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EFFECT OF TETRODOTOXIN AND ETHMOZINE ON ARRHYTHMIAS OF THE DOG'S HEART ISOLATED IN THE LATE STAGE OF EXPERIMENTAL MYOCARDIAL INFARCTION

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Intravenous injection of tetrodotoxin (TTX), a specific blocker of sodium channels, abolishes cardiac arrhythmias arising in dogs 24 h after occlusion of the coronary artery [1]. TTX potentiates antiarrhythmic activity of ethmozine and mexetil (mexiletine) [2], which effectively suppress arrhythmias in this period of experimental myocardial infarction [3, 4]. These results enabled the antiarrhythmic action of these preparations in the late stage of infarction to be explained by their ability to affect the fast inward sodium current. However, the sensitivity of nerve cells to TTX is known to be much higher than that of myocardial cells [5]. During intravenous injection of TTX and antiarrhythmic agents, suppression of arrhythmias may take place as a result of both the direct action of the drugs on the myocardium and their action on the nervous system, activity of which plays a definite role in the development and abolition of arrhythmias in myocardial infarction [6].

The object of the present investigation was to study the action of TTX and ethmozine on cardiac arrhythmias in a dog isolated 24 h after occlusion of the coronary artery. By conducting the experiments in this way, the possible participation of the nervous system in the mechanism of the antiarrhythmic action of the drugs tested could be eliminated.

EXPERIMENTAL METHOD

In experiments on mongrel dogs weighing 10-15 kg, under pentobarbital anesthesia (35 mg/kg, intravenously) and with artificial respiration, the thorax was opened under sterile conditions through the 4th left intercostal space. A myocardial infarct was induced by two-stage occlusion of the left descending coronary artery by Harris' method [8]. When marked ventricular extrasystoles were present 24 h after the operation, the coronary vessels of the infarcted recipient's heart were perfused with blood from a donor dog. Animals weighing 25-40 kg served as donors. Recipient and donor were anesthetized with a mixture of 600 mg/kg urethane and

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TABLE 1. Effect of Isolation of the Heart on Ventricular Extrasystoles Developing 24 h after Occlusion of Coronary Artery (M ± σ)

Expt. No.	Before isolation		After isolation	
	frequency of ventricular extrasystoles	% of ventricular extrasystoles	frequency of ventricular extrasystoles	% of ventricular extrasystoles
1	191±5	100	118±14	96±3,2
2	178±9	99,3±1	182±8	100
3	153±6	100	104±5	94±1
4	167±9	99±0,7	115±9	98±3
5	133±4	100	105±10	100

Note: Results of seven recordings, each for 1 min, before and after isolation of the heart are given.

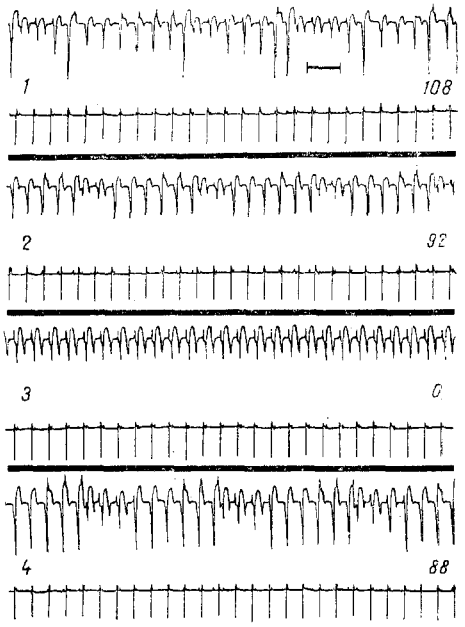

Fig. 1. Action of tetrodotoxin on arrhythmias of isolated heart. 1) Before isolation of heart; 2) 1 h after isolation; 3) after action of TTX for 2 min in a concentration of $4 \cdot 10^{-8}$ g/ml; 4) after rinsing for 3 min. Here and in Fig. 2: top trace shows ECG, bottom electrogram from auricle of right atrium; numbers on right show numbers of ventricular extrasystoles in 21 min; time marker 1 sec.

50 mg/kg chloralose, intravenously. Before isolation of the heart, the animals were given an intravenous injection of heparin (1500 units/kg). The descending aorta, left subclavian artery, and the superior and inferior venae cavae of the recipient were ligated. The donor's arterial blood was taken from the femoral artery into a pressurized reservoir, from which it was supplied under constant pressure (90-100 mm Hg) through a cannula in the brachiocephalic artery into the coronary vessels of the isolated heart. Coronary venous blood from the isolated heart was returned via a catheter inserted through the azygos vein into the right ventricle to the donor. The order of the manipulations during isolation was such that the coronary blood flow was not interrupted throughout the dissections. Blood was supplied to the isolated heart at a constant temperature (37 ± 1°C). The rate of the coronary blood flow varied in different experiments between 30 and 80 ml/min. TTX and ethmozine were dissolved in the donor's arterial blood, taken in a volume sufficient to perfuse the isolated heart for 6-8 min, and injected into the pressurized reservoir. The initial concentrations were $4 \cdot 10^{-8}$ g/ml

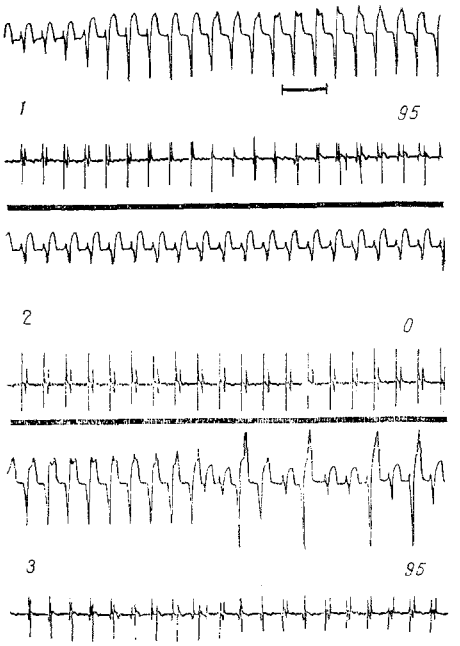

Fig. 2. Action of ethmozine on arrhythmias of isolated heart, 1) 2 h after isolation of heart; 2) after action of ethmozine for 1 min in concentration of $3 \cdot 10^{-5}$ g/ml; 3) rinsing for 5 min.

TTX and $3 \cdot 10^{-5}$ g/ml ethmozine. If no antiarrhythmic effect was obtained (restoration of the sinus rhythm) the concentrations of the drugs were increased in steps of $2 \cdot 10^{-8}$ g/ml TTX and $1 \cdot 10^{-5}$ g/ml ethmozine. Blood containing the test substances was not returned to the donor. The loss of donor's blood was made good with blood taken during isolation of the recipient's heart and also with polyglucin. The donor's blood pressure did not vary in the course of the experiment by more than 20% of its initial value. Intervals between the action of the drugs on the isolated heart were not less than 30 min.

The ECG (lead II) and bipolar electrogram from the auricle of the right atrium were recorded by means of VR-12 amplifiers (Electronics for Medicine) on a two-channel recorder (Gould-Brush Model 2400). Records of the ECG and electrogram for 1 min at intervals of 10 min were obtained for 1 h before and 1 h after isolation of the heart. From the data thus obtained the mean frequency of ventricular extrasystoles per minute was determined and their percentage of the total frequency of cardiac contractions calculated before and after isolation of the heart. During injection of TTX and ethmozine into the perfusing blood the ECG and electrogram were recorded throughout the period of action of the drugs.

EXPERIMENTAL RESULTS

The hearts of five dogs which developed marked and persistent ventricular extrasystoles 24 h after occlusion of the coronary artery were isolated. The effect of isolation of the heart on disturbances of rhythm are given in Table 1 and they show that the frequency of the ventricular extrasystoles was reduced in four experiments after isolation of the heart. This reduction took place as a result, not of partial recovery of sinus rhythm, but of a fall in the general rhythm of the heart, for the number of ventricular extrasystoles as a percentage of the total frequency of cardiac contractions remained virtually unchanged.

It will be clear from Fig. 1, which gives the results of an experiment to study the action of TTX on arrhythmias of the isolated heart, that 24 h after occlusion of the coronary artery marked ventricular extrasystoles had developed, and they continued after isolation of the heart (in fragments 1 and 2 excitation of the atria and ventricles will be seen to be completely asynchronous). TTX, injected into the blood perfusing the coronary vessels of the isolated heart in a concentration of $4 \cdot 10^{-8}$ g/ml completely restored the sinus rhythm. Concentrations of TTX which effectively abolished the arrhythmias ranged in different experiments from $4 \cdot 10^{-8}$ to $1 \cdot 10^{-7}$ g/ml ($6 \pm 2.6 \cdot 10^{-8}$ g/ml; $n = 5$). The antiarrhythmic effect was achieved 4 ± 3.1 min after the beginning of perfusion of the isolated heart with blood containing TTX.

Ethmozine, like TTX, effectively suppressed the arrhythmias of the isolated heart. It will be clear from Fig. 2 that in a concentration of $3 \cdot 10^{-5}$ g/ml it completely abolished ventricular extrasystoles and restored the sinus rhythm. The effective antiarrhythmic concentration of ethmozine lay within the range $(3\text{-}5) \cdot 10^{-5}$ g/ml ($4.4 \pm 0.9 \cdot 10^{-5}$ g/ml; n = 5). An antiarrhythmic effect was observed 1.4 ± 0.6 min from the beginning of perfusion with blood containing ethmozine.

The results showing the presence of ventricular extrasystoles in the isolated heart indicate that activity of the autonomic nervous system is not the decisive factor maintaining the disturbances of the cardiac rhythm in the late stage of infarction. The fall in the frequency of ventricular ectopic excitations after isolation of the heart could be the result of desympathization of the myocardium. The decrease in the maximal frequency of ventricular extrasystoles observed after desympathization of the heart [9] is evidence in support of this view. Abolition of the arrhythmias after injection of TTX and ethmozine into the blood perfusing the isolated heart leads to the conclusion that the antiarrhythmic effect is due to the direct action of these substances on the myocardium and is the result of a reduction in the sodium current in the myocardial tissue.

In the present experiments the TTX concentration required to achieve an antiarrhythmic effect was $4 \cdot 10^{-8}$-$1 \cdot 10^{-7}$ g/ml, which was 100-1000 times less than the TTX concentrations required to depress the sodium current in intact cells of the contractile myocardium and in Purkinje fibers [5, 7]. Restoration of the sinus rhythm after injection of TTX and ethmozine was not accompanied by any marked change in the conduction of excitation in the heart. There are thus grounds for considering that both TTX and ethmozine, in doses giving an antiarrhythmic effect, acted mainly on myocardial fibers damaged by ischemia. This conclusion is supported by direct measurements of the conduction delays in normal and infarcted zones during the action of ethmozine; After injection of the drug conduction was found to be slowed in the zone of infarction but to undergo no significant changes within the intact myocardium [12]. It is thus evident that myocardial fibers injured by ischemia are more sensitive to TTX and to ethmozine than the intact myocardium.

The increased sensitivity of the myocardial fibers in the zone of infarction to TTX and ethmozine may be due to several causes. The first is that depolarization of the membrane of infarcted cells leads to an increase in their sensitivity to TTX and to antiarrhythmic drugs [5, 11]. The second factor affecting the ability of antiarrhythmic drugs to act selectively on cells of the infarcted zone may be changes in the ultrastructure of the layer covering the outer surface of the principal cell membrane, which has been called the "glycocalyx" [10], arising during ischemia. Destruction of the glycocalyx may make the sodium channels more accessible for interaction with TTX and antiarrhythmic drugs.

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