Effects of Propranolol on the Transmembrane Potentials of Ventricular Muscle and Purkinje Fibers of the Dog

By Larry D. Davis, Ph.D., and John V. Temte, Ph.D.

ABSTRACT

Papillary muscle-false tendon tissue preparations isolated from dog hearts were perfused with Tyrode's solution containing propranolol in concentrations ranging from 0.1 to 20.0 mg/liter. Transmembrane action potentials of both ventricular muscle fibers and Purkinje fibers were recorded. With sufficient concentration of drug, the velocity of the upstroke and the overshoot of both fiber types decreased. The curve relating upstroke velocity to level of membrane potential for Purkinje fibers was displaced to the right and down. The ability of both ventricular muscle fibers and Purkinje fibers to respond to rapid frequencies of stimulation was decreased. Repolarization of Purkinje fibers was accelerated by propranolol, but repolarization of ventricular muscle fibers was unaffected. Duration of the effective refractory period of Purkinje fibers decreased; that of ventricular muscle fibers was unchanged. Graded responses and decremental impulse conduction in Purkinje fibers were abolished in the presence of propranolol. Low doses of propranolol which caused no change in the transmembrane potential completely blocked the increase in Purkinje diastolic depolarization normally induced by epinephrine. The possible mechanisms by which propranolol might exert its antiarrhythmic actions on ventricular arrhythmias were discussed.

ADDITIONAL KEY WORDS

cardiac arrhythmias  epinephrine diastolic depolarization
rapid electrical stimulation effective refractory period

A number of currently available drugs can block catecholamine beta-receptors (1-5). Many of these compounds exert actions against certain experimentally-induced cardiac arrhythmias. Dichloroisoproterenol, pronethalol, and propranolol prevent the ventricular arrhythmias induced by catecholamines (6-11) and prevent or terminate the ventricular arrhythmias induced by cardiac glycosides (8-16). Other drugs that are unrelated chemically to the above group but also block beta-receptors (4, 5) prevent the arrhythmias induced by catecholamines but not those elicited by cardiac glycosides (8, 9, 17). Whether these antiarrhythmic effects are secondary to a blockade of beta-receptors or are due to some nonspecific action is uncertain.

The effects of these drugs on cardiac transmembrane potentials have been studied in attempts to determine the mechanisms of their antiarrhythmic actions. Williams and co-workers studied the effects of dichloroisoproterenol, pronethalol, and propranolol (18, 19) on single fibers of isolated rabbit atria. The major effects were a decrease in the maximum rate of depolarization and a decrease in the magnitude of overshoot. Also there was a loss of ability of the tissue to be excited by every stimulus at rapid frequencies, an effect interpreted to indicate a lengthening of the atrial refractory period. The contour of the atrial action potential was affected relatively little. The effects of pronethalol on the action potentials of canine ventricular tissues has

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also been investigated (20, 21). The velocity of the upstroke of both ventricular muscle and Purkinje fibers decreased. The plateau phase of the Purkinje action potential shortened, but the duration of the refractory period lengthened. It is uncertain whether these changes in cardiac transmembrane potential result from blockade of beta-receptors or some nonspecific action of the drug. In the present study, the effects of propranolol on the transmembrane potentials of single ventricular muscle fibers and Purkinje fibers and the effect of propranolol on the response of Purkinje fibers to epinephrine are presented.

Methods

Dogs were anesthetized with 33% cyclopropane in oxygen or sodium pentobarbital, 30 mg/kg, intravenously. The heart was removed, and in oxygen or sodium pentobarbital, 30 mg/kg, the heart was removed, and in oxygen or sodium pentobarbital, 30 mg/kg, and Purkinje fibers to epinephrine are presented. In some experiments, records were obtained simultaneously from two different fibers. Action potentials were monitored continuously on one oscilloscope and could be photographed from a second oscilloscope. The maximum rate of rise of the upstroke of the action potential was determined by electronic differentiation. A detailed description of all recording apparatus used in this study appears elsewhere (22).

Test solutions of propranolol were prepared by adding sufficient propranolol hydrochloride (Inderal, Ayerst Laboratories) to a reservoir of Tyrode’s solution to obtain propranolol concentrations of 0.1 to 20 mg/liter. In the usual experiment, perfusion with control Tyrode’s solution was maintained until a fiber was impaled. Then perfusion with propranolol solution was started and continued for 15 minutes or until changes that occurred in the transmembrane potential had stabilized. Photographic records of action potentials were obtained before and at intervals during treatment with propranolol. Several features of the control and experimental

### TABLE 1
Effect of Propranolol on the Contour of the Transmembrane Action Potentials of Purkinje Fibers

<table>
<thead>
<tr>
<th></th>
<th>Maximum diastolic potential (mv)</th>
<th>Magnitude of action potential (mv)</th>
<th>Time to repolarize to —60 mv (msec)</th>
<th>Duration of action potential (msec)</th>
<th>Maximum upstroke velocity (v/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)*</td>
<td>97.2 ± 13.3</td>
<td>125.4 ± 3.8</td>
<td>191.0 ± 11.2</td>
<td>262.0 ± 12.4</td>
<td>737.8 ± 52.4</td>
</tr>
<tr>
<td>Propranolol 0.3 mg/liter</td>
<td>97.1 ± 3.6</td>
<td>125.4 ± 3.8</td>
<td>182.5 ± 11.2</td>
<td>253.1 ± 12.4</td>
<td>704.4 ± 52.4</td>
</tr>
<tr>
<td>Mean difference</td>
<td>—0.1 ± 0.5</td>
<td>0.0 ± 3.8</td>
<td>—8.5† ± 11.2</td>
<td>—8.9‡ ± 12.4</td>
<td>—33.4 ± 52.4</td>
</tr>
<tr>
<td>Confidence interval†</td>
<td>±1.3 ± 41.1</td>
<td>±3.8 ± 11.2</td>
<td>±11.2 ± 12.4</td>
<td>±12.4 ± 12.4</td>
<td>±52.4 ± 52.4</td>
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<tr>
<td>Control (8)*</td>
<td>98.6 ± 38.3</td>
<td>131.3 ± 131.3</td>
<td>234.7 ± 304.1</td>
<td>304.1 ± 76.7</td>
<td>766.7 ± 52.4</td>
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<tr>
<td>Propranolol 1.0 mg/liter</td>
<td>94.4 ± 31.3</td>
<td>128.3 ± 206.5</td>
<td>205.6 ± 275.9</td>
<td>275.9 ± 73.5</td>
<td>735.5 ± 52.4</td>
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<td>Mean difference</td>
<td>—2.2 ± 2.0</td>
<td>—3.0 ± 20.6</td>
<td>—29.1§ ± 28.2§</td>
<td>—28.2§ ± 52.1</td>
<td>—51.2 ± 75.1</td>
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<tr>
<td>Confidence interval†</td>
<td>±3.6 ± 3.1</td>
<td>±5.4 ± 20.6</td>
<td>±25.5 ± 75.1</td>
<td>±75.1 ± 75.1</td>
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<td>Control (14)*</td>
<td>94.9 ± 35.4</td>
<td>127.2 ± 237.6</td>
<td>237.6 ± 304.5</td>
<td>304.5 ± 76.0</td>
<td>766.0 ± 52.8</td>
</tr>
<tr>
<td>Propranolol 3.0 mg/liter</td>
<td>93.6 ± 30.8</td>
<td>121.1 ± 183.3</td>
<td>185.8 ± 278.5</td>
<td>278.5 ± 62.0</td>
<td>636.0 ± 52.8</td>
</tr>
<tr>
<td>Mean difference</td>
<td>—1.3 ± 4.6§</td>
<td>—6.1§ ± 49.2§</td>
<td>—49.2§ ± 28.4§</td>
<td>—28.4§ ± 130.0§</td>
<td>—130.0§ ± 97.7</td>
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<tr>
<td>Confidence interval†</td>
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<td>±3.9 ± 18.7</td>
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<td>Control (11)*</td>
<td>95.9 ± 33.0</td>
<td>126.5 ± 227.1</td>
<td>227.1 ± 296.4</td>
<td>296.4 ± 79.3</td>
<td>796.3 ± 52.8</td>
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<td>Propranolol 10.0 mg/liter</td>
<td>92.4 ± 22.2</td>
<td>113.1 ± 159.2</td>
<td>159.2 ± 283.3</td>
<td>283.3 ± 556.3</td>
<td>556.3 ± 52.8</td>
</tr>
<tr>
<td>Mean difference</td>
<td>—3.5§ ± 10.8§</td>
<td>—13.4§ ± 67.9§</td>
<td>—67.9§ ± 13.1‡</td>
<td>—13.1‡ ± 240.0‡</td>
<td>—240.0‡ ± 87.9</td>
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<tr>
<td>Confidence interval†</td>
<td>±3.4 ± 4.3</td>
<td>±6.0 ± 29.2</td>
<td>±19.4 ± 87.9</td>
<td>±87.9 ± 87.9</td>
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</tr>
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</table>

*Values are mean of the number of preparations studied (number in parentheses).
†Confidence intervals computed at the 99% level. †P 0.05. §P 0.01. ‡P 0.001.
action potentials were measured and compared statistically. Methods used to make such measurements have been described previously (22). Solutions of epinephrine hydrochloride (Adrenaline, Parke-Davis Laboratories) were prepared by diluting 1 mg with water to a volume of 25 ml. Epinephrine was administered by rapidly injecting 0.2 ml of solution directly at the inflow of the tissue bath.

Results

A. Contour of the Action Potential

Purkinje Fibers.—Forty-three experiments were performed on fibers from 22 hearts. Concentrations of propranolol used were 0.3, 1.0, 3.0 and 10.0 mg/liter. Usually, each preparation was treated with a single concentration of drug and then discarded, but occasionally the effects of two or more successively higher concentrations were determined on the same fiber. It is apparent that several features of the Purkinje transmembrane potential were altered by propranolol (Table I). The occurrence and magnitude of a given change were dose dependent. Therefore, for convenience, the effects of an intermediate concentration (3 mg/liter) will be described in detail and only significant differences observed at other concentrations will be noted.

Action potentials recorded in an experiment on Purkinje fibers in which propranolol was raised to 3 mg/liter are shown in Figure 1. The most prominent change was an acceleration of repolarization. The rate of repolarization during the plateau (phase 2) increased and the onset of the rapid phase of repolarization (phase 3) occurred earlier. These changes greatly accelerated repolarization and decreased both the time required to repolarize to minus 60 mv and total duration of the action potential. Overshoot of the action potential decreased, and the maximum diastolic potential was unchanged; consequently the magnitude of the action potential declined. The maximum rate of depolarization during

![Figure 1](http://circres.ahajournals.org/)

**Figure 1**

Effect of propranolol, 3.0 mg/liter, on the action potential of a Purkinje fiber. A: in control solution. B: 12 minutes in propranolol solution. C: records A and B superimposed by aligning action potential upstrokes. D: 35 minutes after return to control perfusion. The time calibrations in this and subsequent records apply to action potential traces only. The lowest trace is recorded at a sweep speed 10 times that of action potentials and shows small biphasic deflections preceding a large spike. These represent stimulus artifact and a differentiated record of the action potential upstroke, respectively. Magnitude of the large spike is proportional to the maximum rate of depolarization (\(dv/dt\) in volts/sec as shown in calibration).
FIGURE 2
Effect of propranolol, 10.0 mg/liter, on the action potentials of a ventricular muscle fiber (upper action potential) and a Purkinje fiber. A: in control solution. B: 15 minutes in propranolol solution. C: records A and B superimposed. The spike in the lower trace represents the upstroke velocity of the ventricular fiber.

FIGURE 3
Effect of propranolol, 20.0 mg/liter, on the action potentials of a Purkinje fiber and subsequently on that of a ventricular muscle fiber. A: Purkinje fiber in control solution. B: same fiber after 4 minutes in propranolol solution. C: same fiber after 15 minutes in propranolol solution. Between C and D the microelectrode was removed from the Purkinje fiber and inserted in a ventricular muscle fiber. D: the ventricular muscle action potential.

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the upstroke of the action potential decreased.

As seen in Table 1 the acceleration in repolarization was evident but proportionately less at concentrations of 1.0 and 0.3 mg/liter. However, a decrease in the velocity of depolarization was not observed consistently at these lower concentrations. Such a finding suggests operation of different mechanisms to produce the changes in repolarization and depolarization. In four experiments, solutions containing 0.1 mg of drug/liter were applied, and no change in any feature of the transmembrane potential was noted.

Concentrations of propranolol greater than 3 mg/liter in general increased the effects described above (Figs. 2 and 3). Thus, the plateau phase almost disappeared and the repolarization phases resembled those normally observed in atrial muscle fibers. In addition, there was a small but statistically significant decrease in maximum diastolic potential. A further effect of the higher concentrations was to prolong the terminal portion of phase 3, with the result that while the total duration of the action potential was shortened, it was much less than would be anticipated from the greatly accelerated rate of repolarization during the plateau. These changes in the Purkinje transmembrane potential occurred and were stabilized after 12 to 15 minutes of treatment with propranolol and were completely reversible when the fibers were returned to the control solution, but full recovery took much longer than onset (approximately 45 to 60 minutes).

The maximum rate of depolarization of the

<table>
<thead>
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<th>TABLE 2</th>
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<td><strong>Effect of Propranolol on the Contour of the Transmembrane Action Potentials of Ventricular Fibers</strong></td>
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<table>
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<tr>
<th></th>
<th>Resting potential (mv)</th>
<th>Overshoot (mv)</th>
<th>Magnitude of action potential (mv)</th>
<th>Time to repolarize to −60 mv (msec)</th>
<th>Duration of action potential (msec)</th>
<th>Maximum upstroke velocity (v/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (14) *</td>
<td>91.5</td>
<td>29.1</td>
<td>120.5</td>
<td>138.9</td>
<td>184.2</td>
<td>336.8</td>
</tr>
<tr>
<td>Propranolol 0.3 mg/liter</td>
<td>91.4</td>
<td>28.5</td>
<td>120.0</td>
<td>140.6</td>
<td>184.7</td>
<td>322.8</td>
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<tr>
<td>Mean difference</td>
<td>−0.1</td>
<td>−0.6</td>
<td>−0.5</td>
<td>+1.7</td>
<td>+0.5</td>
<td>−15.8</td>
</tr>
<tr>
<td>Confidence interval †</td>
<td>±1.7</td>
<td>±2.5</td>
<td>±3.2</td>
<td>±9.6</td>
<td>±9.6</td>
<td>±45.2</td>
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<tr>
<td>Control (17) *</td>
<td>92.3</td>
<td>28.2</td>
<td>120.2</td>
<td>148.8</td>
<td>196.5</td>
<td>358.5</td>
</tr>
<tr>
<td>Propranolol 3.0 mg/liter</td>
<td>91.0</td>
<td>24.3</td>
<td>115.5</td>
<td>154.0</td>
<td>200.7</td>
<td>296.5</td>
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<tr>
<td>Mean difference</td>
<td>−1.3 ‡</td>
<td>−3.9 ‡</td>
<td>−4.7 ‡</td>
<td>+5.2</td>
<td>+4.2</td>
<td>−60.0 §</td>
</tr>
<tr>
<td>Confidence interval †</td>
<td>±1.6</td>
<td>±2.6</td>
<td>±3.3</td>
<td>±11.6</td>
<td>±11.9</td>
<td>±59.8</td>
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<tr>
<td>Control (16) *</td>
<td>91.8</td>
<td>26.8</td>
<td>118.1</td>
<td>133.9</td>
<td>181.3</td>
<td>366.2</td>
</tr>
<tr>
<td>Propranolol 10.0 mg/liter</td>
<td>89.4</td>
<td>20.5</td>
<td>110.2</td>
<td>144.8</td>
<td>191.7</td>
<td>229.2</td>
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<tr>
<td>Mean difference</td>
<td>−2.4 ‡</td>
<td>−6.3 ‡</td>
<td>−7.9 ‡</td>
<td>+10.8 ‡</td>
<td>+10.4 ‡</td>
<td>−140.0 §</td>
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<tr>
<td>Confidence interval †</td>
<td>±2.9</td>
<td>±3.8</td>
<td>±5.9</td>
<td>±11.8</td>
<td>±11.5</td>
<td>±84.2</td>
</tr>
</tbody>
</table>

Symbols mean the same as in Table 1.
action potential upstroke (dv/dt) is dependent on the level of membrane potential (23). Tests of the relation between dv/dt and membrane potential yield information about membrane responsiveness. In Figure 4, the maximum rate of depolarization is plotted against the level of membrane potential. Data for this graph were obtained by applying test stimuli to the fiber at different times during the repolarization phase and hence at different levels of membrane potential. The curves shown in Figure 4 were obtained during control perfusion and again during perfusion with solution containing propranolol, 0.3, 3, and 10 mg/liter. Treatment with the two higher concentrations of drug shifted the curves to the right and down, an indication of a decrease in the ability of the fiber to depolarize at given levels of membrane potential. This effect on the ability to depolarize is caused by several other agents with antiarrhythmic properties (24, 25).

Ventricular Muscle Fibers.—Forty-seven experiments were performed on tissue isolated from 19 hearts. The effects of three concentrations of propranolol were studied: 0.3, 3, and 10 mg/liter (Table 2). The transmembrane potential of ventricular muscle was affected much less by these concentrations of propranolol than were Purkinje fibers. The most consistent changes were decreases in the maximum rate of the upstroke and in magnitude of overshoot (Fig. 5).

At the highest concentrations of propranolol, there was a small but statistically significant decrease in resting potential, and this effect in combination with the decrease in overshoot led to a decrease in magnitude of the action potential. The repolarization phase of the ventricular action potential was not affected to any appreciable degree by even the highest levels of propranolol (20 mg/liter; shown in Fig. 3, D). Figures 2, 10 and 11 show records obtained in experiments in which Purkinje and ventricular transmembrane potentials were recorded simultaneously before and during treatment with propranolol. It is apparent that concentrations of this agent that produce marked changes in repolarization of Purkinje fibers produce no appreciable change in ventricular repolarization.
Control determination of the effective refractory period of Purkinje fibers. See text for methods and interpretation. Upper action potential is from the proximal fiber and the lower from the distal fiber. Test stimuli were applied to ventricular muscle during phase 3, and propagated action potentials elicited the responses shown. The test stimulus, indicated by the white vertical lines, was given at a time progressively later in the cycle.

Determination of the effective refractory period of Purkinje fibers in 3 (A-C) and 10 (D-F) propranolol, mg/liter. Records are from the same experimental preparation as in Figure 6. Vertical white lines indicate time of test stimulation. The very abrupt appearance of fully propagated activity with only a small delay in application of the stimulus is apparent.

B. DURATION OF THE EFFECTIVE REFRACTORY PERIOD

Purkinje Fibers.—Two methods were used to measure duration of the effective refractory period of Purkinje fibers. Records obtained in seven experiments using the first technique (26) are shown in Figures 6 and...
7. In these experiments both the driving and test stimuli were administered to the tendinous tip of the preparation through the same stimulating electrodes. A test stimulus was applied at a preselected time after a driving stimulus and was given no more frequently than every sixth driving stimulus. Two microelectrodes were located in Purkinje fibers in the false tendon, one very close to the site of attachment of false tendon with papillary muscle (proximal fiber) and the other several millimeters in the false tendon (distal fiber). In this preparation, Purkinje tissue is excited by propagated action potentials from the ventricular muscle (26). Records obtained during a control determination of the refractory period are in Figure 6. Here, stimuli were applied at different intervals during the repolarization phase of different cycles. The earliest premature response occurred in the proximal fiber 190 msec after the upstroke of the action potential. The response was of small magnitude with slow velocity of upstroke and short duration, typical of what has been described as a local or graded response (27). The distal fiber did not respond at this time, indicating failure of propagation of this type of premature response. Records B through G show subsequent trials in which the test stimulus was given progressively later in the cycle, with the result that the magnitude, upstroke velocity, and duration of the

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premature responses increased at both recording sites. In addition, the contours of the responses at the two sites became similar. The time at which the premature response elicited at the distal site abruptly increased in magnitude and assumed a contour similar to that at the proximal site was taken as the start of nondecremental conduction and also was taken as the end of the effective refractory period. In Figure 8 the magnitude of the premature responses recorded at each of the two sites is plotted against the time at which the responses were produced during the cycle by test stimuli. The dashed vertical line indicates the time at which the response at the distal site suddenly became large and marks the end of the effective refractory period. After this point in the cycle, responses of large magnitude and similar contour were obtained at both proximal and distal sites, and the two curves in the graph representing these fibers tend to parallel each other.

Subsequently, propranolol, 3 mg/liter, was added to the solution and after 15 minutes a second determination of the effective refractory period was made. Finally, the concentration of propranolol was increased to 10 mg/liter and a third determination was made. Selected records obtained in these experiments are shown in Figure 7 and data are plotted.

**FIGURE 9**

Determination of the effective refractory period of Purkinje fibers using electronic stimuli. In all records the upper action potential is from a Purkinje fiber close to the stimulating electrode. The lower action potential is from a ventricular muscle fiber at a distance. Records A and B were taken during control perfusion, C and D during treatment with propranolol, 3 mg/liter, and records E and F during treatment with propranolol, 10 mg/liter. For each determination the trial of test stimulation (A, C, E) which immediately preceded that causing the first test response (B, D, F) is shown. See text for discussion. During propranolol treatment a different ventricular fiber was impaled.
Several changes in the effective refractory period are evident. At the lower concentration of propranolol, the effective refractory period was shortened.

It also should be noted from these experiments that the level of membrane potential needed to obtain propagated responses is the same after addition of propranolol as during the control determination. Thus at a concentration of 3 mg/liter the decrease in duration of the functional refractory period closely paralleled the acceleration of repolarization. However, at the larger concentration of propranolol, a higher level of membrane potential was required to obtain propagated responses, with the result that the refractory period was not shortened as much as would be anticipated from the degree of acceleration of repolarization.

The most striking effect of propranolol from the standpoint of a possible mechanism of its antiarrhythmic action is the lack of local, graded responses and decremental conduction. The earliest responses which could be elicited by premature excitation in the presence of propranolol were relatively large and of long duration and with rapid velocity of the upstroke. Furthermore the contours of the action potentials at both recording sites were similar and were propagated. No matter how closely the test stimuli were applied during the repolarization limb of the action potential, local, nonpropagated responses could not be obtained. It was therefore im-

**FIGURE 10**

Determination of the effective refractory period of ventricular muscle fibers treated with propranolol, 3 mg/liter. Records A and B were obtained during control perfusion. Records C and D were obtained after 15 minutes in propranolol solution. Upper action potential is from a ventricular muscle fiber close to the stimulating electrode. Lower potential is from a ventricular fiber at a distance. Records E through G and H through J show control and experimental determinations, respectively, made on a different preparation. Upper action potential is from a ventricular fiber close to the stimulating electrode. Lower action potential is from a distant Purkinje fiber. See text for discussion.
possible in the presence of propranolol to show the phenomenon of decremental conduction described by others (27) for normal cardiac tissue. In Figure 7, B and E show the trial of test stimulation which gave the earliest recorded response in the presence of propranolol. A and D show the trial of test stimulation which preceded by a few milliseconds that shown in B and E. It is evident that a very small delay in application of the test stimulus results in only propagated activity.

The second method used to determine the duration of the effective refractory period of Purkinje and ventricular fibers was that used by Moore et al. (28). Both drive and test stimuli were administered directly to the type of tissue under study. One microelectrode was located in a fiber very close to the site of stimulation (<2 mm), and a second microelectrode was located in a distant fiber of the same or different type to determine if propagation occurred. Figure 9 shows records obtained in an experiment in which Purkinje fibers were stimulated. The distal microelectrode was inserted in a ventricular muscle fiber (lower trace). It is evident that a very small delay in application of test stimulation resulted in the abrupt appearance of propagated activity. Duration of the effective refractory period decreased in the presence of propranolol. The minimal level of membrane potential needed to give propagated action potentials during treatment with propranolol is evident in this figure.

Ventricular Muscle Fibers.—Records obtained in two experiments in which we determined the effects of propranolol, 3 mg/liter, on the ventricular refractory period are shown in Figure 10. In each determination, early application of the test stimulus produced only a local response in the proximal fiber (top trace). A slight delay in application of the stimulus resulted in propagation to the distal recording site. Propranolol caused no change in duration of the ventricular refractory period. Unlike the response of Purkinje fibers, local responses in ventricular muscle were still in evidence while the tissue was exposed to propranolol. Records E and H show the earliest propagated responses which could be obtained during control and propranolol perfusions, respectively. Again there was no change in duration of the ventricular refractory period. During the control determination early application of test stimulation caused excitation of the ventricular fiber without producing a response in the Purkinje fiber. Then, as the stimulus was presented later in the cycle, the Purkinje fiber showed local, and finally propagated, responses. After treatment with propranolol it was impossible to excite the ventricular tissue without also exciting the Purkinje tissue.

C. RESPONSE TO RAPID FREQUENCIES OF STIMULATION

The ability of ventricular muscle and Purkinje fibers to respond rapidly was determined because, during ventricular tachycardia and fibrillation, rapid rates of the action potentials of individual fibers (29, 30) may help to maintain these arrhythmias. Measurement was made by stepwise increases in the frequency of the driving stimulus, beginning at 95/min and continuing to 600/min. Each frequency was applied for approximately 5 seconds and then the next higher frequency was given. Results from two experiments are shown in Figure 11. Record A shows that in the control solution, both fiber types could respond to every stimulus given at 600/min. After treatment with propranolol, 3.0 mg/liter, the fibers failed to respond to each stimulus when the rate was increased from 460 to 600/min (B). Records C through F show potentials recorded after exposure to propranolol, 10 mg/liter. The ability of the ventricular muscle fiber to respond rapidly was further impaired, as shown in F. In addition, a curious response on the part of the Purkinje tissue became apparent at this concentration of propranolol. When the rate of stimulation reached 300/min (C) the fiber responded to the first two stimuli and then responded to every other stimulus only. Finally, when the rate of stimulation reached 375/min the Purkinje fiber ceased to respond, and only small oscillations in the resting potential were evident. Such an effect might
Determination of ability to respond to rapid frequencies of stimulation. A: control. B: propranolol, 3 mg/liter. C-F: propranolol, 10 mg/liter. Upper action potentials are from a Purkinje fiber and lower potentials are from a ventricular muscle fiber. The stimulating electrode was applied to ventricular tissue. The numbers indicate the stimulus rate in pulses per minute. The vertical white bars indicate a change in stimulus rate. Records G-I are from an experiment in which stimuli were applied directly to Purkinje tissue under the influence of propranolol, 10 mg/liter. See text for discussion.

be attributed to a decrease in stimulating effectiveness of the ventricular fibers, which in this preparation caused excitation of the Purkinje fibers. That this was not entirely the explanation is shown in records G-I of Figure 11. Here, stimulation was applied directly to the false tendon, and a Purkinje fiber close to the stimulation site was impaled. When the rate of stimulation reached 150/min the fiber responded to every other stimulus. At a rate of 300/min only every third stimulus elicited an action potential, and finally, when a rate of 375/min was applied, the fiber ceased to respond.

D. EFFECT OF PROPRANOLOL ON THE RESPONSE TO EPINEPHRINE

The effect of low concentrations of propranolol on the response of Purkinje fibers to epinephrine was studied in nine experiments. Initially in each experiment, epinephrine was given by rapidly injecting 0.2 ml of a 1:25 dilution of epinephrine in water directly into the inflow of the tissue bath. Perfusion of the bath continued uninterrupted at 25 ml/min, and presumably this procedure caused a transient rise in the concentration of epinephrine in the bath fluid followed by a fall toward zero. In all experiments prior to treatment with propranolol, administration of epinephrine caused an increase in both rate and magnitude of diastolic depolarization of Purkinje fibers. Repeated injection performed on the same preparation caused the same magnitude of response but the response varied considerably in preparations from different hearts. Records from several experiments are...
shown in Figure 12. The usual effect was an increase of 3 to 5 mV in diastolic depolarization. In some experiments, there was an initial increase in diastolic depolarization and then development of an arrhythmia in which each driven action potential was preceded by a "spontaneous" action potential. It was unknown whether the "spontaneous" action potentials resulted from activity of an ectopic pacemaker or were the result of a disturbance of conduction. Treatment with propranolol, 0.1 mg/liter, in all preparations completely blocked the increase in diastolic depolarization, and the arrhythmias, as described above, were not observed. The concentration of propranolol required to prevent these responses to epinephrine is well below that needed to produce even minimal changes in the transmembrane potential.

**Discussion**

Results presented here show that propranolol causes several changes in the transmembrane action potential of ventricular muscle and Purkinje fibers. Processes involved in depolarization as well as those associated with repolarization were affected. Decreases in velocity of depolarization and in magnitude of overshoot and an impairment in ability to respond to rapid stimulation occurred in both fiber types studied. Similar effects occur in fibers of the rabbit atrium (19). Significant changes in the repolarization processes were limited to Purkinje fibers. Doses of propranolol that greatly accelerate repolarization of Purkinje fibers had relatively little effect on this feature of ventricular muscle fibers in the same preparation. Repolarization of atrial muscle fibers is affected only minimally by propranolol (19).

Our interest in propranolol arose because it exerts antiarrhythmic actions similar to its predecessors, dichloroisoproterenol and pronethalol, and currently is being evaluated for human use. It has been found effective in the management of certain atrial and ventricular...
Use of the information regarding the effects of propranolol on cardiac membrane potentials to explain its antiarrhythmic actions must be approached with caution. It necessitates knowledge of the specific electrophysiological changes causally related to production of the various cardiac arrhythmias. Such information presently is uncertain even for experimentally induced arrhythmias and is quite incomplete for clinical disorders. Hoffman and Cranefield (35) and Hoffman (36) have provided a detailed classification and discussion of those changes in cardiac transmembrane potential currently believed to give rise to cardiac arrhythmias. Such information makes possible a consideration of the mechanisms by which propranolol might exert its antiarrhythmic actions.

Propranolol decreased the velocity of depolarization of both ventricular muscle and Purkinje fibers. In the latter fibers the curve relating upstroke velocity to the level of membrane potential was displaced to the right and down. Similar effects are produced by other antiarrhythmic agents (24, 25) and are considered important with regard to their action. Such changes may prolong the effective refractory period even without prolonging the action potential (25). If, as in the present study, duration of the action potential decreases simultaneously with the reduction in velocity of the upstroke, changes in refractoriness cannot be predicted and must be measured. In this study, duration of the effective refractory period of Purkinje fibers treated with propranolol decreased and that of ventricular muscle fibers was unchanged. Prolongation of the refractory period generally is considered advantageous in combating cardiac arrhythmias (37). Propranolol is not unique among antiarrhythmic agents with respect to its action on shortening the refractory period of Purkinje fibers. Diphenylhydantoin has a similar effect (38).

One possible explanation for the effects of propranolol is that it decreases the ability of the cardiac fiber to follow rapid frequencies of stimulation. During periods of tachycardia or fibrillation, single cardiac fibers have rapid action potentials (29, 30) and probably must have to sustain the arrhythmia. Accordingly, any agent or procedure which limits the ability of the tissue to respond rapidly should tend to terminate or prevent these irregularities.

Another and perhaps more significant action of propranolol that may account for its antiarrhythmic action is the prevention of local responses and decremental conduction in Purkinje fibers. Decremental conduction (39) can be elicited in normal isolated preparations of cardiac muscle and is thought to cause certain types of conduction disturbances. Earlier reviews may be consulted (35, 36) for detailed discussion of the means by which this phenomenon produces disturbances of propagation. Briefly, decremental conduction results in nonuniform spread of the impulse and development of various degrees of conduction block. Unidirectional block of conduction is thought to occur and, in combination with the slowed velocity of propagation, may lead to re-entry of excitation. Any agent or procedure that prevents decremental conduction should therefore be of value in the treatment of arrhythmias. Propranolol is such an agent. It eliminates graded responses in Purkinje fibers and leads to more uniform spread of excitation between the ventricular tissues. Although the mechanism by which it exerts this effect is unknown, the significance of this action is apparent.

The question whether beta-receptor-blocking drugs exert their antiarrhythmic actions by virtue of beta-receptor blockade or these actions are due to nonspecific effects has concerned several investigators. Lucchesi and Hardman (13) showed that the ability of dichloroisoproterenol to block ouabain and acetylstrophanthidin-induced arrhythmias was not temporally correlated with beta-receptor blockade. In addition, compounds structurally related to dichloroisoproterenol but unable to effect beta-receptor blockade also antagonized these arrhythmias. Subsequently, by similar methods, Lucchesi (14, 40) proved the same to be true of pronethalol. Tuttle and
Innes (15) noted that suppression of ouabain-induced arrhythmias by pronethalol is transient and not correlated with the duration of beta-receptor blockade. Somani and Lum and others (8-10, 17) tested the effectiveness of different beta-receptor blocking agents on catecholamine-induced and ouabain-induced arrhythmias. All compounds prevented onset of the catecholamine arrhythmias, but only pronethalol and propranolol terminated ouabain-induced arrhythmias.

Results obtained here may be interpreted to show that propranolol produces changes in the transmembrane potential that are independent of an action on beta-receptors. First, we have shown that a small dose of propranolol, much lower than that needed to produce any change in membrane potential, completely prevented the increase in rate of epinephrine-induced diastolic depolarization of Purkinje fibers. It has not been shown conclusively that this action of epinephrine is a beta-receptor phenomenon. However, the positive chronotropic action of catecholamines in the intact animal is considered to be mediated by beta-receptors (41), and it is known that this response is due largely to an increase in rate of diastolic depolarization of sinoatrial nodal fibers (42). Since Purkinje fibers, too, show diastolic depolarization and can act as pacemakers (39), it seems likely that the augmentation in their diastolic depolarization induced by epinephrine results from an action on beta-receptors. If this assumption is correct, it is demonstrated that the changes in membrane depolarization and repolarization observed in the presence of propranolol are not related to beta-receptor blockade, because the block was established in the absence of changes in membrane potential. Second, we have shown that when the concentration of propranolol is increased, the changes produced in the membrane potential are intensified. If such changes resulted from beta-receptor blockade it seems likely that there would be no further change with higher doses after blockade was effected. Finally, we found that changes in repolarization of Purkinje fibers occurred at lower doses than those needed to elicit a decrease in upstroke velocity. One would expect that if these events were the result of beta-receptor blockade they would occur simultaneously with establishment of the block.

All beta-receptor antagonists tested, regardless of chemical structure, act against catecholamine-induced arrhythmias but only certain of these drugs are effective against digitalis-induced irregularities. This suggests that different mechanisms are operative in both occurrence and antagonism of these arrhythmias. The most apparent and consistent effect produced by catecholamines on electrical events in cardiac tissue is an increase in rate and magnitude of diastolic depolarization of pacemaker fibers. As suggested above, this effect possibly is mediated by beta-receptors. Whatever the mechanism, an increase in diastolic depolarization of Purkinje fibers can produce disturbances in cardiac rhythm. It is generally agreed that the presence of diastolic depolarization confers upon the cardiac fiber the ability to become a pacemaker and thus result in development of an ectopic focus. Recently it has been shown that development of diastolic depolarization can give rise to disturbances in impulse conduction (43), because enhanced diastolic depolarization may lead to decremental conduction with the attendant disturbances in impulse propagation outlined above. We have shown that propranolol prevents the increase in diastolic depolarization caused by epinephrine. One could speculate that this is a means by which beta-receptor-blocking agents prevent epinephrine-induced ventricular arrhythmias.

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