Effect of Verapamil on the Normal Action Potential and on a Calcium-Dependent Slow Response of Canine Cardiac Purkinje Fibers

By Paul F. Cranefield, Ronald S. Aronson, and Andrew L. Wit

ABSTRACT

The effect of verapamil on normal sodium (Na)-dependent and slow calcium (Ca)-dependent action potentials recorded from canine cardiac Purkinje fibers was studied. The Ca-dependent slow response was obtained in fibers exposed to solutions in which all NaCl was replaced by tetraethylammonium chloride and in which Ca ranged from 4 mM to 16.2 mM. Verapamil (0.25-2 mg/liter) had little or no effect on the upstroke of the normal action potential, but such concentrations of verapamil suppressed rhythmic activity and depressed excitability in fibers that showed Ca-dependent slow responses. Spontaneous activity and rhythmic activity evoked by long depolarizing pulses were depressed. Verapamil decreased the amplitude and the upstroke velocity and shifted the threshold potential toward zero in fibers that showed Ca-dependent slow responses. The effectiveness of verapamil varied with the level of Ca; 0.25 mg/liter of verapamil was as effective in suppressing activity in fibers exposed to 4 mM Ca as was 2 mg/liter of verapamil in fibers exposed to 16.2 mM Ca. Although verapamil did not alter the upstroke of the normal Na-dependent action potential, it did depress the plateau and prolong the action potential of fibers exposed to normal Tyrode's solution.

KEY WORDS: calcium blocking drugs, antiarrhythmic drugs, AV node, D-600, nodal tachycardia, cardiac arrhythmias, calcium and cardiac action potential, reentrant arrhythmias, SA node

We believe that reentrant arrhythmias result from slow conduction through discrete segments of partially depolarized fibers (1-5) and that slow conduction in such fibers is a property of the slow response. The slow response is an action potential qualitatively different from the normal action potential (6). It is possible that the slow response depends partly on calcium (Ca) currents (6), and it is nearly certain that it does not depend on the rapid increase in permeability to sodium (Na) that is characteristic of the normal action potential. Therefore, we sought a preparation in which propagated action potentials solely dependent on Ca could be obtained in a stable, reproducible fashion so that the effects of drugs on such action potentials could be examined.

The preparation which was developed consisted of canine cardiac Purkinje fibers exposed to a solution in which all NaCl had been replaced by tetraethylammonium (TEA) chloride and in which Ca varied from 4 mM to 16.2 mM (7). Fibers exposed to such solutions showed stable action potentials for several hours. These action potentials had a slow upstroke and a low conduction velocity; they were blocked by manganese, insensitive to tetrodotoxin, and depressed by the apparently specific Ca-blocking agent, D-600 (7).

In the present paper, the effects of verapamil on these Ca-dependent action potentials are presented. Verapamil was studied because it is a new and clinically effective antiarrhythmic agent (8) with an unknown mode of action. Moreover, verapamil is a Ca-blocking agent closely related to D-600, which is a methoxy derivative of verapamil (9). We found that verapamil depresses spontaneous activity and reduces the overshoot and the upstroke velocity of the action potential in Purkinje fibers whose electrical activity is based on Ca-dependent action potentials; moreover, it does so in concentrations that have no effect on the upstroke of the normal action potential. Many forms of spontaneous activity occur in fibers that show a low resting potential. Verapamil depresses the spontaneous activity of the sinoatrial (SA) node, depresses conduction through the atrioventricular (AV) node, and abolishes arrhythmias that depend on nodal...
reentry (Wit and Cranefield, unpublished observations). Therefore, we suggest that the antiarrhythmic activity of verapamil results from its ability to depress the slow response.

**Methods**

Mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv). The heart was excised and immersed in normal or Na-free Tyrode's solution (7). Bundles of Purkinje fibers (false tendons) were removed from the ventricles and placed in a beaker containing the solution at room temperature (25-27°C). Bundles to be studied were placed in a tissue bath perfused with solution maintained at 36-38°C. Fibers from which Ca-dependent action potentials were obtained were exposed to Na-free solutions for at least 2 hours before any studies were made.

Electrical recording was achieved with glass microelectrodes filled with 3MKCl. Pulses were isolated from the ground and were timed by a digital parallel timing system (10). In the experiments in which prolonged depolarizing pulses were applied, a pulse supplied by a constant-current stimulator was applied via an intracellular microelectrode that was placed in the same fiber about 0.1-0.2 mm from the recording electrode.

Table 1 gives the composition in mmoles/liter of the solutions used in these experiments. Glass-condensed redistilled water was used. Tyrode's solutions buffered with bicarbonate were bubbled with 95% O₂-5% CO₂. The Na-free, TEA-substituted Tyrode's solutions were buffered with 5 mM Tris neutralized to pH 7.2-7.6 with HCl and were bubbled with 100% O₂. The appropriate amount of a fresh stock solution of verapamil was added to the superfusion fluid.

In experiments in which extracellular Ca was increased beyond 16.2 mM, an appropriate amount of 0.5M CaCl₂ was added to the superfusing solution without reducing the TEA concentration; therefore, some hypotonicity resulted. Verapamil is α-isopropyl-α-[N-(N-methyl-N-homoveratryl)-γ-aminopropyl]-3,4-dimethoxyphenylacetonitrile hydrochloride.¹

**Results**

EFFECTS OF VERAPAMIL ON THE ACTION POTENTIAL OF PURKINJE FIBERS EXPOSED TO NORMAL TYRODE'S SOLUTION

The effects of verapamil on the electrophysiological characteristics of canine cardiac Purkinje fibers superfused with normal Tyrode's solution are shown in Figure 1 and Table 2. Figures 1 and 2 and Table 2 illustrate results obtained during experiments in which the same impalement was maintained without interruption throughout the determination of all control values and all changes induced by the drug. The maximum diastolic potential, the amplitude, and the maximum rate of change of potential (Vmax) changed at most by 3%; this change was insignificant because these parameters were measured by maintained impalements of single fibers. Figure 1A shows the action poten-

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1Kindly supplied by the Knoll Pharmaceutical Company.

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TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Maximum diastolic potential (mv)</th>
<th>Action potential amplitude (mv)</th>
<th>APD&lt;sub&gt;50&lt;/sub&gt; (msec)</th>
<th>APD&lt;sub&gt;100&lt;/sub&gt; (msec)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (v/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.5 (87-91)</td>
<td>120.0 (117-122)</td>
<td>169 (150-175)</td>
<td>357 (330-365)</td>
<td>560 (500-650)</td>
</tr>
<tr>
<td>Verapamil (0.5 mg/liter)</td>
<td>90.0 (88-91)</td>
<td>121.0 (118-122)</td>
<td>120&lt;sup&gt;*&lt;/sup&gt; (105-150)</td>
<td>414&lt;sup&gt;*&lt;/sup&gt; (360-430)</td>
<td>556 (480-650)</td>
</tr>
<tr>
<td>Verapamil (1.0 mg/liter)</td>
<td>87.0 (85-91)</td>
<td>118.0 (116-120)</td>
<td>140&lt;sup&gt;*&lt;/sup&gt; (130-160)</td>
<td>580&lt;sup&gt;*&lt;/sup&gt; (400-500)</td>
<td>561 (485-640)</td>
</tr>
<tr>
<td>Verapamil (2.0 mg/liter)</td>
<td>88.5 (86-90)</td>
<td>119.5 (117-121)</td>
<td>132&lt;sup&gt;*&lt;/sup&gt; (120-140)</td>
<td>620&lt;sup&gt;*&lt;/sup&gt; (510-640)</td>
<td>555 (460-610)</td>
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</table>

All values are means, and the range is given in parentheses. The data were obtained from five Purkinje fibers; a continuous impalement was maintained in each fiber. APD<sub>50</sub> = time to 50% repolarization, APD<sub>100</sub> = time to 100% repolarization, and V<sub>max</sub> = maximum rate of depolarization.

<sup>*</sup>Significantly different from control, P < 0.001.

Effects of Verapamil on Action Potentials of Purkinje Fibers Perfused with Normal Tyrode's Solution

The tracing shown in Figure 2A was obtained from the same fiber used for Figure 1C; it was, however, obtained after 40 minutes of exposure to 0.5 mg/liter of verapamil, i.e., 17 minutes later than the record shown in Figure 1C. Also, the sweep speed...
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in Figure 2A is half that in Figure 1C. Immediately after obtaining the record shown in Figure 2A the level of Ca in the Tyrode’s solution was increased from its usual value of 2.7 mM to 8 mM; 15 minutes later (Fig. 2B) the plateau had returned toward normal in terms of the level at which it was initiated as had the slopes of phases 2 and 3 and the total duration. The duration under control conditions (Fig. 1A) was about 80% of the duration seen in Figure 2B.

EFFECT OF VERAPAMIL ON CALCIUM-DEPENDENT ACTION POTENTIALS

As previously reported (7), Purkinje fibers perfused with Na-free, Ca-rich solutions are often spontaneously active. The effect of verapamil on a spontaneously active fiber exposed to 16.2 mM Ca is shown in Figure 3. The control rate of spontaneous activity was 60/min (A); after addition of 1 mg/liter of verapamil, it decreased to 30/min (B) in 13 minutes and to 24/min (C) in 22 minutes. The level of verapamil was then raised to 2 mg/liter; after 7 minutes the rate was 21/min. The slope of phase 4 depolarization, which is 22 mv/sec in A, fell to about 7.8 mv/sec in B and remained at about that level in C; in D it is about 7.4 mv/sec. A reduction in the slope of phase 4 from 7.8 mv/sec to 7.4 mv/sec would reduce the rate from 24/min to about 22.5/min. The slowing between C and D may therefore depend on some additional effect of the drug; there is an apparent change in the threshold potential as judged by the inflection point between the phase 4 depolarization and the upstroke. Although the level of that inflection cannot be judged with precision, in the records shown in Figure 3 the threshold potential is about -37 mv in A, -30 mv in B, -30 mv in C, and -20 mv in D. The slowing thus results from a decrease in the steepness of phase 4 depolarization and from a shift in the threshold potential; both changes are in the direction that causes slowing. There were also losses in amplitude, resting (maximum diastolic) potential, and overshoot: in A resting potential -58 mv, reversal 36 mv, total amplitude 94 mv; in B resting potential -53 mv, reversal 27 mv, total amplitude 80 mv; in C resting potential -52 mv, reversal 25 mv, total amplitude 77 mv; in D resting potential -48 mv, reversal 22 mv, total amplitude 70 mv. These complex changes are typical of those seen in many fibers exposed to verapamil. The reduction in steepness of phase 4 depolarization apparently accounted for much of the slowing; a shift in threshold potential also caused slowing. The shift in threshold potential was, however, accompanied by a shift in resting potential so that the amount of depolarization needed to shift from the resting potential to the threshold potential was 21 mv in A, 23 mv in B, 22 mv in C, and 28 mv in D. The effects on phase 4 depolarization and on threshold explain the slowing: the loss of amplitude and the shift in threshold indicate a reduction of the safety factor of conduction.

The effect on the maximum rate of change of voltage during the upstroke is seen at a fast sweep speed in Figure 4. This figure was obtained during the experiment used for Figure 3 but not at the same time. In Figure 4A, immediately before the drug was added, Vmax was 3.6 v/sec; in B, 6 minutes after the addition of 1 mg/liter of verapamil, Vmax was 1.92 v/sec; in C, 14 minutes after the addition of 1 mg/liter of verapamil, Vmax was 1.85 v/sec; and in D, 6 minutes after the concentration of verapamil was increased to 2 mg/liter, Vmax was 0.85 v/sec. The fall in Vmax was further evidence of a reduction in the ability of the impulse to propagate, i.e., evidence of a diminution in the safety factor of conduction.

Our initial report on fibers exposed to Na-free, Ca-rich solutions (7) examined the properties of spontaneously active fibers. We have since ascertained that some fibers that show a relatively high resting potential (-60 to -80 mv) and that are quiescent become spontaneously active during the passage of a depolarizing current (Aronson and
Records from a fiber exposed to 16.2 mM Ca and zero Na; the horizontal line indicates zero transmembrane potential. A: Record taken in the absence of verapamil. B: Record taken 6 minutes after exposure to 1 mg/liter of verapamil. C: Record taken 14 minutes after exposure to 1 mg/liter of verapamil. D: Record taken 6 minutes after the level of verapamil was raised to 2 mg/liter. There was an obvious decrease in the maximum rate of depolarization after the addition of verapamil. Calibrations: vertical 20 mv and horizontal 0.1 second.

Cranefield, unpublished observations). The records in Figure 5 were obtained from a fiber that was exposed to 16.2 mM Ca and that remained quiescent; its resting potential was about -64 mv. In Figure 5A the passage of a 12-second long depolarizing pulse of 0.15 × 10^-7 amp brought about rhythmic activity; seven impulses appeared during the pulse. During a stronger pulse, 0.2 × 10^-7 amp, ten action potentials appeared (Fig. 5B). The fiber was then exposed to 0.25 mg/liter of verapamil, and the records shown in Figure 5C and D were obtained 20 minutes later. The pulse of 0.15 × 10^-7 amp evoked only two action potentials; the pulse of 0.2 × 10^-7 amp evoked three action potentials. In other experiments this effect was the maximum effect produced by 0.25 mg/liter of verapamil; that dose often markedly reduced the number of action potentials evoked by a depolarizing pulse but never wholly abolished the appearance of multiple responses. Therefore, the low dose (0.25 mg/liter) differs from larger doses which regularly abolish all but the first action potential seen during a depolarizing pulse (Fig. 6). The effects of 0.25 mg/liter of verapamil can be completely reversed by washing out the drug; such reversal is not, however, seen except after prolonged exposure to the drug-free perfusate. A nearly full return to control levels is seen in Figure 5E and F where pulses of 0.15 × 10^-7 amp and 0.2 × 10^-7 amp produced the same frequency of activity as that seen in Figure 5A and B. This reversal of the effect of the drug required 60 minutes of exposure to the drug-free perfusate.

Records obtained from a quiescent fiber exposed to 16.2 mM Ca and zero Na; the top horizontal line indicates zero transmembrane potential, and the bottom trace indicates the application of a depolarizing pulse with strength shown on an arbitrary scale. A and B: No drug was present. During the passage of a depolarizing pulse of 0.15 × 10^-7 amp in A the fiber became spontaneously active. During the passage of a depolarizing pulse of 0.2 × 10^-7 amp in B a higher frequency of spontaneous activity was seen. C and D: Exposure to 0.25 mg/liter of verapamil for 20 minutes reduced the frequency of activity evoked by pulses of 0.15 × 10^-7 amp and 0.2 × 10^-7 amp, respectively. E and F: After 60 minutes of exposure to drug-free perfusate the rates evoked by pulses of 0.15 × 10^-7 amp and 0.2 × 10^-7 amp had returned to the control levels seen in A and B. Calibrations: vertical 20 mv and horizontal 2 seconds.

In Figure 6 the fiber was exposed to 16.2 mM Ca and was quiescent with a resting potential of -60 mv. The fiber became active (Fig. 6A) during passage of a 12-second depolarizing pulse of 0.5 × 10^-7 amp. After 28 minutes of exposure to 2 mg/liter of verapamil the application of a pulse of the same strength to the same fiber produced the same shift in transmembrane potential but only a single response was seen; the sustained rhythmic activity was suppressed. Much stronger pulses also produced only one response and no sustained activity. The single response that was evoked had a much reduced duration compared with that of the first response seen in Figure 6A.
Records were obtained from a quiescent fiber exposed to Na-free solutions containing 16.2 mM Ca. The top horizontal trace indicates zero transmembrane potential. The bottom trace shows the time course of a depolarizing pulse with strength shown in arbitrary units. 

A: Pulse of $0.5 \times 10^{-7}$ amp evoked rhythmic activity before the addition of verapamil. 
B: Depolarizing pulse of the same strength evoked only a single response after the fiber had been exposed to 2 mg/liter of verapamil. 
C: Strength of the depolarizing pulse was $0.12 \times 10^{-7}$ amp, which is the lowest strength capable of exciting the fiber; no verapamil was present. 
D: After the addition of 2 mg/liter of verapamil a pulse of $0.2 \times 10^{-7}$ amp was needed to evoke a single response. 

Vertical calibrations are 20 mv; horizontal calibrations are 2 seconds in A and B and 1 second in C and D.

Figure 6

The effects of weaker depolarizing pulses on the same fiber are shown in Figure 6C and D. In both cases, the pulse was set at the lowest level that would produce a single response ($0.12 \times 10^{-7}$ amp in C). The control record in Figure 6C was obtained prior to the addition of verapamil; the control record in Figure 6D was obtained 10 minutes after the addition of 2 mg/liter of verapamil. The sweep speeds in C and D were greater than those in A and B so that the depolarizing pulse continued beyond the end of the record in C and D. The shift in threshold potential seen in Figures 3 and 4 occurred between Figure 6C and D. Therefore, the current threshold was increased from $0.12 \times 10^{-7}$ amp to $0.21 \times 10^{-7}$ amp. The steady-state voltage displacements in C and D were, however, about the same, suggesting that the membrane resistance might have changed. The action potential in D was markedly shorter than that in C, just as the action potential in B was shorter than the first action potential in A. The records shown in Figures 5 and 6 offer further evidence that verapamil diminishes the tendency to sustained rhythmic activity and also reduces the ease with which a single propagated action potential can be elicited.

The SA node and the AV node are sensitive to much lower levels of verapamil than 2 mg/liter (Wit and Cranefield, unpublished observations). Therefore, considering the clinical effectiveness of a single intravenous dose of 10 mg of verapamil (8, 11), it seems likely that the drug is effective in quite low concentrations. The effect of verapamil on myocardial fibers varies with the concentration of extracellular Ca (9). We have therefore examined the effect of verapamil on fibers exposed to 4 mM Ca rather than to 16.2 mM Ca and have found that such fibers are much more sensitive to verapamil. Figure 7A shows a fiber that was spontaneously active in the presence of 4 mM Ca; the rate was about 16/min. After only 3 minutes of exposure to 0.25 mg/liter of verapamil the spontaneous rate was about 7/min, and there was a regular alternation between action potentials and subthreshold depolarizations. After another 15 minutes (Fig. 7C) the rate was markedly reduced and activity occurred only at irregular intervals. The level of Ca was then raised from 4 mM to 16.2 mM, and the con-

Figure 7

Records from a fiber that was spontaneously active in a solution containing zero Na and 4.0 mM Ca; the horizontal line represents zero transmembrane potential. 

A: Control record obtained in the absence of verapamil. 
B and C: Records showing marked reduction in rate and loss of amplitude (most marked in C) 3 minutes and 18 minutes, respectively, after exposure to 0.25 mg/liter of verapamil. 
D: With 0.25 mg/liter of verapamil still present, the level of Ca was raised to 16.2 mM. Records taken 4.5 minutes later show a partial return to the control frequency of spontaneous activity and a marked increase in amplitude and overshoot. 

Calibrations: vertical 20 mv and horizontal 5 seconds.
Concentration of verapamil was kept at 0.25 mg/liter. After 4.5 minutes the rate increased to about 6/min which is a marked increase over the rate seen in C but far less than the control rate seen in A. In addition, the total amplitude and the overshoot of the action potential increased. These records show that 0.25 mg/liter of verapamil depressed the spontaneous rate of fibers exposed to 4 mM Ca more severely than 2 mg/liter of verapamil depressed the spontaneous rate of fibers exposed to 16.2 mM Ca (Fig. 3). However, an increase in Ca concentration from 4 mM to 16.2 mM by no means wholly reversed the effect of verapamil in a concentration of 0.25 mg/liter.

We also examined the effect of a low concentration of verapamil on the response to depolarizing pulses of a fiber that was quiescent when it was exposed to 4 mM Ca. Prior to exposure to verapamil (Fig. 8A), application of a depolarizing pulse of $0.75 \times 10^{-7}$ amp evoked activity at a rate of 35/min. The fiber was then exposed to 0.25 mg/liter of verapamil. After 50 minutes (Fig. 8B) a stronger depolarizing pulse of $0.16 \times 10^{-6}$ amp evoked only two action potentials separated by an interval corresponding to a rate of about 12/min. This rate was not sustained during the pulse; if it had been, a third action potential would have appeared prior to the end of the pulse. After another 5 minutes (Fig. 8C) application of a still stronger pulse of $0.2 \times 10^{-6}$ amp evoked only one action potential. (The amplitude of the pulse shown on the bottom trace reflects the strength of the pulse before it was increased or decreased by a factor of 10 at the isolation unit. A stronger pulse in B, C, and D is therefore represented by a deflection in the bottom trace smaller than that representing the weaker pulse in A.) Immediately after obtaining the record shown in Figure 8C the level of Ca was raised from 4 mM to 32 mM, and the concentration of verapamil was kept at 0.25 mg/liter. This procedure restored the response to a depolarizing pulse only partially and very slowly. Only after nearly 40 minutes (Fig. 8D) was it possible to evoke more than one action potential by application of a depolarizing pulse. The record shown in Figure 8D was obtained 37 minutes after extracellular Ca was increased to 32 mM. The strength of the pulse was greater than that in Figure 8C ($0.3 \times 10^{-6}$ amp), but the level of depolarization evoked by it was the same (or a little less) than that in C. During the pulse, three action potentials were evoked at a rate of about 12/min. Although the threshold remained high and the spontaneous rate remained well below that seen in A, Vmax of the action potentials was markedly increased. The records seen in Figure 8 offer further evidence that a low level of verapamil inhibits activity of fibers exposed to a low level of Ca.

Concentrations of verapamil that have little effect on the action potential of either the SA node or the AV node greatly prolong the effective refractory period of the AV node, thereby reducing the ability of the AV node to conduct premature impulses and enhancing the tendency for AV block to occur when the rate increases (Wit and Cranefield, unpublished observations). Therefore, we examined the effect of verapamil on the rate at which a quiescent fiber exposed to Na-free, Ca-rich solutions can be driven. It was very difficult to obtain convincing, reproducible results from such studies, since quiescent fibers are very difficult to drive. It is, however, our definite impression that fibers that can be driven at rates as high as 15/min for long periods often cannot be driven at rates as low as 6/min after exposure to 1 mg/liter of verapamil. This finding is, in a certain sense, consistent with the finding that verapamil slows the rate of spontaneously active fibers and reduces the frequency of activity evoked by the application of depolarizing pulses to quiescent fibers.

**FIGURE 8**

Records from a quiescent fiber exposed to zero Na and 4.0 mM Ca. The top horizontal trace represents zero transmembrane potential; the bottom trace in A shows the duration and the strength of a depolarizing pulse, the strength is shown in arbitrary units. In B-D the strength of the depolarizing pulse is shown on a scale in which 1 unit corresponds to 10 units in A: A: Application of a pulse of $0.77 \times 10^{-7}$ amp evoked sustained rhythmic activity. B: Application of a depolarizing pulse of $0.16 \times 10^{-6}$ amp produced two responses 50 minutes after exposure to 0.25 mg/liter of verapamil. C: A pulse of $0.2 \times 10^{-6}$ amp produced only one response after another 5 minutes of such exposure. D: A pulse of $0.3 \times 10^{-6}$ amp evoked three action potentials in the same fiber still exposed to 0.25 mg/liter of verapamil and exposed for 37 minutes to 32 mM Ca. Calibration: vertical 20 mv and horizontal 5 seconds.
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Discussion

Our results show that verapamil at concentrations of 0.5 mg/liter, 1.0 mg/liter, and 2 mg/liter has no effect on the upstroke of a Purkinje fiber exposed to normal Tyrode's solution. Therefore, verapamil, at these concentrations, does not exert its antiarrhythmic effect by altering the upstroke of the normal action potential.

On the other hand, verapamil exerts several depressant effects on the electrical activity of Purkinje fibers exposed to Na-free, Ca-rich solutions. (1) Verapamil suppresses spontaneous activity in fibers that are spontaneously active; (2) it abolishes the sustained rhythmic activity that can be evoked by depolarizing pulses applied to quiescent fiber; (3) it probably increases the threshold and appears to shift the threshold potential toward zero; (4) it reduces overshoot and upstroke velocity; and (5) it appears to reduce the rate at which quiescent fibers can be driven.

The character, stability, and reproducibility of the electrical activity of canine cardiac Purkinje fibers exposed to Na-free, Ca-rich solutions can be summarized as follows. The experiments in this study were conducted on bundles of canine cardiac Purkinje fibers removed from the ventricle and placed in a beaker of Na-free, Ca-rich solution where they remained for 45-300 minutes before being transferred to a tissue bath. Generally, even the first bundle studied during the experiment had been exposed to Na-free solution for 90-120 minutes in the beaker and tissue bath before any study began. Some bundles showed spontaneous activity, others were quiescent. The spontaneously active bundles remained spontaneously active for as long as 4-5 hours. Other bundles were quiescent in the beaker but became and remained spontaneously active following the evocation of one or a few impulses by applied stimuli. Still other bundles were quiescent except when driven by applied stimuli; such bundles showed repetitive activity during the passage of a long depolarizing pulse. Finally, some bundles were inexcitable. Verapamil exerted the same effects on each type of active fiber; namely, (1) it suppressed spontaneous activity or reduced the tendency toward repetitive activity induced in an otherwise quiescent fiber by a depolarizing pulse and (2) it reduced the overshoot and the upstroke velocity in each type of fiber. These findings were invariable in 25 experiments conducted on 75 bundles; in other words, at no time did verapamil fail to exert its effects either on spontaneously active fibers or on activity induced by depolarizing pulses. In addition, the effects of verapamil described above were reversed when the fiber was exposed to verapamil-free solution; reversibility was, however, not rapid nor was it always complete. Because the preparation without verapamil exhibited the variability described above, our findings are qualitative in the sense that we have reported certain important and reproducible changes induced by verapamil without attaching precise average values to measurements such as the extent to which maximal upstroke velocity is changed by the addition of verapamil. The frequency of spontaneous activity is always markedly reduced by verapamil, as is the slope of phase 4 depolarization, whether the activity is truly spontaneous or is induced during a depolarizing pulse; likewise the overshoot and the maximum upstroke velocity are always reduced in either form of activity. Our finding of a shift in the threshold and in the threshold potential in a direction that reduces both excitability and the safety factor of conduction needs to be qualified. Estimates of the threshold potential based on the level at which an inflection occurs in the upstroke are necessarily somewhat subjective. Moreover, the threshold determined by the application of intracellular stimuli can vary with many factors such as accommodation, cable properties, and changes in the liminal length that must be depolarized to the critical level at which excitation becomes self-sustaining. The changes that we have observed are, however, quite marked and we believe that they point to a reduction in the safety factor of conduction.

The effects of verapamil on Ca-dependent action potentials are consistent with other known effects of verapamil. For example, the sensitivity of the SA node and the AV node to verapamil may be accounted for by assuming that the action potentials of these tissues depend on a slow change in permeability similar to that which presumably underlies the Ca-dependent slow response (Wit and Cranefield, unpublished observations). The negative inotropic effect of verapamil (12-14) may be related to a decrease in Ca influx during the action potential. This finding would be consistent with that of Kohlhardt et al. (9) who, using voltage clamps, found that 2 mg/liter of verapamil inhibits the inward Ca current in ventricular muscle fibers. Finally, of course, verapamil exerts powerful antiarrhythmic activity in both experimental and clinical situations (8, 11-13, 15, 16, Wit and Cranefield, unpublished observations). Reentry can occur in...
networks of Purkinje fibers that show slow conduction and unidirectional block within areas of low resting potential (1–5). To the extent that propagation in markedly depolarized fibers depends on slow inward currents carried by Ca, the action potential seen in Na-free, Ca-rich solutions may, as previously discussed (7), serve as a model for the type of action potential found in depolarized fibers. Therefore, the ability of verapamil to depress the excitability of fibers showing the slow response could eliminate the conditions necessary for the genesis of reentrant arrhythmias. A similar effect on AV nodal fibers apparently explains the ability of verapamil to suppress reentrant nodal tachycardias (Wit and Cranefield, unpublished observations). It is also clear that verapamil suppresses a specific form of rhythmic activity that is seen at certain levels of resting potential in fibers exposed to Na-free, Ca-rich solutions. Rapid spontaneous activity seen in partially depolarized ouabain-poisoned fibers exposed to normal levels of Na is suppressed by verapamil. That activity occurs at levels of transmembrane potential at which the normal Na-dependent upstroke is abolished, and it appears to be slow-response activity (Aronson and Cranefield, unpublished observations).

An important implication of our results is that some antiarrhythmic drugs may act primarily on the slow response that occurs at depolarized levels of membrane potential rather than on the rapid Na-dependent response. Damaged and diseased cardiac cells can generate an electrical response that differs fundamentally from that of the normal cardiac cell (1–6). The normal action potential occurs only in fibers that have a relatively high resting potential and results from a brief, large increase in the permeability to Na. In partially depolarized fibers inactivation abolishes the normal upstroke, and the slow response arises as the result of a prolonged, small increase in the permeability of channels that can admit Ca, Sr, and probably also Na (7). It is this response that appears to be blocked by verapamil.

Recently, it has often been suggested that Ca can carry inward current during the cardiac action potential (17–21). Singh and Vaughan Williams (13), suggesting that verapamil might act by blocking Ca currents, were unable to cite evidence that Ca-dependent action potentials "contribute to the development of an aberrant spike." Other studies (1–7) have shown that propagated electrical responses can occur in partially depolarized fibers or in the complete absence of Na. The inhibition of the Ca-dependent action potential by a drug that is a potent antiarrhythmic agent suggests that verapamil acts by suppressing the slow response; it also strengthens the hypotheses that the presence and the properties of the slow response are responsible for many arrhythmias and that the Ca-dependent action potential is a form of the slow response that is useful for studying the effects of drugs. An important question that remains unanswered is whether verapamil and similar agents suppress only the movement of Ca through the channels responsible for the slow response or whether they suppress the movement of all ions through those channels. This question is part of the larger question of whether the slow response in depolarized fibers exposed to a normal ionic environment depends on the movement of Na, of Ca, or of both (6, 7). The sensitivity of the SA node and the AV node to verapamil (Wit and Cranefield, unpublished observations) suggests that there is a similarity between the slow responses of those cells and the Ca-dependent slow responses described in the present article. The fact that verapamil slows the rate of spontaneous activity in the SA node (Wit and Cranefield, unpublished observations), suppresses one form of spontaneous activity in ouabain-poisoned fibers (Aronson and Cranefield, unpublished observations), and depresses spontaneous activity in fibers generating Ca-dependent action potentials indicates that it may exert an antiarrhythmic effect by suppressing spontaneously active foci. Verapamil may exert an equally important antiarrhythmic effect by suppressing reentry via its ability to depress conduction of the slow response. Such depression is evidenced by prolongation of the AV interval in the presence of normal Tyrode's solution (Wit and Cranefield, unpublished observations) and by various effects on Ca-dependent action potentials, e.g., elevation of threshold, reduction of upstroke velocity, reduction of the rate at which the fiber can be driven, and prolongation of refractoriness. We must, however, add three cautionary remarks. First, although the actions of verapamil that we have described obviously can be the result of a reduction in a slow inward current that is carried by Ca, some of the effects might result from verapamil-induced changes in other currents. Verapamil could affect outward currents in the resting fiber or it could change the voltage dependence of outward currents; leakage currents and currents carried by potassium or by chloride might be affected by verapamil. Second, the effect of verapamil on the plateau (Figs. 1 and 2) suggests
that it may affect the initiation and the propagation of premature impulses in fibers exposed to normal Tyrode’s solution. Finally, concentrations of verapamil of 0.5 mg/liter or higher exert a marked negative inotropic effect on normal fibers and on fibers exposed to Na-free solutions as judged by inspection through the dissecting microscope. Although clinically effective doses may produce a negative inotropic action, it is not likely to be a profound effect and it seems likely that the drug exerts its clinical effect at levels lower than 0.5 mg/liter. This finding is suggested by the efficacy in humans of a single intravenous dose of 10 mg (8, 11). The apparently greater in vivo efficacy may be explained by the fact that verapamil appears to be effective at lower doses in vitro if the level of Ca is more nearly that found in the blood. Our finding that 0.25 mg/liter of verapamil depresses activity in fibers exposed to 4 mM Ca as effectively as 2 mg/liter of verapamil depresses activity in fibers exposed to 16.2 mM Ca supports this view.

References
Effect of Verapamil on the Normal Action Potential and on a Calcium Dependent Slow Response of Canine Cardiac Purkinje Fibers
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