ELECTROPHYSIOLOGICAL EFFECTS OF DISOPYRAMIDE ON HYPOXIC RABBIT VENTRICULAR MUSCLE

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Intracellular microelectrode recording techniques were used to elucidate the mechanism of the antiarrhythmic action of disopyramide in an isolated rabbit ventricular muscle perfused by hypoxic Tyrode's solution. Hypoxia induced no significant changes of the resting membrane potential or action potential amplitude but decreased the maximum upstroke velocity of the action potential (dV/dt max) and shortened the action potential duration and the effective refractory period. Disopyramide in a dose of 5 μg/ml induced a significant decrease of resting membrane potential and action potential amplitude of hypoxic muscle, while it did not alter these parameters in oxygenated muscle. Disopyramide depressed dV/dt max in hypoxic muscle as well as in oxygenated muscle. However, there was much greater depression in hypoxic cells. After disopyramide, action potential duration at the 90% level of repolarization and the effective refractory period were prolonged in both hypoxic and oxygenated ventricular muscle. However, disopyramide lengthened the effective refractory period of hypoxic muscle to a much greater degree than that of oxygenated muscle. This resulted in a decrease of disparity in refractoriness. The above differential effects of disopyramide in oxygenated and hypoxic tissue may account for its effectiveness in post-infarction re-entrant arrhythmias.

Disopyramide is a synthetic antiarrhythmic agent which has been reported to be effective in a variety of experimental and clinical arrhythmias. It is effective in experimental ventricular arrhythmias caused by coronary artery occlusion. In fact, clinical reports indicate that disopyramide can suppress ventricular arrhythmias following acute myocardial infarction.

The electrophysiological actions of disopyramide have been studied in isolated myocardial tissues by several workers. However, little information is available on the effect of disopyramide on the ventricular myocardium under abnormal conditions encountered during ischemia such as hypoxia, acidosis, potassium concentration changes and anaerobic metabolites. The present study was undertaken to evaluate the electrophysiological effects of disopyramide on ventricular muscle superfused with hypoxic perfusate to define more clearly the mechanism of its action in post-infarction ventricular arrhythmias.

Key Words:
Disopyramide
Hypoxia
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Re-entry arrhythmia

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METHODS

Albino rabbits weighing 2 kg were anesthetized with sodium pentobarbital, 30 mg/kg i.v., and their hearts were rapidly removed and placed in cooled oxygenated Tyrode's solution. Trabeculae were excised from the right ventricular free wall, mounted in a perfusion chamber and superfused with Tyrode's solution equilibrated with a 95% O₂-5% CO₂ gas mixture at a constant rate of 12 ml/min. The composition of the Tyrode's solution (millimoles per liter) was as follows: NaCl, 136.9; NaHCO₃, 12; NaH₂PO₄, 3.3; MgCl₂, 0.5; CaCl₂, 1.8; KCl 2.7; and dextrose, 11. The temperature of the tissue bath was maintained at 35 ± 0.5°C.

Rectangular pulses 2 msec in duration and twice diastolic threshold in intensity were delivered at a cycle length of 500 msec through Teflon-coated bipolar silver wires. Transmembrane action potentials were recorded with Pyrex capillary microelectrodes filled with 3M KCl. Electrode resistances ranged from 5 to 20 megohms. The electrodes were coupled to silver-silver chloride wires which led into a high-impedance, input capacitance-neutralizing amplifier (WPI model 750). Transmembrane action potentials were displayed on a cathode ray oscilloscope (Nihon Kohden VC9) for monitoring, and recordings were made on Polaroid film from the face of a storage oscilloscope (Tektronix 5113) or with Mingograf 800 (Siemens-Elema). Records of transmembrane potentials were calibrated by interposing a 50 mV signal between the tissue bath and the ground. The effective refractory period was determined by delivering a premature stimulus progressively earlier after every eighth drive stimulus. A second micro-electrode recorded electrical activity on the site 4 mm distal to the stimulating electrodes, and the effective refractory period was defined as the longest stimulus interval at which a premature stimulus does not elicit a propagated action potential.

After the control measurements were made, the preparation was perfused with hypoxic Tyrode's solution equilibrated with a mixture of 90% N₂-5% O₂-5% CO₂ for 30 min. The PO₂ of the hypoxic solution was 90 ± 10 mmHg, while that of the Tyrode's solution equilibrated with 95% O₂-5% CO₂ was 500 ± 40 mmHg. After measurements were repeated, disopyramide in a dose of 5 μg/ml was added to the hypoxic Tyrode's solution. The preparation was exposed to the drug for 30 min, then repeat measurements were made. Because of the difficulty in maintaining steady impalements in a single muscle cell during exposure to different solutions, records were obtained from more than 5 cells during exposure to each solution and the mean values of the action potential characteristics were calculated.

In another series of experiments, disopyramide in the same dose was added to the Tyrode's solution equilibrated with a mixture of 95% O₂-5% CO₂ after the control study. The preparation was exposed to the drug for 30 min, and the measurements were then repeated.

Statistical comparisons were made using the paired t-test or an analysis of variance (Newman-
Fig.1. Effects of disopyramide on action potential of hypoxic ventricular muscle. Left: Control potential recorded during perfusion of oxygenated Tyrode's solution. Middle: Potential obtained following 30 min of exposure to hypoxic solution. Right: Potential obtained 30 min after addition of disopyramide (5 μg/ml) to hypoxic solution. In each section, the middle tracing represents the differentiated signal of the action potential upstroke. The bottom tracing is time marks of 10 msec. The initial portion of the action potential is recorded at a fast sweep speed in the right upper part. The horizontal short line on the left indicates zero potential, and vertical line represents 50 mV. The vertical calibration on the right side represents 50 V/sec. After exposure to hypoxic solution, dV/dt max decreased from 174.5 to 155.0 V/sec. Disopyramide added to the hypoxic solution depressed it further to 82.0 V/sec.

Fig.2. Effects of disopyramide on action potential of oxygenated ventricular muscle. Left: Control action potential. Right: Potential obtained after 30 min of exposure to disopyramide (5 μg/ml). Calibrations and time marks are the same as in Fig. 1. Disopyramide decreased dV/dt max from 170.5 to 152.5 V/sec.

Keul test). Data are expressed as mean ± SD.

RESULTS

The effects of hypoxia and disopyramide on electrophysiological parameters of ventricular muscle are summarized in Table I.

The effects of hypoxia were usually stabilized within 30 min or less after the beginning of the perfusion. Hypoxia decreased dV/dt max and shortened the action potential duration at both the 50% and 90% levels of repolarization and the effective refractory period. Hypoxia induced no significant changes of resting membrane potential or action potential amplitude. We confirmed in another series of experiments (not shown here) that the above action potential characteristics were almost unchanged during further exposure to the hypoxic solution for 30 min with the exception of action potential duration at the 50% level of repolarization in which there was a slowly progressive decrease. Therefore, the effects of disopyramide which were usually apparent within 10 min after the addition of the drug to the hypoxic solution and stabilized within 30 min or less could not have resulted from prolonged exposure to the hypoxic solution.

Disopyramide depressed dV/dt max in both the ventricular fibers superfused with the hypoxic Tyrode's solution and with the oxygenated solution, from 160.3 to 111.6 V/sec in the hypoxic muscle, and from 174.2 to 152.4 V/sec in the oxygenated muscle. There was much greater
duration at the 90% level of repolarization and the effective refractory period in hypoxic ventricular muscle. These two parameters returned to the control values after disopyramide. Figure 3 shows a typical example of the changes in the effective refractory period. There was no significant change in action potential duration at the 50% level of repolarization in hypoxic ventricular muscle. This is presumably due to the progressive decrease of action potential duration at the 50% level of repolarization during hypoxia.

In the oxygenated preparation, disopyramide prolonged action potential duration at both the 50% and 90% levels of repolarization and the effective refractory period. Expressed as percent change, there was greater prolongation of the effective refractory period in hypoxic ventricular muscle than in oxygenated ventricular muscle (15.9 ± 4.7 vs 9.2 ± 4.5%, p < 0.05).

**DISCUSSION**

Disopyramide exhibits electrophysiological effects similar to those of procainamide and quinidine. It prolongs action potential duration and the effective refractory period, and reduces maximum upstroke velocity of the action potential, conduction velocity and spontaneous phase 4 depolarization. However, these effects are observed during studies on isolated myocardial tissues dissected from normal animal hearts and maintained in an oxygenated perfusate of normal composition. The effects of antiarrhythmic drugs are greatly modified by anaerobic metabolites, hypoxia, acidosis, potassium concentration changes and catecholamines. Most clinically important arrhythmias occur in ischemic or infarcted hearts, where these abnormal factors may exist.

Sasyunuk and Kus studied the effects of disopyramide on action potentials in endocardial preparations removed from both normal canine hearts and hearts subjected to total coronary artery occlusion 18 to 24 hours previously. Their attention was focused mainly on the effects of disopyramide on subendocardial Purkinje fibers that survive over the infarcted region and the mechanism of its action in the late phase arrhythmias following obstruction of a coronary artery.

After coronary artery occlusion, the reduction or complete lack of perfusion of the intramural ventricular muscle initiates a series of biochemical and structural changes. The immediate metabolic changes that occur in the ventricular muscle.

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fibers are due to hypoxia and depressed oxidative metabolism. Therefore, in vitro hypoxia may be one of simple and reasonable models for the early stage of in vivo ischemia after coronary artery occlusion. In the present experiments, we studied the effects of disopyramide on ventricular muscle perfused with hypoxic Tyrode's solution to obtain further information regarding the mechanism of its action in early phase arrhythmias.

The effects of hypoxia alone on transmembrane action potential of cardiac fibers have been reported by previous workers and confirmed in the present study. An early and pronounced effect of hypoxia on ventricular muscle fibers is to shorten the action potential duration and the effective refractory period. Maximum upstroke velocity of the action potential is depressed by hypoxia, even in the absence of a decrease in resting membrane potential. It has been reported that resting membrane potential is decreased only after prolonged hypoxia.

There is also a decline in action potential amplitude. In this study, we chose moderate hypoxia (Po2: 90 ± 10 mmHg) and increased dextrose to 11 mM to obtain the steady-state effects of hypoxia.

Disopyramide depressed dV/dt max more markedly in hypoxic ventricular fibers than in oxygenated ventricular fibers. It also lengthened the effective refractory period of hypoxic ventricular muscle to a much greater degree than that of oxygenated ventricular muscle. These differential effects of disopyramides were observed also in vivo during experimental myocardial ischemia. The precise reasons for the variability in responses are not known, though local metabolic changes in the hypoxic or ischemic tissue may enhance the drug effect.

These differences in the effect of disopyramide in oxygenated and hypoxic muscle may account for its effectiveness in early phase arrhythmias after coronary artery occlusion. The immediate abbreviation of the refractory period of ventricular muscle cells in the whole heart following coronary artery occlusion is not uniform. Consequently, there is an increase in the degree of temporal dispersion of a recovery of excitability. Since disparity in refractoriness facilitates the re-entrant mechanism, it seems clear that disopyramide could alter re-entry by decreasing such disparity. Disopyramide is expected to have a more marked effect on conduction in already depressed infarcted areas, because it decreases dV/dt max more greatly in hypoxic tissue. Therefore, it could abolish re-entrant arrhythmias due to ischemia also by transforming the unidirectional block (a prime requirement for re-entry) to a bidirectional block.

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