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ACTION OF THE DIETHYLAMINO ANALOG OF ETHMOZINE ON FAST SODIUM CURRENT PARAMETERS IN NORMAL AND DEPOLARIZED MYOCARDIAL FIBERS

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The diethylamino analog of ethmozine* (DAAE) is a new antiarrhythmic compound of the phenothiazine series synthesized at the Institute of Pharmocology, Academy of Medical Sciences of the USSR. The high antiarrhythmic activity of this compound [6] is due to its ability to induce effective and prolonged inhibition of the fast sodium current of myocardial fibers [2, 4, 5]. Nevertheless, the question of the effect of DAAE on kinetic parameters of the fast sodium current remains unexplained. Investigation of the action of this compound on parameters of the sodium current in depolarized heart tissue also is interesting, for we know that many antiarrhythmic agents, especially lidocaine, selectively inhibit the fast sodium current in ischemized and depolarized myocardial fibers [8, 11, 14-16].

The aim of the present investigation was to study the action of DAAE on the fast inward sodium current and of its kinetic parameters in normal and depolarized myocardial fibers.

EXPERIMENTAL METHOD

The fast inward sodium current was recorded under membrane voltage clamp conditions, using a double sucrose gap by a method similar to that described previously [3, 7, 12]. Isolated trabeculae, obtained from the atria of Rana catesbiana, were used as test objects; the length of the trabeculae was 3-4 mm and their diameter 80-120 μ. A trabecula was perfused in a testing compartment 250 μ wide with Ringer's solution of the following composition (in mM): NaCl 114, KCl 2.7, $CaCl_2$ 1.8, glucose 5, Tris-HCl 10, pH 7.5. Only those trabeculae on which a resting potential of 75-80 mV was recorded after immersion in the perfusion chamber were used in the experiments. The myocardial fibers were depolarized by two methods: by passing a direct current through the fiber under membrane voltage clamp conditions and by increasing the potassium ion concentration in the perfusion fluid to 8-9 mM. The membrane potential in the depolarized fibers was maintained 15 mV

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

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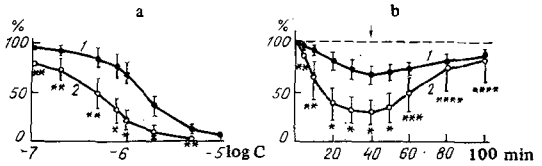

Fig. 1. Action of DAAE on fast inward sodium current of normal (1) atrial trabeculae and trabeculae depolarized by 15 mV (2). a) Dose–effect curves plotted from results of five experiments. Each point corresponds to mean value of current ($M \pm \sigma$) recorded after 30 min. Abscissa, log of concentration of preparation (in g/ml); ordinate, amplitude of current (in %); b) change in amplitude of sodium currents during action of DAAE (8×10^{-7} g/ml) and rinsing. Mean values ($M \pm \sigma$); 1) n = 7, 2) n = 5. Arrow indicates beginning of rinsing. Abscissa, time (in min); ordinate, amplitude of current (in %). *P < 0.001; **P < 0.01; ***P < 0.05; ****P > 0.05. Significance of difference determined by Student's t test.

above the resting potential recorded initially, i.e., within the range from 60 to 65 mV. The level of depolarization of the fibers thus did not lead to any significant decrease in amplitude of the fast sodium currents caused by partial inactivation of the sodium channels. The decrease in amplitude of the current was not more than 20% in all the experiments.

The lasting effect of the slow inward current was abolished by adding the substance D-600 (from Knoll AG, West Germany), a specific blocker of slow channels, to the perfusion fluid in a concentration of 5×10^{-7} g/ml.

During measurement of the steady-state values of inactivation parameters of the sodium currents (h_∞) a conditioning stimulus 1 sec in duration and a testing stimulus 16 msec in duration were applied to the preparation. The interval between stimuli was 2 msec. The amplitude of the testing stimulus corresponded to the maximal value of the current.

Reactivation parameters were measured by two stimuli of equal amplitude, the interval between which varied from 5 to 900 msec. The duration of the conditioning stimulus was 100 msec and of the testing stimulus 16 msec.

The temperature during the experiments was 20-22°C. The frequency of stimulation in all experiments was 0.1 Hz.

EXPERIMENTAL RESULTS

The ability of DAAE in different concentrations to block the fast inward sodium current of normal atrial fibers and of fibers depolarized by 15 mV was determined in two series (five experiments in each series). Dose–effect curves were plotted from the results of these experiments (Fig. 1a). Each point on these curves corresponds to the mean amplitude of the sodium current (in %) after perfusion for 30 min with Ringer's solution containing DAAE. To depress the amplitude of the current by 50% in the course of 30 min of its action a concentration of the compound of 1.5×10^{-6} g/ml was necessary for normal and 4.6×10^{-7} g/ml for depolarized (by 15 mV) atrial fibers. In later experiments the compound was used in a concentration of 8×10^{-7} g/ml, at which the amplitude of the sodium current was reduced within a convenient range for measurement in both normal and depolarized fibers.

The time course of the change in the maximal amplitude of the sodium current under the influence of DAAE in a concentration of 8×10^{-7} g/ml, and also during rinsing with normal Ringer's solution, is shown in Fig. 1b. Acting for 40 min DAAE reduced the current in normal fibers by 33 ± 9% (n = 7) and in depolarized fibers by 72 ± 11% (n = 5). During the action of the compound the amplitude of the current fell steadily, and even after exposure for 40 min to the solution containing DAAE it did not reach a stable level. On rinsing with normal Ringer's solution the currents slowly increased, and after 60 min its value in normal fibers was 84 ± 6% (n = 7) and in depolarized fibers 82 ± 21% (n = 5) of the initial value.

Original traces and current–voltage characteristic curves of the fast sodium current obtained in one of

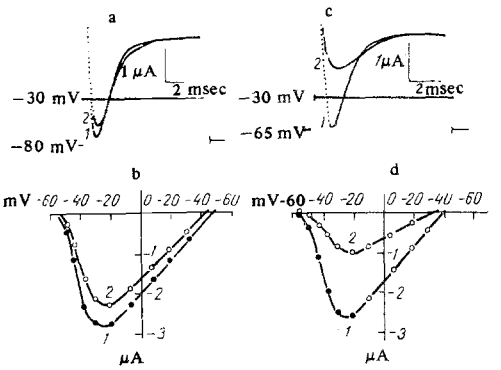

Fig. 2. Changes in fast inward sodium current under the influence of DAAE (8×10^{-7} g/ml) in normal (a, b) and depolarized (by 15 mV; c, d) atrial fiber. a, b) Original recordings of current, c, d) current–voltage characteristic curves: 1) in control, 2) after action of compound for 20 min. Depolarizing step of membrane potential shown below in a and c. Calibration: time 2 msec, current 1 μA. In b and d: abscissa, membrane potential (in mV); ordinate strength of current (in μA).

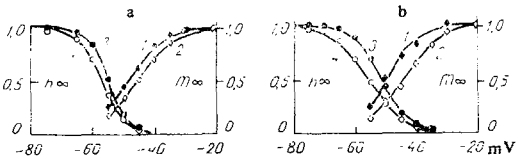

Fig. 3. Steady-state values of parameters of activation (m_∞) and inactivation (h_∞) of fast sodium channels in control (1, 3) and after action of DAAE for 20 min in a dose of 8×10^{-7} g/ml (2, 4) in normal (a) and depolarized (by 15 mV, b) atrial fiber. Abscissa, membrane potential (in mV); ordinate, normalized values of parameter.

the experiments are illustrated in Fig. 2. DAAE (8×10^{-7} g/ml) reduced the amplitude of the sodium current recorded at all values of membrane potential (Fig. 2b, d). The action of the compound was stronger in the depolarized fibers (Fig. 2c, d). Besides reducing its amplitude, DAAE also delayed inactivation of the fast sodium current, as can be seen in the traces in Fig. 2a, c. Under the influence of DAAE for 40 min the time constant of inactivation of the sodium current was increased in normal fibers from 1.00 ± 0.13 to 2.04 ± 0.37 msec ($n = 5$, $P < 0.001$), but in depolarized fibers from 1.06 ± 0.12 to 3.21 ± 0.29 msec ($n = 5$). The values of time constants were found by a graphic method. Rinsing with normal Ringer's solution for 60 min led to partial recovery of the rate of inactivation of fast sodium channels.

In experiments to study the action of DAAE on steady-state values of activation (m_∞) and inactivation (h_∞) parameters it was found that after exposure to the compound (8×10^{-7} g/ml) for 20 min the curve of m_∞ was shifted to the right at the level $m_\infty = 0.5$ along the potentials axis through 2.7 ± 1.4 mV ($n = 5$) for normal and by 5.3 ± 2.1 mV ($n = 5$) for depolarized atrial fibers. The h_∞ curve at the level $h_\infty = 0.5$ was shifted during the same period of action of the compound toward hyperpolarization in normal fibers by 2.3 ± 1.3 mV ($n = 5$) and in depolarized fibers by 7.7 ± 2.3 mV ($n = 5$). After rinsing for 60 min with normal Ringer's solution partial recovery of the parameters m_∞ and h_∞ took place. The results of one experiment to study the action of DAAE on steady-state values of the activation and inactivation parameters (m_∞ and h_∞) are given in Fig. 3.

Reactivation of the fast sodium current is described sufficiently well by a function consisting of the sum of two exponents:

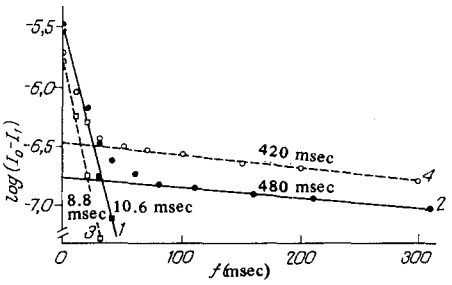

Fig. 4. Typical resolution of fast sodium current reactivation curve into components (data of one experiment on a normal atrial fiber). 1, 2) Fast and slow components respectively with DAAE in concentration of 8×10^{-7} g/ml. Abscissa, time (msec); ordinate, logarithms of difference between currents (in A).

$$I_0 - I_t = I_0 \left[K_1 \exp\left(-\frac{t}{\tau_1}\right) + K_2 \exp\left(-\frac{t}{\tau_2}\right) \right]$$

where I_0 is the maximal value of the current, I_t the amplitude of the current at time t after its complete inactivation; τ_1 and τ_2 the time constants of the fast and slow phases respectively of reactivation; K_1 and K_2 the relative contributions of sodium channels with fast and slow reactivation respectively.

The reactivation parameters τ_1 and τ_2, K_1 and K_2 were determined by a graphic method using a semilogarithmic system of coordinates (Fig. 4). In normal heart tissue the values of these parameters were as follows: in the control $\tau_1 = 11.4 \pm 3.2$ msec, $\tau_2 = 363 \pm 89$ msec, $K_1 = 0.96 \pm 0.02$, and $K_2 = 0.04 \pm 0.02$ ($n = 5$); after exposure for 20 min to DAAE (8×10^{-7} g/ml) the values were: $\tau_1 = 11.1 \pm 2.9$ msec, $\tau_2 = 469 \pm 11$ msec, $K_1 = 0.72 \pm 0.08$, $K_2 = 0.28 \pm 0.08$ ($n = 5$). Under the influence of DAAE the relative contribution of sodium channels with slow reactivation was thus significantly increased ($P < 0.001$) whereas the contribution of channels with fast reactivation was reduced, although changes in the time constants of the two processes were not significant.

Values of the reactivation parameters in myocardial fibers depolarized by 15 mV were as follows: in the control $\tau_1 = 48 \pm 15$ msec, $\tau_2 = 403 \pm 124$ msec, $K_1 = 0.69 \pm 0.17$, $K_2 = 0.31 \pm 0.17$ ($n = 7$); after exposure for 20 min to DAAE (8×10^{-7} g/ml) the values were: $\tau_1 = 49 \pm 16$ msec, $\tau_2 = 405 \pm 59$ msec, $K_1 = 0.42 \pm 0.20$, $K_2 = 0.58 \pm 0.20$ ($n = 7$). The action of the compound on depolarized tissue also was accompanied by an increase in the slow and a decrease in the fast component of reactivation ($P < 0.05$) without any significant change in the time constants of the two processes. On rinsing with normal Ringer's solution for 60 min values of the reactivation parameters, modified under the influence of DAAE, were partially restored. As a result of depolarization of the myocardial fibers, incidentally, there was a significant ($P < 0.001$) increase in the time constant of the fast reactivation phase and a simultaneous decrease in its relative contribution ($P < 0.01$).

The action of DAAE on the fast inward sodium current has a number of characteristic features. In the first place this compound inhibits the amplitude of the sodium current extremely effectively. A reduction of 50% in amplitude in normal heart tissue took place as a result of exposure to DAAE in a mean concentration of 1.5×10^{-6} g/ml for 30 min, whereas ethmozine, according to preliminary observations, had the same effect in the same time in a concentration of 1.3×10^{-5} g/ml. As was shown previously [5, 9], ethmozine does not change the rate of inactivation and reactivation of the fast sodium current. The results of the present investigation show that DAAE delays both these processes. The action of DAAE on the amplitude of the fast sodium current and its kinetic parameters was considerably intensified in depolarized heart tissue. Ethmozine also inhibited the fast sodium current more strongly in depolarized fibers, but it caused no significant changes in the kinetic parameters of the current under depolarization conditions. The very high activity of DAAE was evidently due to the high affinity of this compound for the lipid phase of the cell membrane [1].

Lidocaine is known to bind with structures of sodium channels located on the inner side of the membrane and forming inactivation gates (h-gates) [10]. This property of lidocaine delayed reactivation of the fast sodium current [8]. DAAE also caused delay of reactivation of the sodium current, which suggests the existence of a receptor for this substance, located in the region of the structures forming inactivation gates of the sodium channel. This hypothesis was confirmed by the fact that under the influence of DAAE inactivation of the

fast sodium current was delayed and the curve of the steady-state inactivation parameter (h_∞) was shifted.

The decrease in sodium conductance under the influence of DAAE is mainly due to blocking of sodium channels, by analogy with the action of ethmozine [5, 9]. Binding of DAAE with proteins forming h-gates can only facilitate a reduction in sodium conductance. Potentiation of the blocking action of DAAE in depolarized myocardial fibers suggests that this compound ought to depress the sodium currents selectively in affected areas of the myocardium where, as has been shown [13, 16], the myocardial cells are partially depolarized. This may be manifested as more marked delaying or even suppression of the conduction of excitation in the zone of infarction and in the zone of ischemia compared with the normal myocardium. Delayed reactivation of the fast sodium current under the influence of DAAE points to the possibility of lengthening of refractory periods in different structures of the heart.

Attention is drawn to one other important feature of the action of this compound, namely slow recovery of amplitude of the fast sodium current during rinsing with normal Ringer's solution (Fig. 1b). Rinsing for 60 min did not lead to complete recovery of the current after the action of DAAE, whereas rinsing for 15 min was sufficient for complete recovery of the amplitude of the current after the action of ethmozine [2, 5], lidocaine [8], and other antiarrhythmic agents [7]. This property of DAAE is probably responsible for the longer duration of its antiarrhythmic action.

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