New Mechanism of Antiarrhythmic Drug Action
Increasing L-Type Calcium Current Prevents Reentrant Ventricular Tachycardia in the Infarcted Canine Heart
Candido Cabo, PhD; Heiko Schmitt, MD; Andrew L. Wit, PhD

Background—We studied whether increasing L-type calcium current has antiarrhythmic effects.
Methods and Results—Reentrant circuits in the epicardial border zone (EBZ) of healing canine infarcts were mapped during sustained ventricular tachycardia. The cardiac-specific L-type calcium current enhancer Bay Y5959 prevented initiation of sustained ventricular tachycardia in 7 of 14 experiments. Bay Y5959 caused slowing of conduction in areas of slow nonuniform conduction in reentrant circuits; block eventually occurred. Conduction was not affected in other regions of the circuits or in more normal areas of the EBZ, nor was the EBZ effective refractory period changed. Bay Y5959 also improved conduction of premature impulses so that lines of unidirectional block necessary for VT initiation were not formed, an effect not related to a change in the effective refractory period at the site of block.

Conclusions—Block of conduction caused by enhanced L-type calcium current in reentrant circuits may result from a decreased gap junctional conductance consequent to an increase in intracellular calcium. An increase in L-type calcium current may improve conduction of premature impulses. (Circulation. 2000;102:2417-2425.)

Key Words: tachycardia • myocardial infarction • calcium • ion channels • mapping • antiarrhythmia agents

Drugs currently used to stop reentrant arrhythmias are ion channel blockers. The consequences of increasing transmembrane current flow, in general, have not been investigated. We hypothesized that increasing L-type calcium current might have antiarrhythmic effects on reentry, because it should prolong repolarization. An increase in intracellular calcium resulting from an increased calcium current should also influence other ion channels, providing a different pharmacological profile than potassium channel blockers, which also prolong repolarization. To test this hypothesis, we determined the effects of the calcium promoter Bay Y5959, which increases L-type calcium current by agonistic action on the dihydropyridine receptor, on reentrant excitation.

Methods
We mapped reentrant circuits in the epicardial border zone (EBZ) of healing (4-day-old) infarcted canine hearts during sustained ventricular tachycardias (SVTs; >30 seconds duration) induced by programmed ventricular stimulation. Methods related to infarct production, tachycardia induction, mapping, and effective refractory period (ERP) and conduction velocity determinations have been described.

SVTs were initiated (up to 12 times) by single or double premature premature stimulation protocols and stopped by overdrive pacing. Once the coupling intervals at which SVT could be induced were determined, tachycardia was always induced at those intervals. The dihydropyridine receptor agonist Bay Y5959 (Bayer AG) was then administered in a liposomal carrier system as a continuous intravenous infusion of 30 μg · kg⁻¹ · min⁻¹. The resulting plasma levels were measured in 9 experiments by Bayer AG by use of high-performance liquid chromatographic extraction with UV detection at 245 nm. Reinitiation of SVT was attempted during drug infusion with the same stimulation protocol as control. ERPs and conduction velocities were measured periodically. In 3 of the 7 experiments in which reinitiation of VT was not possible during drug (see Results), the infusion was interrupted and reinitiation was attempted. In 2 experiments, the lipidosomal vehicle alone was infused for 30 minutes before infusion of Bay Y5959 (with the liposomes).

All data are expressed as mean±SD. One-way repeated-measures ANOVA (SigmaStat, Jandel Scientific Software) (significance level of P<0.05) was used for testing the null hypothesis when ERPs and conduction velocities were compared before and after the drug. Paired t test (significance level of P<0.05) was used for testing the null hypothesis when VT cycle lengths were compared before and after the drug.

Results
Effect of Bay Y5959 on VT
In 14 dogs, SVT was initiated during control. Bay Y5959 prevented initiation in 7 by the same stimulation protocol. When VT was prevented, unsustained tachycardia also did not occur. In only 1 of those experiments did a more aggressive protocol (double premature stimuli instead of single) induce VT. In 3 experiments, SVTs could not be induced during the first attempt, 10 minutes after infusion was begun (dose ≈0.3 mg/kg), or thereafter. In the other 4, SVTs with the same QRS morphology were initiated during
drug infusion before prevention at 15 (≈0.45 mg/kg), 21 (≈0.63 mg/kg), 26 (≈0.78 mg/kg), and 88 (≈2.64 mg/kg) minutes. Cycle lengths of SVTs initiated during drug infusion were prolonged (Figure 1A and 1C). In 6 of the 7 experiments in which SVTs were not prevented by Bay Y5959, cycle length was also significantly prolonged (from 202±45 to 237±58 ms; P<0.05) (Figure 1B).

In 3 experiments in which SVT was prevented, drug infusion was interrupted. In 2, SVT could be reinduced within 35 minutes. In the third, SVT could not be reinduced, even after 2 hours. In 2 experiments, the liposomal vehicle alone had no effect on SVT initiation or cycle lengths. In 7 additional experiments in which SVT could not be initiated during control, the drug did not promote induction of any tachycardias.

Effect of Bay Y 5959 on Reentrant Circuits Causing SVT

Figure 2 shows a representative example of an experiment in which SVT was prevented (ECGs in Figure 1C; open triangles in Figure 1A). During control (Figure 2A), the reentrant wave rotated around a horizontal line of functional block (upper thick black line not present during sinus rhythm) (curved black arrows), completing a revolution in 265 ms. Another line of block was located at the Apex-LL margin. It is uncertain whether there is a second reentrant circuit around this line, because activation toward the lateral left ventricle is outside the mapped region. The reentrant wavefront moves transversely to the long axis of the myocardial fibers between the 2 lines of block. Electrograms recorded from 3 rows of electrodes (rectangles on maps) (Figure 2D, left) show fractionation, characteristic of transverse conduction. Figure 2B shows the map of the circuit during infusion of Bay Y5959 (at 10 minutes), when the cycle length of tachycardia had increased to 301 ms (ECG in Figure 1C, middle). Propagation times between isochrones 70 and 180 (above the line of block) were unaltered. However, the drug slowed transverse activation in the right to left direction (isochrones 180 to 300 and 10 to 70) and caused new vertical lines of block between the original horizontal ones. The electrograms (Figure 2D, middle) are in general broader, reflecting the slowed activation. The increased time for activation of this region of 40 ms correlates with the increase in SVT cycle length. Figure 2C shows the map of the circuit 21 minutes after drug infusion (ECG in Figure 1C, bottom). Lines of block caused by the drug were prolonged. A narrow isthmus remains that enabled the reentrant wavefront to complete the circuit. In Figure 2D (right), the block is indicated by the short, thick, oblique lines. This SVT stopped after 45 seconds, conduction blocked at the site at which the isthmus was narrowest. Tachycardia could not be reinitiated.

In each experiment, the area(s) in which Bay Y5959 caused localized slowing and block during reentry had different electrophysiological characteristics than neighboring regions.
Activation during pacing was more irregular and conduction velocity was slower than in adjacent areas (see Figure 4A, circled electrodes during pacing in the same experiment as illustrated in Figure 2). The effect of Bay Y5959 in regions of localized slow conduction was to create block (see thick black line in Figure 5A during pacing in the experiment illustrated in Figure 2).

The cycle length of the SVTs that were not prevented by Bay Y5959 increased in all but 1 experiment (Figure 1B, inverted solid triangles) because of slowing of activation in the circuit similar to that shown in Figure 2, but without block. Figure 3A shows the control map of the circuit in which cycle length did not prolong; a reentrant wave rotated (curved black arrows) around a short line of functional block (thick black line) (cycle length 215 ms). Electrograms a through i (circled on the map) were recorded around the circuit. Toward the apex, there was a long convoluted line of block not involved in the circuit, which was also present during sinus rhythm or pacing. Most of the electrograms recorded in the area between this line of block and the apical margin had a long duration and/or exhibited fractionation. Electrograms s through z (enclosed in squares on the map in Figure 3A and shown below) were recorded at sites adjacent to that line of block. Propagation at those sites was slower than in other areas, as indicated by the wider electrograms.

Figure 3B (top) shows the map of the reentrant circuit in this experiment 33 minutes after drug infusion. Activation in the circuit (curved black arrows and electrograms a through i) is similar to control. Figure 3B (bottom) shows electrograms s through z recorded at sites in the dotted area, which now had a high degree of block. That the major effect of the Bay Y5959 was in an area (stippled on the map in Figure 3B) not crucial for the reentrant circuit explains why the cycle length of tachycardia was not increased.

In summary, Bay Y5959 selectively depressed conduction in specific regions of the EBZ characterized in control by nonuniform slow conduction and fractionated electrograms during pacing. Only when those regions were an integral part of the reentrant circuit did the localized slowing and block caused by Bay Y5959 cause an increase in the cycle length of the tachycardia and termination (Figure 2).

**Effect of Bay Y5959 on Conduction of Premature Impulses Initiating SVT**

Of the 7 experiments in which SVT was prevented, initiation could be mapped in 5 because the propagation of the initiating premature impulse occurred under the electrodes. In 2 of these experiments, lines of unidirectional block of premature impulses during control became bidirectional block after drug, and initiation of reentry was no longer possible. Figure 4A shows the activation pattern of the last basic impulse (CL 300 ms) (V1 in 4D) during control. There was an area (sites c through g) in which conduction was slower than in adjacent regions, which was discussed with
respect to Figure 2. Conduction of a premature impulse (coupling interval 200 ms) (Figure 4B and 4D, V₂) blocked orthodromically at the thick black line. Small electrotonic deflections in the electrograms recorded from electrodes c through g (circled on map; arrowheads in 4D, V₂) were associated with the block. The premature impulse activated those electrodes antidromically after conduction around the ends of the line of block (curved arrows) and activating the distal side. Figure 4C shows conduction of the premature impulse antidromically through the line of block to cause the first reentrant beat (T₁ in Figure 4D) (isochrone 180 in Figure 4B is isochrone 0 in Figure 4C).

After Bay Y5959 (Figure 5A), a line of block (thick black line) occurred during the basic drive (CL 300 ms), which was not present during control (compare with Figure 4A). Conduction of the premature impulse with a 200-ms coupling interval (Figure 5B) blocked orthodromically (thick black line) (electrotonic deflections indicated by arrowheads in electrograms c through g for V₂ in 5C). The premature impulse was unable to propagate to the distal side of the line of block (circled electrodes c through g, stippled area in Figure 5B), resulting in an area of bidirectional block that prevented the initiation of the tachycardia. This is the same region in which block occurred during reentry to terminate tachycardia in Figure 2.

In 3 experiments, lines of unidirectional block of premature impulses that initiated reentry during control were not formed after the drug. Activation of the EBZ during basic drive at a cycle length of 300 ms during control in 1 of these experiments is shown in Figure 6A (electrograms a through e, V₁ in 6D). Propagation of the premature impulse that initiated VT (V₁-V₂ of 200 ms) blocked orthodromically (thick black line in Figure 6B; in 6D, V₂ block between electrograms c and d). Block did not occur in a region that showed evidence of marked conduction depression (see Figure 6A). Propagation proceeded around the line of block and activated the distal side. In Figure 6C, isochrone 10 is the same as isochrone 180 in 6B. The proximal side of the line of block was antidromically reexcited at 74 ms (6D; T₁, electrograms e through b). The rest of the EBZ was activated by counterclockwise- and clockwise-rotating waves (Figure 6C).

In this experiment, the pattern of propagation of the basic impulse (Figure 7A and 7C, V₁) after drug infusion (≈1.05 mg/kg), when tachycardia could no longer be initiated, is similar to the control. The pattern of activation of a premature impulse with a V₁-V₂ coupling interval of 185 ms, from isochrones 50 to 110, is similar to the control (Figure 7B) except that activation is slower. After isochrone 110, there is a marked delay of >40 ms (to isochrone 150) in the same region in which conduction block occurred in the control. Activation on the distal side of this region continues in the orthodromic direction (isochrones 150 to 180); block did not occur (compare electrograms recorded at c and d during V₂ in Figures 6D and 7C). Because activation did not block, reentry
could not occur. The local coupling interval of the premature impulse at electrode c in Figure 7C was 193 ms, 7 ms less than the 200-ms coupling interval at electrode c in control when block occurred (Figure 6D). Therefore, failure of block to occur after drug was not the result of prolongation of the coupling interval in this region. Lines of block at the same location were formed reproducibly for local coupling intervals at electrode c, ranging from 170 to 200 ms in control, but block did not occur at the same range of coupling intervals after drug.

**Effect of Bay Y5959 on ERP and Conduction Velocity**

In the noninfarcted right ventricle, there was a significant increase in the ERP 20 to 40 minutes after drug infusion was started ($\approx$0.6 to 1.2 mg/kg) (Table 1). A further increase in dose (infusion time) did not change the ERP more. There was also a significant increase in the QTc interval from 358±28 to 379±26 ms ($P<0.05$). Changes in sinus cycle length were not statistically significant (control, 358±49 ms; during drug infusion, 378±75 ms). Also, no significant changes occurred in systolic, diastolic, or mean arterial blood pressure.

The mean ERP of the EBZ did not change significantly during drug infusion (Table 1). However, ERPs measured at different EBZ sites either increased (36), decreased (19), or did not change (7). We could not establish any relationship between changes in ERP at individual sites and prevention of reentry, although ERPs were measured in different regions of the reentrant circuits. In general, ERPs could not be determined in the regions in which there was slow and nonuniform conduction with fractionated electrograms where reentrant wave fronts blocked, either in control or after drug, because these regions could not be captured by the external stimuli (maximum current, 10 mA). In other regions of the EBZ with more normal conduction properties, random changes in ERPs occurred (increased, decreased, or showed no change).

Table 2 shows the effect of Bay Y5959 on conduction velocity measured in the areas of the EBZ in which conduction velocity was not severely depressed in control (smooth, biphasic, short-duration electrograms). Conduction velocity in the longitudinal and transverse directions and the anisotropic ratio did not change significantly. However, as previously described, conduction slowed and blocked in regions in which it was depressed.

**Discussion**

**Bay Y5959, an Experimental Tool to Investigate the Antiarrhythmic Consequences of Increasing L-Type Calcium Current**

Bay Y5959 is a dihydropyridine agonist structurally related to nifedipine, which increases the mean opening and closing times of the L-type calcium channel, resulting in a net
increase in calcium current. Bay Y5959 modulation of the Ca channels is strongly voltage-dependent, increasing calcium current only at more negative membrane potentials. Therefore, it has a positive inotropic effect in the heart without causing contraction of vascular smooth muscle. The lack of vascular effects is reflected in the insignificant changes in blood pressure found in the present and earlier studies.

Possible Mechanisms of Antiarrhythmic Effects of Increasing L-Type Calcium Current

Although we have shown that Bay Y5959 has antiarrhythmic effects, the cellular mechanism is still conjecture. An increase in L-type calcium current should prolong the ventricular action potential and ERP and offer an alternative mechanism to blocking delayed rectifier currents for causing a class III antiarrhythmic effect. Indeed, this is the case in guinea pig papillary muscle. We found an increase in ERP in noninfarcted right ventricle and prolongation of the QTc on the ECG. However, even though delayed rectifier blockers have been shown to cause an increase in ERP in the EBZ, Bay Y5959 increased ERP only at some locations and not others. Despite the increase in L-type calcium current caused by the drug in myocytes in the EBZ, the lack of consistent class III effect might be the result of the modulation by intracellular calcium of several ionic channels. An increase in calcium current by Bay Y5959 results in an increase in intracellular calcium, which can inactivate the calcium channel and/or increase current through the calcium-sensitive delayed rectifier channel. The effects of intracellular calcium on the inward rectifier current can also modulate excitability and repolarization. The combination of those effects could explain why an increase in L-type calcium current might not result in an increase in ERP. Differences in handling of intracellular calcium between normal myocytes and myocytes surviving in the EBZ and/or differences in the modulation by intracellular calcium of L-type calcium and delayed rectifier ion channels may explain the different effects of Bay Y5959 on ERPs measured in noninfarcted and infarcted tissues.

Nonuniform anisotropy in the healing infarct border zone is a cause of reentry and may be the result, at least in part, of gap junction remodeling. In areas of slow nonuniform conduction and fractionated electrograms, Bay Y5959 caused further slowing and block. When those areas were important for the maintenance or initiation of VT, tachycardia was prevented. Fractionated electrograms are markers for poor intracellular coupling. We propose that increased block in poorly coupled areas caused by Bay Y5959 was the result of further cell uncoupling as a result of an increase in intracellular calcium, because Bay Y5959 increases calcium transients in border zone myocytes. There is disagreement on the levels of intracellular calcium that might have this effect. Although some studies indicate that moderate alterations in intracellular calcium within the normal physiological range do not alter gap junctional conductance, others suggest a much greater sensitivity of gap junctional coupling to calcium. The effect of intracellular calcium alterations on gap junctional conductance in partially uncoupled preparations has also been shown to be greater than in normally coupled cell pairs, with moderate elevations in intracellular calcium causing further uncoupling. Therefore, it is possible that the increase in intracellular calcium caused by Bay Y5959 in combination with a greater sensitivity to calcium of partially
uncoupled and perhaps remodeled gap junctions in the healing infarct may lead to further uncoupling and block.

The second effect of Bay Y5959 was to improve conduction of premature impulses so that lines of block that were necessary for SVT initiation did not form. This effect occurred in regions in which conduction was not as depressed as in regions in which Bay Y5959 caused block (less uncoupled?). We found no evidence that a decrease in ERP at sites distal to the lines of block was the mechanism. One possible explanation consistent with our results is based on the importance of the L-type calcium current for propagation in cells with Na\textsuperscript{+}-dependent action potentials.\textsuperscript{16–18} In situations in which Na\textsuperscript{+} current may inactivate before local circuit currents depolarize distant cells, the ability of the L-type calcium current to maintain depolarization may determine whether propagation succeeds or fails. This situation might occur during premature stimulation, at sites at which lines of block form in the EBZ. Na\textsuperscript{+} currents in myocytes from the EBZ are reduced because of sodium channel remodeling,\textsuperscript{19} and premature stimulation further reduces the ability of the sodium current to stimulate distal cells, resulting in conduction block. In addition, the L-type calcium current is reduced in myocytes from the EBZ,\textsuperscript{11} most likely reducing its role in supporting propagation of premature impulses with reduced Na\textsuperscript{+} current. Therefore, enhancing L-type calcium current with Bay Y5959 is likely to play a critical role in improving the success of conduction of premature impulses in the EBZ and in prevention of SVT. Although some uncoupling by Bay Y5959 is still expected to occur in these regions, the effects

### TABLE 1. Effect of Bay Y5959 on ERPs in Normal Zone and EBZ

<table>
<thead>
<tr>
<th>BCL = 250 ms</th>
<th>Normal, ms</th>
<th>EBZ, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>131±10 (n=16)</td>
<td>161±21 (n=62)</td>
</tr>
<tr>
<td>20–40 min after Bay Y5959</td>
<td>152±18 (n=16)*</td>
<td>167±27 (n=33)</td>
</tr>
<tr>
<td>60–90 min after Bay Y5959</td>
<td>155±23 (n=11)*</td>
<td>166±18 (n=37)</td>
</tr>
<tr>
<td>90–120 min after Bay Y5959</td>
<td>149±17 (n=8)*</td>
<td>164±27 (n=8)</td>
</tr>
</tbody>
</table>

BCL indicates basic drive cycle length.
*Different from control (P<0.05).

### TABLE 2. Effect of Bay Y5959 on Conduction Velocity in the EBZ

<table>
<thead>
<tr>
<th></th>
<th>BCL=300 ms (n=4)</th>
<th>BCL=200 ms (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L, cm/s</td>
<td>T, cm/s</td>
<td>ANR</td>
</tr>
<tr>
<td>Control</td>
<td>69±6</td>
<td>28±6</td>
</tr>
<tr>
<td>30–60 min</td>
<td>60±9</td>
<td>29±6</td>
</tr>
<tr>
<td>100–130 min</td>
<td>65±11</td>
<td>30±3</td>
</tr>
</tbody>
</table>

L indicates longitudinal propagation; T, transverse propagation; and ANR, anisotropic ratio.
on calcium current to improve propagation predominates over any decrease in conduction caused by the uncoupling.

We discussed earlier that in areas of the EBZ characterized by slow conduction and fractionated electrograms, cell uncoupling by Bay Y5959 may have led to conduction block. However, cell uncoupling may also result in an improvement in conduction. The effects of uncoupling on conduction are bimodal; a small degree of uncoupling can increase the safety factor and improve conduction, whereas further uncoupling decreases the safety factor and leads to block. Therefore, it also must be considered that slight cell uncoupling by Bay Y5959 may sometimes contribute to an improvement in conduction of premature impulses and prevention of VT.

Limitations and Conclusions
Future studies will be needed to determine whether our hypotheses for the mechanisms of antiarrhythmic actions of increasing L-type calcium current are correct. Past experience has also shown that effective drug action on animal models does not always translate into effective clinical antiarrhythmic actions. In addition, the positive inotropic effect that accompanies this mechanism of antiarrhythmic action may not be desirable. Increasing L-type calcium current has also been shown to promote triggered activity and might sometimes be proarrhythmic, although not in our experiments. Therefore, it would be important to target drugs that act specifically in regions of slow activation in reentrant circuits.

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References
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