Two Levels of Resting Potential in Canine Cardiac Purkinje Fibers Exposed to Sodium-Free Solutions

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SUMMARY Canine cardiac Purkinje fibers exposed to sodium-free solutions containing 16 mM CaCl₂, 20 mM tetraethylammonium chloride, 108 mM tetramethylammonium chloride, and 2.7 mM KCl may be quiescent at a resting potential of either -50 mV or -90 mV. The membrane potential of these fibers can be switched from -50 mV to -90 mV by a hyperpolarizing current pulse and from -90 mV to -50 mV by a depolarizing current pulse. The transition from -50 mV to -90 mV depends on a voltage-dependent increase in potassium conductance, that conductance being low at -50 mV and high at -90 mV. A reduction in potassium conductance causes the fiber to depolarize from -90 mV to -50 mV because of the presence of an inward current which apparently is carried mainly by Ca. Fibers that show a high resting potential cannot be excited except by depolarizing stimuli strong enough to move the membrane from -90 mV to a threshold potential of about -40 mV. Fibers that show a low resting potential are more easily excited and may show rhythmic activity sustained by afterpotentials that appear only if the low membrane potential is accompanied by a low potassium conductance. Slow changes in membrane potential also are seen; these changes may result from movements of chloride.

CARDIAC PURKINJE FIBERS bathed in Na-free solutions can show two stable resting potentials, one near -90 mV and one near -50 mV. We now report that within a critical range of Kₐ, the membrane potential can be shifted from either level to the other by the application of hyperpolarizing or depolarizing current pulses. This transition may be governed primarily by a voltage-dependent change in potassium conductance, that conductance being high at the -90 mV level and low at the -50 mV level. This phenomenon assumes special interest because cardiac fibers can produce two distinct types of propagated action potentials. One type, dependent on a rapid increase in sodium conductance, is abolished by voltage-dependent inactivation when the resting potential is low. The other type, called the slow response, depends on an increase in permeability which occurs at membrane potentials between -50 and +10 mV. Various cardiac arrhythmias arise in fibers in which a loss of resting potential causes the rapid upstroke to be replaced by the slow response. The possibility that cardiac fibers are characterized not only by an ability to produce two types of action potential, but also by an ability to display the two levels of resting potential from which those action potentials can arise, is therefore intriguing.

Methods

Mongrel dogs of either sex weighing 15-20 kg were anesthetized with an intravenous injection of pentobarbital sodium (30 mg/kg). The heart was rapidly excised and immersed in Tyrode's solution. Fibers which, had a membrane potential of less than -80 mV in Tyrode's solution were discarded. After equilibration in Tyrode's solution, the fibers were perfused with a sodium-free solution (see below). Fibers were bathed in the Na-free perfusate for at least 20 minutes before any measurement of the resting potential was begun. This equilibration period is sufficient to reduce tissue Na to a steady low level of 3 mmol/kg of wet weight.

Electrical recording and current injection were achieved through glass microelectrodes (tip resistance, 8-30 MΩ; tip potential, less than 5 mV). Recording electrodes were filled with 3 m KCl; current-passing electrodes were usually filled with 0.7-0.85 m potassium citrate; similar results were obtained when electrodes were filled with 3 m KCl. External stimuli were delivered through bipolar, Teflon-coated silver wires, insulated except at their tips. Stimulating current was isolated from ground and delivered by means of a high voltage field-effect transistor circuit; the timing of the current pulses was controlled by a digital parallel timing system. Most records were obtained by directly photographing an oscilloscope (RM 565 or RS103N, Tektronix); some experiments were recorded on a Honeywell 5600 tape recorder and were played back on an oscilloscope. The frequency response of the tape recorder at the speeds used was 5 kHz; this is adequate for recording diastolic depolarization, slow upstrokes seen in Na-free solutions, and slow changes in resting potential.

The composition of the principal solutions used is shown in Table 1. All chemicals except tetraethylammonium chloride (TEA) and tetramethylammonium chloride (TMA) were reagent grade, and all solutions were made up in redistilled, glass-condensed water. TEA was obtained from Eastman Organic Chemicals, from Aldrich, from Matheson, Coleman & Bell. TMA was obtained from Aldrich, or from Matheson, Coleman & Bell. Similar results were obtained with chemicals from all suppliers. TEA solutions were filtered before use. When the ionic composition of one of the principal solutions was varied, e.g., when changing Kₐ, to examine the effects of K on the resting potential, no correction for the change in osmolarity was made.

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TABLE 1  Principal Solutions Used

<table>
<thead>
<tr>
<th>Solution*</th>
<th>NaCl</th>
<th>CaCl₂</th>
<th>KCl</th>
<th>MgCl₂</th>
<th>MnCl₂</th>
<th>TEA</th>
<th>TMA</th>
<th>Dextrose</th>
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<td>Tyrode's</td>
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<td>2.7</td>
<td>4</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.5</td>
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<tr>
<td>Ca, zero Na</td>
<td>0</td>
<td>16</td>
<td>2.7</td>
<td>0.5</td>
<td>0</td>
<td>5-20</td>
<td>123-108</td>
<td>5.5</td>
</tr>
<tr>
<td>Mn, zero Na</td>
<td>0</td>
<td>0</td>
<td>2.7</td>
<td>0.5</td>
<td>5-16</td>
<td>5</td>
<td>140-123</td>
<td>5.5</td>
</tr>
<tr>
<td>Ca, Mn, zero Na</td>
<td>0</td>
<td>16</td>
<td>2.7</td>
<td>0.5</td>
<td>5-16</td>
<td>5</td>
<td>116-99</td>
<td>5.5</td>
</tr>
</tbody>
</table>

TEA = tetraethylammonium chloride; TMA = tetramethylammonium chloride.
* The Tyrode's solution was buffered with 12.5 mM NaHCO₃ and 1.8 mM NaH₂PO₄ and equilibrated with 95% O₂ and 5% CO₂. The Na-free solutions were buffered with 5 mM tromethamine (Tris) adjusted to pH 7.4 at 36°C with HCl and were equilibrated with 100% O₂.

made if the total change was less than 20 mOsm. If the ion change would entail a greater change in tonicity, the amount of TMA in the solution was also changed to maintain a constant osmolarity.

In fibers bathed in 128 mM TEA, the fiber can be switched from one resting potential to the other only during the first 15-20 min after the fiber has been exposed to a Na-free solution.1 In solutions containing 5-20 mM TEA, the rest of the Na being replaced by TMA, two resting potentials can be obtained for at least 2 hours after the removal of Na. When the fiber reverts to showing only one resting potential, it is almost always the low level that is lost. In the experiments here reported, TEA was present at either 5 or 20 mM unless otherwise stated.

Results

TWO LEVELS OF RESTING POTENTIAL

Canine cardiac Purkinje fibers exposed to an Na-free, Ca-rich solution can produce Ca-dependent action potentials that have a threshold potential between −50 mV and −40 mV.4 After a 20-minute exposure to Na-free solution, some fibers are quiescent with a resting potential in the vicinity of −90 mV, others are quiescent with a resting potential in the vicinity of −50 mV, and still others are rhythmically active, showing a maximum diastolic potential of about −60 mV. Fibers that have a resting potential in the vicinity of −90 mV (Fig. 1A, a) can be excited only by long and strong stimuli, becoming repetitively active during the passage of a long depolarizing pulse (Fig. 1A, b). Fibers having a resting potential in the vicinity of −50 mV may respond to a relatively weak and brief stimulus with a burst of impulses or even with sustained rhythmic activity.7 Such activity can be elicited by depolarizing stimuli but is most easily elicited by anodal break stimuli (Fig. 1B, e). Fibers that have been triggered into sustained rhythmic activity (Fig. 1B, f) usually show an increase in their rate of activity during application of a depolarizing pulse (Fig. 1B, g). After the end of a period of increased rate, such fibers often become quiescent (Fig. 1B, h). In many fibers, application of an anodal pulse that is stronger or longer than is needed to trigger repetitive activity causes the fiber to hyperpolarize to a resting potential near −90 mV. In Figure 1B a relatively strong hyperpolarizing pulse (Fig. 1B, i) causes an apparently regenerative hyperpolarization which, however, does not reach a steady state and is not maintained after the end of the pulse. After a longer pulse of the same strength (Fig. 1B, k), a high resting potential is maintained (Fig. 1B, l). These fibers can, therefore, show two resting potentials which differ by some 40 mV.

Transitions between −90 and −50 mV

If a long depolarizing pulse is applied to a fiber that has a high resting potential, the membrane potential may return to the original level in a single step at the end of the pulse (Fig. 1A, c). However, many fibers return to a high resting potential only after an interval of several hundred milliseconds to 10 or more seconds (Fig. 2A). In those fibers,

![Figure 1](https://example.com/figure1.png)  
Changes in membrane potential in response to pulses of depolarizing or hyperpolarizing current. The top trace in each panel shows time marks at 3-second intervals; the bottom edge of the top trace shows the “zero” reference potential. The bottom trace shows applied current in arbitrary units, the upward deflections (b, g) indicating depolarizing pulses and downward deflections (e, i, k) indicating hyperpolarizing pulses; during the intervals a, c, d, f, h, j, and l no current was applied. (K)ₐ = 2.7 mM. Calibrations: vertical, 40 mV; the horizontal calibration corresponds to 4 seconds in panel A and to 10 seconds in panel B.
There are a number of changes that occur more slowly; the shifts from one resting potential to another that have been described above occur within, at most, several seconds. In Figure 1B, for example, the membrane potential does not attain a steady state during either of the hyperpolarizing pulses (Fig. 1B, i and k), nor is it steady after the fiber has been shifted to the high resting potential (Fig. 1B, l). A slow, cumulative change may be seen in fibers in which a resting potential near −50 mV is maintained for only a rather brief period after the end of depolarizing pulses (see Fig. 2A); if such fibers are depolarized repeatedly or by longer pulses they may respond with maintained depolarization or sustained rhythmic activity. In such fibers the entire sequence of membrane potential changes that follows a depolarizing pulse appears at progressively less negative levels after each successive pulse. The difference between a potential from which a fiber will spontaneously return to the vicinity of −90 mV and the potential at which it will remain at a low resting potential may be as little as 4 mV.

Slow changes in membrane potential also are seen after current pulses that cause the resting potential to shift from one level to the other. When the fiber is first shifted to a low resting potential, that potential is usually close to −50 mV and the fiber is easily shifted back to a high resting potential by very weak currents. If, however, the fiber is allowed to remain at the low resting potential, that potential becomes progressively less negative and may fall to as low as −35 mV over a period of 15–90 minutes. As the membrane potential (E_m) declines, increasingly stronger currents are required to shift the membrane back to a high resting potential. If a fiber in which the resting potential has fallen from −50 mV to −35 mV is returned to a high resting potential, that potential is not constant but creeps slowly toward a more negative level. If the fiber then remains at a high resting potential for 5–30 minutes before being shifted back to a low resting potential, that potential is again near −50 mV.

As the low resting potential becomes progressively less negative with the passage of time, it becomes increasingly difficult to trigger long trains of action potentials by a single stimulus. Although long trains of action potentials may be triggered when the resting potential is near −50 mV, once the resting potential had declined to or below −50 mV, a single stimulus evokes one action potential or, at most, a rather brief burst of action potentials. This loss of sustained repetitive activity does not result from a loss of excitability, since single action potentials can almost always be evoked, by either hyperpolarizing or depolarizing stimuli, from resting potentials as low as −30 to −35 mV.

APPARENT MEMBRANE RESISTANCE

Figure 3 shows records from two electrodes in a fiber which shows a low resting potential, one electrode being 1 mm from a current-passing electrode, and the other, 5 mm. In Figure 3A, a 2-second long hyperpolarizing current pulse of $1 \times 10^{-7}$ A evokes small voltage changes at each recording electrode (a) which appear to be consistent with electrotonic spread. When the strength of the hyperpolarizing current is increased (b) to $8 \times 10^{-7}$ A, an apparently regenerative increase in potential carries the membrane beyond the level of the high resting potential (Fig. 3B), i.e., beyond the level maintained after the end of the pulse. When a hyperpolarizing pulse (c) of $1 \times 10^{-7}$ A is again applied to the fiber (Fig. 3C), it can be seen that the resultant steady
state potential changes are smaller and that the electrotonic decrement between the near and distant recording sites is greater than seen in Figure 3A. At a faster sweep it can be shown that the apparent time constant is larger at the low resting potential. These findings, namely that the time constant, the length constant, and ΔE/Δl are all greater when the resting potential is low than when it is high, show that the membrane resistance is greater at the low resting potential and is decreased with the shift to the high resting potential. It will be noted that there is a marked delay between the onset of repolarization at the proximal and distal recording sites in Figure 3C, which suggests that the hyperpolarization may be propagated.

**EFFECTS OF [K]₀**

**Resting Potential High and Sensitive to [K]₀**

Many Purkinje fibers exposed to Na-free solutions containing 16 mM Ca and a mixture of TEA and TMA have a high resting potential when [K]₀ is 2.7 mM. In such fibers an increase in [K]₀ produces a fall in membrane potential (see below, Fig. 7, open circles). When [K]₀ is increased above 10 mM, the change in Eₘ approaches 60 mV for a 10-fold change in [K]₀. These fibers thus behave as if their potassium conductance is high compared to other ionic conductances.

Some fibers that show a high resting potential when [K]₀ is 2.7 mM hyperpolarize when [K]₀ is lowered to about 2 mM; others show no change in potential, and still others depolarize. If [K]₀ is reduced to 0.5 mM, all fibers depolarize markedly to about -50 to -40 mV, at which level they may be quiescent or repetitively active. The concentration at which this marked depolarization occurs in any particular fiber is usually between 0.5 mM and 1.5 mM; once the depolarization has occurred, a further reduction in [K]₀ causes little further change in membrane potential.

**Resting Potential Low and Insensitive to [K]₀**

A few fibers have a low membrane potential when [K]₀ is 2.7 mM, cannot be shifted to a high resting potential by hyperpolarizing pulses, and are relatively insensitive to large changes in [K]₀. An increase in [K]₀ from 2.7 to 10 mM is accompanied by very little change in resting potential in such fibers. Only as [K]₀ is increased above about 10 mM does the resting potential of these fibers vary significantly with changes in [K]₀. Elevation of [K]₀ does not enable such fibers to show two levels of resting potential; we assume that in such fibers the background inward current ("leak" current) is larger than usual. [K]₀ and Two Levels of Resting Potential

About 25% of the fibers that show a high resting potential when [K]₀ is 2.7 mM can be switched to a low resting potential by current pulses. A small reduction in [K]₀ makes it possible to induce two levels of resting potential in an additional 50% of the fibers studied. If [K]₀ is too low that the fiber is depolarized, hyperpolarizing current pulses do not cause a maintained shift to the high resting potential. If [K]₀ is too high the resting potential cannot be switched to the low level by depolarizing pulses. We have never seen a current-induced shift in resting potential when [K]₀ is less than 1.0 mM, nor when it is higher than 5.4 mM; for any particular fiber the range of [K]₀ within which two levels of resting potential can be evoked is considerably narrower. In one fiber exposed to a fixed, low [K]₀ (1.0 mM), the membrane potential repeatedly shifted "spontaneously" from one resting potential to the other.

A moderate increase in [K]₀ can evoke hyperpolarization either in fibers depolarized by low [K]₀ or in fibers that have been switched from a high to a low resting potential by current pulses. Figure 4 shows a fiber in which both resting potentials could be evoked in a solution containing 2.7 mM K. While the fiber was at the low resting potential (-45 mV), [K]₀ was increased from 2.7 mM to 10 mM. The increase in [K]₀ was accompanied by an almost immediate hyperpolarization to -76 mV, followed by a slower depolarization to -64 mV (Fig. 4A). This suggests that the potassium conductance was low at the low resting potential, that the increase in [K]₀ caused that conductance to increase before the solution in the bath was fully equilibrated, so that the value of -76 mV corresponded to a high potassium conductance and a [K]₀ intermediate between 2.7 mM and 10 mM, and that the final level corresponded to a [K]₀ of 10 mM and a high potassium conductance. This interpretation is confirmed by the fact that returning [K]₀ to 2.7 mM caused a further hyperpolarization (Fig. 4B) to -88 mV, i.e., to a level expected if [K]₀ is 2.7 mM and the potassium conductance is high. An increase in [K]₀ to 10 mM then caused a smooth depolarization to -64 mV (Fig. 4). Depolarizing pulses, applied after the resting potential had reached a steady state in the presence of 10 mM [K]₀, were followed by a prompt return to that resting potential; there was no evidence for the presence of a lower resting potential (not shown).

The above interpretation is based on the behavior of a single cell near the surface of the bundle. Cells deep in the bundle will be exposed to the steady state level of 10 mM [K]₀ with a greater lag. Such cells, exposed to, say, 4 mM [K]₀ when surface cells are already exposed to nearly 10 mM [K]₀, would transiently develop a higher resting potential than that of surface cells and might, by electrotonic

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**Figure 3** The effect on membrane potential of hyperpolarizing current pulses. The "zero" reference potential is shown by the upper trace, which also shows time marks at 3-second intervals. The membrane potential records were obtained at 1.0 mm and 5.0 mm from the current-passing electrode. The downward deflections in the lower trace indicate the application of the hyperpolarizing current pulses; the pulses applied at A and C were of the same strength (1 x 10⁻⁴ A). The pulse applied at B was strong enough (8 x 10⁻⁴ A) to cause the fiber to switch from the low to the high resting potential; the time course of the applied pulse in the lower trace in B is partially obscured by the trace showing the membrane potential. A and B do not form a continuous record; B and C do. [K]₀ = 2.7 mM.
FIGURE 4  K-dependent shifts in membrane potential. The upper trace shows the “zero” reference potential and the lower trace shows the membrane potential. In panel A, [K]₀ was changed from 2.7 mM to 10 mM 24 seconds after the beginning of the sweep. In panel B, [K]₀ was changed from 10 mM to 2.7 mM 10 seconds after the beginning of the sweep; the second (lower) sweep in B began 25 seconds after the end of the upper sweep. In panel C, [K]₀ was changed from 2.7 mM to 10 mM 10 seconds after the beginning of the sweep. All records are from a single impalement of the same fiber.

interaction, temporarily increase the resting potential of cells at the surface. The presence of inward-going rectification would, however, tend to reduce this effect.

[K]₀ and Sustained Rhythmic Activity

Sustained rhythmic activity in Purkinje fibers exposed to Na-free solutions results from the fact that each action potential is followed by an early after-hyperpolarization that carries the membrane to about −60 mV and is succeeded by an after-depolarization that carries the membrane to the threshold potential of about −40 mV to evoke the next action potential. For such repetitive activity to occur, the membrane potential must be in the neighborhood of −50 mV. If the resting potential is −90 mV, repetitive activity is seen only during a marked depolarization caused by applied current (Fig. 1A). A fiber that has a high resting potential at a [K]₀ of 2.7 mM can also be depolarized and made repetitively active by lowering [K]₀ (Fig. 5A). If, however, a comparable depolarization is brought about by raising [K]₀, a short train of action potentials may appear as the membrane potential falls to the new level; once depolarized, the fiber can be excited by applied stimuli but will rarely show sustained rhythmic activity.

If a fiber that cannot show a high membrane potential is repetitively active when [K]₀ is 2.7 mM and [K]₀ is raised to 5 or 10 mM, the delayed after-depolarization becomes less steep and the threshold potential becomes both more negative and more sharply defined (Fig. 5B). As the after-depolarization becomes less pronounced, a progressive decrease in rate culminates in quiescence. The maximum diastolic potential of such fibers is relatively little changed by the increase of [K]₀ from 2.7 mM to 10 mM.

INWARD CURRENT

[Ca]₀ and Inward Current

The fact that a decrease in membrane resistance is seen when the fiber is shifted to the high resting potential, taken together with the sensitivity of that potential to changes in [K]₀, suggests that the potassium conductance is high at the high resting potential. This in turn suggests that the shift to the low resting potential results from a reduction in the potassium conductance. Such a reduction would not, however, lead to depolarization unless an inward current were present. In the Na-free solutions used in our study, the cations with a positive equilibrium potential are TEA and TMA, which are relatively large ions and presumably not very permeant, and Ca. The role of Ca in determining the resting potential is difficult to analyze because variations in [Ca]₀ appear to change not only EᵦCa but also the permeability of the membrane. The almost invariable result of raising...
[Ca] in a fiber that can show two resting potentials and is at the low level is a shift to the high level, coupled with a loss of the ability to evoke the low level. Figure 6A shows a resting potential of -52 mV in a fiber exposed to 16 mM [Ca]. Between Fig. 6A and 6B, [Ca] was raised to 25 mM. Within 15 seconds after the increase in [Ca], the membrane potential fell from -52 to -50 mV (Fig. 6D) but then progressively increased, shifting to -92 mV within 100 seconds (Fig. 6C) after the increase in [Ca]. Attempts to reestablish the low resting potential by depolarizing pulses were marked by a delay in the return to -92 mV (Fig. 6D), but the low resting potential was never maintained. In some fibers, the effects of increasing [Ca] were readily reversible, and the low resting potential could be obtained soon after [Ca] was again reduced to 16 mM. In many fibers, however, the membrane could not be shifted to the low resting potential for more than an hour after a return from 25 mM [Ca] to 16 mM [Ca].

Decreasing [Ca] from 16 mM to 8 mM or 4 mM usually causes depolarization of fibers which have a high resting potential in 2.7 mM [K]. In some fibers the membrane potential falls only a few mV and remains sensitive to changes in [K]. The resting potential of other fibers, however, falls to -40 or -50 mV and becomes relatively insensitive to changes in [K]. A reduction of [Ca] to 2 mM is sufficient to depolarize almost all fibers; fibers thus depolarized may be repetitively active.

**Inward Current in the Presence of Mn**

In a further attempt to analyze the inward current in sodium-free solutions we varied [Ca], and [K] in the presence of Mn, an agent which blocks Ca-dependent action potentials, exerts a Ca-like "stabilizing" effect on membrane permeability and is less permeant than Ca. In the absence of Mn, reduction of [Ca] to zero in a solution containing 2.7 mM [K] causes depolarization to or beyond -50 mV. If 5-16 mM Mn is added after that depolarization has occurred, the membrane potential returns to about -90 mV. Similarly, if [Ca] is reduced to zero in the presence of Mn, the membrane potential remains high. These findings suggest that Mn does replace Ca as a membrane stabilizer. In addition, fibers exposed to Ca-free solutions containing 5-16 mM Mn display a more negative resting potential than do the same fibers exposed to 16 mM Ca and do so at all levels of [K]. Fig. 7). Even when [K] is lowered to 0.5 mM, such fibers almost invariably return a resting potential of at least -90 mV. This suggests that Mn either reduces an inward current or increases the potassium conductance.

If Ca is added to Mn-containing solutions while [K] remains at 0.5 mM, the membrane potential falls by 30-40 mV, usually stabilizing near -50 mV. In a fiber that shows two levels of resting potential in the presence of Mn and 2.7 mM [K], if [Ca] is reduced to zero when the fiber is at the low resting potential, the fiber shifts to the high resting potential. These findings indicate that Ca may contribute to the low level current even in the presence of Mn. This conclusion is supported by the finding that, in many fibers exposed to 2.7 mM [K], the resting potential can be switched from -90 mV to -50 mV if both Ca and Mn are present (Fig. 8A) but not if Ca is absent (Fig. 8B). The records shown in Figure 8A also indicate that the low resting potential can be evoked and maintained even when Mn has blocked Ca-dependent action potentials. Verapamil, which resembles Mn in its ability to block Ca-dependent action potentials but which neither stabilizes the membrane nor promotes "sealing over," does not prevent the depolarization that results from lowering [Ca] to zero.

**Discussion**

The phenomena described above are not peculiar to fibers exposed to sodium-free solutions. It is well known that cardiac Purkinje fibers exposed to normal Tyrode's solution can develop action potentials in which repolarization is interrupted by one or more slow response action potentials that have very slow upstrokes and a maximum diastolic potential of about -60 mV. Such activity may terminate in a "spontaneous" return to a high membrane potential from

![Figure 6](image.png)

**Figure 6.** Ca-dependent shift in resting potential. The top trace shows the "zero" reference potential, a deflection in the bottom trace in D indicates the time during which a depolarizing pulse was applied. The middle trace shows the membrane potential; the bottom trace shows time marks at 5-second intervals. In A the fiber was exposed to 16 mM Ca and the resting potential was -52 mV; between A and B, [Ca] was raised to 25 mM. About 15 seconds after [Ca] was increased a small depolarization was seen (B). Some 100 seconds later (C) the fiber shifted to the high resting potential. A sustained low resting potential could not then be evoked although the application of a depolarizing pulse (D) evoked slow response action potentials (reexcitation). Vertical calibration: 40 mV.

![Figure 7](image.png)

**Figure 7.** Effects of [K] on membrane potential. The membrane potential is plotted against log [K]. The fiber was bathed initially in Ca-free solution containing 16 mM Mn and [K] was varied (solid circles). The fiber was then exposed to Mn-free solution containing 16 mM Ca and [K] was varied (open circles). Points marked by an asterisk (*) represent maximum diastolic potential; all other points show resting potentials. All data are from a single impalement of the same fiber.
which normal action potentials with rapid upstrokes arise. Hutter and Noble11 found that sheep Purkinje fibers showing this sort of behavior in normal Tyrode’s solution could be maintained at a high membrane potential by the occasional application of brief anodal stimuli or by replacement of Cl\(^-\) by NO\(_3\)\(^-\). Carmeliet12 found, in sheep Purkinje fibers, that removal of Na followed by removal of K resulted in a membrane potential of —90 mV whereas removal of K followed by removal of Na resulted in a membrane potential of —40 mV. Rhythmic activity at the low membrane potential was enhanced when Cl\(^-\) was replaced by acetylglucininate but when the fiber was returned to a solution containing Cl\(^-\) the oscillations disappeared and the membrane potential returned slowly towards —50 mV. Then, suddenly, within a fraction of a second, the potential returned to —90 mV.12 Carmeliet12 also found that the membrane potential could be shifted from one level to the other by current pulses 2–3 minutes long, i.e., much longer than those used in our study. Marked shifts in membrane potential have been found in canine Purkinje fibers exposed to low temperatures,19 to elevated PCO\(_2\),4 or to ouabain.*

We find that fibers that are quiescent at —50 mV have a high membrane resistance compared to that of fibers that are quiescent at —90 mV. We also find that the resting potential of fibers that are quiescent at —50 mV does not vary with changes in [K]o in the manner predicted by the Nernst equation, whereas that of fibers that are quiescent at —90 mV does do so. In addition, the membrane potential can be shifted from either level to the other by pulses of applied current of the appropriate polarity. These findings suggest that a voltage-dependent increase in potassium conductance governs the transition from —50 to —90 mV, that conductance being low at —50 mV and high at —90 mV. The total current-voltage relationship of cardiac Purkinje fibers is “N-shaped”14–17 and such fibers demonstrate a time-independent outward current (iK) that shows marked inward-going rectification.11–16,17,29 It is also known that elevation of [K]o can increase iK.31 The so-called “pace-maker current” (iK) also shows marked inward-going rectification. It is probable, therefore, that decreases in both iK and iC, cause the low potassium conductance seen at the low resting potential. The N-shaped current-voltage relationship is found at all [K]o but only at relatively low levels of [K]o does it have three intercepts on the voltage axis;31 thus, only at a relatively low [K]o might two levels of resting potential be obtained.

In fibers exposed to a normal ionic environment, repolarization may be initiated, in part at least, by a time-dependent outward current (iK) that is activated at potentials less negative than —40 mV; a recent reconstruction of the Purkinje fiber action potential17 predicts that failure of activation of iK will lead to failure of repolarization. The total current-voltage curve shown in the same study shows two potentially stable resting potentials, at —38 mV and —88 mV, although the authors imply that the —38 mV resting potential is an artifact of their model.

A fall in potassium conductance will lead to depolarization only in the presence of an inward current. Purkinje fibers exposed to a normal ionic environment exhibit a “background” inward current17 so that E\(_{\text{m}}\) is significantly less negative than E\(_{\text{K}}\). The nature of that current is not known; interestingly enough, it need not vanish in Na-free solutions, since removal of Na does not necessarily cause E\(_{\text{m}}\) to move towards E\(_{\text{K}}\). It may be that, under the conditions of our experiments, the background inward current is the same whether the fiber is at —90 mV or —50 mV, and that the shift from one level to the other is mediated solely by a change in potassium conductance. It is also possible that the shift to —50 mV entails some increase in an inward current.

It seems very likely that the background inward current seen under the conditions of our experiments is largely a calcium current, even though the low resting potential does not vary with [Ca]o in the manner that might be expected from the effect of changes in [Ca]o on E\(_{\text{ca}}\). The effect on E\(_{\text{ca}}\) of a reduction of [Ca]o ought to cause hyperpolarization; instead depolarization is seen. We assume that marked lowering of [Ca]o causes normal parts of the membrane to lose their selective permeability and also causes injured areas to become freely permeable to all ions through a loss of the “healing” or “sealing” effect of Ca.16 Mn can presumably reverse both of these effects, replacing Ca both as a “stabilizer” and as promoter of “healing” of damaged areas.

Conversely, the effect on E\(_{\text{ca}}\) of an increase in [Ca]o from 16 mM to 25 mM should cause the low membrane potential to move to less negative levels, whereas the membrane potential actually shifts from the low level to the high level. Increasing [Ca]o presumably increases [Ca], and it is known that an increase in [Ca], increases potassium conductance in snail neurones18 and in cardiac Purkinje fibers.33 Kass and Tsiens4 have reported that an increase in [Ca]o increases iK, and displaces the voltage-dependence of the opening of the
ion channel towards less negative levels. Any of these effects could contribute to a shift from a low to a high resting potential.

In fibers that show a high resting potential, that potential is more negative in Ca-free solutions containing Mn than it is in Mn-free solutions containing Ca. This suggests that Mn can replace Ca as a membrane "stabilizer" but cannot, presumably since it is far less permeant than Ca, make a comparable contribution to an inward current. We cannot, however, be certain that the increase in resting potential seen under these circumstances does not result in part from an Mn-induced increase in potassium conductance.

The finding that neither reduction of $[K]_o$ to 0.5 mM nor the application of depolarizing pulses leads to maintained depolarization in Ca-free solutions containing Mn might also be attributed to an Mn-induced increase in potassium conductance* or even to an Mn-induced decrease in a nonspecific background inward current. However, the addition of Ca to these solutions allows the low resting potential to be obtained in the presence not only of any presumed Mn-dependent increase in potassium conductance but also in the presence of any presumed Ca-dependent increase in potassium conductance (Fig. 8A). This finding, that the low resting potential can be evoked in the presence of Mn and Ca, but not in the presence of Mn alone, clearly demonstrates that Ca supplies a significant part of the inward current necessary for the maintenance of this low resting potential. This in turn suggests that the Ca-dependent inward current in question may not be carried via the Mn-sensitive channel which underlies slow response action potentials, although 5 mM Mn might block Ca-dependent action potentials without abolishing the ability of that channel to carry a Ca current.

One may ask, what ion can carry the inward current needed for the depolarization seen in Ca-free solution and through what channel does that ion flow? The only ions available are TMA and TEA; they might travel through the membrane or they might enter at the site of impalement. It should be noted that the inward current needed to cause depolarization can be very small when the potassium conductance is low; for that reason no very dramatic "sealing" or stabilizing effect of Mn need be postulated to explain its action in Ca-free solutions.

The slow decline in the level of the low resting potential from an initial value near -50 mV to a steady state value closer to -40 mV may be taken as an example of the various slow changes in membrane potential described under Results, all of which seem more likely to result from shifts in Cl than from shifts in K. It is true that a change in membrane potential from -90 mV to -50 mV causes a change in the electrochemical gradient for K favors a loss of K from the cell which in turn might increase $[K]_o$ in the vicinity of the membrane. However, during the low resting potential either is insensitive to or responds to an increase in $[K]_o$ by hyperpolarization. Moreover, the presumptive fall in potassium conductance would be expected to reduce K efflux. Accumulation of K in the extracellular space is thus not a probable cause of the gradual decline in the low resting potential. A more likely cause is a slow redistribution of Cl. The membrane is slightly permeable to Cl at membrane potentials within the range of -50 to -90 mV; and Cl is probably passively distributed across the membrane.* Thus, when the membrane is shifted from a high to a low resting potential, the chloride current is small but outward, i.e., hyperpolarizing. As Cl slowly redistributes, $[Cl]_o$ increases and the hyperpolarizing chloride current decreases, causing the membrane potential to move from -50 mV to less negative levels. If the fiber is then shifted back to the high resting potential, and maintained there for a time, $[Cl]_o$ will fall. If the low resting potential is elicited after $[Cl]_o$ has fallen it would thus once again be -50 mV. We assume that similar shifts in $[Cl]_o$ and the resultant changes in the chloride current, explain many of the slow changes in membrane potential that we have observed. Carmeliet* attributed current-induced shifts from one resting potential to the other to a shift in $[Cl]_o$; in his studies current had to be passed for 2 or 3 minutes to effect a sustained change in membrane potential.

It appears that repetitive activity is seen in fibers that have a membrane potential in the vicinity of -50 mV only if the potassium conductance is high. That conductance is low, e.g., in a fiber depolarized by lowering $[K]_o$ or in a fiber in which the membrane potential is brought from -90 mV to -50 mV by the application of a depolarizing pulse. In a fiber that is depolarized from -90 mV to -50 mV by elevation of $[K]_o$, the potassium conductance is high and repetitive activity does not occur. Repetitive triggered activity is characterized by an after-hyperpolarization that is followed by an after-depolarization. The after-hyperpolarization may well result from a continuation of the increase in potassium conductance that presumably occurs during depolarization. If the potassium conductance then transiently falls below the level characteristic of diastole, an after-depolarization could occur. Sustained rhythmic activity in partially depolarized fibers has been attributed to oscillatory afterpotentials arising from variations in $i_n$. Under the conditions of our experiments, fibers that show sustained rhythmic activity at a low level of membrane potential can be quiescent at a low level of resting potential and can also be switched to a high level of resting potential by hyperpolarizing current pulses. This suggests that the "threshold potential" for the increase of potassium conductance that leads to the fiber assuming a high resting potential is more negative than the range in which variations in $i_n$ might lead to rhythmic activity.

In a previous study we have shown that the resting potential of fibers exposed to Na-free solutions increases when the addition of Na to the perfusate induces electrogenic sodium extrusion. If the fiber is at the low resting potential, the hyperpolarizing current caused by the electrogenic extrusion moves the membrane potential to the level at which a voltage-dependent increase in potassium conductance shifts the fiber to the high resting potential. One might expect this to be a fairly general phenomenon, i.e., one might expect not to find a maintained low resting potential in a fiber that shows marked electrogenic sodium extrusion. Most of the results reported above were obtained in
solutions in which the concentration of TEA was 5–20 mm, the bulk of the Na being replaced by TMA. If all of the Na is replaced by TEA, the low level of resting potential can be obtained only for a relatively brief time after the fiber has been exposed to the Na-free solution. On the other hand, we have never seen a stable low resting potential unless there is at least some TEA in the perfusate. It is known that TEA reduces potassium conductance in cardiac Purkinje fibers and it may be that TEA facilitates obtaining two stable levels of resting potential. On the other hand TEA is not necessary for the appearance of inward-going rectification and two levels of resting potential have been seen in Na-free solutions containing no TEA and in normal Tyrode’s solution (S. Wiedmann, personal communication; D.C. Gadsby and P.F. Cranefield, unpublished observations).

It is believed that some cardiac fibers, such as those of the sinoatrial node, have a low potassium conductance, a low resting potential, and no "fast" sodium channel, so that their action potentials depend solely upon an increase in the permeability of the slow channel. The ability of a fiber to produce an Na-dependent action potential with a rapid upstroke requires not only that the membrane possess "fast channels" but also that it possess a mechanism for maintaining a high resting potential. The acquisition of a relatively high potassium conductance can confer a high resting potential on a fiber that already has the appropriate distribution of ions across its membrane. Cardiac Purkinje fibers that can produce propagated activity based on rapid Na-dependent upstrokes arising from a high resting potential can also produce propagated activity based on slow channel-dependent upstrokes arising from a low resting potential. If such fibers depolarize they may, therefore, produce slow response action potentials of a kind believed to be essential to the genesis of certain arrhythmias. We suggest that the behavior of the K channels that are responsible for the maintenance of a high resting potential presents analogies to an excitable system and may be essential to the genesis of certain arrhythmias.

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