Electrophysiological Actions of Disopyramide Phosphate on Canine Ventricular Muscle and Purkinje Fibers

By Teresa Kus and Betty I. Sasyniuk

ABSTRACT

Disopyramide phosphate is a new antiarrhythmic drug that has been shown to possess significant antiarrhythmic effects in animals and man. In the present investigation, the effects of 2, 5, and 10 \( \mu \)g/ml of disopyramide phosphate were studied on the electrophysiological properties of canine Purkinje fibers and ventricular muscle superfused in vitro. Transmembrane action potentials were recorded from Purkinje fibers in the region of maximum action potential duration (gate), from Purkinje fibers proximal and distal to the gate, and from ventricular muscle. Disopyramide phosphate produced a concentration-dependent decrease in the slope of phase 4 diastolic depolarization of spontaneously beating Purkinje fibers. In all electrically stimulated fibers, the drug decreased the amplitude and the maximum upstroke velocity of the action potential. This depression of phase 0 characteristics was accompanied by a decrease in conduction velocity. In Purkinje fibers located at the gate, a concentration-dependent parallel shift to the right and a depression of the maximum of the membrane responsiveness curve occurred. Effects on action potential duration were variable. Repolarization was altered so that action potentials with dissimilar durations recorded from sites proximal to, at, and distal to the gate became equal. The total action potential duration and the effective refractory period of gate Purkinje fibers were prolonged, but the change in action potential duration was always greater than the change in effective refractory period so that the ratio of the change in duration to the change in refractory period was always greater than one.

Disopyramide (4-diisopropylamino-2-phenyl-2-[2-pyridyl]-butyramide) is a new synthetic antiarrhythmic agent that appears to be effective in a variety of experimental and clinical arrhythmias. It is two to three times more potent than quinidine in abolishing experimental, atrial arrhythmias induced by either aconitine or electrical stimulation (1); it works against experimental ventricular arrhythmias caused by ouabain infusion (1) or coronary artery occlusion (2). In fact, recent clinical reports indicate that disopyramide can suppress both supraventricular (3-6) and ventricular (3, 7-10) arrhythmias of multiple etiology.

The electrophysiological actions of disopyramide have been studied in isolated atrial tissue (10, 11), in which its effects are similar to those of quinidine. However, little information is available on the effects of disopyramide on the electrophysiological properties of ventricular tissue. The present study was undertaken to evaluate the electrophysiological effects of this drug on isolated Purkinje fibers and ventricular muscle to define more clearly the mechanism of its action in ventricular arrhythmias. We sought to determine whether the mode of action of disopyramide differed from the mechanisms of the commonly used antiarrhythmic drugs.

Methods

Sixteen adult mongrel dogs weighing 13.5-22.0 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv) or sodium thiopental (25 mg/kg, iv). Their hearts were exposed through a lateral thoracotomy, removed rapidly, and dissected in cool oxygenated Tyrode’s solution. The preparations that we used consisted of either the right ventricular conducting system including the septal portion of the right bundle branch, the right papillary muscle, and the false tendon with its attachment to the free wall or the left ventricular conducting system including the septal portion of the posterior division of the left bundle branch, the free-running bundle, and the posterior papillary muscle. In some experiments, only the right papillary muscle was used.

The preparations were mounted with small stainless steel pins in a wax-lined Lucite chamber through which Tyrode’s solution, equilibrated with a 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \) gas mixture, was flowing at a constant rate of 12-13 ml/min. The composition of the Tyrode’s solution (mM) was: \( \text{MgCl}_2 \) 0.5, \( \text{NaH}_2\text{PO}_4 \) 0.9, \( \text{KCl} \) 4.0, \( \text{CaCl}_2 \) 1.8, \( \text{NaCl} \) 136.9, \( \text{NaHCO}_3 \) 12, and dextrose 5.5. The temperature of the tissue bath was maintained at 37 ± 0.5°C.

Driving stimuli were delivered to the preparation through Teflon-coated bipolar electrodes of silver wire. The electrodes were positioned either at the most proximal portion of the right or left bundle branch or at the tip.
of the papillary muscle immediately beyond the insertion of the free-running strand. Stimuli were rectangular pulses of twice threshold intensity and 1–2 msec duration delivered by a Tektronix 161 pulse generator and passed through W-P isolation transformers. The pulse generator was triggered by a digitally controlled interval generator (Pulse Train Synthesizer, Schema Versatae). The interval generator permitted the application of a series of basic pulses at equal intervals followed by one to six test pulses delivered at any desired interval. The preparations were stimulated at a basic cycle length of 600 msec except in those experiments in which the cycle length was varied.

Transmembrane action potentials were recorded with glass microelectrodes filled with 3m KCl. Electrode resistances ranged from 5 to 20 megohms. The electrodes were coupled to silver-silver chloride wires (Bionetics) which led into high-impedance, input capacitance-neutralizing amplifiers (Schema Versatae). The outputs of the amplifiers were displayed on a Tektronix 502 oscilloscope for monitoring and either a Tektronix RM 565 or R5103N oscilloscope for recording. Recordings were made on 35-mm film with a Grass C4 kymograph camera. The response of the entire electronic system was calibrated by introducing 150-mv sawtooth pulses ranging from 25 v/sec to 1,000 v/sec into the tissue bath between the recording and indifferent electrodes. The system was linear over this voltage range.

Action potential characteristics were measured either by hand after magnification of the film or by a model 200 signal analyzer (Schema Versatae). The signal analyzer could be operated in two modes. In mode 1, the voltage output from the preamplifiers was fed into the signal analyzer. On command of a trigger pulse which was generated a few milliseconds prior to the tissue response, the analyzer accumulated and stored the resting membrane potential. It displayed this value on a numerical read out (Nixie tubes). The device then determined the absolute maximum excursion of the action potential above the resting membrane potential (amplitude) and displayed this value on another set of Nixie tubes. At the start of the action potential, a 100-kHz oscillator was switched on. As the action potential dropped to some preselected percent of its maximum value, the 100-kHz oscillator was switched off. The interval between the onset of the action potential and a selected percent of repolarization was then displayed on a third set of Nixie tubes. This device was highly accurate, providing instantaneous values for resting membrane potential, amplitude, and duration which were reproducible with an error of less than 1%. In mode 2, the device could be used to measure membrane responsiveness. In this mode, the device accumulated the resting membrane potential of the basic pulses in the same manner as it did in mode 1. It then recorded and stored the value of the membrane potential before the beginning of the test response. In addition, the apparatus recorded and stored the maximum value of dV/dt of the test response. In this mode, the device displayed the resting membrane potential, the potential at which the cell was reactivated, and dV/dt of the corresponding response. In addition to the electronic display, a permanent record of the values was obtained with a Hewlett-Packard 5055A digital recorder.

The membrane responsiveness of Purkinje fibers located at the region of maximum action potential duration was determined in preparations consisting of the posterior division of the left ventricular conducting system. The preparations were stimulated with bipolar electrodes located in muscle just beyond the insertion of the free-running strand into the posterior papillary muscle. Action potentials were recorded from a Purkinje fiber located at the "gate" a few millimeters from the stimulating electrodes. Another microelectrode recorded activity on the free-running strand beyond the gate and distal to the stimulating electrodes. The preparations were stimulated at a basic cycle length of 600 msec. Premature stimuli were introduced after every eighth drive cycle. Premature stimuli were initially introduced late in diastole and then progressively earlier in the cycle. The earliest potential initiated distally which propagated to the Purkinje fiber beyond the gate was measured the effective refractory period of the gate cell. Slightly earlier stimuli caused only local responses in the gate cell and did not propagate to the proximal site.

Measurement of the maximum rate of rise of phase 0 depolarization of each premature response by electronic differentiation allowed the determination of membrane responsiveness.

Powdered disopyramide base was suspended in distilled water at a concentration of 10 mg/ml. The suspension was solubilized by potentiometric titration using a 1:10 dilution of phosphoric acid (ortho 85%) to the first end point. This procedure corresponds to the formulation of di-disopyramide hydrogen phosphate which has a pH of 6.8–6.9. In the initial experiments, this concentrated solution was then diluted with Tyrode’s solution to obtain final concentrations of 2, 5, and 10 μg/ml. In later experiments, a concentrated solution (10 mg/ml) of the phosphate salt of disopyramide (RU3292) supplied by Roussel Laboratories was used. This solution also contained sorbitol (39.2 mg/ml) and sodium mercurothiolate (0.02 mg/ml). No effects of the vehicle alone were observed on the electrophysiological characteristics of Purkinje fibers in two experiments in which it was added to the perfusing medium in quantities that would have contained the maximum concentration of drug used.

All statistical analyses were performed using Student’s t-test based on paired or unpaired observations when appropriate.

Results
EFFECTS ON AUTOMATICITY IN PURKINJE FIBERS

The effects of disopyramide phosphate were examined in spontaneously beating preparations in which automaticity was enhanced by superfusion with Tyrode’s solution containing 2.7 mm K+. The preparations were exposed to 2, 5, and 10 μg/ml of drug. We assumed that these concentrations of disopyramide phosphate were roughly comparable to therapeutic concentrations in man (4).

Perfusion with each concentration was main-

1 Supplies of disopyramide phosphate were kindly made available by Dr. Jacques Gareau, Medical Director, Roussel Laboratories.
tained for 30 minutes. In all preparations and at all concentrations used, disopyramide phosphate caused slowing of spontaneously beating Purkinje fibers by depressing the slope of phase 4 diastolic depolarization. A typical example of the effects of the drug on a spontaneously beating Purkinje fiber located in the septal portion of the posterior division of the left bundle branch is illustrated in Figure 1. During perfusion with control Tyrode’s solution, the spontaneous cycle length was 1,080 msec. Following perfusion with 2 μg/ml of disopyramide phosphate, the cycle length increased to 1,410 msec. Exposure to 5 and 10 μg/ml increased the spontaneous cycle length to 1,670 and 1,830 msec, respectively. The decrease in the spontaneous firing rate was accompanied by a progressive lengthening of the action potentials. The degree of lengthening produced was greater than that which would be expected from an increase in the basic cycle length alone. When preparations that had been perfused with 2.7 mM K+ Tyrode’s solution were electrically stimulated at basic cycle lengths ranging from 400 to 2,000 msec, disopyramide phosphate increased total action potential duration in these cells at all rates of stimulation with the greatest lengthening occurring at the longest basic cycle lengths (unpublished observations). The depression of automaticity by the 5- and 10-μg/ml concentrations was also accompanied by a depression of the action potential amplitude. Following the 2-μg/ml concentration, there was a slight increase in action potential amplitude. It was difficult to assess whether changes in threshold potential had occurred. The spontaneous rate returned toward control values after perfusion with drug-free solution.

**EFFECTS ON TRANSMEMBRANE VOLTAGE CHARACTERISTICS OF PURKINJE FIBERS**

Experiments were performed in a series of preparations obtained from either the right or the left ventricle stimulated at a constant cycle length of 600 msec. The preparations were either exposed to all three concentrations of the drug or the effects of a single concentration were determined and the preparation was discarded. Perfusion with each concentration was continued until the changes in the action potential had stabilized; then, the next higher concentration was administered. Continuous display of action potential parameters enabled us to determine readily when steady-state drug effects had been achieved. In some experiments in which impalements were maintained for a sufficiently long time, the preparations were returned to control Tyrode’s solution and allowed to recover.

The effects of disopyramide were usually apparent within 5 minutes after the beginning of the perfusion and stabilized within 30 minutes or less when the 2- and 5-μg/ml concentrations were used. However, the effects of 10 μg/ml sometimes took several hours to stabilize. Similarly, the electrophysiological changes produced by the 2- and 5-μg/ml concentrations were completely reversible within 30 minutes of perfusion with control Tyrode’s solution. The changes produced by the 10-μg/ml concentration also began to revert to control values within 10–15 minutes; however, it sometimes took several hours of perfusion with drug-free solution for these changes to completely revert to control values.

Figure 2 shows a typical example of the changes observed during a single impalement following exposure to all three concentrations of the drug. Disopyramide phosphate had no significant effect on resting membrane potentials at any concentration. However, there was a progressive decrease in both the amplitude and the rate of rise of the action potential. Characteristic changes were also observed in the repolarization time course of the action potential resulting in a steeper plateau phase and a more gradual phase 3. Changes in
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EFFECTS ON CONDUCTION VELOCITY

The depression of phase 0 characteristics was accompanied by a decrease in conduction velocity. Conduction time was measured between two microelectrodes located at either end of a free-running Purkinje fiber. The action potentials recorded from the two sites were displayed at a fast sweep speed allowing measurement of conduction time between the two action potential upstrokes. The distance between the two microelectrodes was estimated through the eyepiece of the microscope. In three experiments in which such measurements were made, 5 µg/ml of disopyramide phosphate decreased the control conduction velocity of 1.72 ± 0.21 m/sec to 1.63 ± 0.72 m/sec. This decrease represents an average depression of conduction velocity of 5.9 ± 1.4% (P < 0.05).

EFFECTS ON MEMBRANE RESPONSIVENESS

To further examine the effects of disopyramide phosphate on maximum upstroke velocity in Purkinje fibers, the effects of the drug were examined in four experiments on the relationship between the maximum upstroke velocity of phase 0 of the action potential and the level of membrane potential at which the action potential is initiated. This relationship provides information concerning the ability of a cell to respond to stimuli at different levels of membrane potential. Figure 3, the results from a typical experiment, shows the effect on membrane responsiveness produced by 2, 5, and 10 µg/ml of disopyramide phosphate applied successively. The preparation was exposed to each concentration of the drug until steady-state effects on action potential characteristics were obtained. The membrane responsiveness curve was then determined, and the preparation was exposed to the next higher concentration of the drug. A single impalement was maintained throughout all of the determinations.

Disopyramide phosphate produced a concentration-dependent decrease in the maximum and a parallel shift to the right of the membrane responsiveness curve, indicating a decrease in the ability of the membrane to depolarize rapidly on premature stimulation. There was a shift in the value of \( V_h \) (the membrane potential at which \( dV/dt \) is half of its maximum value) to more negative potentials. \( V_h \) under control conditions was -66.5 mv. The

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**FIGURE 2**

Effects of disopyramide phosphate (DP) on action potential characteristics of Purkinje fibers. **Top Left:** Control potential recorded from the region of the gate in the right ventricular conducting system. **Top Right:** Potential obtained following 30 minutes of exposure to disopyramide phosphate (2 µg/ml). **Bottom Left:** Potential obtained after 30 minutes of exposure to disopyramide phosphate (5 µg/ml). **Bottom Right:** Potential obtained after 30 minutes of exposure to disopyramide phosphate (10 µg/ml). In each section, the bottom traces represent the differentiated signal of the action potential upstrokes, and the horizontal lines indicate zero potential. The vertical and horizontal calibrations in the bottom right corner represent 50 µsec and 50 msec, respectively. The vertical calibration to the left of the differentiated signal represents 500 u/sec. The action potential characteristics are as follows: (1) control: resting membrane potential (RMP) = -85.7 mv, action potential amplitude (AMP) = 122.9 mv, \( dV/dt \) = 390 u/sec, action potential duration to 50% repolarization (50% APD) = 308 msec, and action potential duration to 90% repolarization (90% APD) = 343 msec; (2) disopyramide phosphate (2 µg/ml): RMP = -85.7 mv, AMP = 122.9 mv, \( dV/dt \) = 390 u/sec, 50% APD = 255 msec, and 90% APD = 333 msec; (3) disopyramide phosphate (5 µg/ml): RMP = -85.2 mv, AMP = 121.2 mv, \( dV/dt \) = 360 u/sec, 50% APD = 238 msec, and 90% APD = 343 msec; and (4) disopyramide phosphate (10 µg/ml): RMP = -85.0 mv, AMP = 117.6 mv, \( dV/dt \) = 330 u/sec, 50% APD = 207 msec, and 90% APD = 339 msec.

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Action potential duration occurred within 5–10 minutes of exposure to the drug, but changes in \( dV/dt \) began to occur somewhat later (15 minutes).

The effects of disopyramide phosphate on phase 0 characteristics of Purkinje fibers in all of the experiments in which successful impalements were maintained throughout are summarized in Table 1. Disopyramide phosphate had no significant effects on resting membrane potential at any concentration. Although resting membrane potential did decrease slightly in two experiments following exposure to 10 µg/ml, these changes were not significant. However, the drug produced a concentration-dependent decrease in both the action potential amplitude and the maximum rate of rise of the action potential upstroke.
TABLE 1

Effects of Disopyramide Phosphate on Phase 0 Characteristics and Repolarization Time Course of Purkinje Fibers

<table>
<thead>
<tr>
<th></th>
<th>Resting membrane potential (mv)</th>
<th>Action potential amplitude (mv)</th>
<th>dV/dt (v/sec)</th>
<th>Action potential duration (msec)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>To 50% repolarization</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gate</td>
</tr>
<tr>
<td>N 9 Control</td>
<td>-83.9 ± 2.3</td>
<td>127.0 ± 1.3</td>
<td>465 ± 39</td>
<td>243 ± 6</td>
</tr>
<tr>
<td>% Change</td>
<td>+2.7 ± 1.7</td>
<td>-1.3 ± 0.6</td>
<td>-9.6 ± 2.0†</td>
<td></td>
</tr>
<tr>
<td>N 6 Control</td>
<td>-82.4 ± 1.3</td>
<td>124.1 ± 0.8</td>
<td>413 ± 21</td>
<td>217 ± 6</td>
</tr>
<tr>
<td>% Change</td>
<td>+0.4 ± 0.7</td>
<td>-4.1 ± 0.7†</td>
<td>-16.5 ± 1.8†</td>
<td></td>
</tr>
<tr>
<td>N 6 Control</td>
<td>-82.2 ± 3.0</td>
<td>125.0 ± 1.3</td>
<td>433 ± 39</td>
<td>243 ± 6</td>
</tr>
<tr>
<td>% Change</td>
<td>+0.6 ± 1.7</td>
<td>-9.4 ± 3.9†</td>
<td>-29.9 ± 4.9†</td>
<td></td>
</tr>
</tbody>
</table>

All results are expressed as means ± SE. N = number of experiments, and DP = disopyramide phosphate.

* P < 0.05.
† P < 0.01.

Values obtained following exposure to 2, 5, and 10 μg/ml of disopyramide phosphate were -67.5 mv, -69.0 mv, and -70.0 mv, respectively.

EFFECTS ON REPOLARIZATION TIME COURSE OF PURKINJE FIBERS

Myerburg et al. (12) have previously shown that Purkinje fiber action potential duration increases progressively from the proximal bundle branches and reaches a maximum several millimeters from the insertion of the false tendon into the papillary muscle. Beyond this region of maximum action potential duration, referred to as the gate, the action potential duration becomes progressively shorter. The gate determines the functional refractory period of the ventricular conducting system under normal conditions. A breakdown in the gating system was assumed by Myerburg et al. (12) to play a role in the generation of reentrant arrhythmias. However, Wittig et al. (13) and Rosen et al. (14) have suggested that the disparity in action potential duration might facilitate the generation of reentry. Therefore, the effects of disopyramide phosphate on the repolarization of Purkinje fibers and ventricular muscle throughout the conducting system were investigated to determine if the drug had quantitatively different effects on repolarization in each of these fibers.

Figure 4 illustrates a representative example of the differential effects of the drug on repolarization of Purkinje fibers. Each section shows superimposed traces of Purkinje fiber action potentials recorded simultaneously from a region proximal to and at a region of maximum action potential duration. Under control perfusion, there was a marked difference in action potential duration between these cells. Following perfusion with 2 μg/ml of disopyramide phosphate, all phases of the action potential were prolonged in the proximal cell. However, in the gate cell, there was an abbreviation of the action potential at the 50% level of repolarization. At the two higher concentrations, 5 and 10 μg/ml, disopyramide phosphate produced a very small further increase in the plateau phase of the proximal cell but shortened this phase in the gate cell even more. At the 90% level of repolarization, there was further lengthening in both cells, but a greater prolongation occurred in the proximal cell. These changes effectively diminished the marked differences in action potential duration seen under control conditions, resulting in almost complete equalization of action potential duration.

The effects of disopyramide phosphate on repolarization of Purkinje fibers in all of the experiments are summarized in Table 1. The data from cells proximal and distal to the gate have been combined and referred to as nongate, since the changes observed in these cells were similar. Disopyramide phosphate decreased the action potential duration at the 50% level of repolarization in
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Purkinje fibers located at the gate but increased it in cells located proximal and distal to the gate. Expressed as percent change, there was a progressively greater abbreviation of the plateau phase of the gate cells at each higher concentration but a prolongation of this phase in the nongate cells. At the 90% level of repolarization, disopyramide phosphate increased the action potential duration in all Purkinje fibers. However, there was a consistently greater mean prolongation in the nongate cells.

The effect of disopyramide phosphate on action potential duration at various levels of repolarization was further explored by mapping action potential durations at various sites along the entire ventricular conducting system in four additional experiments. Action potentials were recorded sequentially at 1-2-mm intervals from the septal portion of the posterior division of the left bundle branch and along the free-running strand to its insertion into the posterior papillary muscle. These experiments further confirmed a differential effect of the drug at various sites within the ventricular conducting system. The greatest degree of lengthening of total action potential duration was observed in the Purkinje fibers most proximal and most distal to the gate and in ventricular muscle. Greater uniformity of action potential duration throughout the ventricular conducting system in the presence of the drug was most evident at the 50% and 75% levels of repolarization. In fact, at the 50% level of repolarization, there was very little difference in duration between Purkinje gate cells and ventricular muscle cells.

**FIGURE 3**

Effect of disopyramide phosphate (DP) on membrane responsiveness of a Purkinje fiber gate cell. dV/dt (v/sec) is plotted on the ordinate, and the membrane potential (mv) at which each action potential was initiated is plotted on the abscissa. Circles = experimental points obtained under control conditions, and squares, triangles, and diamonds = experimental points obtained following exposure to 2, 5, and 10 μg/ml of disopyramide phosphate, respectively.

**FIGURE 4**

Differential effects of disopyramide phosphate (DP) on repolarization of Purkinje fibers. Each section shows superimposed traces recorded from a Purkinje fiber located proximal to and at the region of the gate. Δ50% and Δ90% refer to differences in action potential duration at the 50% and 90% levels of repolarization, respectively. Vertical and horizontal calibrations in the top right corner indicate 25 mv and 50 msec, respectively.
EFFECTS ON EFFECTIVE REFRACTORY PERIOD

The effects of disopyramide phosphate on the effective refractory period of Purkinje fibers located at the gate were studied in four preparations. The effective refractory period was defined as the shortest interval in milliseconds between the basic response and the premature response in the gate cell which propagated to a site beyond the gate. The results of a typical experiment are shown in Figure 5. Disopyramide phosphate prolonged the total action potential duration of the gate cell from 360 msec under control conditions to 390 msec after 2 μg/ml, 405 msec after 5 μg/ml, and 410 msec after 10 μg/ml. The drug also progressively prolonged the effective refractory period from 295 msec under control conditions to 320 msec after 2 μg/ml and 325 msec following exposure to both 5 and 10 μg/ml. However, the change in action potential duration was always greater than the change in effective refractory period at each concentration of the drug so that the ratio of these changes was always greater than one. Since membrane responsiveness of Purkinje fibers at the gate sites is shifted to the right by disopyramide phosphate, the first extrasystole which propagated beyond the gate occurred at a progressively higher membrane potential with increasing concentrations of the drug. However, the rate of rise of this first extrasystole was not necessarily correspondingly improved. The absolute refractory period, which was defined as the shortest time interval between the basic response and a premature response in the gate cell which did not propagate beyond the gate, was also prolonged by disopyramide phosphate.

EFFECTS ON VENTRICULAR MUSCLE

The effects of disopyramide phosphate were also examined on the action potential characteristics of ventricular muscle in six preparations. Figure 6 shows the results from a typical experiment. The preparation was exposed to 2, 5, and 10 μg/ml of the drug during a single impalement of a ventricular muscle fiber recorded from the tip of the right papillary muscle. Disopyramide phosphate caused a progressive decrease in the amplitude and the rate of rise of the upstroke of the action potential, and it increased action potential duration at both the 50% and 90% levels of repolarization. Because of the difficulty in maintaining steady impalements in a single muscle cell during exposure to three different concentrations of the drug, experiments were done in which a group of 20 fibers were impaled successively at the tip of the right papillary muscle during control perfusion. Ventricular muscle cells located in this region have fairly uniform characteristics. The preparation was then exposed to 2 μg/ml of the drug, and records were obtained from another group of cells. The measurements were repeated after exposure to 5 and 10 μg/ml of disopyramide phosphate. The numbers above the traces indicate the total action potential duration of the gate cell in msec. Right: The effective refractory period of the gate cell under the conditions indicated in the corresponding left section. In each section, the number above the zero potential lines indicates the effective refractory period of the gate cell. The number below these lines indicates the membrane potential of the gate cell at which the first extrasystole propagated beyond the gate. Calibrations are the same as they are in Figure 2.

As shown in Table 2, disopyramide phosphate did not alter the resting membrane potential of

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\text{Table 2: Summary of Data on a Group of 20 Cells.}
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\begin{tabular}{|c|c|c|}
\hline
Concentration & APD & ERP \\
\hline
Control & 360 & 295 \\
2 μg/ml & 390 & 320 \\
5 μg/ml & 405 & 325 \\
10 μg/ml & 410 & 330 \\
\hline
\end{tabular}
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Control DP - 2 µg/ml

FIGURE 6

Effects of disopyramide phosphate (DP) on action potential characteristics of ventricular muscle. Top Left: Control action potential. Top Right: Potential obtained following exposure to 2 µg/ml of disopyramide phosphate. Bottom Left: Potential obtained following exposure to 5 µg/ml of disopyramide phosphate. Bottom Right: Potential obtained following exposure to 10 µg/ml of disopyramide phosphate. The bottom traces in each section are the differentiated signals of the action potential upstrokes. The horizontal lines are the zero potential lines. Calibrations are the same as they are in Figure 2.

There was a progressive decrease in action potential amplitude which was not statistically significant. However, there was a significant decrease in dV/dt of these cells. The drug significantly prolonged action potential duration at both the 50% and 90% levels of repolarization. All of the changes occurred in a concentration-dependent manner.

Discussion

Previous studies with disopyramide phosphate in intact animals and man have demonstrated alterations in the electrophysiological properties of the whole heart at doses which have significant antiarrhythmic effects against experimental and clinical arrhythmias (2, 15, 16). The present study defines further the effects of this drug on the electrophysiological properties of isolated canine Purkinje fibers and ventricular muscle. We studied the effects produced by 2, 5, and 10 µg/ml of the drug. These concentrations were chosen on the basis of a study by Desruelles et al. (4) in man; they reported a range of plasma levels of 1.91 to 6.5 µg/ml following the administration of oral doses of 300 mg/day for the treatment of atrial arrhythmias. Another study in man (17) has shown that peak serum levels of 8–14.1 µg/ml are attained 3 hours after oral doses of 100 mg.

It has been emphasized repeatedly that ventricular arrhythmias can occur as a result of alterations in automaticity, conduction, or both (18). Suppression of automaticity has been considered to be one of the essential electrophysiological properties of antiarrhythmic drugs and has been demonstrated with all clinically effective antiarrhythmic agents with the exception of bretylium (19). Disopyramide phosphate significantly depressed automaticity in spontaneously beating Purkinje fibers. This effect appeared to be due largely to a marked decrease in the slope of phase 4 diastolic depolarization in these fibers. Following perfusion with the lowest concentration, depression of phase 4 was accompanied by an improvement in the action potential amplitude with no significant alterations in other action potential parameters. However, after perfusion with the higher concentrations, depression of phase 4 was also accompanied by a significant depression of the action potential amplitude and prolongation of the action potential duration. This action of disopyramide differs from that of lidocaine. The latter drug exerts powerful effects on the

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td>Effects of Disopyramide Phosphate on Action Potential Characteristics of Ventricular Muscle</td>
</tr>
<tr>
<td>Resting membrane potential (mv)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>DP (2 µg/ml)</td>
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<tr>
<td>DP (5 µg/ml)</td>
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<td>DP (10 µg/ml)</td>
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</table>

All results are expressed as means ± se. DP = disopyramide phosphate. n = 20.

* P < 0.05.
† P < 0.01.

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automaticity of Purkinje fibers without significantly altering other action potential parameters (20). Disopyramide's actions are similar, however, to the reported effects of procainamide (21). The mechanism by which disopyramide phosphate suppresses automaticity is uncertain. Pacemaker activity in Purkinje fibers has been attributed to several mechanisms, namely, (1) a decrease in potassium conductance during diastole, (2) an increase in sodium conductance during diastole, and (3) a decrease in the activity of a sodium pump during diastole (22). No information is available about which of these conductances is altered by disopyramide phosphate.

Weidmann (23) originally suggested that the principal mode of action of quinidine and procainamide is a prolongation of the effective refractory period of Purkinje fibers out of proportion to any increase in action potential duration. Previous studies on the electrophysiological actions of various antiarrhythmic drugs in isolated cardiac tissues have stressed this property as an important requisite for antiarrhythmic effectiveness (24). In our experiments, disopyramide phosphate did not cause a greater prolongation of the effective refractory period relative to the action potential duration in Purkinje fibers located in areas of maximum action potential duration. The greater increase in effective refractory period relative to the drug-induced lengthening of the action potential duration seen with drugs like quinidine and procainamide has been attributed to a decrease in membrane responsiveness. It has been postulated that because of reduced responsiveness repolarization has to proceed to a more negative value before reexcitation can occur, resulting in a prolongation of refractoriness independent of any change in duration. This action is thought to favor the propagation of premature beats, since the first propagated potential occurring at a more negative membrane potential is assumed to have a better upstroke velocity. Disopyramide phosphate also caused a concentration-dependent depression of the maximum and a parallel shift to the right of the membrane responsiveness curve. However, it did not produce the expected effects on refractoriness. In fact, changes in effective refractory period tended to lag behind changes in action potential duration. Furthermore, the first propagated potentials did not necessarily have more rapid upstroke velocities. Rosen et al. (21) have also found that changes in action potential duration are greater than changes in refractoriness in Purkinje fibers perfused with blood and exposed to procainamide in therapeutic concentrations. They did not comment on this discrepancy with previous concepts. These results suggest that in this respect disopyramide phosphate is again similar to procainamide but may differ from other antiarrhythmic drugs. However, we think that the concept of net prolongation of refractoriness as a requisite for antiarrhythmic effectiveness should be reevaluated. A drug that causes a parallel shift to the right and a depression of the maximum of the membrane responsiveness curve would not necessarily be expected to cause a net prolongation of the effective refractory period. Such a prolongation can occur (1) if the drug causes only minimum changes in action potential configuration but depresses responsiveness and (2) if the drug eliminates the tail of the membrane responsiveness curve. Disopyramide phosphate significantly alters action potential configuration, and elimination of the tail of the membrane responsiveness curve has only been demonstrated in the presence of lidocaine and diphenylhydantoin (20, 25, 26). A drug that significantly delays sodium reactivation kinetics can alter the relationship between effective refractory period and action potential duration, but there is no evidence available to suggest that disopyramide phosphate alters sodium reactivation.

Net prolongation of refractoriness in individual fibers is probably of lesser importance for antiarrhythmic effectiveness than is alteration of refractoriness throughout the ventricular conducting system for drugs like disopyramide phosphate and procainamide. The effects of disopyramide phosphate on the repolarization time course in Purkinje fibers depended on the site within the conducting system from which the potentials were recorded. Repolarization was modified in such a way that a greater similarity in contour developed between proximal, gate, and distal sites. The plateau phase of the action potential was decreased only at gate sites. At all other sites, including ventricular muscle, the plateau phase was prolonged. Total action potential duration was prolonged at all sites; however, a much greater lengthening occurred at sites proximal and distal to the gate. Since the ionic mechanisms responsible for the different phases of repolarization in cardiac Purkinje fibers and ventricular muscle are still uncertain, it is difficult to speculate on the basis for the differential effects of disopyramide phosphate on repolarization in these fibers. However, one consequence of this action was to diminish the nonuniform repolarization which normally occurs in Purkinje fibers and to reduce the discrepancy between action potential durations.
in Purkinje fibers and ventricular muscle. However, this action of disopyramide phosphate was not as pronounced as that which has been demonstrated to occur with lidocaine (13). The effects produced are again more similar to the changes that occur with procainamide (14).

Disopyramide phosphate had significant depressant effects on phase 0 characteristics that were accompanied by a depression of conduction velocity. However, the depressant effects of the drug were apparent later than the changes in refractoriness.

These effects of disopyramide phosphate permit a speculation concerning its mechanism of action in the abolition of reentrant arrhythmias. Since unidirectional block and disparity in refractory periods are the hallmarks of a reentry mechanism, it seems clear that disopyramide phosphate could alter reentry by decreasing the disparity in refractory periods and by further slowing conduction through the depressed pathway, resulting in a bidirectional conduction block. It also seems apparent that, as with procainamide, changes in conduction may be the more important mechanism for abolition of reentry.

The fact that disopyramide phosphate bears some structural resemblance to procainamide may account for the similarity in their electrophysiological actions. Moreover, the fact that drugs with similar electrophysiological actions are often effective against a similar spectrum of arrhythmias suggests that disopyramide phosphate may be effective against the same clinical arrhythmias as procainamide. However, this drug has certain advantages over the latter drug. Its half-life is approximately twice as long as that of procainamide (17). Although clinical studies with this compound are still in the investigational stage, studies to date indicate that disopyramide phosphate is well tolerated during chronic oral therapy (6, 9). Future studies may show that disopyramide phosphate is useful in situations in which procainamide is effective but not well tolerated.

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