Electrophysiological Effects of Diphenylhydantoin on Canine Purkinje Fibers

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ABSTRACT
The effects of diphenylhydantoin (DPH) were studied on isolated, perfused Purkinje fibers over a range of concentrations from $10^{-8}$ to $10^{-4}$ M. The time course of repolarization of the transmembrane action potential shortened due to abbreviation of all phases of repolarization. The effective refractory period also shortened during exposure to DPH, but to a lesser extent than the action potential. As a result the earliest effective test stimulus elicited action potentials with greater amplitude and $dv/dt$ of phase 0 than under control conditions. In driven fibers with normal action potentials, DPH had little effect on the amplitude or rate of rise ($dv/dt$) of phase 0 of the action potential. In driven fibers which were partially depolarized, or those with low $dv/dt$ of phase 0 despite normal resting potentials, DPH caused an increase in the rate of rise of phase 0 of the action potential. DPH caused a decrease in the firing rate of normal automatic fibers by decreasing the slope of phase 4 depolarization. In automatic fibers which showed generalized diastolic depolarization and decreased maximum diastolic potential, DPH caused an increase in the latter as well as a decrease in the slope of phase 4 depolarization.

ADDITIONAL KEY WORDS
membrane responsiveness effective refractory period excitability
diastolic depolarization extrasystole automaticity
antiarrhythmic activity ouabain catecholamines
dogs

Diphenylhydantoin (DPH) has been found effective in abolishing a variety of cardiac arrhythmias produced in laboratory animals or encountered in the clinic. It is most effective against experimental ventricular arrhythmias induced in laboratory animals by a variety of means. These include ventricular tachycardia caused by ligation of the anterior descending coronary artery (1), arrhythmias caused by ouabain infusion (2), arrhythmias caused by administration of epinephrine and cyclopropane (3), and the ventricular arrhythmias caused by hypothermia (4). DPH also is effective against aconitine and delphinine induced atrial arrhythmias (5); this observation is of particular interest, since these arrhythmias are unresponsive to treatment with quinidine and procaine amide. This fact suggests that there are differences between the modes of action of DPH and the commonly used antiarrhythmic drugs of which quinidine is the prototype.

In the clinic, DPH has been found effective in controlling ventricular arrhythmias occurring after myocardial infarction (6, 7), open heart surgery (8), and ventricular arrhythmias induced by digitalis excess (7, 9). Most of these arrhythmias respond when DPH plasma levels are 10 to 18 µg/ml; a few respond at levels of 3 to 4 µg/ml (10). Its use has suggested to some that its mechanism of action
might differ from that of quinidine-like drugs (8, 11), although most authors have concluded that its actions are much the same (12).

Most ventricular arrhythmias are best understood in terms of alterations in automaticity, conductivity, and associated changes in the electrophysiological characteristics of Purkinje fibers. The present study was undertaken to evaluate the effects of DPH on the transmembrane potentials of isolated,perfused canine Purkinje fibers. The experiments include observations on the effects of a wide range of concentrations of DPH on fibers which had normal resting potentials and also on fibers which had been partially depolarized by experimental interventions. Use of isolated preparations eliminates extrinsic neural and humoral factors. It was, therefore, thought that the results of these studies would show whether the antiarrhythmic action of DPH was central or peripheral and would clarify some of the contradictory findings obtained with this drug in studies of the excitability of the heart in situ. DPH had sufficient effect on automaticity, excitability, and conductivity of these isolated preparations to suggest that its predominant antiarrhythmic action is peripheral, and to permit us to propose a mechanism for this action.

Methods

Mongrel dogs weighing 10 to 19 kg were anesthetized with sodium pentobarbital (28 to 30 mg/kg, iv). The heart was rapidly removed and dissected in cool, oxygenated Tyrode solution. False tendon (Purkinje fiber) preparations were obtained from both ventricles and were stored in cool, oxygenated Tyrode solution until studied.

Tyrode solution, gassed with 95% oxygen, 5% carbon dioxide, was infused into the perfusion chamber at constant selected rates between 5 to 10 ml/min. Temperature was maintained at 36 ± 1°C except when the effects of cooling to 29°C were examined. The same gas mixture was used to directly aerate the chamber. The preparation was held in place by fine stainless steel pins which passed through the peripheral portion of ventricular muscle attached to the Purkinje fibers and into the layer of wax lining the bottom of the chamber.

The composition of the Tyrode solution, in millimoles per liter was: NaCl, 137; KCl, 3.0; NaH₂PO₄, 1.8; CaCl₂, 2.7; MgCl₂, 0.5; dextrose, 5.5, and NaHCO₃, 12. Twice distilled, deionized water was used in the preparation of all solutions. The pH of the Tyrode solution gassed with 95% oxygen and 5% carbon dioxide was 7.4 at 37°C.

Drugs used in the study were added to separate reservoirs of gassed Tyrode solution in the desired concentrations. Sodium EDTA (5 × 10⁻⁸ M, final concentration) was added to solutions containing catecholamines to slow their oxidation and inactivation. Concentrated solutions (1.8 × 10⁻⁴ M) of diphenylhydantoin sodium were prepared either in commercial diluent (10% alcohol, 20% propylene glycol, pH 11.0) or in alkaline saline containing no diluent (10⁻⁴ M). Dilutions were then made in Tyrode solution to obtain concentrations of 10⁻⁸ to 10⁻⁴ M.

Driving stimuli were delivered to the Purkinje fiber or ventricular muscle by means of Teflon-coated bipolar electrodes of silver wire (0.020 in. diameter). The stimuli were generated by a combination of Tektronix waveform and pulse generators arranged so that a basic drive stimulus (S₁) and intermittent single or trains of test stimuli (S₂) could be delivered to the preparation through the same electrodes (Fig. 1). The basic drive rate, drive and test stimulus duration and amplitude, recurrence of test stimuli, and interval between drive stimuli and test stimuli were independently variable. The stimuli were isolated from ground by isolation units (Type ISA 100, Bioelectric Instruments). In ex-
Experiments where test stimuli were used, an electronic counter (Hewlett-Packard, 523 DR) was used to indicate the interval between S₁ and S₂ with an accuracy of ±0.5 msec. The duration of S₁ was generally 3 msec, and its amplitude was twice threshold. The duration of S₂ was usually 5 msec, and its amplitude was three to four times diastolic threshold. S₂ was used to determine the effective refractory period (ERP). Also, by intermittently delivering S₂ during and after the repolarization of an action potential induced by S₁, the relationship between membrane potential and the rate of rise of the premature action potential upstroke (membrane responsiveness) could be obtained for the cell being studied.

Microelectrodes were machine-pulled from capillary glass and filled with 3 M KCl. Tip diameters were less than 1 μ. Electrodes with a resistance of 15 to 25 megohms were coupled to Ag-AgCl wire which led into high impedance capacitance neutralizing amplifiers (NF 1, Bioelectric Instruments). The outputs of the neutralizing amplifiers were led either single-ended or differentially into a differential amplifier (Type 2A63, Tektronix) and displayed on the upper beam of a dual beam cathode ray oscilloscope (505 RM, Tektronix).

Surface electrograms were obtained from closely spaced silver wires which were insulated to the tips, mounted in glass, and placed via a micromanipulator on the Purkinje fiber and/or ventricular muscle. The electrograms were amplified by solid state operational amplifiers (Nexus 2LV-1) and were displayed on the dual beam oscilloscope.

Action potential amplitude, rate of rise, dv/dt of phase 0, and the input capacitance neutralization were checked with each oscilloscope sweep against a repetitive calibration. Every sweep of the recording oscilloscope triggered a time-base amplifier (2B67, Tektronix) which delivered a linear sawtooth pulse with a rising slope that could be varied through a wide range. This calibration pulse was adjusted to 100 mv with respect to a standard calibrator and, with each sweep of the oscilloscope, was injected into the fluid in the tissue chamber via a 3 M KCl-filled indifferent electrode. The calibrating sawtooth pulse followed the same electrical path to the differential amplifier as the signals generated by the tissue preparation. The calibration signals and action potentials from the output of the neutralizing amplifiers were differentianed by an operational amplifier (3A8, Tektronix) with a time constant of 2 msec. Figure 2 demonstrates the calibration procedure used to measure the rate of rise (dv/dt) of phase 0 of the action potential. This system was linear from 0 to 1000 v/sec. Panel A shows both traces sweeping at a rapid velocity; 100-mv sawtooth pulses of known rate of rise are displayed upward; from left to right the rising velocities are 500, 200, 100 and 50 v/sec. The electronic differentiation of these pulses is displayed downward, and the amplitude of the differentiated spike can be seen to be linearly related to the rate of rise of the sawtooth. In panel B the arrangement used during experiments is shown. The lower beam sweeps at a lower velocity so that the sawtooth pulse appears as a single upward spike. The upper beam sweeps rapidly and displays the same sawtooth and its electronic differentiation. Panel C shows a record taken during an experiment. The lower beam sweeps at a lower velocity so that the sawtooth pulse appears as a single upward spike. The upper beam sweeps rapidly and displays the sawtooth and the differentiation of both the sawtooth and phase 0 of the action potential. Calibrations are shown for voltage (millivolts) and maximum rate of rise of voltage (volts per second) in each panel. Time calibrations were obtained from a time mark generator (Type 184, Tektronix) and were displayed on the zero potential reference line.

The signals displayed on the cathode ray oscilloscope were viewed directly through a
camera mount (Reflexor Camera Mount, Bio-electric Instruments) and from the screen of a closed circuit television monitor. Recordings were made on 35-mm film with a kymograph camera (C4, Grass) or on Polaroid film from the face of a slave oscilloscope (564 RM, Tektronix).

**Results**

**EFFECT OF DPH ON ACTION POTENTIAL CHARACTERISTICS**

In concentrations of $10^{-8}$ to $10^{-6}$ M, DPH had little effect on the resting potential and overshoot of normal Purkinje fibers stimulated at constant rates, and the rate of rise of phase 0 was either unaffected or slightly decreased. However, the action potential duration shortened prominently, i.e., by 40 to 80 msec at a drive cycle length of 800 msec. This shortening was due predominantly to an increased slope of phase 2 and varied with the concentration of the drug. In $10^{-8}$ to $10^{-6}$ M DPH, the shortening of phase 2 was prominent, and little change was noted in the later phases of repolarization. At higher concentrations of DPH ($10^{-6}$ and $10^{-5}$ M), the slope of phase 2 increased still further, the inflection between phases 2 and 3 became less distinct, and the slope of phase 3 repolarization was decreased so that the voltage-time course of repolarization assumed a smooth upward concavity. Occasionally, at concentrations higher than $10^{-5}$ M, the duration of phase 3 was so prolonged that the total action potential was longer than with lower concentrations. The shortening was less when commercial diluent was used.

Figure 3 shows the effect of DPH on the duration of the action potential of Purkinje fibers. Open circles show measurements made on a number of different Purkinje fibers under control conditions; solid circles indicate measurements made on the same fibers during exposure to DPH in a concentration of $10^{-6}$ M. Note the decrease in action potential duration at any given cycle length during exposure to DPH.
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Figure 4 shows that the difference between control and test values was greater at slower rates. All data shown in Figures 3 and 4 were obtained from fibers perfused at 37°C. In similar experiments at a temperature of 29°C, the action potentials were longer under control conditions, and the shortening during DPH administration (10⁻⁸ to 10⁻⁶ M) was much more marked, in some instances being as much as 160 msec.

EFFECT OF DPH ON THRESHOLD FOR ELECTRICAL STIMULATION AND CONDUCTION

The current requirement for stimulation was estimated for 12 Purkinje fibers and, for ventricular muscle, in five experiments. In each case the threshold for stimulation was reduced by 20 to 60% during exposure to 10⁻⁸ to 10⁻⁶ M DPH.

The conduction velocity of normal preparations was not significantly affected by DPH. It decreased by a very small amount in fibers in which DPH caused a slight decrease in the rising velocity of the action potential. Observations on conduction velocity were also made for a group of fibers which were partially depolarized by stretch, cold, hypoxia, or ouabain administration and whose action potentials displayed decreased rates of rise of phase 0. Under these conditions, conduction velocity usually is slowed, and automaticity may be enhanced; arrhythmias are often noted in such preparations. Under these conditions, DPH (10⁻⁸ to 10⁻⁶ M) had more marked effects. The resting potential, amplitude of action potential, rate of rise of phase 0, and conduction velocity all increased and, in some instances, to a remarkable degree. Figure 5 shows the effect of DPH on a partially depolarized Purkinje fiber. This state was induced by prolonged exposure to in vitro conditions and cooling to 29°C. It can be seen that as the rate of rise and amplitude increased and action potential duration decreased, there was a pronounced decrease in conduction time. Records were obtained at low and high sweep velocities while the preparation was driven at a constant cycle length of 1200 msec. The maximum rate of rise (dv/dt) of phase 0 of the action potential in volts per second is indicated by the amplitude of the differentiated spike on the bottom trace of the record taken at low sweep velocity. The second trace from the bottom is a surface electrogram recorded from the Purkinje fiber 2 cm from the site of the microelectrode. The control records are shown in panel A, and the records taken during exposure to 10⁻⁹ M DPH are shown in panel B.

Figure 6 shows records from an experiment done at 37°C on an unbranched partially depolarized Purkinje fiber. Two microelectrodes were introduced into cells of the fiber, and bipolar electrodes were placed on the

A CONTROL

B DPH 10⁻⁸ M

FIGURE 5
Effect of DPH (10⁻⁸ M) on a partially depolarized Purkinje fiber. The fiber was studied at (29°C). See text for discussion.

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FIGURE 6
Effects of DPH on conduction velocity in a partially depolarized Purkinje fiber. See text for discussion.
fiber half the distance between the microelectrodes. Improvement of the electrical characteristics of this fiber during exposure to DPH led to a decrease in conduction time without any change in sequence of activation. The fiber was stimulated at one end at a cycle length of 850 msec. Transmembrane potentials were recorded through microelectrodes at sites near to (upright action potential) and distant from (inverted action potential) the site of stimulation. The surface electrogram was recorded through bipolar silver electrodes placed halfway between the two microelectrodes. Slow recordings made on moving film are shown in panels A and C. Records made at a high sweep velocity, panels B and D, show details of the depolarization sequence, the interelectrode conduction time (interval between the action potential upstrokes), and the time relationship of the surface electrogram to the upstrokes of the proximal and distal action potentials. The maximum rate of rise of phase 0 of the action potential is represented by the amplitude of the differentiated spike on the bottom trace. Note the slow conduction under control conditions, indicated by the distance between the action potential upstrokes in panel B, and the increase in conduction velocity in the presence of 10^-6 M DPH, indicated by the narrowing of the distance between the action potential upstrokes in panel D. The sequence of activation of the fiber, from proximal microelectrode to electrogram to distal microelectrode, is, in both cases, normal.

Similar results were obtained in studies of other depolarized fibers, regardless of what intervention was used to induce the state of depolarization.

**EFFECT OF DPH ON FREQUENCY, DEPOLARIZATION VELOCITY RELATIONSHIP**

The relationship between the maximum upstroke velocity of Purkinje fibers and the rate of stimulation was evaluated on one cell which remained impaled for the entire sequence in 15 experiments. The basic drive cycle length was varied from 2000 to 200 msec. After each change in driving frequency, the rate of rise was observed until it became stable; then records were taken. Most fibers did not begin to show significant decreases in the rate of rise of phase 0 until the cycle length was shortened to 400 msec. Under control conditions, the fibers failed to follow the drive with a 1:1 response at cycle lengths below 200 msec when the stimulus was set at one and one-half to two times threshold. Most fibers showed an oscillation in dv/dt of phase 0 when driven at rapid rates. The reading of dv/dt under these conditions was taken as the average of the range. After DPH administration, there was little change in the cycle length at which dv/dt of phase 0 fell or the time taken to return to normal dv/dt on switching from short to long cycles. The maximum follow rate was, however, increased.
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In five experiments the action potential was noted to have a normal rate of rise at long drive cycles under control conditions, but, as the cycle length was decreased, the upstroke velocity fell abruptly. Figure 7 illustrates findings from a fiber typical of this group. This fall in dv/dt was not due to the encroachment of stimuli on incomplete repolarization from the previous action potential because, at a cycle length of 800 msec, repolarization was complete well before the next stimulus occurred. After addition of DPH, the dv/dt at long cycle lengths was unchanged. However, the decrease in upstroke velocity was not noted until much shorter cycle lengths were reached than under control conditions. In this type of fiber, the rate at which the fiber followed a rapid driving stimulus with 1:1 responses was much increased in the presence of DPH, and less time was required for the action potential to resume normal dv/dt after sudden increases in the length of the drive cycle than under control conditions. In fibers with action potential having low upstroke velocities even at long cycle lengths, a rapid fall in dv/dt was always noted as the cycle length was shortened. The rate of rise of the action potential was clearly increased in such fibers, even at slow driving rates, during exposure to DPH.

EFFECT OF DPH ON “MEMBRANE RESPONSIVENESS”

Previously studied antiarrhythmic drugs have profound depressant effects on the relationship between the rate of rise of phase 0 of the action potential and the membrane potential from which the action potential arises. The effects of DPH on this relationship were studied in 12 fibers. The observations in each case were made from a single impalement. Very little effect was seen in normal fibers until very high concentrations (5 x 10^{-5} to 10^{-4} M) of DPH were employed. At such concentrations the curve relating dv/dt to membrane potential was, in some cases, shifted to the right, indicating a small decrease in the “membrane responsiveness.” In fibers which were slightly to moderately depressed, the curve was uniformly shifted to the left during exposure to DPH, indicating an increase in membrane responsiveness. The magnitude of the shift in the curve to the left was greatest at concentrations of 10^{-8} to 10^{-6} M, and the curve slowly moved back toward its control position as the concentration of DPH was increased to 10^{-4} M. By the time this concentration had been reached, the curve was only slightly to the left of its control position. In several experiments, when the curve was prominently displayed to the left by DPH, normal Tyrode solution was allowed to perfuse the fiber for an hour, and the relation-
ship was determined once again. In normal Tyrode the curve approached control values after one hour. A second perfusion with Tyrode containing DPH caused a second shift of the curve to the left. In three fibers a single impalement was held long enough to repeatedly assess this relationship, and in each case consistent shifts in the curve, as previously described, were seen when DPH was added or withdrawn. It was often noted during this procedure that during perfusion with DPH the fiber could not be stimulated at the lower membrane potentials (below about 65 to 70 mv) so that the lower portion of the curve often could not be obtained in the presence of the drug.

Figure 8 demonstrates the effect of DPH on the rate of rise of phase 0 of premature action potentials in relation to the membrane potential from which arose the premature action potential, or membrane responsiveness. This study was conducted on a slightly depressed fiber. Examination of the control plot of upstroke velocity as a function of membrane potential reveals that the line has a decreased slope when compared with curves from normal fibers. Determinations were made on action potentials elicited at selected levels of membrane potential by test stimuli applied at selected times during and after repolarization.

After adding DPH, the points on the curve were displaced to the left, indicating that the upstroke velocity of test action potentials, arising from any given membrane potential, was greater than under control conditions. Also the slope of the line describing this relationship was steeper during DPH perfusion. It was noted in this and several other experiments that there was less scatter of points on the steep portion of the curve after DPH than under control conditions.

In initial experiments with DPH dissolved in commercial diluent, the curve relating the rate of rise of phase 0 to the membrane potential shifted to the right more frequently than for fibers perfused with DPH dissolved in saline. Therefore, the effects of diluent alone were determined in three experiments using a concentration equal to that present when 10^-5 to 10^-4 M DPH perfused the fiber. Figure 9 shows the typical effect of this concentration of diluent on membrane responsiveness. During exposure to diluent, the curve shifts to the right, and the maximum dv/dt achieved when the depolarization arose from a normal resting membrane potential was depressed. Action potentials were elicited in the same manner as in the experiment shown in Figure 8. The solid curve drawn through the open circles represents control data. The broken curve drawn through the solid triangles represents measurements made after exposure for twenty minutes to the commercial diluent supplied with DPH for parenteral use (ethanol, 10%, and

![Figure 9](http://circres.ahajournals.org/)

Effect of diluent on the relationship between maximum rate of rise (dv/dt) of action potentials, in volts per second (v/sec), and the level of membrane potential in millivolts (mv). See text for discussion.

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propylene glycol, 40%). Note the shift of the curve to the right and the decrease in the maximum rate of rise of the action potential phase 0 at normal membrane potentials after exposure to the diluent.

**EFFECT OF DPH ON EFFECTIVE REFRACTORY PERIOD**

The effective refractory period (ERP) of the isolated Purkinje fiber was obtained by analyzing the responses to stimulation through a bipolar surface lead. Test stimuli were applied at selected intervals during every seventh drive cycle. Responses were recorded through a microelectrode placed in a cell within 1 mm of the stimulating electrodes. The test stimulus was moved earlier in the cycle until the earliest test stimulus eliciting a propagated response was determined (ERP). These measurements were repeated during DPH infusion.

The ERP consistently shortened after exposure to DPH in concentrations from $10^{-8}$ to $10^{-5}$ M. The decrease became progressively less pronounced in increasing concentrations, and at $10^{-4}$ the ERP usually was only slightly less than the control value. This was

**FIGURE 10**

Effect of DPH on action potential duration and ERP. Superimposed action potentials are shown. The solid line represents an action potential recorded under control conditions; the broken line is an action potential recorded in the presence of DPH ($10^{-7}$ M). The upward spikes on the bottom trace indicate the do/dt of phase 0 of the earliest propagated action potential under control conditions (solid line) and during perfusion with DPH (broken line). Solid circles on each action potential represent the ERP, the earliest point at which a propagated action potential could be elicited by a test stimulus. Stimulus parameters were identical under both conditions. The triangle on the action potential recorded during DPH perfusion indicates the point at which the ERP would have been expected if it occurred at the same membrane potential as under control conditions. The relationship between ERP and action potential duration changed during DPH perfusion so that, although the ERP shortened, it was longer relative to the time course of repolarization than under control conditions. The earliest propagated action potential was thus initiated at a higher membrane potential and had a greater amplitude and do/dt of phase 0. ERP = effective refractory period.
similar to the effects of the drug on action potential duration. However, it was noted that the abbreviation of the ERP was relatively less than the shortening of action potential duration. This relationship was noted in all the preparations studied and over a wide range of drug concentrations. The action potential shown in Figure 10 shortened 80 msec during DPH perfusion; the ERP shortened by 25 msec. Under control conditions the earliest action potential was elicited by the test pulse at a membrane potential of 59 mv and had a rate of rise of 160 mv. However, a striking finding was obtained during perfusion with DPH. The lowest membrane potential at which a propagated action potential could be elicited was at 71 mv; this extrasystole had a rate of rise of 350 v/sec. Thus, the change in the ERP was less than the change in action potential duration. How this combination of effects might explain, in part, the effectiveness of the drug as an antiarrhythmic agent will be discussed.

EFFECTS OF DPH ON AUTOMATICITY

The effects of DPH on automaticity were observed in studies on Purkinje fiber preparations which included: (1) fibers which were beating automatically at stable rates with normal action potentials; (2) automatic fibers which had developed generalized diastolic depolarization so that their action potentials were low in amplitude and upstroke velocity; (3) fibers in which catecholamines or digitalis had enhanced automaticity.

Fibers having normal action potentials and beating spontaneously responded to DPH with a decrease in the spontaneous rate due to a decrease in the rate of phase 4 depolarization. In many preparations even though the action potential had a normal amplitude and a rate of rise during regular stimulation, generalized diastolic depolarization developed when stimulation was slowed or stopped. The generalized diastolic depolarization led to a decrease in the amplitude and the rate of rise of phase 0. These changes were not merely time-dependent. This fact was established by stimulating the fiber early after a spontaneous depolarization or by hyperpolarizing the fiber with an electrical pulse. Both of these maneuvers allowed the fiber to fire from a higher membrane potential. Under these circumstances, fibers showing generalized diastolic depolarization had a more nearly normal amplitude and rate of rise of phase 0, which was appropriate to the membrane potential at the time of activation. Figure 11 shows the effect of DPH on such a fiber. After exposure to DPH, there was a decrease in the rate of phase 4 depolarization. Maximum diastolic potential increased, and a reversal of the generalized diastolic depolarization occurred. These changes led to an increase in action potential amplitude, an increase in the rate of rise of phase 0, and the development of an overshoot. Further increases in DPH concentration led to cessation of spontaneous firing with maintenance of a normal resting potential and normal excitability. Similar effects of DPH were noted in eleven such experiments.

DPH had similar effects on fibers in which automaticity had been enhanced by exposure to ouabain or isoproterenol. One additional observation was made in studies utilizing isoproterenol. Catecholamines usually have marked restorative effects on in vitro Purkinje fibers having normal action potentials and beating spontaneously responded to DPH with a decrease in the spontaneous rate due to a decrease in the rate of phase 4 depolarization. In many preparations even though the action potential had a normal amplitude and a rate of rise during regular stimulation, generalized diastolic depolarization developed when stimulation was slowed or stopped. The generalized diastolic depolarization led to a decrease in the amplitude and the rate of rise of phase 0. These changes were not merely time-dependent. This fact was established by stimulating the fiber early after a spontaneous depolarization or by hyperpolarizing the fiber with an electrical pulse. Both of these maneuvers allowed the fiber to fire from a higher membrane potential. Under these circumstances, fibers showing generalized diastolic depolarization had a more nearly normal amplitude and rate of rise of phase 0, which was appropriate to the membrane potential at the time of activation. Figure 11 shows the effect of DPH on such a fiber. After exposure to DPH, there was a decrease in the rate of phase 4 depolarization. Maximum diastolic potential increased, and a reversal of the generalized diastolic depolarization occurred. These changes led to an increase in action potential amplitude, an increase in the rate of rise of phase 0, and the development of an overshoot. Further increases in DPH concentration led to cessation of spontaneous firing with maintenance of a normal resting potential and normal excitability. Similar effects of DPH were noted in eleven such experiments.

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fibers which show marked, generalized diastolic depolarization. In the presence of DPH, isoproterenol maintained this ability. It was further noted that in the presence of DPH, high concentrations of isoproterenol (up to $5 \times 10^{-6}$ M) caused only small increases in rate. Thus, DPH not only allowed extremely high concentrations of catecholamines to be given without toxic effects, but also it dissociated the hyperpolarizing effect from the chronotropic effect.

**Discussion**

Weidmann (13) studied a number of drugs (cocaine, procaine amide, quinidine, and diphenhydramine HCl) having local anesthetic actions, several of which are used to protect hearts against extrasystoles and fibrillation. Employing these drugs in rather high concentrations, he found that they had several properties in common: (1) spontaneous activity was abolished; (2) the membrane potential of resting fibers did not drop below 72 mV before impulse conduction was blocked; (3) the size of the "overshoot" and the rate of rise of the action potential were greatly decreased for a given resting potential. Since DPH had been found extremely effective in abolishing ventricular arrhythmias, and since all previous antiarrhythmic agents including pronethalol and propranolol (14) have quinidine-like properties, it was assumed that most of these quinidine-like effects could be demonstrated for DPH. This assumption is largely unsubstantiated.

Purkinje fibers with characteristics normal for in vitro conditions were observed for long periods of time (up to 12 hours) while being perfused with high concentrations of DPH (up to $10^{-4}$ M). The usual result was little or no change in resting potential, overshoot, or rate of rise of the action potential. This finding contrasts markedly with those of Weidmann (13) obtained by using quinidine (10 $\mu$g/ml) and procaine amide (50 $\mu$g/ml) to perfuse Purkinje fibers. He found that during 1 to 2 hours of drug dosage, the resting membrane potential decreased, and the rate of rise and overshoot of the action potential for any given membrane potential progressively decreased. Similar findings have been obtained in guinea pig and rabbit right ventricular muscle fibers (15) and rabbit atrium (16, 17).

It has been observed that the amplitude and rate of rise of cardiac action potentials may be frequency-dependent and that the demonstration of drug effects on rate of depolarization in cardiac tissues depends on the rate of stimulation during study (15 and 18-20). In the case of quinidine, it has been demonstrated (16, 19) that "normal" values for maximum rate of depolarization can be obtained if the tissue is driven at a low rate; also, early in the course of exposure to quinidine, cardiac fibers showed a greater decline in maximum rate of depolarization than in action potential amplitude. When driven at decreasing cycle lengths, the maximal rate of depolarization falls off much more quickly in the presence of quinidine. In this study the effect of DPH on rate of rise of the action potential as a function of driving frequency showed one of two responses: (1) the relationship was relatively unchanged compared to control, or (2) the rate of rise of the action potential actually declined less as a function of frequency than under control conditions. Thus DPH, in contrast to quinidine, either did not increase or even decreased the frequency-dependent changes in depolarization rate.

Weidmann (13) studied the effects of quinidine and procaine amide on the relationship between the maximum depolarization rate and the membrane potential at which a Purkinje fiber was activated. He found that this relationship shifted to the right, i.e., at a given membrane potential the maximum rate of depolarization obtained was much less during quinidine or procaine amide perfusion than under control conditions. He suggested that this indicated that these drugs largely inactivated the sodium carrying system at a relatively high membrane potential (21). DPH had little effect on this relationship when studies were conducted on fibers which were considered normal. Only slight shifts to the
left or right were seen. In fibers that had low rates of depolarization or were partially depolarized, however, there was often a striking shift in the curve to the left, an increase in the slope of the curve, and occasionally an increase in the maximum depolarization rate attained at the highest membrane potential. These findings suggest that, in those fibers which have become partially depolarized, DPH partially reverses the inactivated state of the sodium carrying system.

Two other related differences between the action of quinidine-like drugs and DPH were seen. One was the effect of DPH on conduction velocity. Quinidine is known to depress the velocity of conduction in Purkinje fibers and ventricular muscle. DPH, on the other hand, either had little effect or increased the conduction velocity in Purkinje fibers. When amplitude and rate of rise were relatively unaffected by DPH, the conduction velocity was unchanged; when amplitude and velocity of depolarization were increased, as occurred in partially depolarized fibers during DPH perfusion, the conduction velocity was increased. Since amplitude and rate of rise of the action potential are important determinants of conduction velocity, these results are to be expected.

Another difference between the actions of DPH and the quinidine-like antiarrhythmic drugs is the effect on stimulation threshold. The quinidine-like drugs cause an increase in diastolic threshold in both mammalian atrium and ventricle. It has been suggested that this may be related to the ability of quinidine to slow “complete recovery” in cardiac muscle after depolarization. DPH usually lowered the threshold for stimulation in Purkinje fiber preparations. Similar observations have been made in situ ventricular and atrial muscle. If a slow process of “complete recovery” after depolarization exists, it may be that DPH accelerates it in a manner similar to its effect of shortening the time course of repolarization of the action potential.

DPH was noted to consistently accelerate repolarization of Purkinje fibers. Although the mechanism by which this is accomplished has not been demonstrated, there are many possibilities. Either an increase in the rate of rise of potassium conductance after depolarization or an increase in the rate of fall of sodium conductance during the plateau would give the observed result.

It is of great interest to note this shortening of the action potential by an agent which demonstrates antifibrillatory actions. Agents which shorten the action potential previously have been associated with an increased likelihood of extrasystoles and fibrillation, in both atria and ventricles. Burn et al. discussed atrial fibrillation induced by electrical stimulation or aconitine in the presence of acetylcholine. They felt that fibrillation was due to different cardiac fibers getting out of phase in the presence of shortened repolarization so that re-excitation could occur. They felt that any factor which prolonged repolarization would arrest this process. In their discussion of effects of calcium and cyclopropane on Purkinje fibers, Tempte et al. noted that cyclopropane produced a calcium-dependent acceleration of repolarization. They reasoned that because the functional refractory period of Purkinje fibers usually persists until the membrane potential repolarizes to approximately —60 mv, the functional refractory period of Purkinje fibers might be shorter during administration of cyclopropane. Furthermore, they suggested that shortening of the refractory period, per se, was a condition favoring the initiation and maintenance of re-entrant activity, and that the increased ventricular irritability observed during cyclopropane anesthesia was due to this shortening. It should be emphasized, however, that neither group included any measurements of the functional refractory period under their experimental conditions; a shortening of the functional refractory period was assumed because of the acceleration in repolarization. The findings in the present study have shown that DPH shortens both the time course of repolarization and the effective refractory period of Purkinje fibers.
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Purkinje fibers. These might seem to be paradoxical properties of an agent which has been shown to decrease the likelihood of re-entrant activity and ventricular fibrillation under many circumstances, including arrhythmias induced by cyclopropane and catecholamines.

An answer to this apparent paradox may be found in a further consideration of the relationship between the shortening of the effective refractory period and the acceleration of repolarization caused by DPH. Normally, near the very end of repolarization, fibers recover their excitability (ability to respond to monopolar cathodal stimuli) at a time when repolarization is not quite complete. Because the fiber has not completely repolarized, less additional depolarization may be required from the stimulating cathode to bring the fiber to threshold. Any action potential evoked by stimulation before completion of full repolarization, even if evoked in the period of supernormality, is abnormal in terms of the rate of rise of phase 0, amplitude, and general configuration. Such an action potential will either propagate with reduced velocity or will "wait" at its site of origin moving very slowly until the surrounding tissue has repolarized further (23, 31). During DPH administration the time course of repolarization shortens more than the time course of the recovery of excitability. Although this change is rather small, it has the effect of shifting the earliest response to stimulation to a higher membrane potential. This has the effect of increasing both the amplitude and velocity of depolarization of extrasystoles, and therefore the conduction velocity of premature action potentials elicited by stimuli applied during the relatively refractory or supernormal periods is also increased. Thus these action potentials not only become more effective stimuli at the outset, but they propagate into tissue that is more fully repolarized than would have been the case under control conditions. The fact that the recovery of excitability is shortened to a lesser extent than the time course of repolarization would also, in the whole heart, tend to reduce the complications arising from the marked temporal variations in recovery of membrane polarization and excitability at different sites.

Again, DPH differs in this respect from drugs having quinidine-like actions. Quinidine tends to lengthen the time course of depolarization rather than shorten it. However, in certain concentrations quinidine can be seen to prolong the recovery of excitability more than it prolongs the action potential (32). This tends to bring the relationship between recovery of resting potential and recovery of excitability into a relationship similar to that seen with DPH. This one similarity between the actions of DPH and quinidine may point to the crucial nature of the relationship between recovery of normal membrane potential and recovery of excitability and the importance of changes in this relationship in determining the effectiveness of a drug as an antiarrhythmic agent.

Any attempt to understand an antiarrhythmic drug must include a consideration of its effects on automaticity. Automaticity has been carefully defined by Hoffman and Cranefield (33).

It has been pointed out by Hoffman that it is likely that any arrhythmias due to enhancement of normal automaticity will be associated with some local decrease in conductivity (34, 35). This results from the fact that if enhanced automaticity is due to increased phase 4 depolarization, the resulting decrease in diastolic membrane potential, if of sufficient magnitude, will not only decrease the amplitude and velocity of depolarization but also slow conduction and even produce block (34).

The effect of DPH on normal fibers was to moderately decrease the slope of phase 4 depolarization without any notable effect on resting potential or threshold potential. In fibers which were partially depolarized and automatic, in addition to the effect on phase 4, there was an increase in the maximum diastolic potential during exposure to DPH. These fibers, therefore, gave rise to action potentials which had greater amplitude and rate of rise during exposure to DPH. The extent of the
decrease in the slope of diastolic depolarization ranged from slight, in most instances, to complete in an occasional fiber which showed cessation of automatic firing. Although there was cessation of automaticity, responses still could be obtained with electrical pulses, demonstrating that the fibers were excitable although not automatic. In the partially depolarized fibers, although the increase in maximum diastolic potential caused by DPH may play an important role in decreasing firing rate, the decrease in slope of phase 4 depolarization seems to be the most important factor.

When fibers had been exposed to toxic concentrations of ouabain or catecholamines or were partially depolarized due to stretch or prolonged in vitro conditions, DPH caused an increase in maximum diastolic potential and a decrease in phase 4 depolarization. Consequent to these changes, the rate of firing decreased. Despite slower rates in the presence of DPH, these fibers almost invariably fired from a higher membrane potential than under control conditions; the action potential consequently had a greater amplitude and rate of rise of phase 0. If the decrease in membrane potential before exposure to DPH had been great enough to cause a decrease in conduction velocity or altered sequence of activation, these changes were reversed as the maximum membrane potential, and membrane potential at which the fiber fired increased. Also, administration of catecholamines to depolarized fibers in the presence of DPH allowed their hyperpolarizing action to occur with only minimal increases in automaticity.

When compared to the quinidine-like drugs, the effects of DPH on automaticity seem less marked. DPH seldom abolished automaticity completely; this was so especially in preparations which had been unstimulated throughout the study. Quinidine frequently has this effect and may cause Purkinje fibers to become inexcitable. We saw no evidence of DPH-induced inexcitability, nor with DPH did we see the depolarization and increased automaticity that occur in Purkinje fibers exposed to toxic levels of quinidine or procaine amide. It should be pointed out, however, that because of its limited solubility we did not study concentrations of DPH in excess of $10^{-4}$ M.

Many of the observed effects of DPH would be expected to decrease the likelihood of re-entrant rhythms. It has been pointed out (36, 37) that the primary determinants of a re-entry path are the refractory period of the constituent fibers of the path and the conduction velocity in the path. Hoffman et al. (38) have pointed out that the existence of decremental conduction not only explains occurrence of functional block in any part of the conducting system but also, because of the slowed conduction, removes any requirement for a minimum path length in re-entry. Hoffman and Cranefield (33) have pointed out that two aspects of the electrical activity of specialized cardiac fibers appear to be involved in the production of arrhythmias due to conduction disturbances, decremental conduction, and unidirectional block.

We studied Purkinje fibers which, for a variety of reasons, had arrived at the state in which they showed the properties associated with decremental conduction. Fibers in this condition, whether driven or spontaneously beating, almost invariably responded to DPH with an increase in amplitude and rate of depolarization of the action potential and a decrease in stimulus requirement. When these changes occurred, conduction velocity invariably increased. This increase in conduction velocity would make the minimum required length for the pathway of re-entry much longer than under original conditions. Even small increases in conduction velocity would be expected to abolish the conditions needed to sustain a re-entrant rhythm.

The effects of DPH on Purkinje fibers under in vitro conditions seems sufficient to suggest an explanation for its antiarrhythmic effects on ventricular arrhythmias in vivo. Most important in this regard would seem to be its effects on: (1) the relationship between action potential duration and refractory period duration, (2) automaticity, and (3) par...
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Electrophysiological Effects of Diphenylhydantoin on Canine Purkinje Fibers
J. THOMAS BIGGER, Jr., ARTHUR L. BASSETT and BRIAN F. HOFFMAN

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