The Effect of Antiarrhythmic Drugs on Depressed Conduction and Unidirectional Block in Sheep Purkinje Fibers

ROBERT W. WALD, MENASHE B. WAXMAN, AND EUGENE DOWNAR

SUMMARY We studied the effect of therapeutic concentrations of lidocaine, procainamide, quinidine, propranolol, and diphenylhydantoin on two models of depressed conduction and unidirectional block produced by asymmetric focal cooling and crushing in sheep Purkinje fibers. All drugs were shown to induce reversible deterioration of conduction. Unidirectional block was converted to bidirectional block with each drug. Improvement of conduction was rare and conversion of unidirectional block to bidirectional conduction never was observed. These experiments suggest that all five drugs may act by a uniform mechanism of action in some reentrant ventricular arrhythmias involving a zone of depressed conduction or unidirectional block within the Purkinje network. Circ Res 46: 612–619, 1980

DESPITE extensive studies directed at the electrophysiological effects of the antiarrhythmic drugs (Vaughan-Williams, 1970; Hoffman and Bigger, 1971; Hoffman et al., 1975), direct experimental evidence about their mode of action in reentrant arrhythmias remains scarce and the proposed mechanisms of action remain inferential. Unidirectional block has long been established as a conceptual requisite for the initiation of reentrant arrhythmias (Garrey, 1914; Schmitt and Erlanger, 1928–1929). It has been postulated that some of the antiarrhythmic drugs, such as procainamide and quinidine, convert regions of unidirectional block to bidirectional block, whereas others, such as lidocaine and diphenylhydantoin, may improve conduction and reestablish bidirectional conduction in these regions (Hoffman and Bigger, 1971). This study was undertaken to determine the effect of lidocaine, procainamide, quinidine, propranolol, and diphenylhydantoin on depressed conduction and unidirectional block induced experimentally in sheep Purkinje fibers by asymmetric cooling and crushing (Downar and Waxman, 1976).

Methods

Free-running Purkinje fiber bundles from freshly excised sheep hearts obtained from an abattoir were mounted in a silicone rubber tissue bath (capacity 15 ml) and superfused (30 ml/min) with oxygenated (95% O₂, 5% CO₂) Tyrode's solution (in mM: NaCl, 137; KCl, 3.0 or 5.4; MgCl₂, 0.5; NaH₂PO₄, 1.8; NaHCO₃, 12; CaCl₂, 1.35; dextrose, 5.5) at 37 ± 0.5°C. Twenty-four preparations were studied at [K⁺], = 3.0 mM and 36 at [K⁺], = 5.4 mM. Asymmetric conduction properties were produced either by asymmetric segmental cooling or crushing (Downar and Waxman, 1976). Fibers were stimulated with bipolar silver electrodes placed on each side of the block. A manual switch allowed the side from which the preparation was stimulated to be changed without interruption of the basic cycle. Rectangular pulses of 1.5 × threshold voltage were delivered by two Grass SD-9 stimulators (with isolated outputs) which were in turn driven by a Digitimer D4030 crystal clock digital programmer. Stimulus cycle length (CL) was varied between 250 or 300 and 1000 msec. Intracellular action potentials were recorded from two sites separated by 1.5–2.5 cm and located on each side of the blocked zone using standard glass microelectrodes filled with 3 M KCl (tip resistance = 10–30 MΩ). Signals were amplified with Grass P18 DC microelectrode amplifiers with capacity compensation, displayed on a Tektronix 565 and Gould Advance OS4000 digital storage oscilloscope, and recorded on a Philips Analog 7 FM tape recorder and a Siemens Mingograph 800 ink-jet recorder. Conduction time (CT) across the zone of block was measured directly from the digital storage oscilloscope screen.

Induction of Directionally Asymmetric Conduction Disturbances

Asymmetric cooling or crushing was used in separate experiments to induce stable directionally asymmetric conduction disturbances in Purkinje fibers (Fig. 1). Asymmetric cooling was achieved by placing the fiber across a triangular copper stage attached at its base to a 16 G stainless steel U-shaped tube. Pressurized CO₂ was allowed to escape at rates which were controlled by a needle valve through a small nozzle into the steel U-tube, thus producing graded cooling by the Joule-Thompson
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FIGURE 1 Schematic illustration of the two techniques of inducing unidirectional block in Purkinje fibers: asymmetric crushing (top) and cooling (bottom). Bipolar silver electrodes were used for antegrade (Sa) and retrograde (Sr) stimulation.

effect. The triangular shape of the copper stage allowed the overlying fiber to be cooled in an asymmetrical manner. The base of the triangle, by virtue of its direct attachment to the cooling probe and smaller surface area relative to its mass (allowing less heat exchange with the warm bath solution), was the coldest region of the stage. Heat exchange with the warm bath caused the stage temperature to rise gradually toward the apex. Thus the temperature gradient along the overlying fiber was relatively abrupt at the base and gradual at the apex of the triangle. Asymmetric crushing was performed with a micromanipulator-controlled, asymmetrically beveled Perspex probe which, when lowered perpendicularly onto the fiber, produced an abruptly severe lesion from the direction of its straight edge and a more gradual lesion from the direction of its beveled edge.

Conduction impairment of varying degrees of severity was induced in a total of 60 preparations. Cooling or crushing was increased very gradually while CT in both directions was continuously monitored at a stimulus CL of 300 msec. The following drugs were then added one at a time to the superfusate: lidocaine, 1-5 mg/liter; procainamide, 1-5 mg/liter; propranolol, 0.5-5 mg/liter; quinidine, 1-5 mg/liter; and diphenylhydantoin, 1-5 mg/liter. Increasing concentrations of each drug (incremented in steps of 1 mg/liter within the range of 1-5 mg/liter) were used and studied during 30 minutes; test of each concentration was followed by a 60-minute wash-out period. CT's in both directions were continuously monitored throughout the procedure. The results were accepted only if the initial degree of block was restored at the end of the wash-out period. Each drug was tested at least twice at each concentration on at least two preparations.

Drug Studies

The stability of induced conduction block was first established by allowing a 30-minute equilibration period. Following this, CT's were measured in both directions at CL's ranging from 250 or 300 to 1000 msec. The following drugs were then added:

- Lidocaine, 1-5 mg/liter
- Procainamide, 1-5 mg/liter
- Propranolol, 0.5-5 mg/liter
- Quinidine, 1-5 mg/liter
- Diphenylhydantoin, 1-5 mg/liter

Results

Effect of Cooling and Crushing on Conduction

Prior to cooling or crushing, all fibers conducted well (1:1 at CL 250 or 300 msec) in both directions. Conduction time across the 1.5- to 2.5-cm distance between microelectrodes ranged from 2.0 to 5.6 msec. There usually was a small difference between antegrade and retrograde CT ranging from 0 to 2.6 msec. Although CT at slower pacing rates was generally shorter (the difference between CT at CL 400 and 1000 msec ranged from 0. to 0.5 msec), about 50% of the fibers exhibited some shortening of CT (up to 0.6 msec) as the CL was decreased from 400 to 300 or 250 msec. Gradual application of cooling or crushing resulted in a gradual increment in CT. The degree of CT prolongation always was different in the two directions and reflected the asymmetry of the applied lesion rather than the asymmetry of the control CT. As the severity of cooling or crushing was progressively augmented, CT increased further and, eventually, rate-related conduction block developed in one or both directions. At this point, fibers were able to conduct 1:1 during stimulation at slower rates but 2:1 or higher block ratios were observed at faster rates. The CT of the conducted impulses shortened suddenly with the onset of block. For any given conduction ratio, the CT was more rate sensitive during block than during 1:1 conduction. Non-integer ratios (e.g., 3:2) and alternating ratios (e.g., 3:1 alternating with 2:1)
often were observed but usually were transient. The stimulation rate at which 1:1 conduction broke down was different in the two directions but did not consistently reflect the asymmetry in CT’s, that is conduction block did not always occur at slower rates in the direction exhibiting the longer CT. These points are illustrated in Table 1 which contains the results of one of the experiments.

The 18 preparations in which complete block was induced in one direction generally conducted very well in the other direction. In 12 of the 18 fibers conduction in the “good” direction was 1:1 at CL 300-1000 msec, while, in the remaining six, 2:1 block occurred at CL 400 msec or less. Figure 2 illustrates these findings for a preparation with complete retrograde block, 1:1 antegrade conduction at CL 300 msec or longer and 2:1 antegrade block at CL 250 msec.

Effect of Antiarrhythmic Drugs on Fibers with Partial Asymmetric Block

The effect of incremental concentrations of lidocaine, procainamide, propranolol, quinidine, and diphenylhydantoin on CT and on conduction ratio at CL 250 or 300 to 1000 msec was studied in 30 fibers in which partial depression in conduction had been induced.

Irrespective of the \([K^+]_o\) (3.0 or 5.4 mM) and provided the conduction ratio was unchanged, all drugs prolonged CT in both directions to an extent that was directly proportional to drug concentration with the exception of one fiber in which CT at CL of 700 msec or longer was shortened slightly by lidocaine, 1 and 2 mg/liter. This fiber exhibited spontaneous automaticity at a rate of about 60/min at \([K^+]_o = 3.0 \text{ mM}\). CT shortening induced by lidocaine in this fiber was accompanied by simultaneous suppression of automaticity. However, even in this fiber CT was prolonged at shorter CL’s and by higher lidocaine concentrations (Table 2). Whereas conduction ratio at long CL’s frequently was unchanged, it almost always deteriorated at short CL’s. Complete unidirectional block was induced by drug superfusion in six preparations (Table 1). Improvement in conduction ratio was never observed with any of the drugs tested.

Effect of Antiarrhythmic Drugs on Fibers with Complete Unidirectional Block

The effect of incremental concentrations of each drug on complete unidirectional block was tested in 18 preparations. Unidirectional block was converted to bidirectional block after superfusion with each of the drugs tested. Deterioration of conduction in the “good” direction was gradual but generally quite rapid after addition of the drug to the superfusate (Fig. 3). At lower drug concentrations, block in the “good” direction was not complete and conduction continued to occur at variable ratios depending on the driving CL (Fig. 4). The degree of block could,

<table>
<thead>
<tr>
<th>Direction of stimulation</th>
<th>Stimulating cycle length (msec)</th>
<th>Pre-crush</th>
<th>Post-crush</th>
<th>Lidocaine, 2 mg/liter, 5 min</th>
<th>Lidocaine, 2 mg/liter, 30 min</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
<td>400</td>
<td>500</td>
<td>600</td>
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<tr>
<td>A</td>
<td>1.1</td>
<td>2:1</td>
<td>3:1</td>
<td>2:1</td>
<td>3:1</td>
<td>1.1</td>
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<tr>
<td>R</td>
<td>1:1</td>
<td>3:1</td>
<td>5:1</td>
<td>3:1</td>
<td>5:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Complete block</td>
<td>2:1</td>
<td>3:1</td>
<td>5:1</td>
<td>3:1</td>
<td>5:1</td>
<td>1:1</td>
</tr>
<tr>
<td></td>
<td>Complete block</td>
<td>2:1</td>
<td>3:1</td>
<td>5:1</td>
<td>3:1</td>
<td>1:1</td>
</tr>
</tbody>
</table>

Table 1: Conduction Ratio and Conduction Time across a Zone of Depressed Conduction during an Experiment with Lidocaine, 2 mg/liter, at \([K^+]_o = 3.0 \text{ mM/liter}\)

Abbreviations: A = antegrade, R = retrograde. Conduction ratios are expressed as number of applied stimuli per number of active responses propagated across the zone of depressed conduction. Conduction times are expressed in msec. Depression of conduction in this preparation was induced by crushing. Pre-crush and post-crush observations were obtained prior to and 30 minutes after crushing. The post-crush observations served as control during the drug study. Lidocaine was removed from the superfusate after 30 minutes, and the observations during washout were made 30 minutes later. Note that, following washout, there was a return in conduction properties to control.
however, always be increased by increasing the drug concentration and eventually complete bidirectional block was obtained with each drug (Table 3).

Drug-induced conversion of unidirectional to bidirectional block was always preceded by prolongation of conduction across the blocked zone. Figure 5 illustrates this in an experiment in which complete unidirectional block was created by crushing. Prior to drug intervention, control conduction time across the zone of unidirectional block was 15.25 msec at a BCL of 300 msec (panel A). During superfusion with lidocaine, 2 mg/liter, progressive prolongation of conduction time was observed up to a maximum of 20.5 msec just prior to the onset of 2:1 block (panel B). With the onset of block (panel C), conduction time shortened slightly to 19.5 msec, indicating that a small proportion of it was due to rate-related encroachment on the relative refractory period of an intervening structure. The degree of block progressed to complete block (panel D) with further drug superfusion. Conduction was later restored during wash-out of the drug and conduction time returned to its control value (panel E).

**TABLE 2**  Effect of Lidocaine, 3–5 mg/liter, on Conduction Time*

<table>
<thead>
<tr>
<th>Driving cycle length (msec)</th>
<th>600</th>
<th>700</th>
<th>800</th>
<th>900</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.2 ± 1.7</td>
<td>11.9 ± 1.9</td>
<td>12.4 ± 2.0</td>
<td>12.6 ± 1.7</td>
<td>12.5 ± 1.7</td>
</tr>
<tr>
<td>Lidocaine 3 mg/liter</td>
<td>13.6 ± 1.5</td>
<td>14.6 ± 1.5</td>
<td>14.7 ± 2.3</td>
<td>15.0 ± 1.9</td>
<td>15.2 ± 1.9</td>
</tr>
<tr>
<td>Lidocaine 5 mg/liter</td>
<td>17.8 ± 1.8</td>
<td>19.8 ± 1.8</td>
<td>20.5 ± 2.5</td>
<td>19.8 ± 1.8</td>
<td>19.8 ± 1.8</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* Data from two preparations in which complete block was induced are excluded. Conduction times expressed as mean ± SE in msec, and statistical differences calculated by paired Student’s t-test.
FIGURE 4 Conversion of unidirectional 1:1 antegrade conduction (control panel) to 2:1 antegrade conduction after a 30-minute superfusion of propranolol, 1 mg/liter (middle panel). This effect was reversed and antegrade conduction was restored to 1:1 (right panel) following drug wash-out. The driving frequency (BCL) is identical in all three panels. The solid bars indicate the side of stimulation.

Reversible improvement of conduction in the originally blocked direction was never observed to occur during drug superfusion. This included the periods of wash-in and wash-out of the drugs during which the effective concentration of the drugs within the bath was below the concentration being tested. When improvement did occur during an experiment, control unidirectional block could not be reestablished with drug wash-out, and improvement was then attributed to instability of the block. Experiments in which the block was unstable were deemed invalid. When unidirectional block reverted to bidirectional conduction during an experiment, it could often be reestablished and stabilized during superfusion with drug-free solution by readjusting the degree of cooling or crushing.

Discussion

Unidirectional block, the most extreme form of directional asymmetry of conduction, has been a long-recognized property of cardiac tissues (Engelmann, 1894; Erlanger, 1906; Cranefield et al., 1971) which plays an important role in the concept of reentry (Garrey, 1914; Schmitt and Erlanger, 1928-1929; Han, 1971, Wit et al., 1972a, 1972b). Since zones of tissue that possess this property may be an integral part of reentrant circuits, the effect of antiarrhythmic drugs on conduction across these zones is salient to our understanding of their mechanisms of action. Cooling and mechanical crushing were two of the first methods described to produce block in cardiac tissues (Engelman, 1894; Drury,
FIGURE 5 Recordings of transmembrane potentials from two Purkinje cells 2.5 cm apart, one proximal (top tracing) and another distal (bottom tracing), to a zone of stable retrograde unidirectional block produced by asymmetric crushing. The preparation is paced antegradely—that is, in the direction of intact conduction—at a BCL of 300 msec. The panels on the left are time-expanded records of those on the right. During control (panel A), conduction time between the two cells is 15.25 msec. During superfusion with lidocaine 2 mg/l, conduction time increases to 20.5 msec (panel B) just prior to the onset of 2:1 block (panel C) which causes a slight decrease in the conduction time to 19.5 msec. During wash-out of the drug (panel E), 1:1 conduction is restored and conduction time returns to control values. The ramps preceding the stimulus artifact in the panels on the left are 100 volts/sec calibration signals.

1925; Schmitt and Erlanger, 1928–1929). Directional asymmetry of conduction has been attributed to asymmetry of the applied lesion (Schmitt and Erlanger, 1928–1929; Katz, 1946). The techniques used in our experiments allow accurate control over the directional asymmetry and severity of the induced lesion. The resultant depression in conduction is: (1) variable over a wide range of severity, (2) reversible within certain limits, (3) stable over prolonged periods of time, and (4) directionally asymmetrical in a predictable manner (Downar and Waxman, 1976).

All five drugs tested in this study converted unidirectional block to bidirectional block at concentrations generally deemed to be therapeutic. Conversion to bidirectional conduction never was observed. These observations were as expected in the case of procainamide, quinidine, and propranolol. These drugs have been uniformly shown to depress membrane responsiveness, decrease excitability, and slow conduction (Weidman, 1955a; Hoffman, 1957; Gettes et al., 1962; Davis and Temte, 1968; Rosen et al., 1972). Although the concentrations at which propranolol was shown to exert these depressant effects on normal Purkinje fibers (3 mg/liter) (Davis and Temte, 1968) was much higher than the usual antiarrhythmic plasma levels, these actions may occur at lower concentrations in injured fibers (Wit et al., 1975). Conversion of unidirectional block to bidirectional block by lidocaine and diphenylhydantoin has more intricate implications. Some investigators have demonstrated improvement in membrane responsiveness and conduction velocity by low concentrations of lidocaine in normal Purkinje fibers (Bigger and Mandel, 1970a, 1970b) and therapeutic concentrations of diphenylhydantoin in partially depolarized Purkinje fibers (Bigger and Mandel, 1968), suggesting that one mechanism by which lidocaine and diphenylhydantoin abolish arrhythmias is by conversion of zones of unidirectional block to bidirectional conduction (Bigger and Mandel, 1968, 1970a, 1970b). It should be pointed out that improvement in membrane responsiveness by lidocaine and diphenylhydantoin has not been observed universally (Davis and Temte, 1969; Singh and Vaughan-Williams, 1971), and other investigators have demonstrated a decrease in responsiveness at therapeutic concentrations of these drugs (Singh and Vaughan-Williams, 1971). Singh and Vaughan-Williams have attributed these apparent differences in observed effect to differences in potassium concentration in the superfusate. Although we did not study the effect of drugs on membrane responsiveness, each drug prolonged conduction time or caused block in our models irrespective of whether $[K^+]_o$ was 5.4 or 3.0 mM.

Once stable complete unidirectional block has been generated in a fiber, it is difficult to assess whether failure to improve conduction in the blocked direction is due to an inherent drug action or an exceedingly severe depression of conduction which would mask any tendency to improvement during treatment with a drug. Although conduction in the "good" direction was uniformly depressed by each drug, the marked differences in conduction properties in the two directions after the lesion was created do not allow an easy extrapolation of the observations from one direction to the other. To resolve this question, we performed a series of experiments on fibers in which conduction was par-
Bigger JT, Mandel WJ (1970a) Effect of lidocaine on conduction in canine Purkinje fibers and at the ventricular muscle-Purkinje fiber junction. J Pharmacol Exp Ther 172: 239-254
Brennan FJ, Cranefield PF, Wit AL (1978) Effects of lidocaine on slow response and depressed fast response action potentials of canine cardiac Purkinje fibers. J Pharmacol Exp Ther 204: 312-324
Drury AN (1925) Further observations upon intra-auricular conduction block produced by pressure or cooling. Heart 12: 143-169
Garrey WE (1914) The nature of fibrillary contraction of the heart. Its relation to tissue mass and form. Am J Physiol 33: 397-414
Hoffman BF (1957) The action of quinidine and procaine amide on single fibers of dog ventricle and specialized conducting system. J Pharmacol Exp Ther 120: 113-114

References

Changes in Renal Vascular Reactivity at Various Stages of Deoxycorticosterone Hypertension in Rats

KATHLEEN H. BERECEK, MARTHA STOCKER, AND FRANZ GROSS

SUMMARY We studied the possible contribution of increased vascular reactivity to the development of deoxycorticosterone acetate (DOCA) hypertension in rats. Changes in vascular reactivity were studied in isolated, constant-flow perfused kidneys of male Sprague-Dawley rats post unilateral nephrectomy which received a single subcutaneous implant of Silastic containing 100 mg/kg of DOCA and were given 0.9% NaCl plus 0.2% KCl solution to drink. Age- and sex-matched control rats (CR) received Silastic implants. The hypertensive rats were studied at 4 days (prehypertensive stage) and 61 days (chronic hypertensive stage) after implantation. At an average of 4 days, blood pressure in DOCA-treated rats did not differ significantly from that measured prior to implantation, and renal vascular resistance was similar to that in the matched controls. However, renal vascular reactivity to norepinephrine (NE), vasopressin (ADH), and angiotensin II (A II) was enhanced in the DOCA-treated rats. Dose-response curves for kidneys of these prehypertensive rats showed a parallel leftward shift, reduced ED₅₀, and decreased threshold dose. After an average period of 61 days, blood pressure in the DOCA-treated rats was 189.2 ± 3.5 mm Hg, and renal vascular resistance at maximal vasodilation was significantly greater (P < 0.0001) than in CR. Renovascular reactivity to NE, ADH, and A II was markedly enhanced. Dose-response curves were characterized by a leftward shift, steeper slopes, increased maximal responses, decreased ED₅₀, and threshold doses. Hence, enhanced vascular reactivity clearly precedes and may initiate the rise in arterial pressure in DOCA-treated rats. The initial increase in response to vasoconstrictor substances is attributed to an enhanced sensitivity of vascular smooth muscle, whereas, in the chronic stage of hypertension, structural changes in the resistance vessels, secondary to the rise in arterial pressure, are the main mechanisms responsible for the intensified reactivity. Circ Res 46: 619-624, 1980

INCREASED vascular reactivity to pressor agents is considered a primary characteristic of chronic deoxycorticosterone acetate (DOCA) hypertension in the rat (Finch and Haeuser, 1974; Beilin et al., 1970). Recently, longitudinal studies of changes in whole-body vascular reactivity in the DOCA-hypertensive pig revealed an enhanced reactivity prior to a significant rise in arterial pressure (Berecek and Bohr, 1978). This finding suggested that the increase in vascular reactivity participates in the development of DOCA hypertension.

In the current study, the temporal relationship between the development of hypertension and the appearance of changes in vascular reactivity was studied in isolated, perfused kidneys of DOCA-hypertensive rats. The use of such a preparation, devoid of extrinsic neural and humoral control, permits a more direct analysis of alterations in the responses of the resistance vessels. The animals
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