The effects of milrinone on conduction, reflection, and automaticity in canine Purkinje fibers

Jorge M. Davidenko, M.D., and Charles Antzelevitch, Ph.D.

ABSTRACT Milrinone is a newly developed analogue of amrinone possessing potent positive inotropic action. Electrophysiologic actions of the drug have not been reported. In this study microelectrode techniques were used to assess the electrophysiologic effects of milrinone in canine false tendons homogeneously superfused with either normal or high-K Tyrode's solution and in Purkinje fibers mounted in a three-compartment chamber in which the central segment was depressed with an "ischemic" solution. Milrinone (0.2 to 20 μg/ml) caused no major changes in the action potential characteristics, refractoriness, or conduction velocity in fibers exposed to normal Tyrode's solution, but markedly improved conduction and abbreviated or eliminated postpolarization refractoriness in the ischemic gap preparations. The drug also exerted important effects on reflected reentry generated in these preparations. Depending on the initial level of block, milrinone (1) suppressed the arrhythmia, (2) shifted its frequency dependence, or (3) created the conditions that allowed reflection to occur. Similar results were obtained in homogeneously depressed fibers. At similar concentrations, milrinone caused a relatively small enhancement of automaticity. Thus, in addition to its inotropic actions, milrinone produces important electrophysiologic effects. By restoring or improving conduction through areas of depressed conductivity, the drug may exert either antiarrhythmic or arrhythmogenic effects.


MILRINONE (Win 47203), a new analogue of the cardiac bipyridine amrinone, has been shown to exert potent inotropic and vasodilatory effects. These actions, demonstrated in a variety of animal preparations1,2 and recently in man,3–5 indicate an important potential use for milrinone in the long-term treatment of congestive heart failure.

Although the effects of milrinone on the mechanical properties of cardiac and smooth muscle have been investigated in some detail, studies of the actions of the drug on the electrical properties of heart tissues are lacking.

Our study was designed to assess the effects of milrinone on action potential morphology, impulse conduction, automaticity, and arrhythmias in normal and depressed preparations of isolated cardiac Purkinje fibers. The effect of the drug on reflected reentry was evaluated with the use of the "ischemic gap" preparation previously developed in our laboratory.6

The results demonstrate an important effect of this drug on the electrophysiologic properties of depressed fibers. By improving impulse conduction across areas of depressed conductivity, milrinone was shown to exert an important influence on the manifestation of reentrant arrhythmias.

Methods

Free-running false tendons were dissected from hearts removed from dogs anesthetized with sodium pentobarbital (35 mg/kg iv). Unbranched preparations were placed in a tissue bath and superfused with Tyrode's solution containing in mM 137 NaCl, 3 to 4 KCl, 0.9 NaH2PO4, 20 NaHCO3, 1.8 CaCl2, and 0.5 MgSO4 (pH 7.4). The solution was bubbled with a 95% O2–5% CO2 mixture and the temperature was maintained at 37 ± 0.5° C. Transmembrane potentials were recorded from one or more sites along the tissue with glass microelectrodes filled with 2.7M KCl (10 to 20 MΩ direct-current resistance) connected to a high-input impedance amplification system (WPI). The amplified signals were displayed on a Tektronix oscilloscope, photographed with a Grass kymographic camera, and recorded on an 8-channel FM tape recorder (Vetter). The fibers were stimulated from one end with rectangular pulses (1 to 3 msec duration and two to three times threshold intensity) applied through a pair of thin silver electrodes that were insulated except at the tips.

Ischemic gap preparation. The experimental setup was similar to that described earlier.6 Unbranched fibers were threaded through holes preformed in two rubber partitions separating the three compartments of a tissue bath. The central compartment (gap) was 1.5 to 2.0 mm wide. The three compartments were independently perfused with normal Tyrode's solution. Stimuli were applied to the proximal tissue segment and transmembrane recordings were obtained from the proximal,
gap, and distal segments of the preparation. After a 1 hr equilibration period, if all three segments displayed normal electrical activity, the gap was perfused with an "ischemic" solution that contained 15 to 21 mM KCl and 10 mM lactic acid (pH 6.8) and was bubbled with a 95% N₂-5% CO₂ mixture. No correction for isotonicity was made in this modified Tyrode’s solution. Starting with a concentration of KCl of 15 mM, we carefully titrated the [K⁺]₀ until the desired level of conduction impairment was achieved.

Conduction curves relating the impulse conduction time across the gap to the prematurity of responses elicited at the proximal site were obtained by scanning the basic cycle with single stimuli delivered once every tenth or fifteenth cycle. Frequency scans were performed by increasing the driving basic cycle length in 10 or 20 msec steps every 30 sec.

The functional refractory period (FRP) of the system is defined in this study as the shortest attainable D₁-D₂ interval, where D₁ is the distal action potential resulting from transmission of a basic proximal response (P₁) across the gap and D₂ is the distal response to a premature proximal beat (P₂) transmitted across the gap. The effective refractory period (ERP) is defined as the longest P₁-P₂ interval at which P₂ fails to propagate across the gap.

Homogeneous superfusion. In six experiments we exposed the fibers along their entire length to either the ischemic solution described above or to Tyrode’s solution containing 12 to 22 mM K⁺. Depending on the experimental protocol, the [K⁺]₀ was elevated to levels at which gross conduction impairment was manifest or to levels necessary to render the tissue inexcitable. In some experiments nonhomogeneous propagation, as evidenced by discrete step delays, and reentrant activity were observed. In these cases the preparations were carefully mapped so that microelec-trode recordings could be obtained from regions proximal and distal to the site of major conduction delay.

Depolarization-induced automaticity. A standard-current clamp method was used. The central compartment of a three-compartment bath was perfused with a solution consisting of purified sucrose (300 mM) and dextrose (5 mM) dissolved in deionized water equilibrated with 100% oxygen: CaCl₂ (0.1 mM) was added to the sucrose solution to prevent cellular uncoupling. The proximal segment was isolated with Tyrode’s solution containing 20 to 30 mM KCl and the distal compartment, which contained the test segment, was perfused with normal Tyrode’s solution. A constant-current unit under computer control (designed by Dr. Elharrar, Indianapolis) delivered 0.1 to 80 μA of current through Ag-AgCl electrodes placed in the two outer compartments. Transmembrane potentials were recorded differentially from the test segment and the applied current was measured as the voltage drop across a 10 kΩ resistor placed in series with the negative output of the constant-current unit. The test segment (1 to 3.0 mm long) was depolarized to progressively more positive levels for a period of 4 to 5 sec after each train of 25 basic beats. Basic stimuli were applied to the test segment through bipolar surface electrodes.

Drugs. Milrinone was prepared by dissolving 10 mg in 0.4 ml of 1.0N HCl and adding 9.6 ml of H₂O to yield a stock solution of 1 mg/ml. Final concentrations in the perfusate ranged between 0.1 and 20 μg/ml. The vehicle alone produced no effects on the electrophysiology of the preparations. d- Propranolol (Ayerst) was dissolved in Tyrode’s solution and verapamil (Knoll) was dissolved in ethanol and diluted in Tyrode’s solution.

Statistics. The results are expressed as mean ± SD. The significance of the difference between two means within the same experimental group were analyzed by t test for paired data.

Results

In 16 experiments we examined the actions of milrinone on the action potential characteristics of Purkinje fibers bathed in normal Tyrode’s solution. The preparations were stimulated at a basic cycle length of 800 msec. In the concentration range of 0.2 to 1.0 μg/ml, the drug produced no major changes after a 30 min exposure period (table 1). At higher concentrations (10 to 20 μg/ml) milrinone significantly prolonged action potential duration but produced no changes in any of the other parameters. The conduction velocity remained unaltered at these concentrations and changes in ERP were consistent with the changes in action potential duration (four experiments).

In contrast, low concentrations of milrinone caused marked alterations in the action potential characteristics of slow or depressed fast responses observed in K-depolarized fibers. For example, in a fiber bathed in Tyrode’s solution containing 15 mM K⁺ and 10 mM lactic acid, the addition of 0.2 μg/ml milrinone resulted in an increase in action potential amplitude (27%), duration (20%), and dV/dt (25%). The maximum diastolic potential increased only slightly (~ 51 to ~ 54 mV). The activity returned toward the control situation

| TABLE 1 |
| Effects of milrinone on normal Purkinje fibers |

<table>
<thead>
<tr>
<th>Concentration</th>
<th>MDP (mV)</th>
<th>OS (mV)</th>
<th>Amp (mV)</th>
<th>APD (msec)</th>
<th>dV/dt</th>
<th>Plateau voltage</th>
</tr>
</thead>
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<tr>
<td>n (µg/ml)</td>
<td></td>
<td></td>
<td></td>
<td>30%</td>
<td>50%</td>
<td>90%</td>
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<tr>
<td>9 Control</td>
<td>-88 ± 1</td>
<td>28 ± 5</td>
<td>116 ± 6</td>
<td>273 ± 60</td>
<td>305 ± 66</td>
<td>370 ± 79</td>
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<td>0.2-1</td>
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<td>29 ± 5^a</td>
<td>117 ± 7</td>
<td>278 ± 57^b</td>
<td>311 ± 65</td>
<td>378 ± 85</td>
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<tr>
<td>7 Control</td>
<td>-90 ± 2</td>
<td>32 ± 8</td>
<td>122 ± 9</td>
<td>249 ± 54</td>
<td>297 ± 74</td>
<td>366 ± 89</td>
</tr>
<tr>
<td>10-20</td>
<td>-89 ± 4</td>
<td>32 ± 7</td>
<td>121 ± 11</td>
<td>266 ± 61^a</td>
<td>321 ± 86^a</td>
<td>412 ± 110^a</td>
</tr>
</tbody>
</table>

Plateau voltage was measured at 100 msec after the onset of the upstroke.

Data are mean ± SD.

MDP = maximum diastolic potential; OS = overshoot; Amp = amplitude; APD = action potential duration.

^a Difference significant at 1% level; ^b difference significant at 5% level.

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after 45 min of washout. These actions of the drug were dose dependent, and higher concentrations produced still greater improvement. In two experiments we pretreated the fibers with 1 μg/ml of propranolol. After a period of 60 min, the \([K^+]_0\) was raised to 11 mM. In both cases, the response to an isoproterenol (0.01 μg/ml) challenge was negative, yet the effects of 0.2 μg/ml milrinone were maintained. The subsequent addition of 2 μg/ml verapamil to these preparations abolished the transmembrane response.

The effect of milrinone was even more pronounced on fibers that remained depressed after a long period of equilibration in normal Tyrode’s solution (figure 1). Figure 1, A, illustrates transmembrane activity recorded from one such preparation displaying a maximum diastolic potential of −50 mV, an amplitude of 60 mV, and a maximum dV/dt of 52 V/sec. Before drug the activity remained stable for a period of 70 min. The addition of 0.2 μg/ml milrinone restored transmembrane activity to a near-normal level; the maximum diastolic potential, amplitude, and dV/dt were −87 mV, 115 mV, and 345 V/sec, respectively, after 15 min of drug exposure (figure 1, B). After 60 min of washout (figure 1, C) the activity returned toward the control level. In three other spontaneously depressed preparations milrinone effected similar although less remarkable changes.

In addition to enhancing depressed responses, milrinone proved to be effective in restoring activity to fibers rendered inexcitable by K depolarization. Figure 2 illustrates results of an experiment in which the stimulation intensity was maintained at a level three times threshold and extracellular K+ was gradually increased until complete inexcitability was achieved. Figure 2, A, was recorded at a \([K^+]_0\) of 18 mM and the transmembrane trace in B, recorded 10 min after the addition of 0.2 μg/ml milrinone, illustrates the restoration of activity. Low concentrations of the drug (therapeutic levels, 0.1 to 0.4 μg/ml) were effective in activating the depolarized tissue only when the \([K^+]_0\) approximated that just necessary to produce inexcitability. When the \([K^+]_0\) substantially exceeded this marginal level, higher concentrations of the drug (5 to 20 μg/ml) were required to restore regenerative activity.

In a series of 11 experiments we examined the effects of milrinone on conduction, refractoriness, and reflection in a preparation consistent with the conditions likely to impair impulse conduction in diseased hearts, i.e., an ischemic gap preparation. The characteristics of conduction across the ischemic gap are illustrated in figure 3. Two superimposed sweeps of the oscilloscope are pictured in each panel, depicting activity in the proximal (top), gap, and distal (bottom) segments of the three-compartment preparation. The

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

**FIGURE 1.** The effect of milrinone on a spontaneously depolarized Purkinje fiber. Each panel shows the transmembrane potential (top) and dV/dt (bottom). A, Recorded from a Purkinje fiber exposed to normal Tyrode’s solution. B, Recorded 10 min after the addition of milrinone (0.2 μg/ml). C, Recorded after 60 min of washout. The same impalement was maintained throughout.

![Figure 2](http://circ.ahajournals.org/)

**FIGURE 2.** Restoration of an active response by milrinone in a K-inactivated Purkinje fiber. A, Transmembrane activity recorded from a Purkinje fiber superfused with Tyrode’s solution containing 18 mM KCl. B, Recorded 15 min after the addition of 0.2 μg/ml milrinone.
feature of this system, is apparent. The ERP and FRP were 240 and 260 msec, respectively.

The transmembrane potential recorded from the gap had a two-component upstroke; the first occurred shortly after the proximal action potential and the second succeeded the upstroke of the distal response. As demonstrated in earlier studies, in the ischemic gap preparation this is the sine qua non of electrotonically mediated conduction, indicating the presence of a short inexcitable cable at the recording site within the gap.3 Under these conditions, the local circuit current generated by the proximal response passed through the intercellular nexal connections of the inactivated cells. This electrotonic current, if of sufficient intensity upon reaching excitable tissue beyond the depressed zone, gradually depolarizes the distal tissue to its threshold level, thus maintaining continuity of conduction across the inexcitable region. Propagation therefore stops and then resumes after a considerable step delay.

Figure 3, B, illustrates the activity recorded after adding 0.2 μg/ml milrinone to the gap perfusate. Although propagation across the gap remained electrotonically mediated, the ERP and FRP decreased to 180 and 200 msec, respectively. In figure 3, C, recorded after an increase in the concentration of milrinone to 0.5 μg/ml, the earliest elicited proximal response succeeded in propagating across the gap. Postrepolarization refractoriness was eliminated: the FRP was reduced to 175 msec. In this instance, the upstroke of the gap response preceded the upstroke of the distal action potential, indicating the participation of an active response (i.e., a slow response — membrane potential was -50 mV) within the gap. Conduction time of basic beats, which was 30 msec at control, was 20 msec after exposure to 0.2 μg/ml milrinone and 3.5 msec after 0.5 μg/ml of the drug.

The magnitude of the drug-induced improvement in conduction depended not only on the concentration of milrinone, but also on the predrug level of conduction impairment. Figure 4 illustrates the results of an experiment in which the initial level of block was greater than that shown in figure 3, A. The [K+] o of the ischemic solution was 22 mM. Milrinone (1 μg/ml) produced a marked improvement in both conduction and refractoriness, with the ERP being abbreviated from 520 to 340 msec and the FRP from 590 to 405 msec.

Reflection. Conduction through areas of depressed conductivity may be so delayed as to permit retrograde transmission of the distal impulse via the same blocked segment in time to reexcite the proximal tissue, thus generating a reflected response. This type of reentrant

![FIGURE 3. Effect of milrinone on refractoriness and conduction in an ischemic gap-Purkinje fiber preparation. The top, middle, and bottom traces in each panel represent the intracellular recordings obtained from the proximal, central, and distal compartments, respectively. The preparation was driven at a basic cycle length of 1000 msec. Each panel illustrates two superimposed sweeps showing the earliest propagated premature beat and the latest that failed to propagate across the gap. A. Control; the gap segment was superfused with Tyrode’s solution containing 18 mM K+ and 10 mM lactic acid. B. Recorded after adding milrinone (0.2 μg/ml) to the central compartment perfusate. C. Recorded after an increase in the concentration of milrinone to 0.5 μg/ml; postrepolarization refractoriness was abolished.

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activity is sensitive to changes in frequency because conduction time across such regions is frequency dependent. Figure 5 illustrates an example of the frequency-dependent alterations of impulse transmission across the ischemic gap and the resultant changes in arrhythmic patterns. In each panel the top, middle, and bottom traces represent activity recorded from the proximal, gap, and distal segments, respectively. Under control conditions, at a basic cycle length of 400 msec, only 1 of every 3 stimulated beats conducted across the ischemic gap. Each distal response propagated in the retrograde direction and reexcited the proximal tissue, thus generating closely coupled reflections in a pattern of quadrigeminy. At slower frequencies the percentage of conducted beats increased, giving rise to an increase in the incidence of arrhythmic activity. Patterns of trigeminy and bigeminy were observed at basic cycle lengths of 800 and 1000 msec. Milrinone (0.2 μg/ml) added to the gap perfusate improved conduction across the gap. At a basic cycle length of 400 msec, 50% of the stimulated proximal beats successfully propagated to the distal site and conduction time became too brief to permit manifest reentry. At a basic cycle length of 800 msec propagation of the stimulated beats was 1:1, with delays long enough to permit reflection of each propagated beat. At still lower frequencies (basic cycle length of 1000 msec) conduction remained 1:1, but anterograde conduction time was brief and no manifest reflections occurred. A complete frequency scan of this preparation showed a shift of the reflection zone (i.e., the range of frequencies at which reflected reentry was manifest) to lower basic cycle lengths. Similar results were obtained in four other experiments. In one of these, the milrinone-induced shifts in conduction and reflection were negated by the addition of verapamil (2 μg/ml) to the gap perfusate. Proximal-to-distal conduction after verapamil was slightly poorer than that under control conditions.

In five experiments we studied the cumulative or differential action of milrinone on the three segments of the ischemic gap preparation. Figure 6 is a summary of the results of an experiment in which milrinone was sequentially added to the gap, proximal, and distal perfusates. The graphs plot the percentage of conducted beats and the incidence of reflected reentry as a function of the driving cycle length. Milrinone (0.2 μg/ml) added to the gap perfusate shifted the reflection zone to higher frequencies and limited its range. The same concentration of the drug added to the proximal perfusate produced a further improvement in conduction and a further shift of the reflection zone to the left. Finally, when all three segments were exposed to the drug, only occasional reflected beats were observed at the highest frequencies (basic cycle lengths of 250 and 300 msec). In two experiments in which the drug was initially added to one of the outer compartments, the greatest effect was observed upon introduction of the drug to the gap perfusate. The extent of the milrinone-induced action on the tissues in the outer compartments depended to some degree on the length of the segments. Due to depolarizing electrotonic influence from the gap, the degree of depolarization of the proximal and distal tissues is a function of their respective lengths. In two of the five experiments in this group, the proximal segment was relatively short (<2.5 mm) and depolarized. In both cases, relatively large effects were observed after exposure of this segment to the drug.

The results presented thus far indicate that the influence of milrinone on the manifestation of arrhythmias

FIGURE 4. The effect of milrinone on conduction in an ischemic gap preparation that manifested a relatively high level of predrug conduction impairment. A, Conduction times of test responses (P2–D2) are plotted as a function of prematurity (P1–P2). B, Relationship between “input” (P1–P2) and “output” (D1–D2) intervals from the same experiment. Milrinone abbreviated conduction time and markedly shortened both ERP and FRP.

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depends on (1) the stimulation frequency and (2) the portion(s) of the preparation exposed to the drug. Figure 7 presents the following two additional variables: (1) the predrug level of conduction impairment and (2) the drug concentration. In this example, the initial level of block was high; none of the impulses elicited on the proximal side succeeded in propagating across the gap (figure 7, A). Milrinone (5 μg/ml gap perfusate), by restoring conduction, created conditions under which reflection could occur (figure 7, B). A reflection zone extending from a basic cycle length of 700 to 1500 msec was created where none existed before. At higher concentrations of the drug (10 and 20 μg/ml) conduction was further improved and arrhythmic activity was abolished (figure 7, C and D). At the highest concentration (panel D) proximal-to-distal conduction time was 3 msec and it appeared that propagation was not electrophysiologically mediated.

**Homogeneous superfusion.** Conduction delay, block, and reentry were also observed in false tendons homogeneously superfused with a K⁺-rich Tyrode’s or ischemic solution. Figure 8 illustrates a representative example. The top and bottom traces were recorded 4.5 and 2.0 mm from the stimulating electrodes. At a basic cycle length of 400 msec only subthreshold depolarizations were recorded at the distal site (figure 8, A). Milrinone (0.2 μg/ml) restored conduction and facilitated reentry in this preparation (figure 8, B) and at higher concentrations (panel C) the drug further improved conduction and abolished the reentrant activity. In three other experiments, when under predrug conditions conduction was less impaired and reentry was present, the drug improved conduction and abolished the arrhythmia.

**Depolarization-induced automaticity.** Using current-clamp techniques we assessed the actions of milrinone on automaticity occurring over a wide range of membrane potentials. Figure 9 illustrates results of a representative experiment. The two columns show transmembrane activity at two levels of membrane potential (−80 and −40 mV) recorded from the test segment of a sucrose gap preparation. The first 2 beats in each panel are the last of a train of 25 beats stimulated at a basic cycle length of 500 msec. A 5 sec current pulse was introduced 500 msec after the last driven beat. In the absence of drug (top panels) depolarization to −80 mV yielded a pacemaker with an average cycle length of 1200 msec; at −40 mV the cycle length was 460 msec. Milrinone (10 μg/ml; middle panels) effected only small changes in the automaticity of the prepara-
tions. In comparison, a low concentration of isoproterenol (0.05 µg/ml; lower panels) produced a much greater effect.

A complete voltage scan was performed in this and six similar experiments. We observed only minor milrinone-induced (0.2 to 10 µg/ml) changes in the automatic responses. In one experiment in which unusually slow automatic activity occurred at potentials positive to −60 mV, milrinone (5 µg/ml) produced acceleration in spontaneous beating at these positive voltages but exerted no effect on automatic activity at more negative potentials.

**Discussion**

Milrinone, a recently synthesized nonglycosidic, nonadrenergic cardiotonic drug with potent positive inotropic activity, has been shown to exert important electrophysiologic actions in isolated cardiac Purkinje fibers. Apparent therapeutic concentrations of the drug (0.1 to 0.4 µg/ml), although they had practically no effect on normal fibers, markedly improved conduction and action potential characteristics in spontaneously depressed preparations and in fibers exposed to a simulated ischemic environment. Milrinone also restored electrical activity to K-inactivated preparations. This action of the drug has also been described for the parent compound, amrinone.

**Ischemic gap preparation.** Ventricular arrhythmias that occur in the early periods of experimental coronary occlusion have been shown to be secondary to severe conduction impairment in the ischemic zones. Some identified components contributing to the electrophysiologic abnormalities of the early phase of ischemia (elevated concentrations of K⁺, lactate, and H⁺) were added to the gap perfusate to simulate an ischemic environment within the central segment of false tendon, thus creating a narrow zone of depressed conductivity. As we have previously shown, major conduction delays across the ischemic gap are due to the electrotonically mediated transmission of impulses across an inexcitable region within the gap. The central segment is strongly depolarized by the ischemic solution, but the membrane potential increases gradually with distance from the maximally depolarized central zone toward the normally polarized regions at either end, providing zones at the gap borders at which slow or depressed fast responses are possible. Any agent affecting the length of the inexcitable segment or the depressed active responses at the gap boundaries will also affect the propagation of impulses across the gap.

The ability of milrinone to improve conduction (figures 5 through 7) and abbreviate refractoriness (figures 3 and 4) across the gap may be explained by its ability to enhance the activity of depressed responses (figure...
FIGURE 7. Dose-dependent effect of milrinone on conduction and reflection in an ischemic gap preparation. Each panel pictures the transmembrane activity from the proximal (top), gap (middle), and distal (bottom) segments. The central compartment was superfused with Tyrode's solution containing 20 mM KCl and 10 mM lactic acid. A. At a basic cycle length of 900 ms complete block was observed. B. The addition of milrinone (5 µg/ml) to the gap perfusate allowed for conduction of some beats. Slow anterograde propagation facilitated the reexcitation of the proximal site (reflection) resulting in a pattern of trigeminy. C. Milrinone concentration was raised to 10 µg/ml. Conduction of the impulse through the gap was 1:1 and no reflections were observed. D. A further increase in the concentration of milrinone (20 µg/ml) restored prompt conduction across the gap, apparently eliminating the inexcitable gap within the centrally depolarized tissue.

1) and restore activity to inexcitable tissues (figure 2). These actions of the drug may serve to (1) narrow the inexcitable zone, (2) augment the action potentials at the proximal end of the inexcitable cable (source), and (3) improve the excitability at the distal end (sink). Local circuit current intensity provided to the sink thus increases because of a greater input at the source and a shorter distance for the current to decay between source and sink.

Reflection. There is a critical dependence of the success or failure of reflection on the magnitude of conduction delays in both directions across the gap. Because excitability of cardiac tissue continues to recover for hundreds of milliseconds after an action potential, impulse conduction delay across the inexcitable gap is a sensitive function of time or frequency.7,14,15 The incidence and pattern of reflected reentry therefore depend greatly on frequency.6,14-17 It is therefore not surprising that the effect of milrinone on the manifestation of reflected reentry also depends on the rate of stimulation. In a given preparation, the drug may produce antiarrhythmic, arrhythmogenic, or no effects depending on the basic driving rate of the preparation in which it is being used.

For example, in the experiment illustrated in figure 5, milrinone exerted an antiarrhythmic effect at a basic cycle length of 1000 msec, but exerted an arrhythmogenic effect at a basic cycle length of 800 msec. If in the course of drug therapy the heart rate were to accelerate from a basic cycle length of 1000 to 800 msec, the basic arrhythmic manifestation (bigeminy) would not change. Figure 6 offers another example. After the addition of milrinone to the gap segment, the reflection zone shifts to the left such that an antiarrhythmic action is present at basic cycle lengths within a range of 600 to 900 msec, an arrhythmogenic action is present between 300 and 500 msec, and little or no effect is observed at basic cycle lengths of 500 to 600 msec.

These results emphasize the importance of considering heart rate in the assessment of antiarrhythmic drug efficacy.

The overall effect of milrinone on manifest reentry depended on (1) the predrug conduction characteristics, (2) the concentration of drug employed, and (3)

FIGURE 8. The effect of milrinone on conduction and reentry observed in a false tendon homogeneously superfused with Tyrode's solution containing 20 mM KCl and 10 mM lactic acid. Top and bottom traces were recorded 4.5 and 2.0 mm from the stimulating electrodes. Basic cycle length was 400 ms. A. Control; only subthreshold depolarization were recorded at the distal site. B. Recorded 15 min after adding milrinone (0.2 µg/ml); the drug induced reentrant activity. C. An increase in milrinone concentration (0.5 µg/ml) further improved conduction and abolished reentry.
actions on arrhythmic manifestations depend on variables such as the initial state of conduction, drug concentration, and frequency.

**Homogeneously superfused Purkinje fibers.** The characteristics of conduction observed in false tendons homogeneously superfused with ischemic solution were similar to those recorded from the ischemic gap preparations. Two-component upstrokes, foot potentials, and step delays, all of which are manifestations of nonhomogeneous propagation, were observed with careful titration of [K⁺]₀ and longitudinal mapping of the preparation. In this preparation milrinone produced similar effects on conduction, refractoriness, and reentry as in the ischemic gap model.

**Depolarization-induced automaticity.** Most positive inotropic and dromotropic agents enhance normal automaticity in cardiac tissues and facilitate the occurrence of abnormal automatic activity.¹⁸,¹⁹ It was therefore of interest to determine whether milrinone, at concentrations that affect conduction, also enhanced automaticity. Low concentrations of the drug produced no important changes in automatic activity at any level of membrane potential. Although higher concentrations enhanced automaticity at all levels of membrane potential, the effect was small when compared with that of a low concentration of isoproterenol.

**Ionic mechanisms.** Although elucidation of the ionic mechanisms underlying the actions of milrinone is beyond the scope of this study, the results provide some insight. The electrical activity elicited by milrinone in K-inactivated fibers is consistent with slow response activity resulting from activation of the slow inward current.²⁰,²¹ The enhancement of depressed responses observed in K-depolarized fibers is likewise attributable to an increase in slow-channel activity since the maximum diastolic potential was almost always more positive than −55 mV. Moreover, these actions of the drug were observed in the presence of propranolol and suppressed with the slow-channel-blocking agent verapamil. Thus, the electrophysiologic actions of milrinone may be explained by an increase in the intensity of slow inward current through a mechanism other than β-adrenergic–receptor stimulation. Possible mechanisms for this effect include a direct drug-induced facilitation of transmembrane calcium movement through the slow channels or, as suggested in a recent report, an indirect facilitation through inhibition of cyclic adenosine monophosphate phosphodiesterase.

**Clinical implications.** Our results suggest that therapeutic concentrations of milrinone, by facilitating impulse propagation across ischemic zones, may alter the manifestation of reentrant arrhythmias. As with lido-
caine and verapamil, either antiarrhythmic or arrhyth-
mogenic effects may be produced.

Our present and past experiences with these and similar preparations suggest that the heart rate dependence of the arrhythmia can provide the basis for selection of those individuals that might benefit from milrinone therapy and also aid in screening individuals in whom the drug might initiate or exacerbate arrhyth-

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J M Davidenko and C Antzelevitch

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