found to inhibit the response to ACh application but not to change the characteristics of the response to L-glutamate application (Figs. 3 and 4). The results demonstrate unequivocally that the receptor sites for ACh and L-glutamate are different, although they possess similar structural elements.

Further investigation of the properties of ACh and glutamate receptor systems of the subesophageal ganglion of Z. guanensis showed that both are insensitive to scopolamine, atropine, and hexamethonium.

The presence of ACh and L-glutamate receptors sensitive to cholinergic ligands with nicotinic specificity (tubocurarine and cytisine) was thus demonstrated for the first time on the basis of electrophysiological data after microapplication of ACh and L-glutamate to the central parietal zone of the subesophageal ganglion of the mollusk Zachrysia guanensis. Modification of cytosine, by introducing a three-dimensional substituent at the nitrogen atom, gives azidocytisine, which inhibits the function of the ACh receptor but does not affect the glutamate receptor. The structures of the recognition sites of the receptors are evidently closely similar and differ in the zone of recognition of the ammonium group (CBC) of endogenous ligands.

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# CHARACTERISTICS OF THE PROTECTIVE ACTION OF ETHACIZINE ON THE ISCHEMIC MYOCARDIUM


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Ethacizine, a diethylamine analog of ethmozine, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, differs from ethmozine in its stronger and more lasting antiarrhythmic action [2], as has been confirmed by clinical trials [4, 5].

The aim of this investigation was to study the effect of ethacizine on the size of an experimental myocardial infarct and to shed light on some of the pathogenetic mechanisms of that effect.

## EXPERIMENTAL METHOD

Experiments were carried out on chinchilla rabbits weighing 2.0-2.5 kg. A myocardial infarct was produced by ligation of the anterior interventricular branch of the left coronary artery in its upper third. The operation was performed under pentobarbital anesthesia (30 mg/kg, intravenously), using a trans-sternal approach to the


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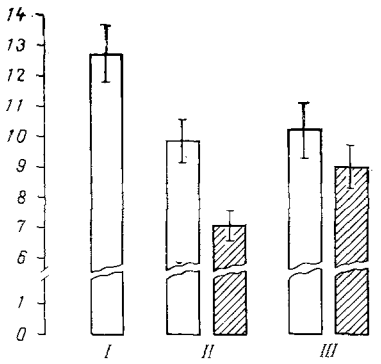


Fig. 1. Effect of ethacizine (II) and ethmozine (III) on size of experimental myocardial infarct in rabbits (in % of total weight of left ventricle). Unshaded columns – effect of 1-day cycle of injection of drugs, shaded columns – effect of late 6-day course of treatment.

heart and maintaining spontaneous respiration. Ethacizine and ethmozine, used as a drug for comparison, were injected by two schemes. In accordance with the first, the compounds were injected intravenously starting with the 30th minute after occlusion, 5 times at intervals of 30 min. The sessional dose for ethacizine was 0.3 and for ethmozine 1.0 mg/kg. The second scheme of administration consisted of a 6-day course of intravenous injections, the cycle beginning 24 h after ligation of the coronary artery. The compounds were injected 3 times a day at intervals of 4 h. The sessional dose of ethacizine was 0.3 and of ethmozine 3.0 mg/kg. When the last scheme was used, the animal received twice the quantity of the drugs every day. The rabbits were killed 7 days after coronary occlusion and the size of the focus of necrosis was measured planimetrically [9]. The size of the lesion was represented as the ratio of the weight of necrotic tissue to the total weight of the left ventricle, expressed in per cent. To study the effect of the compounds on energy metabolism in the ischemic focus, the ATP content was determined in conventionally intact myocardium and in the zone of occlusion by kits from Boehringer (West Germany). The action of ethacizine and ethmozine on permeability of the blood-humoral barrier and membranous structures of the conventionally intact and ischemic myocardium of the left ventricle was studied by means of a modified sulfacyl test [3]. To assess the effect of the compound a coefficient of permeability of sodium sulfacyl (the ratio between the concentration of the indicator in the myocardium and its concentration in blood plasma) for the two zones separately were used. In each experiment, changes in the sulfacyl concentration in the ischemic myocardium, expressed as percentages of its concentration in the conventionally intact part of the left ventricle, was calculated in each experiment, in order to give a more accurate idea of changes in distribution of the indicator among the zones chosen for analysis. Changes in the ATP concentration and the permeability of the membranous structures in the two zones were determined 3 h after coronary occlusion in untreated rabbits and in animals receiving the compounds in accordance with the first scheme described above. Chlorpromazine, tested by the same indicators to disclose any possible nonspecific action of the phenothiazine derivatives studied, was injected by the same method (sessional dose 1.0 mg/kg, intravenously). The significance of differences was calculated by Student's t test.

## EXPERIMENTAL RESULTS

Ethacizine and ethmozine reduced the size of the focus of necrosis by 22.8 and 20.5% respectively when injected during the first few hours after ligation of the coronary artery. A long course of treatment led to a decrease in size of the lesion by 44.9 and 29.9% respectively compared with the control (Fig. 1). With an increase in duration of injection, the effects of the two compounds were thus potentiated, despite a considerable interval (24 h) between coronary occlusion and the beginning of the injection cycle.

The biochemical investigation showed that ethacizine significantly increased the ATP concentration both in the conventionally intact zone (by 36.20%) and in the ischemic myocardium (by 72.5%), whereas ethmozine had no appreciable effect on the ATP concentration. Chlorpromazine, under these same conditions, caused an increase in the ATP concentration in the zone of ischemia only (by 35.1%). Ethacizine also had a more marked membrane-stabilizing action, and in the sulfacyl test it significantly reduced both the coefficient of permeability of the indicator in the ischemic myocardium and the increase in its absolute concentration in the occlusion zone

TABLE 1. Effect of Ethacizine and Compounds for Comparison of Permeability of Membranous Structures and ATP Concentration in Conventionally Intact and Ischemic Myocardium

| Preparation | Coefficient of permeability of | | Sulfacyl concentration in zone of ischemia, % | ATP concentration (in μmoles/g) in | |
|---|---|---|---|---|---|
| | intact myocardium | ischemic myocardium | | intact myocardium | ischemic myocardium |
| Control | 41,5±0,9 | 51,1±1,2 | +23,0±2,0 | 3,04±0,21 | 1,31±0,16 |
| Ethacizine | 41,4±0,8 | 46,7±1,2* | +12,9±2,7* | 4,14±0,25* | 2,26±0,18* |
| Ethmozine | 42,0±0,9 | 49,0±0,9 | +16,8±1,9 | 2,93±0,28 | 1,40±0,06 |
| Chlorpromazine | 41,2±1,1 | 47,5±0,6* | +15,6±2,3* | 3,33±0,12 | 1,77±0,10* |

Legend. Asterisk indicates significant difference from control.

(Table 1). In these experiments ethmozine caused no statistically significant changes of permeability, although a tendency was observed for the sulfacyl concentration in the zone of ischemia to fall compared with that in the intact myocardium ($0.05 < P < 0.1$). Chlorpromazine significantly reduced the increase in indicator concentration in the ischemic area (Table 1).

The decrease in size of the infarct discovered under the influence of ethacizine and ethmozine may be due to several physiological and biochemical factors, including the effects of the compounds on the supply of energy to ensure survival of myocytes in the peri-infarct zone, and also stabilization of the membranous structures of the myocardium during ischemia.

As was pointed out above, ethacizine significantly increased the ATP concentration not only in the ischemic focus, but also in the conventionally intact zone, and this must have had some influence on the functional capacity of the heart as a whole. Improvement of the energy supply to the ischemic tissue also has been observed under the influence of certain other phenothiazine derivatives, including chlorpromazine [8], as our own results confirmed.

A definite role in the protective effect of ethacizine may be played by its marked membrane-protective action. A significant, although weaker, effect also was given by chlorpromazine, in agreement with existing data [6]. Considering the decrease in permeability of membranous structures for sulfacyl only in the occlusion zone, it can be postulated that the compounds tested acts on neurohumoral mechanisms of ischemic damage to the membranous structures of the cells. The ability to inhibit phospholipase activity, characteristic of other phenothiazine derivatives [1, 7], may also be of definite importance in this case.

Comparison of the character of action of the antiarrhythmic drugs of the phenothiazine series tested in these experiments with that of chlorpromazine may suggest the presence of a common component, connected with the presence of a common chemical structure, in the cardioprotective effect of these compounds.

It can be concluded from these results that the most active of the substances tested was ethacizine. The drug limits the size of the infract, if given by either of the two therapeutic schedules. It increases the ATP concentration in the ischemic and the conventionally intact zones of the myocardium, and also reduces permeability of cardiomyocyte membranes.

Ethacizine thus not only has a marked antiarrhythmic effect, but it also has a significant protective action on the ischemic myocardium, evidence of its promising role in the treatment of myocardial infarction.

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