ANTIARRHYTHMIC AND ELECTROPHYSIOLOGICAL PROPERTIES OF ETMOZIN AND ITS DIETHYLAMINO ANALOG

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The effect of the antiarrthymic drug etmozin and its diethylamino analog (DAA-etmozin) was compared in dogs with ventricular arrhythmias caused by ligation of the coronary artery. Both compounds were shown to abolish ventricular arrhythmias. However, DAA-etmozin had a more rapid and prolonged action. The electrophysiological properties of etmozin and DAA-etmozin were studied by the voltage clamp method on frog atrial trabeculae. Both compounds were shown to reduce the fast inward sodium current; DAA-etmozin had a stronger and more prolonged action.

KEY WORDS: antiarrhythmic activity; ectopic beats; etmozin and its diethylamino analog; fast inward sodium current.

It was shown previously that acyl derivatives of phenothiazine possess antiarrhythmic activity [1, 8]. One such compound, the Soviet preparation etmozin, has already been used clinically [2, 4]. The further study of the relationship between the chemical structure of 10-acylamino-derivatives of phenothiazine and their antiarrhythmic action led to the discovery of a compound which, according to several tests, has stronger antiarrhythmic activity than etmozin itself. The diethylamino analogs of etmozin (DAA-etmozin) were shown to be twice as active and to act for twice as long [5, 7]. In experiments on a model phospholipid membrane, DAA-etmozin showed twice the affinity of etmozin for the membrane [3].

The object of this investigation was to compare the effect of etmozin and its analog on ventricular disturbances of the cardiac rhythm in waking animals with experimental myocardial infarction and also to study the electrophysiological properties of these compounds.

EXPERIMENTAL METHOD

Experiments were carried out on dogs weighing 10-16 kg. The animals were anesthetized with pentobarbital sodium (35 mg/kg) intravenously. Under aseptic conditions the thorax was opened at the level of the fourth intercostal space. The pericardium was divided and the descending branch of the left coronary artery mobilized at the level of the apex of the left auricle. Two-stage ligation was carried out by the method described in [9]. The wound was closed in layers; a control recording of the ECG in standard lead II was carried out after 24 h. Substances for testing were injected intravenously in doses of 1-3 mg/kg (etmozin) and 0.5-1.5 mg/kg (DAA-etmozin). The numerical results were subjected to statistical analysis [6].

Ionic currents were recorded on isolated atrial trabeculae of Rana ridibunda. The preparations, 75-120 μ in diameter and 3-5 mm long, was placed in a perfusion chamber with a double sucrose gap [11]. To record the transmembrane potential (TMP) and apply current to the preparation, low-ohmic (under 5 kΩ) extracellular Ag-AgCl electrodes with agar bridges were used. To clamp the TMP and record ionic currents, the Dagan (USA) electronic circuit was used. The output voltage of the amplifier with negative feedback, clamping the TMP, was $\pm$ 90 V and the amplification factor 25,000. When the currents were recorded the assigned TMP was stabilized on the preparation for less than 100 μsec. The testing compartment of the chamber (200 μ wide) with a double sucrose gap was perfused with Ringer's solution of the following composition (in mM): NaCl 114,

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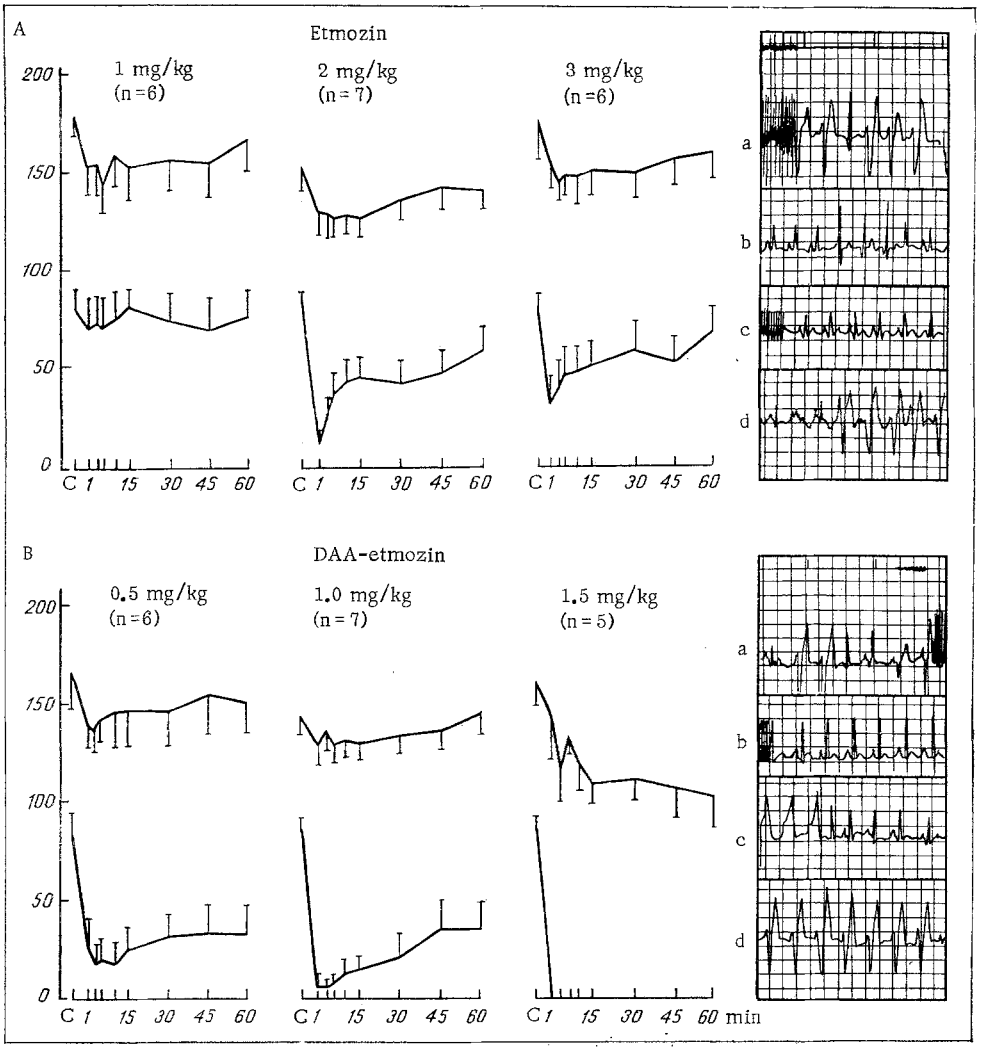

Fig. 1. Effect of etmozin and DAA-etmozin in doses of 1, 2, and 3 mg/kg on ventricular arrhythmias in waking dogs after 24 h. A: left side – graph showing effect of etmozin on total number of beats (top curve) and percentage of ectopic beats (bottom curves). n) Number of animals, *) $P < 0.05$ [not shown in Russian original]; right side – ECG of dog in standard lead II: a) 24 h after ligation of coronary artery (background); b) 2 min after injection of etmozin in dose of 2 mg/kg, c) 10 min after, d) 15 min after injection. ECG recorded at speed of 25 mm/sec, but for great illustrativeness the ECG of some experiments was recorded at a speed of 2.5 mm/sec. C) Control. B: left side – graph showing effect of DAA-etmozin (legend as in Fig. 1A); right side – ECG of dog in standard lead II: a) background, b) 1 min after injection of DAA-etmozin in dose of 1 mg/kg, c) 45 min after, d) 60 min after injection.

KCl 2.7, $CaCl_2$ 1.8, Tris-HCl 10, glucose 6.5, pH of the solution 7.5-7.6. All experiments were carried out at 20-22°C. The fast inward sodium current in the control and under the influence of etmozin and DAA-etmozin in concentrations of $5 \cdot 10^{-6}$/ml was recorded in the presence of substance D-600 to suppress the slow inward Ca^{++} currents.

EXPERIMENTAL RESULTS

Etmozin, in doses of between 1 and 3 mg/kg, given to waking dogs with ventricular arrhythmias, reduced the total number of cardiac contractions on average by 21%. As Fig. 1A shows, with an increase in the dose of etmozin the percentage of ectopic beats was reduced. A 100% effect (complete suppression of the ectopic rhythm) developed only after administration of etmozin in a dose of 2 mg/kg. The action of the compound began

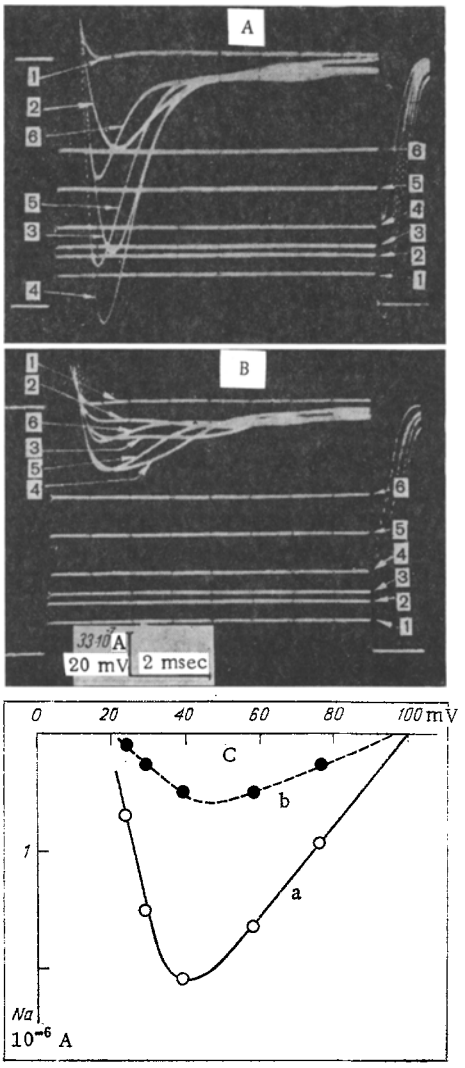

Fig. 2. Effect of DAA-etmozin on fast inward sodium currents (I_{Na}). A and B) Family of curves of currents recorded at different values of TMP before and after action of DAA-etmozin ($5 \cdot 10^{-6}$ g/ml) for 10 min. On right side in A and B, numbers 1-6 denote voltages of depolarization steps (in mV): 15, 25, 30, 40, 60, and 80; on left side corresponding outward currents are shown. C) Voltage-current curves plotted for peak values of currents taken from A and B. a) Control; b) action of DAA-etmozin. Calibration for A and B shown in B: potential 60 mV, current $6.6 \cdot 10^{-7}$ A, time 2 msec.

after 2-3 min and lasted 10-15 min, after which the ventricular extrasystoles and tachycardia reappeared. Etmozin in a dose of 3 mg/kg did not cause complete disappearance of the arrhythmia. In some animals the compounds aggravated arrhythmia and caused vomiting and motor excitation. In Fig. 1A four fragments of the EEG from one experiment are shown; clearly the compound acted after 2-3 min and its effect ceased after 15 min.

Since ED_{50} for DAA-etmozin was only half that for etmozin itself, doses of 0.5-1.5 mg/kg were chosen for testing [7]. In a dose of 1 mg/kg, DAA-etmozin reduced the total number of heart beats in dogs with myocardial infarction on average by 30%. As Fig. 1b shows, the percentage of ectopic beats fell immediately after injection of the drug. The anti-arrhythmic action of DAA-etmozin increased with an increase in its dose. In some animals the compound completely abolished the disturbances of rhythm in a dose of as little as 0.5 mg/kg and the effect lasted more than 1 h. Fragments of an ECG of one experiment are illustrated in Fig. 1B; clearly DAA-etmozin in a dose of 1 mg/kg abolished the arrhythmia imediately after injection and the effect lasted for about 1 h.

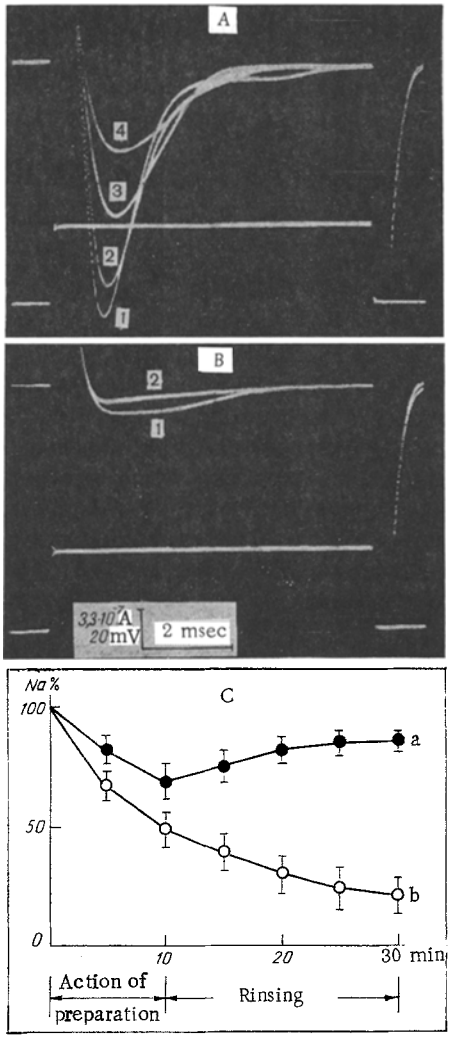

Fig. 3. Comparison of action of DAA-etmozin and etmozin in concentrations of $5 \cdot 10^{-6}$ g/ml on fast inward sodium currents. A) Decrease in I_{Na} under influence of DAA-etmozin: 1) control; 2, 3, 4) after action of DAA-etmozin for 15 min; curve 2) I_{Na} 5, 10, 15, and 20 min after beginning of rinsing preparation with normal Ringer's solution. In A and B: currents during depolarization step of 40 mV from resting potential level. Calibrations for A and B shown in B: current $6.6 \cdot 10^{-7}$ A; time 2 msec. C) Change in peak value of fast inward current under influence of etmozin (a) and DAA-etmozin (b) followed by rinsing preparations with normal Ringer's solution. Values of current in control taken as 100%. Each point represents results of five experiments.

The effect of DAA-etmozin in a concentration of $5 \cdot 10^{-6}$ g/ml on the fast inward sodium current (I_{Na}) recorded at different values of TMP is shown in Fig. 2A, B. The voltage-current characteristic curve plotted for six values of inward current taken from Fig. 2A and B is illustrated in Fig. 2B. Clearly DAA-etmozin considerably reduced the amplitude of I_{Na} after acting for 10 min.

The results of one experiment to study the action of DAA-etmozin on the value of I_{Na} and its dynamics during rinsing with normal Ringer's solution are illustrated in Fig. 3A and B. The combined results for changes in the sodium current under the influence of DAA-etmozin and etmozin, followed by rinsing, are shown in Fig. 3C. Clearly DAA-etmozin and etmozin itself, after acting for 10 min, reduced I_{Na} by 50 and 30% respectively. Unlike etmozin, however, rinsing out DAA-etmozin for 20 min did not lead to restoration of the sodium current. DAA-etmozin thus had the ability of etmozin to produce the fast inward sodium current but its action on I_{Na} lasted longer.

This comparative study of the effect of etmozin and its diethylamino analog showed that the latter has advantages. DAA-etmozin, while similar in the character of its electrophysiological properties, has a stronger and more prolonged action on the fast inward sodium current. In experiments on dogs with ventricular arrhythmias arising 24 h after two-stage ligation of the left descending coronary artery, DAA-etmozin produces a rapidly increasing, more stable, and more prolonged effect. If it is recalled that the activity of antiarrhythmic drugs on this model of arrhythmia indicates their possible clinical efficacy more accurately, DAA-etmozin can be regarded as a highly promising compound for further study.

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