Actions of Diphenylhydantoin on the Electrical Properties of Isolated Rabbit and Canine Atria

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ABSTRACT

The effects of diphenylhydantoin (DPH), 10^{-4} to 10^{-6} M, were studied on the various types of cells in the isolated right atrial preparation and on the specialized cells of Bachmann's bundle in atrial preparations obtained from puppies. High concentrations of DPH (10^{-3} M) slowed sinoatrial rate but lower concentrations had little effect. These chronotropic effects of DPH were direct. DPH in concentrations above 10^{-4} M decreased the slope of phase 4 depolarization in cells in the sinoatrial node and venous automatic tissue. DPH in concentrations of 10^{-4} to 10^{-5} M had no effect on transmembrane action potentials recorded from any cell studied; concentrations of 10^{-5} M, however, prolonged the terminal phase of repolarization. High concentrations (10^{-4} M) also prolonged the effective refractory period of Bachmann's bundle and ordinary atrial and perinodal fibers by an average of 10%. DPH markedly increased the dv/dt of phase 0 of the action potential and membrane responsiveness of ordinary atrial and specialized Bachmann's bundle fibers under control conditions and produced more striking increases when these two variables had been decreased by ouabain. DPH was also proved capable of reversing ouabain-induced sinoatrial block. This unique ability of DPH to enhance membrane responsiveness and to improve conduction in the absence of significant effects on the effective refractory period and automaticity of atrial cells suggests that this may be the sole mechanism of its antiarrhythmic action.

ADDITIONAL KEY WORDS automaticity transmembrane potentials effective refractory period antiarrhythmic activity resynchronization membrane responsiveness acetylcholine atropine catecholamines

Recent reports indicate that diphenylhydantoin sodium (DPH) is effective in abolishing many ventricular arrhythmias (1-11). Among the atrial arrhythmias, only supraventricular tachycardia, paroxysmal atrial tachycardia, and digitalis-induced atrial arrhythmias usually respond to DPH therapy (6, 8, 10, 11). These observations suggest that DPH does not affect atrial tissue to the same extent as ventricular fibers or that the mechanisms responsible for atrial and ventricular arrhythmias differ. In addition, although DPH is less effective in the treatment of supraventricular arrhythmias than either quinidine or procainamide, DPH is effective in abolishing cocaine-induced arrhythmias which fail to respond to quinidine (12). This may mean that the mechanisms of antiarrhythmic action of DPH differ from that of quinidine and procainamide.

Clinical and laboratory studies suggest that some of the cardiac effects of DPH may be mediated through the autonomic nervous system (10, 13, 14, 15, 16). Williams reported that oral anticonvulsant doses of DPH caused...
sinus bradycardia in two patients presumed to have normal hearts (17). Unger et al. (18). Colleran et al. (19), and Conn (6) have reported bradycardia or atrioventricular block after attempts to convert atrial flutter or supraventricular tachycardia with intravenously administered DPH. Tachycardia occurring in awake dogs following intravenous administration of DPH has been interpreted as being due to an anticholinergic effect of this drug (16). On the other hand, Baudouin et al. (14) ascribed vagomimetic actions to DPH to account for part of its effects on the heart.

Little information is available on the effects of DPH on electrophysiological properties of isolated atrial tissue, a preparation which is free from extrinsic autonomic influences. The present study was initiated to provide information on the effects of DPH on the many types of atrial cells and on sinoatrial (S-A) nodal rate. Specific procedures were utilized to determine whether the effects of DPH on sinus rate were direct or resulted from an interaction with the autonomic mediators. Furthermore, experiments were performed to provide information on how DPH alters the effects induced by digitalis in this preparation.

**Methods**

Young mongrel dogs (1.5 to 3.0 kg) were anesthetized with pentobarbital sodium, 25 to 35 mg/kg iv or ip, and rabbits (1.8 to 2.5 kg) were stunned by a blow on the head. The hearts were excised quickly and dissected in cool modified Tyrode's solution. Preparations were then pinned to the wax-lined bottom of a 30-ml lucite tissue chamber. Preparations of canine atria containing Bachmann's bundle were studied by methods similar to those described by Wagner et al. (20), and the rabbit right atrial preparations were studied in a manner similar to that described by Young and co-workers (19). The modified Tyrode solution which continuously perfused the tissue chamber at a constant temperature and flow rate contained (in mM): NaCl, 137.0; KCl, 3.0; NaH₂PO₄, 1.8; CaCl₂, 2.7; MgCl₂, 2.7; dextrose, 5.5; and NaHCO₃, 12.0. All solutions were made with deionized, twice-distilled water. Oxygenation and control of pH were achieved by bubbling the Tyrode solution in the reservoir bottles and the tissue bath with a mixture of 95% O₂ and 5% CO₂. The tissues were maintained at 35.0°C ± 0.6°C during the experiment.

Drugs were added in increasing amounts to the reservoir bottles containing Tyrode's solution to provide the desired concentrations; the preparations were not washed free of the drug between concentrations. Recordings were obtained from the tissue under control conditions and after the effects produced by the test solution had stabilized. Powdered DPH was dissolved in alkaline distilled water and then further diluted in Tyrode's solution to final concentrations of 10⁻³ M to 10⁻⁸ M. Other drugs used in this study were: atropine, 10⁻⁶ M to 10⁻² M; propranolol, 1.2 X 10⁻⁴ M; MJ 1999 (4-[2-isopropylamino-1-hydroxyethyl] methane sulfonamide), 10⁻³ M; ouabain, 4 X 10⁻⁷ M; and isoproterenol HCl, 10⁻⁸ M to 10⁻⁴ M.

Although a few cardiac arrhythmias respond to plasma concentrations of DPH near 4 μg/ml and concentrations up to 30 μg/ml have been achieved in unsuccessful attempts to convert atrial flutter or fibrillation to normal sinus rhythm in man, the majority of cardiac arrhythmias in man respond at plasma concentrations between 10 and 18 μg/ml. Thus the therapeutic concentration range would be about 2 to 6 x 10⁻⁶ M. Toxic central nervous system symptoms occur at concentrations near 8 x 10⁻⁶ M. We feel, however, that it would be unjustified to compare these concentrations attained in clinical usage to concentrations utilized in these in-vitro studies since the protein binding, complex tissue distributional gradients, hepatic metabolism, and interferences of dose administration are all important in the intact animal or man, and all of these factors are absent in studies performed in the tissue bath. Exposure in-vivo and in-vitro concentrations would therefore be difficult to estimate in anything but the most general terms. For this reason, we have thought it best to study this drug over a wide range of concentrations.

Transmembrane potentials were recorded through machine-pulled glass microelectrodes filled with 3M KCl. The resistance of the microelectrodes was 15 to 35 megohms. The potentials were led through Ag-AgCl wires in contact with 3M KCl to amplifiers with high input impedance and variable capacity neutralization (Bionlectric Instrument Co., NV-1). The output from the NF-1 was fed into a differential amplifier and displayed on a dual-beam cathode-ray oscilloscope (Tektronix, RM-565). Extracellular records of electrical activity were obtained through close bipolar electrodes made of Tellurium-coated Ag wires. Signals were filtered and amplified with a differential amplifier and displayed on the dual-beam oscilloscope.
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The maximum rate of rise (dv/dt) of phase 0 of the transmembrane potential recorded from the sinus node was estimated from a tangent drawn to the maximum slope of phase 0; this determination was reproducible to within ±5% on any series of measurements. The maximum dv/dt of phase 0 of the transmembrane potential recorded from the other types of atrial fibers was obtained by electronic differentiation in a manner previously described (22). Ten- and 100-msec time marks provided by a time-mark generator (Tektronix type 184) were displayed on the oscilloscope trace. The oscilloscope image was viewed by a television camera and displayed on a monitor screen. The image on the oscilloscope was also viewed directly and simultaneously photographed with a camera (Grass Model C-4) on 35-mm film. The film was enlarged, and the magnified images were measured.

For analysis of S-A nodal rate, bipolar surface electrograms were recorded from the crista terminals of the spontaneously beating right atrial preparations of the rabbit. The signal was simultaneously displayed on the dual-beam oscilloscope and a slave oscilloscope (Tektronix type RM 564) and was used to trigger an electronic counter (Hewlett-Packard 522 DR). The average duration of 200 consecutive cycles was obtained and expressed to the nearest 0.1 msec. Transmembrane potentials were recorded from the S-A node to validate a 1:1 relationship between the electrical activity of the S-A node and the crista terminalis recording site. Rates were sampled under control conditions and following exposure to graded concentrations of DPH with and without pretreatment with isoproterenol, acetylcholine, atropine, propranolol, or MJ 1999. In addition, rates were recorded from atropinized, catecholamine-depleted preparations both before and after exposure to DPH. These preparations were obtained from rabbits pretreated with reserpine, 3.5 mg/kg im, 48 hours before they were killed (23).

Antegrade Stimulation.—In the cristal part of the S-A ring bundle was used in this study to denote cells located near the sinus node and between it and the crista terminals; these cells included those in the cristal part of the S-A ring bundle (21) and adjacent area of the crista terminalis. Transmembrane potentials recorded from these cells were characterized by a more rapid upstroke velocity during phase 0 than that recorded from sinus node fibers.

Definitions

Effective Refractory Period.—The end of the effective refractory period is the earliest time during repolarization when a propagated action potential can be evoked by a stimulus (24).

Membrane Reactivation.—Membrane reactivation is used to refer to the relationship of the interval between responses to S1 and S2 and the maximum dv/dt of phase 0 of the action potential evoked by S2. This relationship is used to describe recovery from refractoriness in the perinodal cell (see below).

Membrane Responsiveness.—Membrane responsiveness is used to refer to the relationship between membrane potential at the time of excitation by S1 and the maximum dv/dt of phase 0 of the resultant action potential. Thus in the former relationship the abcissa is the interval of the cycle in msec while in the latter the abcissa is the membrane potential in mV at the time of excitation.

Action Potential Duration.—Action potential duration was measured from the onset of depolarization to 90% repolarization.

Depressed Preparations.—Preparations which were classified as being depressed were characterized by a slow spontaneous rate of 60/min or less and by low values for the resting potential and amplitude and dv/dt of phase 0 of the transmembrane potentials recorded from the various types of atrial fibers. Such a state could be induced by the following experimental procedures: stretch; mechanical trauma; toxic concentrations of acetylcholine, propranolol, or potassium.

Perinodal Cell.—The term perinodal cell was used in this study to denote cells located near the sinus node and between it and the crista terminals; these cells included those in the cristal part of the S-A ring bundle (21) and adjacent area of the crista terminalis. Transmembrane potentials recorded from these cells were characterized by a more rapid upstroke velocity during phase 0 than that recorded from sinus node fibers.
TABLE 1
Effects of DPH on S-A Nodal Rate with and without Pretreatment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control</th>
<th>After drug</th>
<th>10^-8 M</th>
<th>10^-7 M</th>
<th>10^-6 M</th>
<th>10^-5 M</th>
<th>10^-4 M</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>(10)</td>
<td>117 ± 4.9</td>
<td>117 ± 4.9</td>
<td>117 ± 4.9</td>
<td>116 ± 5.5</td>
<td>112 ± 5.4</td>
<td>99 ± 4.5</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>(5)</td>
<td>118 ± 5.5</td>
<td>81 ± 7.0</td>
<td>113 ± 5.5</td>
<td>112 ± 5.7</td>
<td>111 ± 5.3</td>
<td>107 ± 5.3</td>
</tr>
<tr>
<td>Propranolol</td>
<td>(5)</td>
<td>113 ± 6.5</td>
<td>113 ± 5.5</td>
<td>113 ± 5.5</td>
<td>112 ± 5.5</td>
<td>112 ± 5.5</td>
<td>110 ± 4.3</td>
</tr>
<tr>
<td>Atropine</td>
<td>(5)</td>
<td>113 ± 5.5</td>
<td>125 ± 3.5</td>
<td>125 ± 3.5</td>
<td>124 ± 3.0</td>
<td>123 ± 2.7</td>
<td>109 ± 4.3</td>
</tr>
<tr>
<td>Atropine + propranolol</td>
<td>(7)</td>
<td>123 ± 8.5</td>
<td>117 ± 7.4</td>
<td>117 ± 5.5</td>
<td>118 ± 5.1</td>
<td>117 ± 5.1</td>
<td>116 ± 5.6</td>
</tr>
<tr>
<td>Atropine + M5199</td>
<td>(4)</td>
<td>137 ± 5.6</td>
<td>136 ± 6.0</td>
<td>130 ± 6.0</td>
<td>131 ± 4.9</td>
<td>128 ± 3.8</td>
<td>111 ± 2.4</td>
</tr>
</tbody>
</table>

Figures are mean rates ± SEM. Significant difference by t-test for paired samples.
*P < 0.025; †P < 0.01.

Repolarization of these fibers differed from other types of atrial fiber and a definite hyperpolarization terminated phase 3. Recovery from refractoriness in these cells was more dependent on time (membrane reactivation) than on membrane potential (Strauss, H. C., and Bigger, J. T., Jr., unpublished observations).

Results

The effect of DPH on the rate of the S-A node is shown in Table 1. Data are shown for several concentrations of DPH under control conditions and after exposure to acetylcholine, atropine, propranolol, M5199, isoproterenol, and reserpine. In ten untreated preparations, DPH in low concentrations (10^-8 to 10^-7 M) had no significant effect on the S-A nodal rate. High concentrations of DPH (10^-5 M) slowed the sinus rate by 16%. This decrease in heart rate noted during infusion of 10^-4 M DPH remained stable over a period of 2 hours. When DPH (10^-4 M and 10^-3 M) was added to two depressed preparations, sinus arrest occurred. In one other experiment DPH (10^-4 M) markedly intensified existing sinus bradycardia. This action of DPH was reversed by the addition of epinephrine (3 x 10^-5 M) to the tissue bath.

To evaluate the proposed anticholinergic properties of DPH (15, 16), we examined its effects on preparations exposed to constant concentrations of acetylcholine or a β-receptor blocking agent. In five experiments (Table 1) the slow S-A rate produced by perfusion with Tyrode’s solution containing acetylcholine (5.5 x 10^-6 M) was not altered by the addition of DPH in concentrations from 10^-8 to 10^-4 M. Furthermore, pretreatment with DPH (10^-5 M) failed to block the bradycardia caused by acetylcholine (5.5 x 10^-5 M). In the presence of propranolol (1.2 x 10^-5 M), low concentrations of DPH (10^-8 to 10^-7 M) exerted a slight depressant effect on rate. Under these same circumstances, high concentrations of DPH (10^-4 M) progressively slowed the sinus rate over a period of 1 hour to a rate 15% lower than that obtained following β-receptor blockade. This depressant effect on rate was similar to that obtained in the absence of β-receptor blockade.

In five experiments (Table 1), low concentrations of DPH (10^-4 to 10^-5 M) did not alter the sinus rate after treatment with atropine (2 x 10^-4 M). High concentrations (10^-3 M) progressively slowed the sinus rate over a period of 1 hour to a rate 14% lower than that obtained following treatment with atropine. In seven other sinus node preparations (Table 1) pretreated with atropine (2 x 10^-4 M) and either propranolol (1.2 x 10^-5 M) or M5199 (10^-4 M), low concentrations of DPH had an insignificant effect on the sinus rate; this action was similar to that seen in the untreated preparations. High concentrations of DPH progressively slowed the sinus rate over a period of 1 hour to a
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It should be noted that none of these pretreatment regimens significantly altered the response of the sinus rate to any given concentration of DPH.

The effects of DPH (10^-5 M) on the dose-response relationship for isoproterenol were examined using controls to adjust for changes in sensitivity of the effector system (25). Results obtained from six experiments (Fig. 1) indicate that DPH did not block the positive chronotropic effects of isoproterenol at any concentration.

The effects of a wide range of concentrations of DPH were studied using the atropine-treated sinus node from rabbits treated with reserpine. As can be seen in Table 1 the results were similar to those obtained from preparations pretreated with atropine and β-receptor blocker. At no time during the course of these studies was S-A block encountered even when marked sinus node depression had been caused by high concentrations of DPH.

TRANSMEMBRANE POTENTIAL CHARACTERISTICS

The effects of DPH (10^-4 to 10^-8 M) on transmembrane potentials recorded from several types of atrial fibers were evaluated in 16 experiments. Cells studied included those from the S-A node and perinodal area. The specialized atrial fibers of Bachmann’s bundle (20) and ordinary atrial fibers of the right atrial appendage were also studied (Fig. 2).

The slope of phase 4 depolarization of potentials recorded from the sinus node and adjacent venous automatic cells was unaffected by DPH at 10^-4 to 10^-6 M. A slight decrease in the slope of phase 4 was noted during exposure to a concentration of 10^-5 M and a greater decrease at 10^-6 M. Since the maximum diastolic potential and the slope of phase 4 depolarization were not significantly altered by DPH at concentrations from 10^-5 to 10^-7 M (Fig. 2), and since these concentrations did not change the sinus rate, it is most likely that the threshold potential of the sinus pacemaker cells was similarly unaffected.
Effects of DPH (10^-6M) on transmembrane potentials recorded from several types of atrial fibers. Records are shown of transmembrane potentials recorded from the S-A node (la and lb), a perinodal fiber (2a and 2b), and an ordinary atrial fiber (3a and 3b); all of these action potentials were recorded from rabbit right atrial preparations. The transmembrane potentials shown in 4a and 4b were recorded from a specialized atrial fiber in a canine Bachmann’s bundle preparation. Time marks are repeated every 100 msec in panel 1 and every 10 and 100 msec in the other panels. The middle traces record the transmembrane action potentials and the bottom trace of panels 2 through 4 shows, first, the calibrating sawtooth pulse and its first time derivative and then the first time derivative (dv/dt) of phase 0 of the action potential. The amplitude of the dv/dt of the calibration pulse represents 100 v/sec in panels 2 and 3 and 500 v/sec in panel 4. The resting potential, dv/dt of phase 0, duration and amplitude of the action potential, maximum diastolic potential and slope of phase 4 depolarization in the case of the S-A node (panel 1) were measured before (a in each panel) and during (b in each panel) exposure to DPH. Exposure to DPH did not alter these parameters in action potentials recorded from the sinus node and perinodal fibers. In panels 3a and 3b (ordinary atrial fiber), exposure to DPH increased dv/dt from 240 v/sec to 330 v/sec. In panels 4a and 4b, the action potential amplitude in the specialized fiber in Bachmann’s bundle increased slightly and the dv/dt increased from 600 v/sec to 800 v/sec during exposure to DPH.

Resting potential and amplitude and dv/dt of phase 0 of the action potentials recorded from the sinus node and perinodal area were unchanged by DPH at concentrations from 10^-8 to 10^-5M (Fig. 2). In concentrations greater than 5 x 10^-5M, DPH caused a small decrease in resting potential and amplitude and dv/dt of phase 0 of the action potential. In the case of the specialized Bachmann’s bundle fibers and ordinary atrial fibers, DPH (10^-4 to 10^-3M) caused an impressive increase in the dv/dt of phase 0 of the action potential without a significant effect on the other parameters (Fig. 2). This increase was more marked for cells whose transmembrane potentials gave lower than normal values for resting potential and amplitude and dv/dt of phase 0. Concentrations of DPH greater than 5 x 10^-5M did not further increase these values but instead were without effect or caused a small decrease in resting potential and amplitude and dv/dt of phase 0 of the action potential.

At concentrations from 10^-4 to 10^-3M, DPH had no effect on the configuration of phases 1, 2, and 3 of transmembrane potentials recorded from cells of the sinus node, the perinodal area, specialized Bachmann’s bundle fibers, and fibers of the appendage (Fig. 2). In the case of Bachmann’s bundle fibers and ordinary atrial fibers, concentrations of DPH greater than 5 x 10^-5M caused a very slight prolongation of the terminal phase of repolarization and thus action potential duration. The changes in action potential duration which normally are caused by changes in rate were unaltered by DPH; this is shown for ordinary atrial fibers (Fig. 3, A) and fibers of Bachmann’s bundle (Fig. 3, B).

EFFECTIVE REFRACTORY PERIOD

The effects of DPH on the effective refractory period of the various atrial cells were
studied in ten experiments. In low concentrations (10^{-8} to 10^{-6}M), DPH did not change the effective refractory periods of specialized Bachmann's bundle fibers, ordinary atrial fibers of the atrial appendage, or perinodal fibers. Higher concentrations (10^{-4} M) prolonged the effective refractory period of these various cells by 10% or less.

**MEMBRANE RESPONSIVENESS AND MEMBRANE REACTIVATION**

In 12 experiments we studied the effects of a wide range of concentrations of DPH on the membrane responsiveness of specialized fibers in Bachmann's bundle and ordinary atrial fibers and on membrane reactivation of perinodal fibers. Typical S-shaped curves relating dV/dt of phase 0 to membrane potential are illustrated for ordinary and specialized atrial fibers in Figures 4 and 5. In all experiments at concentrations of 10^{-4} to 10^{-5}M, DPH increased membrane responsiveness at any given level of membrane potential, as demonstrated by the shifts of the curve upward and to the left. A subsequent increase in concentration of DPH to greater than 5 \times 10^{-5}M shifted the S-shaped curves downward and to the right, and, in a few instances, to levels below those recorded under control conditions. Membrane reactivation of the perinodal fibers was insensitive to the effects of DPH at concentrations from 10^{-6} to 10^{-3}M (Fig. 6). In concentrations greater than 5 \times 10^{-5}M, DPH caused a small depression of membrane reactivation, with a small shift of the curve downward and to the right.

**DIGITALIS**

The effects of DPH on transmembrane potentials of atrial fibers exposed to high concentrations of ouabain were examined in five experiments. In each experiment, the transmembrane potentials and dV/dt of phase 0 were recorded during maintained impalements of fibers of the atrial appendage under control conditions, during exposure to ouabain, and to ouabain and DPH. The maximum dV/dt of phase 0 of the action potentials under control conditions was 124 \pm 16 v/sec.
Effect of DPH (10⁻⁶M) on the membrane responsiveness of a specialized Bachmann's bundle fiber. The abscissa shows the membrane potential at the time of excitation and the ordinate shows the maximum dv/dt of phase 0. Curves were plotted from data obtained under control conditions (solid circles) and during exposure to DPH (open circles). Note the marked shift of the curve upward and to the left during exposure to DPH signifying an enhancement of membrane responsiveness. Following washout of DPH, the curve was shifted downward and to the right.

(bain exposure by 113% to 151 ± 12 v/sec (P < 0.01) within 10 minutes.

The effect of DPH on ouabain-induced S-A block was examined in four experiments. Following exposure to ouabain (4 × 10⁻⁷M), conduction block developed between recording sites in the S-A node, crista terminalis, and right atrial appendage (Fig. 7). After addition of DPH (10⁻⁵M), the electrograms increased in amplitude, and conduction was progressively improved so that sinus impulses propagated to the recording sites in both the crista terminalis and right atrial appendage. This reversal of ouabain-induced block persisted until the experiments were terminated.

Discussion

The use of preparations of the sort studied in these experiments eliminates the necessity of considering reflex and centrally mediated autonomic influences in the interpretation of our results. However, it has been demonstrated that intra-atrial autonomic nerve terminals are plentiful in the rabbit heart and that these nerve terminals are capable of releasing their stored transmitters (26, 27, 28, 29), acetylcholine and norepinephrine, which produce their characteristic effects on interacting with appropriate receptors.

DPH had no significant effect on S-A nodal rate until high concentrations were employed. At these high concentrations (10^{-9}M), it caused a 16% decrease in S-A nodal rate. This could be attributed to: (a) a vagomimetic effect; (b) a sympatholytic effect; or (c) a direct effect. Our experiments exclude the first two possibilities. Rosati et al. (16) have postulated that the increase in heart rate and acceleration of atrioventricular conduction caused in dogs by DPH is due to its anticholinergic properties. However, we observed that the slow sinus rate produced by acetylcholine was not altered by the addition of DPH (10^{-8} to 10^{-9}M), nor did prior exposure to these concentrations of DPH block acetylcholine-induced sinus bradycardia. When β-receptor blockade was employed to remove any complicating sympathomimetic effects which might have been operating, a significant decrease in S-A nodal rate was seen at lower concentrations of DPH. This decrease was attributed to properties of propranolol other than that due to β-receptor blockade, since this finding was not present in the S-A node obtained from rabbits treated with reserpine. We also looked for possible sympathomimetic effects of DPH. In our experiments on the sinus node treated...
Effect of DPH (10^(-4)M) on ouabain-induced S-A block. Records of 100-msec time lines are seen in the top traces of each panel. Records of transmembrane potentials recorded from the S-A node and ordinary atrial fiber (ATRIUM) are seen in the second and third traces of the panels. Recordings made under control conditions (top panel) demonstrate the normal sequence of activation from the sinus node to the crista terminalis and atrial fiber recording sites. Within 40 minutes of onset of perfusion with ouabain (4 × 10^(-7)M), the cycle length in the spontaneously beating preparation lengthened from 460 to 490 msec, partial block developed between the sinus node and crista terminalis recording sites, and complete failure of impulse propagation to the atrial recording site occurred. Fifteen minutes after the addition of DPH to the ouabain perfusate, the cycle length increased to 855 msec, 1:1 conduction was reestablished to the crista terminalis recording site, the amplitude of the electrogram recording was increased, and one impulse was conducted to the atrial recording site (A arrow). Fifteen minutes later the cycle length had decreased to 690 msec and 1:1 conduction was reestablished to the atrial recording site (bottom panel). In addition, note the marked shortening of the atrial action potential presumably as a result of the vagal effects of ouabain. Furthermore, the time between the recorded sinus action potential and the atrial action potential decreased implying that the pacemaker had shifted, altering the sequence of activation seen under control conditions.

with atropine, there was no evidence of any positive chronotropic effect. The demonstrated lack of a positive chronotropic effect on atropine-treated preparations exposed to DPH indicates a lack of sympathomimetic effect. Thus, the tachycardia observed in awake dogs by Rosati et al. (18) and attributed to anticholinergic effects would seem more likely to be explained by a reflex response to the hypotensive effects of DPH administered intravenously or to pain and anxiety.

The decrease in S-A nodal rate seen during exposure to high concentrations of DPH could not be explained by a β-receptor blocking action of this drug (Fig. 2). Furthermore, DPH would not seem to block the release of norepinephrine from postganglionic symp-
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pathetic nerve terminals, since the decrease in S-A nodal rate seen with DPH was unchanged in experiments in which norepinephrine had been depleted by prior treatment with reserpine.

DPH has been cited as a cause of sinus bradycardia and cardiac arrest in man (10, 17, 18, 19) and in dogs (30). Examination of the reported cases of sinus bradycardia and cardiac arrest due to DPH therapy (10, 17, 18, 19) suggests that these effects are more common in patients with heart disease. We attempted to see if cardiac catecholamine depletion, which may occur in congestive heart failure (31), made the heart more sensitive to the actions of DPH. Results from our experiments do not substantiate this hypothesis since sinus slowing caused by DPH was only slightly more prominent in rabbits pretreated with reserpine than in controls. Experimental procedures which cause sinus-node depression, such as stretch, mechanical trauma, or toxic concentrations of acetylcholine, propranolol, or potassium, made the sinus node more susceptible to the slowing caused by toxic concentrations of DPH. In fact, sinus arrest was noted in two instances. However, S-A block was not seen during exposure to toxic concentrations of DPH, even in previously depressed sinus nodes. This finding contrasts markedly with the effects of other depressants of atrial tissue such as digitalis or potassium (32, 33).

Our results indicate that low and moderate concentrations of DPH had no significant effects on S-A nodal rate and that the decrease in S-A nodal rate seen with high concentrations results from a direct effect on the S-A nodal cells. When the sinus node had been depressed by experimental conditions, it became more susceptible to the negative chronotropic effects of DPH. DPH (10^-8 to 10^-5M) did not alter phases 1, 2, and 3 of the action potentials of any types of atrial cell studied, even though changes in action potential duration were sought over a wide range of stimulation rates (34). One explanation given for the shortening of action potential duration in Purkinje and ventricular muscle fibers is an increase of potassium conductance (g_K) (32). An increase in g_K in rabbit atrial fibers induced by exposure to acetylcholine has been shown to cause marked shortening of the action potential (35). It would thus seem that DPH has little effect on the g_K of atrial fibers. Several possibilities can be proposed to reconcile this apparent contradiction: 1) DPH-induced changes in g_K might be obscured by concomitant changes in conductances of other ions in atrial cells; 2) the shortening of action potentials recorded from Purkinje and ventricular muscle fibers may not be due to an increase in g_K, but to an increase of an ionic current such as Ca^{++}, which may be absent in atrial cells. This latter possibility is not entirely unreasonable since considerable inward current attributable to Ca^{++} has been shown to occur in voltage clamp experiments on sheep Purkinje fibers in low Na solutions (36). In addition, experiments by Lu et al. (37) on rabbit atrial muscle indicate that the Ca^{++} current is of relatively small importance in atrial cells.

The slope of phase 4 depolarization of potentials recorded from the sinus node and adjacent venous automatic tissue was unaffected by DPH in concentrations of 10^-8 to 10^-4M while high concentrations (10^-3M) caused some decrease in the slope. On the other hand, DPH decreased the slope of phase 4 depolarization in action potentials recorded from canine Purkinje fibers at a concentration of 10^-5M (22). Thus, automatocity in the isolated canine Purkinje fiber was more sensitive to this action of DPH than was the rabbit sinus node and adjacent venous automatic tissue. This greater sensitivity of automatocity in canine Purkinje fiber to DPH has been demonstrated for other cardioactive agents such as potassium (24). This suggests that DPH would be more effective in the treatment of automatic ventricular arrhythmias than automatic atrial arrhythmias. On the other hand, one can only speculate on the effects of DPH on phase 4 depolarization in ectopic atrial pacemaker cells, as we could
not find a suitable model for study of this problem.

Bigger et al. (22) have shown that DPH may cause a temporal dissociation between changes in action potential duration and the effective refractory period since the effective refractory period of Purkinje fibers was not shortened as much by DPH as action potential duration. The present study not only failed to demonstrate significant effects of DPH on action potential duration but also failed to demonstrate an effect on the effective refractory period in the specialized Bachmann's bundle fibers, ordinary atrial fibers, or in the perinodal fibers. Only in high concentrations (10^{-4}M) did DPH slightly prolong the effective refractory period in these fibers. These findings are unlike those for quinidine, which in low concentrations prolongs the effective refractory period of atrial muscle fibers (36).

The dv/dt of phase 0 of the action potential and the membrane responsiveness of ordinary atrial fibers and specialized Bachmann's bundle fibers were increased by DPH (10^{-5} to 10^{-6}M). These changes were greater in fibers which showed depression of these parameters before exposure to DPH. Findings similar to these were previously reported for canine Purkinje and ventricular muscle fibers (22). These findings again are unlike those found for quinidine, which in low concentrations decreases the dv/dt of phase 0 of action potentials recorded from ordinary atrial muscle cells (39). It has been shown that there is a decrease in resting membrane potential and maximum dv/dt of phase 0 of the action potential when Purkinje fibers are exposed to hypoxia (40). The time necessary to achieve this decline in resting potential and decrease in maximum dv/dt of phase 0 after the onset of hypoxia is nearly doubled in the presence of DPH (41). This ability of DPH to enhance the dv/dt of phase 0 of the action potential is seen in many types of cells of several species under varied conditions. This finding suggests that this drug may increase the "sodium carrier" activity over a wide range of electrochemical gradients or may, directly or indirectly, cause an increase in the rate of sodium-potassium exchange pumping. The latter mechanism has been suggested for brain by Woodbury et al. (48). The effect of DPH on conduction velocity was not assessed because of the nonlinear branching characteristics of atrial fibers (24). However, one would expect that conduction velocity might increase after treatment with an agent that increases the dv/dt of phase 0 of the action potential (24).

The lack of effect of DPH on membrane reactivation of perinodal cells and the dv/dt of phase 0 of action potentials recorded from the perinodal area and sinus node suggests that the changes in ionic conductances responsible for the upstroke of these action potentials are insensitive to this agent. These observations would tend to support the concept that the ionic mechanisms responsible for depolarization of the sinu node are different from those which depolarize ordinary atrial fibers (24, 37). Our recent observations on perinodal fibers would tend to indicate that this type of cell more closely resembles sinus node fibers than ordinary atrial muscle with regard to the ionic basis for their depolarization.

Toxic effects of digitalis on heart muscle might be due to depression of the sodium pump, or a postulated reduction in availability of the sodium carrier, or both (43). These two factors would lead to a loss of resting potential and amplitude and dv/dt of phase 0 of the action potential and would be reflected in depressed conduction. We found that DPH reversed the depression of dv/dt of phase 0 in ordinary atrial fibers caused by ouabain. DPH also reversed ouabain-induced S-A block. This finding again suggests that DPH has the ability to enhance membrane responsiveness by one of the previously postulated mechanisms of action. By whatever mechanism this increase in dv/dt of phase 0 of the action potential is brought about, it has been demonstrated here that the result can be an improvement in conduction.
EFFECTS OF DPH ON ATRIA

We have demonstrated that when the sinus node had been depressed by experimental conditions, it became more susceptible to the negative chronotropic effects of DPH. Although this experimental model may not be completely comparable to the situation observed in the patient with abnormal S-A function, it seems reasonable to administer DPH with caution to such patients. This suggestion may be particularly pertinent when administering it intravenously, since the solvent for DPH contains propylene glycol. Intravenous administration of propylene glycol alone has been reported to cause marked sinus bradycardia, leading to nodal or multifocal ectopic ventricular rhythms and hypotension and even asystole (44). Further, one might speculate that some reported cases of cardiac arrest occurring coincident with DPH administration may be due to the drug, or its solvent, or both. Either agent given in large or in usual doses to patients with depressed sinus node function might cause marked sinus bradycardia with the emergence of ectopic rhythms or sinus arrest with subsequent failure of subsidiary pacemakers to emerge. Should such mechanisms lead to the occurrence of sinus bradycardia or sinus arrest during exposure to DPH, administration of catecholamines might be beneficial since catecholamines were noted to have marked restorative effects in preparations with sinus node depression induced by DPH.

Depressed conduction may be a primary prerequisite for the initiation and/or perpetuation of some atrial arrhythmias by allowing reentry (45, 46). Under such circumstances, prolongation of refractoriness or improvement of conduction might abolish the arrhythmias (24). Although DPH was shown to increase membrane responsiveness and thereby conductivity, it did not significantly prolong the effective refractory period. Furthermore, DPH did not significantly decrease the slope of phase 4 depolarization in cells of the sinus node and adjacent venous automatic tissue, except in high concentrations. Thus, the ability of DPH to enhance membrane responsiveness in certain types of atrial fibers, without significantly suppressing atrial automaticity or prolonging atrial refractoriness, suggests that its sole mechanism of action in abolishing atrial arrhythmias may be its ability to improve conduction. This unique property of enhancing membrane responsiveness without complicating effects on automaticity or refractoriness may be of further use in studying the mechanism of atrial arrhythmias. Similarly, depressed conduction leading to S-A block or reentry may in part be responsible for the occurrence of digitalis-induced atrial arrhythmias in man. Thus, it is not unreasonable to assume that the ability of DPH to enhance membrane responsiveness and improve conduction in the presence of toxic concentrations of ouabain, as shown in the rabbit, may be related to the efficacy of this drug in the conversion of digitalis-induced atrial arrhythmias in man (6,10,11).

References


Actions of Diphenylhydantoin on the Electrical Properties of Isolated Rabbit and Canine Atria
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