: Tris-aspartate 140 mM, EGTA 2 mM, pH 7.3; t = 20°C.

Kapron cord (diameter 100 μ), introduced inside the V-shaped tube, so that the cell became accessible for intracellular perfusion.

A special electronic system, enabling compensation of linear leakage and series resistance, was used for clamping the membrane voltage and measuring ionic currents. The frequency of stimulation was 1 Hz. The experiments were carried out at room temperature.

EXPERIMENTAL RESULTS

The results of an experiment in which the action of DAAE on the current–voltage characteristics of I_{Na} was studied are illustrated in Fig. 1. In this case the substance was added from the outer side of the membrane. As Fig. 1 shows, DAAE in a concentration of 8×10^{-6} g/ml effectively depressed I_{Na} over the whole range of membrane potential. Subsequent rinsing with normal solution for 15 min led to partial recovery of the amplitude of I_{Na}. The absence of reversal potential on the current–voltage characteristic curves of I_{Na}, shown in Figs. 1-3, was due to the fact that the intracellular solution contained no Na^+ ions. It must be pointed out that besides a decrease in amplitude of I_{Na} under the influence of DAAE, the kinetic parameters of I_{Na} were changed from the outer side of the membrane: the inactivation constant τ_h of the sodium current was increased, indicating that DAAE affects the process of I_{Na} inactivation. As Table 1 shows (cell No. 1), at the 5th minute of action of DAAE from the outer side of the membrane τ_h increased from 3.9 to 4.8 msec. During rinsing, when the amplitude of I_{Na} was restored, τ_h continued to rise (Table 1, cell No. 1). Previously the writers showed under similar conditions [4] that ethmozine has virtually no effect on the kinetic parameters of I_{Na}. The same results also are obtained when ethmozine was tested on I_{Na} of atrial trabeculae of the frog heart [3]. It was concluded from these results that ethmozine depresses I_{Na} on account of a decrease in maximal conductance of Na^+ ions. DAAE evidently has a more complex influence on I_{Na}.

TABLE 1. Action of DAAE on Inactivation of I_{Na} of a Single Rat Heart Cell

Cell No.	Control – τ_h, msec	Action of DAAE (8×10^{-6} g/ml) from outside: time, min	τ_h, msec	Action of DAAE (8×10^{-6} g/ml) from inside: time, min	τ_h, msec	Rinsing: time, min	τ_h, msec
1	3,9	5	4,8			8	6,4
2	1,6			6	τ_{h_1} = 1,6 τ_{h_2} = 3,6		
3	3,7			34	5,95	70	10,95
4	τ_{h_1} = 4,1 τ_{h_2} = 12,4			45	15		

Legend: Inactivation constants calculated from the equation $I_{Na}(t) = I_{Na_{max}}\left(K_1 e^{-\frac{t}{\tau_{h_1}}} + K_2 e^{-\frac{t}{\tau_{h_2}}}\right)$, where $I_{Na}(t)$ is the amplitude of I_{Na} at time t_1; $I_{Na_{max}}$ the maximal amplitude of I_{Na}; K_1 and K_2 are coefficients calculated from the experimental data.

Experiments were carried out to study the action of DAAE on I_{Na} when the substance was introduced from the inner side of the cell membrane. In this case DAAE to begin with caused delay of I_{Na} activation. Data on the intracellular action of DAAE on I_{Na} are given in Fig. 2, I. The experiments showed that after the action of the substance for 6 min from inside the cell there was virtually no change in the amplitude of the maximal I_{Na}, whereas inactivation was prolonged. In this experiment inactivation in the control was well described by an equation containing one exponent:

$$I_{Na}(t) = I_{Na_{max}} \cdot \exp\left(-\frac{t}{\tau_{h_1}}\right).$$

The value of τ_{h_1} was 1.6 msec (Table 1, cell No. 2). After the action of DAAE, a second exponent had to be introduced into the equation in order to describe the time course of inactivation:

$$I_{Na}(t) = I_{Na_{max}}\left[0.9 \exp\left(-\frac{t}{\tau_{h_1}}\right) + 0.1 \exp\left(-\frac{t}{\tau_{h_2}}\right)\right].$$

τ_{h_1} and τ_{h_2} were 1.6 and 3.6 msec respectively (Table 1, cell No. 2). The coefficients obtained in the equation may perhaps reflect the number of sodium channels for which the corresponding inactivation constant is characteristic. In the experiment whose results are shown in Fig. 2, I, under the influence of DAAE acting from the inner surface of the membrane, 10% of channels exhibited delayed inactivation. Depending on the duration of action of DAAE from the inner side of the membrane, the contribution of the slower components was increased. As Table 1 (cell No. 4) shows, after 45 min of action of the compound the fast component, i.e., channels with fast inactivation, disappeared completely. It was impossible to restore inactivation even by prolonged rinsing to remove DAAE (Table 1, cell No. 3). The amplitude of I_{Na} was inhibited less effectively by the action of DAAE inside the cell than by its extracellular action.

Like ethmozine [3, 4], DAAE acting from the outer side of the membrane reduced the amplitude of I_{Na}. In this respect the two compounds were similar. From the inner side of the membrane DAAE mainly affected I_{Na} inactivation, which was virtually never observed during the action of ethmozine [3]. In this respect the actions of ethmozine and its analog differed.

Experiments on the giant squid axon [9] showed that structures of the channel responsible for inactivation of I_{Na} are located on the inner side of the membrane. Treatment of the axon membrane from inside by the proteolytic enzyme pronase led to complete disappearance of the inactivation process, whereas activation and the amplitude of I_{Na} showed little change. Accordingly experiments were carried out to study the effect of pronase from inside the membrane on I_{Na}. As Fig. 3 shows, after the action of the enzyme for 10 min I_{Na} inactivation was protracted, whereas the amplitude of the currents was virtually unchanged. It can thus be postulated that the structural units of the sodium channel in heart cells responsible for inactivation are located in-

side the cell. The action of DAAE and pronase from inside the sarcolemma (Fig. 2, I; Fig. 3) is very similar and evidently directed toward the same site in the membrane.

It can be postulated on the basis of these results that DAAE, compared with ethmozine, has an additional site for its action on the sarcolemma. This feature of DAAE is evidently responsible for its longer and more effective antiarrhythmic action.

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EFFECT OF GUTIMIN ON THE MYOCARDIAL FATTY ACID UPTAKE DURING PROLONGED DISCONNECTION OF THE HEART FROM THE CIRCULATION IN DOGS WITH MODERATE HYPOTHERMIA

I. F. Matyushin, G. A. Boyarinov, and Yu. A. Bogdarin

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Gutimin (guanylthiourea) is used in medicine as an antihypoxic agent [1, 3, 4, 8]. Experiments have demonstrated its positive effect on ATPase activity in the myocardium, brain, and erythrocytes [1, 6].

Considering that the main sources of energy for heart muscle are fatty acids, in both the free and the bound state [9-11], the effect of preventive administration of gutimin on myocardial fatty acid uptake was studied when the heart was disconnected from the circulation in dogs with moderate hypothermia.

EXPERIMENTAL METHOD

Experiments were carried out on 48 male dogs weighing 12-20 kg, kept on a diet consisting mainly of animal fat. The dogs were not fed for 24 h before the experiments. After premedication (trimeperidine, atropine), under endotracheal ether-oxygen anesthesia (stage III_2) together with relaxants, the animals were cooled by a combination of the Kholod-2F apparatus and ice and snow packs covering the trunk. When the rectal temperature was reduced to 30°C thoracotomy was performed in the sixth right intercostal space, and the heart was disconnected from the circulation by application of tourniquets to the atrial veins (groups 3 and 4). In the experimental series (groups 5 and 6) gutimin was injected intravenously in a dose of 20 mg/kg before cooling and in a dose of 45-50 mg/kg 25-30 min before occlusion under similar experimental conditions. Prolonged disconnection of the heart from the circulation (for 60 min) was carried out in both series. Measures to restore cardiac activity included cardiac-massage, artificial ventilation of the lungs, intra-arterial injection of

Central Research Laboratory, S. M. Kirov Gor'kii Medical Institute.

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