EFFECT OF ETHMOZINE ON ACTION POTENTIAL AND FORCE OF CONTRACTION OF THE GUINEA PIG MYOCARDIUM

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KEY WORDS: ethmozine; action potential; force of contraction; sodium-calcium exchange.

Ethmozine — 2-carbethoxyamino-10-(3-morpholylpropionyl)-phenothiazine hydrochloride — is an effective antiarrhythmic agent recently introduced into clinical practice. Its antiarrhythmic activity under both experimental and clinical conditions exceeds that of other antiarrhythmic agents of the same group (quinidine, novocainamide, lidocaine) [1]. Electrophysiological studies of the action of ethmozine on dog Purkinje fibers [10] and the frog myocardium [2] were carried out previously. Their results suggested that the antiarrhythmic action of ethmozine is linked with its ability to slow the rate of rise of the action potential (AP) by blocking the fast inward sodium current.

In the present investigation this cycle of research was continued on the working mammalian myocardium, and attention was concentrated on the effect of ethmozine on the force of contraction.

EXPERIMENTAL METHOD

The papillary muscle from the right ventricle of a guinea pig was placed in a perfusion chamber (volume 0.5 ml, perfusion rate 10 ml/min) and perfused with Tyrode solution of the following composition (in mM): NaCl - 118.4, KCl - 2.7, $NaHCO_3$ - 25, NaH_2PO_4 - 1.2, $MgCl_2$ - 1.2, $CaCl_2$ - 1.8, glucose-10; the pH of the solution oxygenated with carbogen (95% O_2 + 5% CO_2) was 7.4 and its temperature 36 ± 0.5°C. The preparation was stimulated by square pulses 1 msec in duration and of twice the threshold intensity; the frequency of stimulation varied from 0.1 to 0.8 Hz. Flat Ag—AgCl electrodes not touching the preparation were used for stimulation. The force of contraction was measured with a 6MKh1S mechanotron (USSR). Transmembrane action potentials were recorded with glass microelectrodes filled with 3 M KCl. The maximal rate of rise of AP ($\dot{V}_{max}$) was obtained by means of a differential amplifier, linear up to 500 V/sec. All parameters measured were displayed on the screen of a "Tektronix-5115" oscilloscope (USA); the force of contraction was recorded continuously on a "Servogor" (USA) automatic writer. The action of ethmozine on the slow calcium AP was studied during elevation of the K^+ ion concentration in the perfusion fluid to 18 mM without osmotic compensation [7]. The ethmozine used was obtained from the Laboratory of Pharmacology of the Cardiovascular System, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR. The experimental results were analyzed by the paired t test and expressed as M ± m.

EXPERIMENTAL RESULTS

The action of ethmozine was studied within the concentration range from 1×10^{-7} to 1×10^{-4} g/ml. The typical action of ethmozine in a concentration of 3×10^{-5} g/ml is shown in Fig. 1a. Its effect on myocardial electrical activity was to depress $\dot{V}_{max}$ significantly without causing any appreciable change in the duration of AP or the resting potential. On average in eight experiments, with this high concentration of ethmozine the duration of AP was reduced to 95 ± 6% of the control and changes in the resting potential also lay within the limits of scatter of the experimental data: +0.5 ± 0.7 mV, whereas $\dot{V}_{max}$ was lowered to 42 ± 3% of the control value. All of these results were obtained by comparison of pairs.

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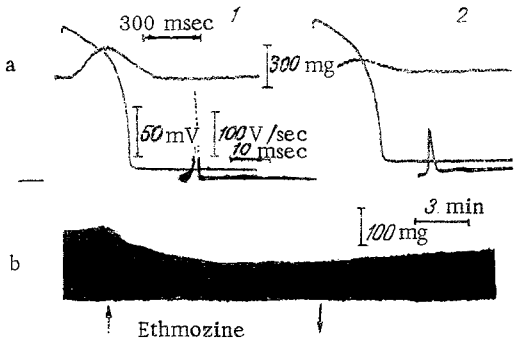

Fig. 1

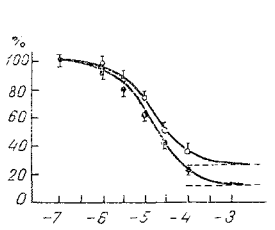

Fig. 2

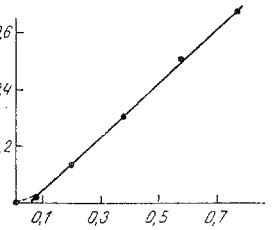

Fig. 3

Fig. 1. Action of 3×10^{-5} g/ml ethmozine on force of contraction and AP of myocardial fibers: a) AP, $\dot{V}_{max}$, and single contraction cycles recorded in control (1) and after action of ethmozine for 12 min (2); b) continuous trace of force of contraction. Frequency of stimulation 0.8 Hz.

Fig. 2. Relative values of force of contraction (empty circles) and of $\dot{V}_{max}$ (filled circles), normalized against control values for each experiment (ordinate), as a function of logarithm of ethmozine concentration in perfusion fluid (abscissa). Points on graph are mean values for each concentration (M ± m). Continuous lines obtained by theoretical approximation by the equation:

$$1-y=\Delta y_0/\left(1+\frac{K_{Dy}}{[eth]}\right),$$

where y is the relative value of the parameter recorded ($\dot{V}_{max}$ or force of contraction), Δy_0 the maximal depression of the parameter by ethmozine, K_{Dy} the apparent dissociation constant. The best approximation (by the method of least squares) was obtained with the following values of the constants: for $\dot{V}_{max}$ $\Delta y_0 = 0.9$, $K_{dy} = 1.52 \cdot 10^{-5}$ g/ml; for the force of contraction $\Delta y_0 = 0.75$, $K_{dy} = 1.48 \cdot 10^{-5}$ g/ml.

Fig. 3. Correlation between reduction of force of contraction (ordinate) and reduction in $\dot{V}_{max}$ (abscissa) with different concentrations of ethmozine (points taken from graph in Fig. 2); r = +0.998.

Changes in $\dot{V}_{max}$ under the influence of different concentrations of ethmozine are illustrated in Fig. 2.

By the 20th minute of action of ethmozine (3×10^{-5} g/ml) a decrease in the force of contraction was observed, as shown by the single cycles illustrated in Fig. 1a. Fig. 1b shows a continuous trace of the force of contraction, reduced by the action of ethmozine. On average for this concentration, the force of contraction fell to 49 ± 2.1% of the control. Inhibition of the force of contraction by different concentrations of ethmozine is shown in Fig. 3. Correlation between depression of $\dot{V}_{max}$ and the fall in the force of contraction is shown in Fig. 3. The two were found to be directly proportional with a coefficient of linear correlation r = +0.998.

The theoretical approximation of the experimental points in Fig. 2 was based on the hypothesis that blocking of sodium channels (C) by ethmozine (E) is described by the binding equation $C + E \rightleftarrows E \cdot C^*$, and that it can be determined both as a decrease in $\dot{V}_{max}$ and as inhibition of the force of contraction. The better agreement between the theoretical curves and experimental points obtained by approximation indicates that the decrease in both $\dot{V}_{max}$ and the force of contraction was in fact directly proportional to the number of blocked sodium channels. This conclusion is evident in the case of $\dot{V}_{max}$. This result for the force of contraction can be explained on the basis of the hypothesis of the existence of sodium-calcium exchange, capable of regulating both contraction [6] and relaxation [9] of the myocardium. In agreement with this hypothesis, inhibition of sodium conductance expressed as a decrease in $\dot{V}_{max}$ under the influence of ethmozine, leads to a decrease in the intercellular Na^+ ion concentration, to inhibition of the work of the sodium-calcium exchange mechanism and, as a result, to a decrease in the number of Ca^{++} ions entering the cell by means of this mechanism with each excitation cycle.

The best approximation was achieved with the following values of the constants (Fig. 2): maximal depression of the force of contraction 0.75, $\dot{V}_{max} = 0.9$; the apparent dissociation constants, moreover, virtually coincided, being 1.48×10^{-5} and 1.52×10^{-5} g/ml respectively. Incomplete inhibition of $\dot{V}_{max}$ by ethmozine can most likely be explained on the grounds that when the fast sodium current is completely blocked, the rate of rise of AP is determined by the slow calcium current, which is not inhibited by ethmozine (see below). The value of $\dot{V}_{max}$ of this "calcium" AP is approximately 20 V/sec [5], i.e., 10% of the normal AP of the guinea pig heart (192 ± 8 V/sec, n = 15). Incomplete inhibition of the force of contraction by ethmozine (25% of the control value remained) was evidently due to the fact that some Ca^{++} ions enter the myoplasm in response to excitation by means of a mechanism that is independent of Na^+ inflow, for example, with the slow calcium current, or is released from the sarcoplasmic reticulum.

However, the fall in the force of contraction lagged behind the fall in $\dot{V}_{max}$: With an ethmozine concentration of 3×10^{-5} g/ml the corresponding time constants were 142 ± 12 and 88 ± 9 sec. This lag, according to the adopted hypothesis, can be explained on the grounds that changes in the intracellular Na^+ ion concentration, which is the regulating parameter of sodium-calcium exchange, lag behind the decrease in the Na^+ inflow into the cell, because intracellular sodium plays the role of buffer.

Our experiments also revealed correlation between the inhibitory action of ethmozine and the frequency of excitation of the preparation. Changes in the value of $\dot{V}_{max}$ under the influence of 3×10^{-5} g/ml of ethmozine were found during switching from a frequency of stimulation of 0.8 to 0.1 Hz and vice versa. A considerable increase in $\dot{V}_{max}$ was observed at a low frequency of stimulation, despite the constant presence of ethmozine in the solution. On average, $\dot{V}_{max}$ was reduced by ethmozine in this concentration from 192 ± 8 V/sec (n = 15) to 84 ± 9 V/sec (n = 7) with a frequency of stimulation of 0.8 Hz. A fall in the frequency of stimulation to 0.1 Hz led to the virtually complete restoration of the normal rate of rise of AP (186 ± 12 V/sec, n = 5). Similar results were obtained by other workers for quinidine and lidocaine [3, 4]. Instead, however, another interesting phenomenon was observed (Fig. 4b): Under the influence of ethmozine the negative inotropic effect of a fall in the frequency of stimulation also was reduced. At a frequency of 0.1 Hz the force of contraction was virtually the same whether ethmozine was present (in a concentration of 3×10^{-5} g/ml) (54 ± 8 mg, n = 5) as when it was absent (64 ± 6 mg, n = 7), although with a frequency of stimulation of 0.8 Hz ethmozine significantly lowered the force of contraction: 225 ± 18 mg, n = 15, in the control; 95 ± 16 mg, n = 8, under the influence of ethmozine. These data also can be explained by the hypothesis of sodium-calcium exchange: A fall in the frequency of stimulation reduces the inhibitory action of ethmozine on sodium conductance, and this explains the decrease in the negative inotropic effect at a low frequency of stimulation.

To verify that the reduction in the force of contraction under the influence of ethmozine was attributable entirely to its ability to block sodium channels, i.e., that it has no effect on the inflow of Ca^{++} ions into the myoplasm via slow calcium channels, the effect of a high concentration of ethmozine (3×10^{-5} g/ml) on the slow calcium AP was studied [7]. The results of eight experiments showed that ethmozine has virtually no effect on the slow response (the reduction of the AP overshoot averaged 2.5 ± 4 mV).

The results of the present investigation are in agreement with the previous hypothesis that the antiarrhythmic action of ethmozine, like that of other antiarrhythmic drugs of the first group, is linked with its ability to reduce the rate of rise of AP as a result of inhibition of sodium conductance. This conclusion is based on the fact that ethmozine affects only $\dot{V}_{max}$ and does not change the other parameters of AP. Moreover the results of the present investigation serve to link the inhibitory action of ethmozine on sodium permeability with a reduction in the force of contraction of the working myocardium.

It must be pointed out, however, that the negative inotropic action of ethmozine was observed in concentrations much higher than therapeutic levels. For instance, when ethmozine was given in a daily dose of 500 mg, sufficient to suppress ventricular arrhythmias, the maximal plasma ethmozine concentration was 597 ± 48 ng/ml [1], which is approximately equal to 0.6×10^{-6} g/ml. In the present experiments, even with the higher concentration of ethmozine (1×10^{-6} g/ml), the force of contraction did not differ significantly from the control (by 1.6 ± 2.7%, n = 5), although depression of $\dot{V}_{max}$ was already considerable (7.3 ± 1.2%, n = 5). The negative inotropic effect of ethmozine, which the present experiments revealed, is thus not significant within the therapeutic range of its concentrations, but may be manifested in higher concentrations.

The results of this investigation confirm the conclusions of studies with procaine, benzocaine [11], and quinidine [8], showing that a decrease in the flow of Na^+ ions into the myocardial cell under the influence of antiarrhythmic agents of the first group may be accompanied by a negative inotropic effect. The mechanism of this negative inotropic effect is evidently linked with depression of sodium-calcium exchange.

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EFFECT OF THE CENTRAL α-ADRENOBLOCKER IEM-611 ON ALCOHOL AND ALDEHYDE DEHYDROGENASES IN RAT LIVER

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During the formation of an attachment to alcohol an important role is played by the activity of neurotransmitter systems [2, 4, 5] and the enzymes of ethanol and acetaldehyde metabolism [11]. Depending on whether animals have a predisposition for drinking water or ethanol, significant differences are found in the functioning of their corresponding enzyme systems [1, 3]. Pharmacological intervention directed toward the functional state of either of these systems (neurotransmitter or enzyme) may affect ethanol consumption and the development of addiction to it [7, 9].

The aim of the present investigation was to study the effect of the central α-adrenoblocker IEM-611 (p-di-isopropylaminophenylacetic acid β-phenylisopropylamide) on ethanol consumption and to estimate the activity of alcohol dehydrogenase (AlDH – E.C. 1.1.1.1) and aldehyde dehydrogenase (AdDH – E.C. 1.2.1.3) in the liver of animals preferring water or ethanol, and receiving or not receiving the drug.

EXPERIMENTAL METHOD

Experiments were carried out on 150 noninbred male albino rats weighing 200-250 g and divided into three groups: 1) animals preferring water (19% of the total number of rats), 2) intermediate group (56%), 3) rats preferring ethanol (13% of the total number of animals). Selection was carried out in the course of 10 days when the animals were allowed free choice between 15% ethanol solution and water. The rats were kept in individual cages measuring

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