Manganese and Electrogenic Phenomena in Canine Purkinje Fibers

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ABSTRACT

Studies were performed on canine cardiac Purkinje fibers to evaluate the effects of manganese on membrane electrogenesis. The results indicate that manganese has a calciumlike effect on the excitatory sodium current and inhibitory effects on potassium conductance and slow inward current. The inhibitory action of manganese on potassium conductance was suggested by the following observations. (1) Manganese caused an initial increase in action potential duration largely due to a lengthening of the plateau and decreases in the rates of phase 3 and terminal repolarization. (2) Manganese increased the rate of diastolic depolarization. (3) Manganese blocked the initial fall in maximum diastolic potential accompanying rapid stimulation. (4) Manganese in high concentrations caused generalized depolarization which was reversed by rapid stimulation and by increased extracellular potassium concentrations. The action of manganese to block slow inward current was indicated by the eventual shortening of the plateau and by the elimination of responses initiated from low levels of membrane potential (< -55 mv). In addition to these effects, manganese also reduced membrane excitability, eliminated arrhythmic beats occurring during low-frequency electrical stimulation, and caused membrane hyperpolarization which was blocked by tetrodotoxin.

KEY WORDS: excitability, responsiveness, arrhythmic beats, overdrive stimulation, tetrodotoxin, potassium conductance, slow inward current

The use of manganese as a selective inhibitor of calcium current has become widespread since Fatt and Ginsborg (1) first observed that manganese blocks the calcium-dependent action potentials of crustacean skeletal muscle. Studies on cardiac cells have shown that manganese inhibits a slow inward current presumably carried by calcium and probably responsible in part for the plateau of the action potential. Recent reviews of these investigations have been published by Reuter (2), Trautwein (3), and Weidmann (4).

The effects of manganese on ionic currents other than those involving calcium are less well understood. Recent evidence indicates that manganese decreases potassium conductance in frog skeletal muscle (5), and a similar effect has been suggested for various types of atrial and ventricular myocardial cells (6-8). In cardiac Purkinje fibers, manganese reportedly has little effect on potassium current (9), but its action on the excitatory sodium current is unknown.

The main objective of this study was to assess the extent to which manganese influences those electrogenic phenomena most directly related to sodium and potassium currents in free-running cardiac Purkinje fibers. The results indicate that manganese has effects other than blocking calcium current.

Methods

Hearts were excised from healthy adult dogs anesthetized with either sodium pentobarbital (60 mg/kg, iv) or ether. Strands of Purkinje fibers with attached pieces of papillary muscle were quickly removed from both ventricles and placed in oxygenated Tyrode's solution at room temperature. Tissue samples were pinned to a paraffin block in a flow-through (10 ml/min), Plexiglas chamber (10 ml) and superfused with Tyrode's solution equilibrated with a 96% O2-4% CO2 gas mixture. The pH and the temperature of the bathing solution were held constant during each experiment (pH range 7.2 to 7.3, temperature range 35 to 37°C).

Normal Tyrode's solution had the following millimolar...
composition: NaCl 137, dextrose 5.5, NaHCO\(_3\) 12.0, KCl 2.7, MgCl\(_2\) 0.5, and CaCl\(_2\) 1.8. Manganese Tyrode’s solutions were made by adding either MnCl\(_2\) or MnSO\(_4\) to normal Tyrode’s solution to obtain concentrations of 2.5, 5.0, and 10.0 mM. There was no difference in the physiological effects of the two forms of manganese. Solutions containing 7.2 mM calcium were made by adding CaCl\(_2\) to normal Tyrode’s solution. Potassium Tyrode’s solutions were prepared by replacing the NaCl in normal Tyrode’s solution with an osmotic equivalent of KCl to give 5.4, 16.2, and 21.6 mM KCl with NaCl concentrations of 145.3, 135.5, and 130.1 mM, respectively. Tetrodotoxin (TTX) was applied to the preparation by adding it in appropriate concentration to normal Tyrode’s solution. For each experiment, 1 mg of crystalline TTX (Sanko Co., Ltd.) was dissolved in 10 ml of distilled water; from this solution further dilution was made in physiologic salt solution.

Membrane potential was recorded with intracellular glass microelectrodes filled with 3M KCl and having resistances ranging from 10 to 20 megohms. The maximum upstroke velocity of the initial spike was determined using an electronic differentiator (resistance 0.5 megohms, capacitance 100 pfarads). Records were taken on a polygraph or photographed from an oscilloscope. The tissue was electrically stimulated on the endocardial surface with a square-wave pulse through a glass electrode filled with Tyrode’s solution and placed 5-15 mm from the recording electrode. Unless otherwise indicated, the stimulus frequency was 90/min.

Several characteristics of the Purkinje fiber action potential were measured based on the following definitions.

\[ V_{max} = \text{Maximum diastolic potential.} \]
\[ V_{min} = \text{Membrane potential from which the action potential originates (minimum diastolic or take-off potential).} \]
\[ V_{os} = \text{Difference between zero membrane potential and the peak of the action potential (overshoot potential).} \]
\[ V_{ap} = \text{Overshoot potential plus the minimum diastolic potential.} \]
\[ V_{max} = \text{Maximum rate of depolarization of the initial spike (phase 0).} \]
\[ V_{2}, V_{3}, V_{r} = \text{Maximum rate of depolarization during the plateau (phase 2), phase 3, and terminal repolarization (10), respectively.} \]
\[ T_{min} = \text{Time required for the action potential to repolarize to a level equal to Vmin.} \]
\[ T_{ss} = \text{Time required for the action potential to repolarize to \(-60\) mv.} \]
\[ V_{n} = \text{Amplitude of the notch preceding the plateau (measured as the difference [mv] between the maximum notch depth and the point of intersection of the initial spike with a line tangent to phase 2).} \]
\[ V_{m} = \text{Resting membrane potential in nonbeating cells and maximum diastolic potential (Vmax) in electrically driven cells.} \]

To reduce the possibility that the technique used for measuring membrane responsiveness might influence the results, two methods were employed—S2 analysis and potassium depolarization.

\[ S2 \text{ Analysis.} \]

Action potentials and \( V_{max} \) were recorded from cells stimulated at a basic frequency of 90/min (S1), and an extra stimulus (S2) was applied every fifteenth beat. The S2 stimulus was timed to occur at various points during repolarization of the action potential. Responsiveness curves representing the relationship between \( V_{max} \) and \( V_{min} \) (11) were plotted from the data obtained from the extra responses during control periods and 15-20 minutes after the application of manganese.

\[ \text{Potassium Depolarization.} \]

Action potentials and \( V_{max} \) were recorded from a cell stimulated at 90/min as it gradually depolarized (\(-10\) mv/min) during superfusion with Tyrode’s solution containing 21.6 mM KCl. Following potassium depolarization, the cell was repolarized to its control level in Tyrode’s solution containing 2.7 mM potassium. After a 15-minute recovery period, manganese Tyrode’s solution was applied for 15 minutes, and the cell was then depolarized again in manganese Tyrode’s solution having an elevated potassium concentration. Responsiveness curves were constructed from the \( V_{max} \)-\( V_{min} \) data obtained during control periods and in the presence of manganese. To maintain sodium concentration (190.1 mM) and osmolarity (339.8 mosmols) constant in all solutions, KCl was substituted for sucrose.

In some experiments the stimulus frequency was abruptly increased from 12 to 60/min and maintained at the rapid level for 2 minutes. The period of rapid stimulation was defined as overdrive stimulation.

In several figures presented in this paper, action potential records were retraced and superimposed to lend clarity to the experimental observations. Data from sample populations are presented as means \( \pm \) se. Student’s t-test was used for the comparison of sample means.

**Results**

**ACTION POTENTIAL CONFIGURATION**

Figure 1 shows the effects of 2.5 mM and 5.0 mM manganese on the action potential configuration of Purkinje fibers; the vertical lines below each record are proportional to the maximum upstroke velocity. The 2.5 mM manganese hyperpolarized the membrane 5-10 mv and continued to do so for superfusion times as long as 25-30 minutes. A notch appeared at the beginning of the plateau, and the action potential duration increased largely through a lengthening of the plateau and a decrease in the slope of phase 3. In most cells the rate of terminal repolarization was also slowed. The plateau was frequently displaced upward a small amount with little change in slope. For superfusions longer than 15 minutes, the slope of phase 3 and terminal repolarization continued to fall and the plateau was displaced downward below control by a few millivolts. In addition to the changes occurring during repolarization, the initial upstroke velocity was depressed in spite of membrane hyperpolarization. In the sequence shown, for ex-
Effects of 2.5 mM (top) and 5.0 mM (bottom) manganese on the action potential configuration of Purkinje fibers as a function of time. Both the top and the bottom section show superimposed action potentials recorded from the same cell during control periods (T = 0) and during superfusion with manganese at the indicated times (T). The vertical lines below the tracings represent the output of the electronic differentiator and are proportional in height to the maximum upstroke velocity (Vmax) of the respective action potentials. Stimulation frequency = 90/min. Calibration marks are indicated at the right of the top section: vertical calibration = 100 mV for voltage scale and 500 mV/sec for differentiator scale, and horizontal calibration = 0 msec.

The initial effects of 5.0 mM manganese were similar to those of 2.5 mM manganese, i.e., hyperpolarization, decreased Vmax, little change in Vos, development of a notch, increased action potential duration, decreased Vr, and decreased Vt. Later the plateau was depressed, and the cells sometimes depolarized slightly (3-4 mV).

The effects of 10.0 mM manganese are illustrated in Figure 2, which shows the slow- and fast-sweep action potential records of a single Purkinje cell in control solution (A) and at three different times following the application of 10.0 mM manganese (B-D). The early effects of 10 mM manganese (Fig. 2B) were similar to those of 2.5 and 5.0 mM manganese. Later the plateau was greatly depressed in amplitude and duration and the membrane potential was decreased back to the control level (C); the upstroke velocity, however, remained below control. Figure 2D shows that after 50 minutes of exposure the cell had depolarized to −69 mV. Note that the initial spike was fairly well maintained but that the plateau was abolished. The large negative afterpotential following the spike indicates a persistent slowing of phase 3 and terminal repolarization (Fig. 2D). Continued exposure to this concentration frequently resulted in depolarization to inexcitability at −50 to −60 mV. The time required for generalized depolarization to occur varied directly with the stimulation frequency.

The notch, which appeared at all three manganese concentrations, was occasionally present under control conditions. When this situation occurred, the application of manganese led to the disappearance of the original notch followed by the development of a similar but larger notch. Coincident with the development of this notch was a marked increase in the rate of phase 1 repolarization.
The cell hyperpolarized to -95 mV, and then gradually depolarized to about -65 mV. These results indicate that manganese depressed membrane excitability possibly by altering the resting membrane potential, displacing the threshold toward less negative potentials, or both. A similar result was obtained in two other experiments.

**EXCITABILITY**

Following the application of manganese, it was frequently observed that the stimulus intensity became inadequate and had to be increased. To further assess this finding, strength-duration curves were determined by measuring the minimum stimulus intensity at each of several stimulus durations required to produce an extra propagated response. The preparation was driven at 90/min, and test stimuli were applied to the surface of the Purkinje bundle after a fixed interval late in diastole. An example of this analysis is shown in Figure 4. The experimental curves, measured in the same cell at different times during application of 10.0 mM manganese, were shifted upward and to the right. Indicated next to each curve is the Vmin at the time the curve was measured. The cell hyperpolarized to -95 mV, and then gradually depolarized to about -65 mV. These results indicate that manganese depressed membrane excitability possibly by altering the resting membrane potential, displacing the threshold toward less negative potentials, or both. A similar result was obtained in two other experiments.

**RESPONSIVENESS**

The finding that Vmax decreased even though the membrane was hyperpolarized suggests that the normal relationship between upstroke velocity and membrane potential is altered by manganese. To explore this relationship in greater detail, extra responses were initiated during repolarization while the take-off potential and the upstroke velocity of each response were recorded. Figure 5 shows tracings of action potentials recorded from the same cell during the control period and 15 minutes after the application of 10.0 mM manganese. Two extra responses are superimposed on the repolarization limb of each action potential. This record indicates that manganese does not decrease Vmax uniformly at all levels of Vmin. In fact, as responses were initiated from less polarized levels, Vmax and Vos were actually increased in the presence of manganese until around -54 mV where they were again depressed. For example, when Vmin was -69 mV, manganese increased Vmax from 95 V/sec to 339 V/sec. In contrast, the response initiated from -54 mV was markedly depressed in the presence of manganese.

Figure 6 demonstrates similar results obtained from an experiment in which potassium depolarization was used to change Vmin. The action potentials were recorded from the same cell during continuous monitoring throughout the experimental procedure. Each section of the figure contains two superimposed tracings, one taken before (solid line) and one taken 15 minutes after (broken line) exposure to 10.0 mM manganese. Figure 6A shows the typical response to manganese Tyrode's solution containing 2.7 mM potassium. Note in particular the reduction of Vmax and the depression of the plateau with no decrease in Vmin. Figure 6B, C, and D show records taken during the depolarization caused by superfusion with Tyrode's solution containing 21.6 mM potassium. Superimposed action potentials in each section of the figure were selected from control and experimental trials to
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FIGURE 4

Typical strength-duration curves for a Purkinje fiber exposed to 10 mM manganese. The curve connecting the solid circles was generated under control conditions. The remaining curves were generated at the following times during manganese superfusion: open squares = 8 minutes, open circles = 20 minutes, and open triangles = 29 minutes. The millivolt value next to each curve represents the membrane potential at the time the curve was generated.

FIGURE 5

Effects of manganese on responses initiated during the repolarization of a Purkinje fiber. Potentials were recorded from the same cell during a control period and after exposure to 10 mM manganese for 15 minutes. The basic stimulation frequency was 90/min. Each section shows one normal response and two extra responses. The millivolt value next to each extra response indicates the take-off potential (Vmin) and the value next to the overshoot of the faster rising extra response indicates the maximum upstroke velocity (Vmax) of that response. Calibration marks are indicated at the right of the figure: vertical calibration = 100 mv, and horizontal calibration = 100 msec.

have the same values of Vmin. Note that as the cell depolarized manganese continued to depress the plateau but enhanced rather than suppressed Vmax and Vos of the initial spike.

Typical results from the responsiveness analysis are illustrated graphically in Figure 7. The curves on the left show Vmax and Vos as functions of Vmin before and during the application of 10.0 mM manganese. In this example, manganese depressed Vmax at membrane potentials negative to -82 mv but enhanced it at less negative potentials. The depressant effect of manganese on responses elic-
Effects of manganese on action potentials generated from potassium-depolarized Purkinje fibers. All records were taken from the same cell stimulated at a frequency of 90/min. The solid tracings represent records taken under control conditions, and the broken tracings represent records taken after equilibration in 5.0 mM manganese Tyrode's solution for 15 minutes. The vertical lines below each section of the figure represent the output of the electronic differentiator and are proportional in length to the maximum upstroke velocity (Vmax). Records in A were taken in normal Tyrode's solution (2.7 mM KCl), and those in B, C, and D were taken during depolarization in potassium-rich Tyrode's solution (21.6 mM). Calibration marks for all records are indicated at the right of section A: vertical calibration = 100 mV for voltage scale and 1000 V/sec for differentiator scale, and horizontal calibration = 700 msec.

As noted from low levels of membrane potential is reflected in the relationship between Vos and Vmin (Fig. 7), i.e., in this cell under control conditions Vos was independent of Vmin at values of Vmin less than -60 mV, but in the presence of manganese Vos was markedly attenuated in this voltage range.

To summarize, manganese decreased Vmax at high levels of membrane potential, increased it at intermediate levels, and reduced or abolished the responses initiated from low levels of membrane potential. Manganese-induced changes in Vos followed a similar pattern. All effects were independent of the method used to vary Vmin.

To compare the effects of manganese on responsiveness with those produced by calcium, a similar analysis was carried out in the presence of elevated extracellular calcium. An increase in calcium from 1.8 to 7.2 mM resulted in a decrease in Vmax at the most polarized levels but an increase in Vmax at lower membrane potentials (Fig. 7). Overshoot on the other hand was increased over control at all take-off potentials. The similar action of manganese and calcium to shift the membrane responsiveness curve was quantified by measuring the maximum downward and leftward displacements of each curve in a series of 14 experiments. Manganese caused a downward shift of 131 ± 7.73 v/sec (P < 0.001, N = 8) occurring at a mean Vmin of -96 ± 0.88 mV and a leftward shift of -4.6 ± 0.24 mV (P < 0.001, N = 8). A fourfold increase in extracellular calcium (1.8 to 7.2 mM) caused a downward shift of 84 ± 8.16 v/sec (P < 0.01, N = 6) at mean Vmin of -98 ± 1.02 mV and a leftward shift of -5.2 ± 0.21 mV (P < 0.001, N = 6).

The action of manganese to depress the plateau and block the formation of low-amplitude responses in depolarized cells (Fig. 5 and refs. 2-4) has been attributed to inhibition of a slow inward calcium current. This finding, along with the observation that manganese and high calcium had similar effects on membrane responsiveness, suggests that in canine Purkinje fibers manganese has...
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1.8mM Ca
1.8mM Ca + 5.0mM Mn
7.2mM Ca

FIGURE 8
Comparative effects of manganese and calcium on upstroke velocity (Vmax), overshoot potential (Vos), and plateau of a Purkinje fiber action potential. The same fiber was equilibrated in each of the following solutions before being depolarized by 21.6 mM potassium: normal Tyrode's solution containing 1.8 mM calcium (solid curve), Tyrode's solution containing 7.2 mM calcium (dotted curve), and Tyrode's solution containing 5.0 mM manganese and 1.8 mM calcium (dashed curve). The action potentials represented by these curves were selected from records taken during depolarization to have the same Vmin (-73 mv). The vertical lines below the tracings are proportional to the upstroke velocities of the respective action potentials. Calibration marks are indicated at the right of the figure: vertical calibration = 75 mv for voltage scale and 750 v/sec for differentiator scale, and horizontal calibration = 100 msec.

As previously noted, manganese hyperpolarized Purkinje cells bathed in normal Tyrode's solution. It is possible that the underlying mechanism for this effect is the inhibition of a steady-state sodium leak current. If this mechanism is operative, any condition tending to reduce sodium leak should reduce the manganese-induced hyperpolarization. Two types of experiments were conducted to test this possibility. In the first, preparations were equilibrated in Tyrode's solutions containing elevated potassium concentrations. It was assumed that a decrease in membrane potential produced by elevated extracellular potassium would reduce the driving force for steady-state sodium leak current. Following equilibration at each potassium concentration, manganese was added to the bathing solution and the change in membrane potential was recorded. Table 1 summarizes the results obtained from five experiments. At all levels of extracellular potassium tested, the addition of potassium depolarization (KCl = 21.6 mM) following equilibration in each of the following solutions: (1) 1.8 mM calcium Tyrode's solution, (2) 7.2 mM calcium Tyrode's solution, and (3) 1.8 mM calcium plus 5.0 mM manganese Tyrode's solution. A Vmin of -73 mv was selected for comparison of the records, since at this membrane potential Vmax was increased by high calcium and manganese. Note the opposing effects of high calcium and high manganese on the plateau and the slope of phase 3 and their similar action on Vmax and Vos.

MEMBRANE HYPERPOLARIZATION

Table 1

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>$[K^+]_b$ (mM)</th>
<th>Vm (mv)</th>
<th>Difference (mv)</th>
<th>P</th>
</tr>
</thead>
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<td></td>
<td>Control</td>
<td>Manganese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.7</td>
<td>-95.3 ± 0.8 (8)</td>
<td>-101.0 ± 0.3 (9)</td>
<td>5.7</td>
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<tr>
<td>2</td>
<td>2.7</td>
<td>-92.1 ± 0.8 (8)</td>
<td>-96.2 ± 0.9 (7)</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>-95.7 ± 0.6 (6)</td>
<td>-101.8 ± 0.7 (7)</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>16.2</td>
<td>-68.9 ± 0.9 (6)</td>
<td>-73.6 ± 0.8 (7)</td>
<td>4.7</td>
</tr>
<tr>
<td>1</td>
<td>16.2</td>
<td>-62.2 ± 0.8 (7)</td>
<td>-66.5 ± 0.3 (6)</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>16.2</td>
<td>-63.4 ± 0.6 (10)</td>
<td>-67.5 ± 0.6 (14)</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>21.6</td>
<td>-49.9 ± 0.5 (7)</td>
<td>-53.6 ± 0.3 (6)</td>
<td>3.7</td>
</tr>
<tr>
<td>5</td>
<td>21.6</td>
<td>-44.9 ± 0.7 (6)</td>
<td>-48.0 ± 0.6 (7)</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Data were obtained during a control period and after a 15-minute incubation in 2.5 mM manganese. Values are reported as means ± SE. The number of cells analyzed is given in parentheses. An unpaired difference analysis was used to determine P values. The cells were electrically driven at 90/min except in experiments carried out in 21.6 mM potassium and in experiment 5 in which the cells were quiescent. $[K^+]_b$ = extracellular potassium concentration, and Vm = resting membrane potential of nonbeating cells and maximum diastolic potential of electrically driven cells.
manganese (2.5 mM) resulted in an increase in membrane potential. When the same preparation was equilibrated and tested at more than one potassium concentration, manganese produced significantly less hyperpolarization at higher extracellular potassium concentrations. For example, in experiment 1 manganese produced hyperpolarizations of 5.7, 4.3, and 3.2 mV when it was applied to a preparation equilibrated in Tyrode’s solution containing 2.7, 16.2, and 21.6 mM potassium, respectively. Similar results were obtained with higher concentrations of manganese (5 and 10 mM).

To further test the possibility that manganese blocks the steady-state sodium leak current, the ability of TTX to modify the manganese effect on $V_m$ was examined. The concentration of TTX ($2 \times 10^{-6}$ g/ml) was less than that required to block the action potential-generating mechanism but sufficient to block the steady-state sodium leak permeability (12). Preparations were equilibrated for 40 minutes in 16.2 mM potassium to stabilize membrane potential and eliminate spontaneous firing. In the first of two experiments, exposure to TTX hyperpolarized the cells from $-59.9 \pm 0.6$ mV to $-67.1 \pm 0.9$ mV ($P < 0.001$), and the addition of 2.5 mM manganese had no further effect on $V_m$. In the second experiment, manganese was added before TTX. The usual manganese-induced hyperpolarization from $-63.3 \pm 0.5$ mV to $-67.5 \pm 0.6$ mV ($P < 0.001$) occurred; there was no further increase in $V_m$ on application of TTX. From these data it would appear that manganese and TTX hyperpolarize Purkinje cells through a similar mechanism, presumably by decreasing a steady-state sodium leak current.

**DIASTOLIC DEPOLARIZATION**

Figure 9 illustrates the typical effect of manganese on the time course of diastolic depolarization. Records shown in Figure 9A are from a preparation in which the microelectrode remained in the same cell throughout the entire experiment. The preparation was stimulated continuously at a frequency of 12/min. The slow rate was selected to allow adequate time for diastolic depolarization to develop. The application of 2.5 mM manganese caused a progressive increase in $V_{max}$ and a decrease in $V_{min}$ with a resultant increase in the amount of diastolic depolarization ($V_{max} - V_{min}$) occurring between beats. Records B and C, demonstrating the same effect in another preparation, were taken at a fast paper speed to show the voltage-time course of diastolic depolarization. In B, taken during control conditions, the basic stim-

![Figure 9](http://circres.ahajournals.org/)

**FIGURE 9**

Effect of manganese on diastolic depolarization in Purkinje fibers. All records shown represent the amplified lower portion of successive action potentials and intervening diastolic intervals. The horizontal line under each section of the figure is a $-100$-mV reference line. The point on each record closest to this line is the maximum diastolic potential ($V_{max}$) and the point just prior to the upstroke of each action potential is the minimum diastolic potential ($V_{min}$). A: Records taken from the same cell stimulated at a frequency of 12/min during equilibration in Tyrode’s solution containing 2.5 mM manganese. The time at which each record was taken is indicated in seconds under each trace. The horizontal calibration below the last number $= 30$ seconds. Records B and C were obtained from a preparation in which the basic stimulation frequency of 90/min was interrupted by 30 seconds of no stimulation. B: Record taken under control conditions. C: Record taken 33 minutes after the application of 2.5 mM manganese. Note that in B the quiescent interval was interrupted by three spontaneous beats. Calibration marks are indicated at the lower right corner of section C: horizontal calibration for B and C $= 15$ seconds, and vertical calibration for all records $= 25$ mV.

The time at which each record was taken is indicated in seconds under each trace. The horizontal calibration below the last number $= 30$ seconds. Records B and C were obtained from a preparation in which the basic stimulation frequency of 90/min was interrupted by 30 seconds of no stimulation. B: Record taken under control conditions. C: Record taken 33 minutes after the application of 2.5 mM manganese. Note that in B the quiescent interval was interrupted by three spontaneous beats. Calibration marks are indicated at the lower right corner of section C: horizontal calibration for B and C $= 15$ seconds, and vertical calibration for all records $= 25$ mV.

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spontaneous beats during the 30-second pause in stimulation, indicating a decrease in spontaneous automaticity. This effect will be discussed later. The effect of manganese on diastolic depolarization was less evident at faster stimulation rates due to the shortening of the diastolic interval.

**Vmax During Overdrive Stimulation**

The observation that manganese increased the amount of diastolic depolarization may be explained through a decrease in potassium conductance, electrogenic sodium pump activity, or both. One approach to the study of these mechanisms is the use of rapid stimulation as an experimental procedure. The initial effect of rapid stimulation is a decrease in Vmax due to transient accumulation of extracellular potassium followed by an increase in Vmax resulting from the activation of the electrogenic sodium pump (13). The same mechanisms may be responsible for a simultaneous decrease in the rate of diastolic depolarization seen during and after overdrive. In the following experiments, the simultaneous application of rapid stimulation and manganese was employed to determine the extent to which manganese affects these phenomena. Figure 10A shows the control response to an increase in stimulation frequency from 12 to 60/min. During rapid stimulation, Vmax initially decreased (depolarization) and then increased (hyperpolarization) above the preoverdrive level. On return to the slower stimulation frequency, Vmax increased further before returning gradually to the preoverdrive level. Note also that the extent of diastolic depolarization was reduced following overdrive largely due to a decrease in the rate of depolarization in the latter half of diastole, confirming the observations of Vick (14) and Vassalle (13). Figure 10B shows the effect of 2.5 mM manganese on frequency-dependent changes in Vmax and diastolic depolarization. Prior to overdrive, manganese caused an increase in both Vmax and diastolic depolarization, as noted previously. During overdrive the degree of initial depolarization was reduced and late overdrive hyperpolarization was increased. Postoverdrive hyperpolarization was similar to control. These changes in Vmax were statistically significant \( P < 0.05, N = 5 \).

**Generalized Depolarization**

Manganese in high concentrations caused generalized depolarization of Purkinje cells. The possibility that a marked reduction in potassium conductance is responsible for this effect was tested by using overdrive stimulation as a means of manipu-
lating the potassium system in the presence of a depolarizing concentration of manganese. It has been suggested that the initial effect of overdrive stimulation is to increase extracellular potassium concentration which in turn increases potassium conductance (13). Furthermore, shorter cycle length per se may also result in a net increase in potassium conductance (15, 16). Under normal circumstances it would appear that the reduction in potassium equilibration potential resulting from the accumulation of extracellular potassium has a dominant influence resulting in early overdrive depolarization. If, however, potassium conductance is reduced by manganese to the extent that generalized depolarization occurs prior to rapid stimulation, an overdrive-induced increase in potassium conductance might result in early hyperpolarization. Figure 11 shows the results obtained from such an experiment. Under control conditions, the usual Vmax changes were observed during and after rapid stimulation. The preparation was then superfused with 10 mM manganese Tyrode’s solution. At 5-minute intervals the basic stimulation frequency of 12/min was interrupted for 2 minutes of stimulation at 60/min. By 14 minutes into the experiment no generalized depolarization had occurred and the changes in diastolic depolarization and Vmax were identical to those observed in 2.5 mM manganese (Fig. 10). Later the cell depolarized and by 30 minutes preoverdrive Vmax was reduced to -75 mv. At this time the initial effect of rapid stimulation was a marked hyperpolarization occurring over the first few rapid beats. Following overdrive, Vmax decreased with each beat, returning quickly to the preoverdrive level. Similar results were obtained in four other experiments. One element of the proposed mechanism for explaining these results assumes that overdrive stimulation increases the extracellular potassium concentration which in turn increases potassium conductance thereby reversing the decrease in potassium conductance caused by manganese. To test this assumption more directly, preparations were exposed to depolarizing concentrations of manganese. When sufficient depolarization had occurred, the potassium concentration of the Tyrode’s solution was elevated. The results of three experiments are pre-

![Figure 11](https://example.com/figure11.png)

**FIGURE 11**

Effect of rapid stimulation on a manganese-depolarized Purkinje fiber. All records are from the same cell. The center record of each section shows the amplified lower portion of the action potential and the diastolic interval recorded continuously during an overdrive sequence. Each sequence included a pre- and a postoverdrive interval at a stimulation frequency of 12/min and an overdrive interval of approximately 2 minutes at a frequency of 60/min. The horizontal line below each record is a -100-mv reference line. The two vertical marks below each record indicate the time at which the fast sweep records shown on the right and left sides of the figure were taken. The horizontal mark beside each action potential tracing indicates zero potential. Calibration marks are indicated at the lower right corner of the figure: vertical calibration = 25 mv for the center records and 100 mv for the action potential records, and horizontal calibration = 30 seconds for the center records and 375 msec for the action potential records.
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Effect of Extracellular Potassium Concentration on Purkinje Fibers Depolarized by Manganese

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Control [K+]o (mM)</th>
<th>Control Vm (mv)</th>
<th>[Mn+]o (mM)</th>
<th>Manganese Vm (mv)</th>
<th>Test [K+]o (mM)</th>
<th>Test Vm (mv)</th>
<th>Difference* (mv)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.7</td>
<td>-81.3 ± 0.3 (7)</td>
<td>5</td>
<td>-54.9 ± 0.8 (7)</td>
<td>5.4</td>
<td>-67.2 ± 0.5 (7)</td>
<td>12.3</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>2.7</td>
<td>-94.7 ± 0.5 (14)</td>
<td>10</td>
<td>-69.2 ± 1.0 (6)</td>
<td>5.4</td>
<td>-77.7 ± 0.7 (7)</td>
<td>8.5</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>-99.0 ± 0.7 (7)</td>
<td>10</td>
<td>-47.4 ± 0.6 (6)</td>
<td>16.2</td>
<td>-59.4 ± 1.0 (11)</td>
<td>12.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means ± se. The number of cells analyzed is given in parentheses. The cells were nonbeating in experiment 1 and stimulated at a frequency of 90/min in experiments 2 and 3. [K+]o = extracellular potassium concentration, [Mn+]o = manganese concentration, and Vm = resting membrane potential of nonbeating cells and maximum diastolic potential of electrically driven cells.

* Difference (unpaired) between the Vm following manganese depolarization and the Vm following the elevation of the extracellular potassium concentration. P values indicate the significance of this difference.

In every cell examined, an increase in extracellular potassium concentration following manganese-induced depolarization resulted in hyperpolarization.

Figure 11 also shows the change in action potential configuration associated with overdrive during control and manganese superfusion. For each section of the figure (control, 14 minutes, and 30 minutes), the action potential record on the left was taken just prior to the onset of rapid stimulation, the one on the right just before returning to the slower stimulation frequency. Note that before generalized depolarization occurred (14 minutes), typical manganese-induced changes in the action potential were observed and the effect of overdrive on action potential configuration was similar to control. After generalized depolarization (30 minutes), however, the preoverdrive action potential was markedly depressed. At the end of overdrive the action potential was restored toward normal due to the drive-induced increase in Vmin.

ARRHYTHMIC BEATS

Throughout the series of experiments using overdrive stimulation, arrhythmic beats were often observed during slow stimulation before and after overdrive. Although overdrive stimulation per se failed to block the extra beats, manganese markedly reduced their number. In fact, in most trials the presence of manganese completely eliminated extra beats.

Discussion

The series of alterations in membrane potential resulting from the application of manganese to Purkinje fibers indicates a complex effect of this cation on the electrogenic process. The present findings show that manganese has calcium-like actions on the excitatory sodium current and the threshold potential and a nonspecific inhibitory action on sodium leak and potassium conductances. In addition, these findings support the hypothesis that manganese blocks a slow inward current important to the genesis of the plateau phase.

Manganese and calcium have similar effects on the fast sodium current, since both cause a leftward and downward shift in the membrane responsiveness curve. This action of manganese is similar to that of other polyvalent ions (17-19). The ability of calcium to displace sodium inactivation and responsiveness curves is well established for nerve (20) and heart membranes (21). Wiedmann (21) has found that an increase in calcium from 2.6 to 10.4 mM shifts the responsiveness curve of sheep and calf Purkinje fibers by -5.6 mv, which is in accord with our value of -5.2 mv for an increase in calcium from 1.8 to 7.2 mM.

The ability of manganese to enhance the initial spike of an action potential generated from low membrane potentials has been reported to our knowledge in only one other study (22). Using cow Purkinje fibers depolarized with 10.8 mM potassium, Carmeliet and Vereecke (22) noted that 2.0 mM manganese simultaneously depressed the plateau and increased the overshoot of the initial spike.

In addition to a leftward shift, we found that calcium and manganese both depressed Vmax at the highest levels of membrane potential (downward shift). With regard to calcium, this finding contrasts with that in the study of Wiedmann (21) in which Vmax was unchanged by elevated calcium at membrane potentials greater than -90 mv. Although we cannot account for this difference in results, there are several studies using both nerve membranes (17, 20, 23) and cardiac cells (10, 24) which indicate that elevated extracellular calcium can depress sodium conductance and sodium current (\(i_{Na}\)).

From these examples and our own results, it seems reasonable to conclude that elevated extra-
cellular calcium concentration can depress upstroke velocity at high membrane potentials. This conclusion tends to further support our view that manganese has a calciumlike action on the initial spike of Purkinje fibers, since we found that manganese also depressed \( V_{\text{max}} \) at the most negative membrane potentials.

Other studies of manganese using cardiac and skeletal muscle from various animal species have also shown that manganese either has no effect or depresses the initial spike of fully polarized cells. In most species in which the initial spike of the action potential is thought to involve only sodium current (frog skeletal muscle [5, 25, 26], sheep Purkinje fibers [9], rabbit atria [27] and ventricle [28], and rat ventricle [29]) overshoot is unchanged or only slightly reduced by low concentrations of manganese. Our results on overshoot are in general accord with these findings (Fig. 3). Higher concentrations are reported to block the initial spike (27).

In cardiac cells whose initial spike has two components (early \( i_{\text{Na}} \), late \( i_{\text{Ca}} \)), which include frog atrium (7, 30), frog ventricle (31), and guinea pig ventricle (29), manganese (1-4 mM) inhibits the calcium component with only a small or no depressive effect on the sodium component. At higher concentrations, manganese may block \( i_{\text{Na}} \) (30, 31).

The action of manganese on the slow inward current is in sharp contrast to its action on the fast sodium channel. Several investigators using a variety of techniques have demonstrated that the slow inward current is sensitive to extracellular calcium concentration and blocked by manganese, other heavy metal cations, and verapamil (2-4). The present results show that low-amplitude responses \( (V_{\text{min}} < -55 \text{ mV}, V_{\text{max}} < 30 \text{ v/sec}) \) occurring in Purkinje fibers maintained in normal physiological solution when a second stimulus is applied before repolarization is complete can be blocked by manganese. The fact that these responses can be elicited in free-running, nonmanipulated fibers lends credence to the contention of Cranefield and his co-workers (32-34) that slow-conducting, calcium-dependent spikes may be important in the genesis of reentrant arrhythmias. It is of interest in this regard that manganese effectively eliminated the arrhythmic beats commonly observed in the slowly driven preparations. Although no direct evidence was obtained regarding the antiarhythmic action of manganese, at least three possibilities can be deduced from our findings: (1) the elimination of low-amplitude calcium spikes and thus the elimination of slow-conducting potentials, (2) the leftward shift in the membrane responsiveness curve yielding more favorable conditions for rapid conduction of sodium spikes, and (3) the decrease in excitability leading to depression of spontaneous automaticity.

The hyperpolarization produced by manganese is similar to that produced by TTX (12) and by removal of sodium (16, 35) from the extracellular fluid. The increase in \( V_{\text{m}} \) under these conditions can be accounted for by a net reduction in inward sodium leak current with \( V_{\text{m}} \) moving closer to the potassium equilibrium potential. The fact that the hyperpolarizing action of manganese was reduced or abolished when sodium leak current had been reduced or blocked indicates a similar mechanism for manganese. Calcium also is reported to increase membrane potential by the same mechanism (21, 36, 37). It seems unlikely that the manganese-induced hyperpolarization is due to an increase in electrogenic pump activity, since it occurs under conditions which exist when the pump is presumably electroneutral, i.e., in the nonbeating preparation and in preparations equilibrated in Tyrode's solution containing elevated extracellular potassium concentrations (13, 16).

The repolarization process in Purkinje fibers results from a complex interaction of several ionic currents. The action of manganese to alter the time course of repolarization reflects the ability of this cation to modulate one or more of these currents. As pointed out earlier, for example, manganese blocks a slow inward calcium current which may participate in the genesis of the plateau phase. In studies on guinea pig myocardial cells, Ochi (6) observed that manganese depresses the rate of rise and decay of the slow inward current as well as its magnitude, an effect that might explain the bimodal action of manganese on plateau duration. If in the canine Purkinje fiber manganese initially slows the rise and fall of the slow inward current more than it affects the current's peak magnitude, the plateau might remain unchanged in slope but be displaced in time, causing an increase in action potential duration. At higher concentrations more complete blockade of this current would result in shortening of the plateau, as noted in the present study and by Vitek and Trautwein (9) in sheep Purkinje fibers. A decrease in the rate of activation of the slow inward current might also explain the increase in the rate of initial repolarization (phase 1) and the formation of a notch at the beginning of the plateau. Such changes would be expected if a decrease in slow inward current increased the dynamic outward current (9). It is of interest in this regard to note that an increase in extracellular
calcium has the opposite effect on the rate of initial repolarization (10).

The bimodal action of manganese on action potential duration also has been demonstrated in rabbit ventricular muscle by Takeya and Reiter (28). They concluded that manganese first increases and then decreases a slow inward current involving sodium.

A third possible mechanism for the bimodal action of manganese on plateau duration may involve reductions in both potassium current and slow inward current. Rougier et al. (7) in their study of frog atria suggested that the initial lengthening of the action potential by manganese might be due to a decrease in potassium conductance. In Purkinje fibers the termination of the plateau is thought to depend on the inactivation of slow inward current and on the initiation of a delayed outward current. Noble and Tsien (15, 38) have identified an outward current, $i_{x}$, which is presumably carried by potassium and operates in approximately the same voltage range as the slow inward current; $i_{x}$ is inactivated at -50 mV where a second potassium current, $i_{K1}$, is activated and carries the membrane potential to the maximum diastolic potential. A delay in activation or a decrease in magnitude of $i_{x}$ would bring about an elevation and a lengthening of the plateau. The eventual depression and shortening of the plateau would result from the eventual abolition of slow inward current.

The action of manganese on $i_{K}$ kinetics may be reflected through the progressive slowing of terminal repolarization and the progressive increase in early diastolic depolarization. A manganese-induced shift in the $i_{K}$-Vm relationship to a less polarized level with an increase in the time constant of $i_{K}$ activation and a decrease in the time constant of $i_{K}$ inactivation would result in the observed changes. The effects of manganese on terminal repolarization and diastolic depolarization are strikingly similar to those produced by calcium (10) and norepinephrine (16, 39), agents thought to alter $i_{K}$ kinetics in the aforementioned way (40, 41). It is unlikely that the increase in diastolic depolarization results from a decrease in electrogenic pump activity for the following reasons. (1) The effect occurs at slow stimulation frequencies (12/min) when the pump is presumably electroneutral (Fig. 9). (2) A change in pump activity would alter the time course of diastolic depolarization late in the cycle (13), whereas the major effect of manganese occurred early in diastole (Fig. 9).

The action of low concentrations of manganese to reduce early depolarization and increase late hyperpolarization occurring during overdrive may also depend on reduced potassium conductance. Carpentier and Vassalle (42) have demonstrated a similar effect for low concentrations of norepinephrine and have pointed out that a reduction in potassium conductance alone allows the outward current generated by the electrogenic sodium pump to be more effective in polarizing the membrane throughout the time course of rapid stimulation. In the case of norepinephrine, this effect may also depend on an increase in pump activity per se.

The action of high concentrations of manganese to depolarize Purkinje fibers may indicate a further reduction in potassium conductance. The depolarization produced by a decrease in potassium conductance would increase the difference between the potassium equilibrium potential ($E_{K}$) and $V_{m}$ (43-45). Under the circumstance of markedly reduced potassium conductance, interventions causing an increase in potassium conductance would be expected to increase $V_{m}$ toward $E_{K}$. An increase in extracellular potassium concentration (43, 46) or rapid stimulation (13, 16) is expected to increase potassium conductance, and both caused hyperpolarization of manganese-depolarized cells. An increase in extracellular potassium concentration also reduces $E_{K}$, thus limiting the extent to which a simultaneous increase in potassium conductance increases membrane potential.

It is of interest to note that the early phase of marked hyperpolarization occurring during rapid stimulation has been observed under other circumstances in which a reduction in potassium conductance was suspected as a cause of generalized depolarization. For example, Ruzyllo and Vick (45) have demonstrated this phenomenon in preparations incubated in solutions containing low potassium concentrations, and Vassalle and Carpentier (16) have demonstrated the same effect in cells depolarized by norepinephrine. On the other hand, if the cell has been previously depolarized by reducing $E_{K}$ as would be the case when extracellular potassium concentration is elevated and during glycoside-induced depolarization, then drive-induced hyperpolarization does not occur (45, 47).

In conclusion, these findings indicate that manganese exerts a wide range of effects on membrane activity in the Purkinje fiber. The use of manganese as a selective inhibitor of slow inward current must be considered in this context. Finally, the modulation of sodium and potassium currents by manganese makes it a potentially useful tool for
delineating the role of these ions in electrogenic phenomena.

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