Two Levels of Resting Potential in Cardiac Purkinje Fibers

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ABSTRACT In an appropriate ionic environment, the resting potential of canine cardiac Purkinje fibers may have either of two values. By changing the external K concentration, [K]0, in small steps, it was shown that, in the low (1 mM) Cl, Na-containing solutions used in this study, the two levels of resting potential could be obtained only within a narrow range of [K]0 values; that range was usually found between 1 and 4 mM. Within the critical [K]0 range the resting potential could be shifted from either level to the other by the application of small current pulses. It was shown that under these conditions the steady-state current-voltage relationship was "N-shaped," and that a region of both negative slope, and negative chord, conductance lay between the two stable zero-current potentials. The negative chord conductance was largely due to inward sodium current, only part of which was sensitive to tetrodotoxin (TTX). Under appropriate conditions, the negative chord conductance could be abolished by several experimental interventions and the membrane potential thereby shifted from the lower to the higher resting level: those interventions which were effective by presumably diminishing the steady-state inward current included reducing the external sodium concentration, adding TTX, or adding lidocaine; those which presumably increased the steady-state outward current included small increases in [K]0, brief depolarizations to around -20 mV, or the addition of acetylcholine chloride.

INTRODUCTION

It is well known that as the external potassium concentration, [K]0, is raised above about 10 mM, the resting potentials of both skeletal and cardiac muscle fibers fall approximately as predicted by the Nernst relation for a potassium electrode. As [K]0 is gradually reduced below this level, however, the resting potential, Vr, becomes progressively less negative than the potassium equilibrium potential because of a small inward current, usually carried by sodium ions, and is better approximated by the Goldman (1943), Hodgkin and Katz (1949) equation (Adrian, 1956; Weidmann, 1956). According to that equation, Vr will monotonically increase to a maximum (negative) value as [K]0 approaches zero. However, the resting potentials of cardiac Purkinje fibers (Weidmann, 1956) and frog skeletal muscle fibers (Akiyama and Grundfest, 1971) may fall to about -50 mV when potassium ions are omitted from the external solution. This behavior is not predicted by the Goldman equation, although it may be accounted for if, for example, the potassium permeability is assumed not to remain constant, but to decline at low values of [K]0.
Another experimental finding which is not predicted by the Goldman equation, and which is probably related to the depolarization seen when \([K]_o\) is lowered, is the possible existence of two levels of resting potential at a single fixed value of \([K]_o\) (see Wiggins and Cranefield, 1976). Thus it is known that, partly as a result of inward-going rectification, the steady-state membrane current-voltage relationship in these fibers is "N-shaped" (Adrian and Freygang, 1962; Dudel et al., 1967b) and may therefore, under certain conditions, intersect the voltage axis at three points. Two of these zero-current points may then be possible resting potentials, between which the membrane potential may be shifted by the application of current pulses of appropriate magnitude and polarity.

The aims of the present study were to determine changes in resting potential following small changes in \([K]_o\) over a wide concentration range in order to define the experimental conditions necessary for the demonstration of two levels of resting potential, and to investigate the nature of the steady-state, "background" inward current responsible for the depolarization at low \([K]_o\).

For this purpose, very thin unbranched bundles of Purkinje fibers were perfused in a modified Hodgkin-Horowicz (1959) fast-flow system with solutions in which chloride ions were usually replaced by the larger isethionate and methylsulfate ions. The major advantages of this procedure are that: \([K]_o\) may be changed rapidly and, therefore, independently of the intracellular potassium concentration; complications arising from cellular chloride movements are absent; and pacemaker activity is, apparently, largely abolished.

**MATERIALS AND METHODS**

Small bundles of Purkinje fibers, usually 100-300 μm in diameter and 2-5 mm long, dissected from the right ventricles of dog hearts, were used in these experiments. The bundles were suspended between two fine entomological pins in the narrow channel of a flow chamber similar to that designed by Hodgkin and Horowicz (1959). The rapid perfusion system described here was initially developed for use with skeletal muscle fibers (Gadsby et al., 1977). The pins were passed through connective tissue at each end of the bundle and pushed into the Sylgard (no. 184, Dow Corning Corp., Midland, Mich.) floor of the channel. The preparation was positioned in midstream so that it was surrounded by flowing solution except where it rested lightly on two 100-μm diam, stainless steel wires which afforded support during the insertion of microelectrodes.

The chamber was connected by a short section of silicone rubber tubing to one output of a two-position valve (no. 86410, Hamilton Company, Reno, Nev.). This valve had two inputs and two outputs; the second output served as a drain for the removal of stagnant fluid from an input line immediately before switching that line to the chamber. The Teflon rotor of the valve was modified to permit fast switching between the two inputs to occur without any concomitant change in the rate of perfusion through the chamber. This minimized the mechanical disturbance associated with rapid solution changes and thus greatly facilitated continuous intracellular recording. Each input to this "final" valve was supplied by the output from a four-position distribution valve (no. 86414, Hamilton). Each of these valves had four inputs and one output, and was fitted with spring-loaded stops, allowing any one of several test solutions to be quickly selected for switching to the experimental chamber. Flow-limiting bypasses in the fluid supply lines to the distribution valves allowed selection of two different flow rates: perfusion
was maintained at the lower rate of 5 ml/min but the higher rate of 20 ml/min could be used when fast solution changes were required. Fluid changes near the middle of the channel occurred with a half-time of less than 50 ms at the higher flow rate, and were about 10 times slower at the lower rate. This was measured by switching between Cl-containing and Cl-free solutions while recording the time course of establishment of the liquid junction potential between two broken microelectrodes, one filled with agar-Tyrade's, and the other with 3 M KCl, positioned in midstream with their tips in close proximity.

Conventional glass microelectrodes filled with 3 M KCl (resistances, 15-30 MΩ; tip potentials less negative than −5 mV) were used for potential recording; similar microelectrodes were filled with 2-3 M K-citrate for current injection. The reference half-cell incorporated a sintered Ag/AgCl/Pt-black pellet (Annex Research) and was connected to the chamber, downstream from the preparation, via a flowing 3 M KCl junction. This electrode was placed alongside the suction tube which continuously removed perfusate from the chamber. Also adjacent to this tube was a 3 M KCl-agar bridge containing an Ag/AgCl electrode; the latter was attached to an operational amplifier which held the chamber at virtual ground and served to monitor applied currents. A constant-current generating circuit delivered current pulses of variable waveform: e.g., rectangular, and either rising or falling linear ramps. In some experiments (see, e.g., Fig. 4) currents which increased linearly with time to a maximum and then declined linearly at the same rate were used; these will be referred to as “double ramps.” In addition to photographing oscilloscope traces, records were made with a rectilinear pen recorder (Gilson Medical Electronics, Middleton, Wis.; frequency response, 75 Hz).

The Tyrode's solution with which the preparations were equilibrated during dissection and mounting in the chamber contained (mM): NaCl, 137; KCl, 4; NaHCO₃, 12; NaH₂PO₄, 1.8; MgCl₂, 0.5; CaCl₂, 2.7; dextrose, 5.5. This solution was bubbled with 95% O₂-5% CO₂. The virtually Cl-free solution contained (mM): Na-isethionate (Koch-Light, Colnbrook, Bucks., England), 146; K-methylsulfate (Hopkin & Williams, Chadwell Heath, Essex, England), 4; MgCl₂, 0.5; Ca-methanesulfonate (made with methanesulfonic acid; Eastman Kodak Corp., Rochester, N. Y.), 2.7; dextrose, 5.5; HEPES (N-2-hydroxy-ethylpiperazine-N’-2-ethanesulfonic acid; Sigma Chemical Co., St. Louis, Mo.; adjusted to pH 7.3), 5. This solution was bubbled with pure oxygen. To alter the K concentration, Na-isethionate was replaced with K-methylsulfate, or vice versa. The Na concentration was sometimes lowered, in Cl-containing solution buffered with 5 mM Tris or HEPES, by substitution with Tris⁺ (Tris [hydroxymethyl] aminomethane; Sigma). The following drugs, when used, were added from refrigerated, concentrated stock solutions: tetrodotoxin (TTX, Sigma); acetylcholine chloride (Sigma); atropine sulfate (Amend, Irvington, N. J.); lidocaine hydrochloride monohydrate (xylolcaine; Astra Pharmaceuticals, Worcester, Mass.).

The temperature of the solutions was monitored close to the preparation by means of a small thermistor bead (no. 32A 130, VEGO, Springfield, N.J.) and was kept between 35° and 37°C.

**RESULTS**

The Dependence of the Resting Potential on [K]o

Fig. 1 shows typical responses of the membrane potential of a fiber in a thin bundle to small step changes in [K]o over the range 0-4 mM in low-Cl solutions. At the beginning of the sweep [K]o was 4 mM and the resting potential was
steady at $-90$ mV. The membrane hyperpolarized when $[K]_o$ was reduced to 2 mM but further reduction, to 1 mM, was followed by an abrupt depolarization which gave rise, at about $-60$ mV, to an action potential. After the upstroke of that action potential the membrane repolarized only to about $-35$ mV. On switching to K-free solution a further depolarization was seen which was readily reversed on returning to 1 mM $[K]_o$. When $[K]_o$ was raised to 2 mM, however, the membrane potential did not return to the level previously seen in that solution (close to $-100$ mV) but increased by only a few millivolts. In contrast, the subsequent change in $[K]_o$ from 2 mM to 4 mM was followed by a large, abrupt increase in membrane potential and, within a few seconds, the resting potential was again steady at $-90$ mV; this can be seen from the thickening of the initial 10 s of the potential trace which includes the start of a second sweep. The resting potential of this fiber therefore could have one of two values, 55 mV apart, at a $[K]_o$ of 2 mM, depending on whether that concentration was reached via 4 mM or 1 mM.

The relationship between the resting potential and log $[K]_o$ obtained over a more extensive concentration range in another fiber is shown in Fig. 2. Each point represents a single measurement. A given impalement was maintained throughout several solution changes but the electrode was periodically withdrawn to monitor possible changes in its tip potential. After each change in $[K]_o$ at least 30 s were allowed for equilibration in the extracellular spaces; the steady potential was then noted before switching to a different $[K]_o$. It can be seen that the membrane potential increased from $-0$ to $-100$ mV as $[K]_o$ was reduced over the range 150–2 mM. At zero $[K]_o$, the membrane potential was near $-30$ mV. At both 1 and 2 mM $[K]_o$, however, two levels of resting potential, differing by about 60 mV, could be recorded.

The broken line shows $E_k$ as a function of log $[K]_o$ and was determined by means of the Nernst equation, taking the intracellular potassium concentration, $[K]_i$, to be 155 mM and the temperature to be 36.5°C; its slope is 61.5 mV per 10-fold change in $[K]_o$. The measured potentials approach this line when $[K]_o$ exceeds 8 mM. The continuous line shows the resting potential, $V_r$, calculated by using the Goldman, Hodgkin, Katz equation,
in which $R$, $T$, and $F$ have their usual meanings, the sum of the external concentrations of potassium and sodium, $[K]_o + [Na]_o$, was taken to be 150 mM, and the intracellular concentrations of potassium and sodium, $[K]_i$ and $[Na]_i$, were assumed to be 155 and 20 mM, respectively. The constant, $\alpha$, represents the ratio of the constant-field permeability coefficients for sodium and potassium, $P_{Na}/P_{K}$, and was taken to be 0.01 (cf. Hodgkin and Horowicz, 1959). This curve provides a better fit to the experimental points over a wider $[K]_o$ range than the Nernst relation but clearly cannot, if $\alpha$ is constant, simultaneously fit both the higher and the lower levels of membrane potential recorded at 1 mM and 2 mM $[K]_o$.

Two Levels of Resting Potential and the Steady-State Current-Voltage Relationship

As shown in Fig. 1, a small change in $[K]_o$ may be sufficient to shift the resting potential from the higher to the lower level, or vice versa, but this change must be to a concentration outside the limited range of $[K]_o$ within which both levels may be found. In the experiment of Fig. 1, for example, it was necessary to depolarize the fiber in 1 mM $[K]_o$ before the lower of the two levels of resting potential could be obtained at 2 mM $[K]_o$. The critical range of $[K]_o$ over which both potential levels could be recorded varied between preparations. For
example, two levels were seen at both 1 mM and 2 mM in the fiber of Fig. 2, but only at 2 mM in that of Fig. 1; more usually the range extended to 4 mM. Within that critical range the existence of two resting potentials at a single value of \([K]_o\) could be demonstrated more directly by applying small current pulses of appropriate polarity via a second microelectrode inserted, within 100 \(\mu\)m of the recording electrode, in the midregion of the fiber.

Fig. 3A shows the result of injecting 15 nA constant-current pulses of 1 s duration into a fiber exposed to 4 mM \([K]_o\) whose resting potential was initially \(-49\) mV. During the first pulse the current was inward and the membrane potential increased to \(-96\) mV; the potential subsequently declined to \(-91\) mV and remained at that level until the start of the second pulse. This current was outward, and it caused a regenerative depolarization which gave rise to an action potential. The end of the pulse occurred during the “plateau” of this action potential and was accompanied by an abrupt repolarization of only 15 mV after which the membrane potential very slowly returned to its initial level of \(-49\) mV. (Note that the upstroke of the action potential was followed by a marked “notch” even though the external solution contained only 1 mM Cl. This confirms recent findings in solutions in which large anions other than isethionate were substituted for Cl ions [Kenyon and Gibbons, 1977].)

Fig. 3B shows a similar response to a hyperpolarizing current pulse and was recorded, in a different fiber, at a faster sweep speed to illustrate the time course of the potential changes. The resting potential of this fiber was initially \(-48\) mV. During the current pulse the membrane potential increased slowly.
and then more rapidly, before reaching a steady level of $-102 \text{ mV}$. Shortly after the pulse, the potential was steady at the new resting level of $-92 \text{ mV}$.

The ability of the membrane potential to show two resting levels, i.e. two levels at which the net membrane current is zero (Figs. 1–3), together with the characteristic, “regenerative,” nature of the time course of the potential change from the lower to the higher of these levels (Fig. 3 B) suggests that, under these conditions, the steady-state current-voltage relationship crosses the voltage axis with a positive slope at two points, between which it crosses a third time in a region of negative slope conductance. In order to verify this suggestion the steady-state current-voltage characteristic was determined under conditions in which two levels of resting potential could be obtained. For this purpose, small, slowly rising and falling, double ramps of current (see Materials and Methods) were applied via an intracellular microelectrode, while the resulting potential changes were recorded nearby with a second microelectrode. Typical results from an experiment of this kind are shown in Fig. 4. The maximum rate of change of current in this experiment was about 1.5 nA/s. Each of the insets, a and b, shows superimposed records of the current and potential changes during two runs (1 and 2, inset a; 3 and 4, inset b) using currents of opposite polarity. The current was outward throughout runs 1 and 4, and inward throughout runs 2 and 3. The current-voltage relationship was simultaneously recorded with an X-Y plotter and the graph in Fig. 4 is a tracing of the resulting curves. The fiber was continuously superfused with 4 mM K, low Cl solution; the resting potential was initially $-47 \text{ mV}$. During the rising phase of the first double ramp (Fig. 4a, 1; amplitude +30 nA) the membrane slowly depolarized to $-30 \text{ mV}$. During the declining phase this change was reversed, although the potential was some 2–3 mV more negative at all currents than it was during the rising phase (Fig. 4, graph) and returned to $-47 \text{ mV}$ only some 30 s after the end of the ramp. The second current pulse (Fig. 4a, 2; amplitude $-75 \text{ nA}$) caused the potential to increase steadily to about $-55 \text{ mV}$, then change abruptly to $-90 \text{ mV}$, and thereafter rise slowly to $-102 \text{ mV}$. As the current returned to zero, the potential slowly fell to $-88 \text{ mV}$ and remained steady at that level until the start of the third pulse. During this double ramp (Fig. 4b, 3; amplitude $-80 \text{ nA}$) the potential increased to $-95 \text{ mV}$ and then returned to $-88 \text{ mV}$. At the resting potential of $-88 \text{ mV}$ a pulse of outward current was applied (Fig. 4b, 4; amplitude +30 nA), initially causing the potential to decline slowly until, at about $-80 \text{ mV}$, a more rapid depolarization gave rise to an action potential; the membrane repolarized only to $-35 \text{ mV}$, however, and thereafter the potential changes were similar to those observed during the previous pulse of outward current (run 1). For simplicity these changes have been omitted from the graph. Shortly after the end of the pulse the resting potential was close to its initial value of $-47 \text{ mV}$.

The shifts in the zero-current potential associated with pulses 2 and 4 are directly comparable to those caused by the rectangular pulses in Fig. 3 A, but additional information is provided by the steady-state current-voltage relationship shown in Fig. 4. A major point of interest is the hysteresis between current ramps 2 and 4. The hysteresis results from the presence of a region of negative
slope conductance and allows the extent of that region to be defined, at least approximately. In the present example, the abrupt potential changes seen at about -10 and +10 nA indicate that the negative slope extends from about -80 to -60 mV. Since appropriate steady-state measurements cannot be made in this region by the present method, the short broken line connecting these coordinates was added by hand in order to complete the experimentally determined steady-state current-voltage relationship.

The net ionic current at any potential is the sum of inward and outward components. Since the equilibrium potential for potassium ions is probably close to -100 mV under the present conditions (see Fig. 2, broken line), at potentials positive to this, any K current must be outward. The net ionic current at -60 mV in Fig. 4, however, is inward; its magnitude, about -10 nA in this instance, therefore provides a minimum estimate of the inward current at that potential. Since that current must be carried by an ion with an equilibrium potential positive to -47 mV, the most likely candidates are Na⁺ and Ca²⁺. Experiments concerning the nature of that current are described below.

The Nature of the Steady-State Inward Current

If appreciable inward current is carried by sodium ions then their replacement
by the larger, less permeant Tris ions should result in a reduction of that current and, consequently, in a hyperpolarization of the membrane. Fig. 5A illustrates the effect, on a fiber with an initially low resting potential, of brief exposures to a solution in which Na ions were replaced by Tris+. During the first exposure, which lasted 2 s, the membrane rapidly hyperpolarized by ~40 mV and, a few seconds later, the potential was steady at −90 mV. A much smaller, transient hyperpolarization of ~2 mV accompanied a subsequent 5-s exposure to Na-free solution, after which a small transient depolarization occurred.

![Diagram](image_url)

**Figure 5.** Effects of reducing [Na]₀ and/or adding 2 × 10⁻⁶ g/ml TTX on the lower level of resting potential. In A, the downward deflections in the upper line show when Na ions were briefly replaced by Tris⁺; the lower trace is a chart recording of the membrane potential (4 mM K, Cl-containing solution, Tris buffer; preparation A7-29). In B, the bar indicates the period of exposure to TTX (4 mM K, low Cl solution; preparation A7-16). In C, the upper line shows when [Na]₀ was reduced from 146 mM to 109.5 mM by partial replacement with Tris⁺. The potential trace is a chart recording and the bar below it indicates the period of exposure to TTX (4 mM K, Cl-containing solution, HEPES buffer, [Ca]₀ 0.5 mM; preparation A9-14).

The abrupt shift in resting potential in Na-free solution suggests that the net inward current near −60 mV was abolished when sodium ions were omitted from the external solution, thereby shifting upwards a current-voltage relationship like that illustrated in Fig. 4 until only a single zero-current intercept, near −90 mV, remained. This result shows that at least some of the inward current in the range −60 to −90 mV is carried by sodium ions. The maintenance of the higher resting potential after readmission of Na indicates that the steady-state current-voltage relationship in that solution did indeed have two stable zero-current potentials; the smaller potential changes resulting from the subsequent brief exposure to Na-free solution are consistent with the membrane conductance being greater at the more negative of those zero-current potentials. The explanation for the transient depolarization on readmission of Na ions is not
known but it may reflect changes in intracellular ionic composition since its magnitude was found to increase with the duration of the exposure to Na-free solution.

Since the threshold for activation of the excitable “fast” sodium channel is near −60 mV, a steady inward current component could flow at potentials just positive to this if some or all of those channels undergo only incomplete inactivation. It was therefore of interest to investigate the effect of a high concentration of tetrodotoxin (TTX) on the “lower” level of resting potential. As shown in Fig. 5B, the application of $2 \times 10^{-6}$ g/ml TTX for 15 s to a fiber exposed to a 4 mM K, low Cl solution, in which its resting potential was initially −36 mV, caused an immediate hyperpolarization; the potential increased by almost 20 mV before abruptly shifting to the “higher” resting level where it remained after removal of the TTX. Reapplication of TTX to this fiber 4 min later at the new resting potential of −87 mV caused no further change in that potential (not illustrated). In terms of the arguments used above, this result suggests that in this instance TTX temporarily reduced the steady inward current by an amount sufficient to abolish the more positive zero current intercepts.

Additional experiments were carried out to determine whether the effects of a reduction in $[\text{Na}]_0$ and the addition of TTX are equivalent or supplementary. An example is shown in Fig. 5C. This fiber was equilibrated in 4 mM K, CI-containing Tyrode’s solution; its resting potential was −50 mV. The addition of TTX ($2 \times 10^{-6}$ g/ml) caused the membrane to hyperpolarize by 8 mV. The fiber was then exposed to a solution in which 25% of the sodium ions had been replaced by Tris ions; that solution contained no TTX, in spite of which a further 15-mV hyperpolarization was recorded. In a subsequent run on the same fiber, the corresponding potential increases were 8.5 mV and 18 mV, respectively. The immediate further hyperpolarization seen in Fig. 5C on reducing $[\text{Na}]_0$ suggests that this intervention caused the decline of an inward current which had not been blocked by $2 \times 10^{-6}$ g/ml TTX. However, a further question concerns the relative magnitudes of these two effects. Since the hyperpolarization in response to reducing $[\text{Na}]_0$ was initiated at a more negative membrane potential than that in response to adding TTX, it might be argued that the larger magnitude of the former effect results from the nonlinearity in this region of the current-voltage relationship. It is unlikely that the difference can be entirely accounted for in this way, however, since in two control runs the 25% reduction in $[\text{Na}]_0$ by itself hyperpolarized this fiber from the −50 mV resting potential by 13 mV and 13.5 mV, respectively (not illustrated).

A further possible component of the steady-state inward current must be considered since experimental evidence suggesting incomplete inactivation of the slow inward current has recently been obtained (Gibbons and Fozzard, 1975; Kass et al., 1976). The threshold for activation of that current, however, is thought to be positive to −40 mV (see, e.g., Reuter, 1973) and, for this reason, its contribution to the steady-state inward current near −60 mV (Fig. 4) might be expected to be negligible (but cf. McAllister et al., 1975). Indeed, in fibers at the “lower” level of resting potential in low chloride solutions, the
equimolar replacement of Ca$^{++}$ by either Mn$^{++}$ or Mg$^{++}$, both of which are probably relatively poor carriers of slow inward current (Kohlhardt et al., 1973; Delahayes, 1975), caused only small and slow changes in membrane potential, Mn$^{++}$ tending to hyperpolarize and Mg$^{++}$ to depolarize. Explanations based on the ability of these ions to carry slow inward current would seem to be unnecessary since these small effects are consistent with the efficacy with which these ions mimic the "stabilizing" influence of calcium ions on cell membranes, viz., Mn$^{++}$ > Ca$^{++}$ > Mg$^{++}$ (see, e.g., Jenden and Reger, 1963).

Apparently, then, in the presence of normal [Na]$_o$, the inward current which helps to maintain the lower level of resting potential is carried predominantly by sodium ions via at least two pathways, only one of which is sensitive to TTX. However, Fig. 6 serves to illustrate that even in the absence of extracellular sodium and calcium ions, the magnitude of the remaining inward current may be sufficient to depolarize the membrane when potassium ions are also omitted from the external solution. In this experiment sodium isethionate was replaced with an osmotic equivalent of sucrose, and calcium ions were replaced with manganous ions so that, apart from K$^+$, the cation content of the external solution was 2.7 meq/liter Mn$^{++}$ and 0.5 meq/liter Mg$^{++}$. At 4 mM [K]$_o$ the resting potential, after equilibration in this solution, was about -80 mV. Immediately after the switch to zero [K]$_o$ the membrane rapidly hyperpolarized by about 12 mV and then depolarized with a complex time course during the next 30 s to a new steady level of about -25 mV. The hyperpolarization presumably reflects the increase in $E_K$ during the initial moderate decline of the K$^+$ concentration in the extracellular space (cf. the change from 4 mM to 2 mM [K]$_o$ in Fig. 1), just as the subsequent depolarization presumably reflects the reduction in $P_K$ as the K$^+$ concentration declined further. On switching back to 4 mM [K]$_o$ this sequence is reversed so that the abrupt repolarization results from the increase in $P_K$, and the subsequent slower decline in potential from the return of the K$^+$ concentration in the extracellular space to its original level. In order to account for the depolarization at zero [K]$_o$ under these
conditions, an inward current must be assumed to flow, and it is presumably carried by either Mn\(^{++}\) or Mg\(^{++}\), or by both.

**Outward Current and the Lower Level of Resting Potential**

We have seen that the resting potential can be shifted from the lower to the higher level by interventions which reduce the steady-state inward current, e.g., by lowering [Na]\(_o\), or by applying TTX. Since, as a result of inward-going rectification, the steady-state outward potassium current is expected to be small at the lower level of resting potential, interventions which enhance that outward current will tend to increase the resting potential, and a sufficient enhancement may cause the potential to shift from the lower to the higher resting level.

It is known, for example, that the characteristics of inward-going rectification in cardiac and skeletal muscle fibers are such that when the electrochemical potential gradient for K ions is large and outward, an elevation of [K]\(_o\) may result in an increase in outward K current at a given potential, in spite of the smaller driving force (Hall and Noble, 1963; Noble, 1965). In this context, the small increase in resting potential seen when [K]\(_o\) was raised from 0 to 1 mM, or from 1 mM to 2 mM (Figs. 1 and 2) might be recalled. This change was seen consistently and may have been caused, at least in part, by a small increase in P\(_K\). A more striking example of this behavior is provided by the greater hyperpolarization seen after raising [K]\(_o\) from 2 mM to 4 mM in Fig. 1; this potential change is presumably associated with a larger increase in P\(_K\).

An increase in outward current of some seconds' duration may also be elicited by applying pulses of depolarizing current in the "plateau" range of membrane potential, as was demonstrated by McAllister and Noble (1966, see their Fig. 7). Using the same technique we were often able to induce a maintained hyperpolarization in fibers whose resting potential was initially steady at the lower level, as illustrated in Fig. 7 A. During the constant-current pulses the depolarization rose to a peak and then declined, presumably as the potassium conductance increased. The first pulse was followed by an afterhyperpolarization of almost 20 mV which decayed via a damped oscillation about the steady potential level; the second, larger pulse was followed by a greater hyperpolarization which caused an abrupt and maintained shift of the membrane potential to the more negative resting level.

A large and specific increase in potassium permeability of a different nature may be produced in cells of the sino-atrial node and atrium by the muscarinic action of acetylcholine (e.g., Burgen and Terroux, 1958; Harris and Hutter, 1956). Acetylcholine also has been reported to decrease the automaticity of Purkinje fibers (Bailey et al., 1972; Tse et al., 1976). It seemed worthwhile, therefore, to see whether acetylcholine would hyperpolarize Purkinje fibers whose resting potentials were at the lower level. As shown in Fig. 7 B (upper record) the application of 1.1 \(\times 10^{-4}\) M acetylcholine (ACh) for 20 s to a fiber in 4 mM K, low Cl solution caused a rapid and maintained hyperpolarization of about 45 mV from the initial resting potential of \(-42\) mV. Reapplication of the same concentration of acetylcholine at the new, higher level of resting potential produced a further hyperpolarization of less than 2 mV (not illustrated). The
lower record (Fig. 7 B) shows the response of the same fiber to a much lower concentration of acetylcholine (2.2 × 10⁻⁶ M), this time in 2 mM K, low Cl solution. The resting potential was initially −40 mV, and the acetylcholine caused a rapid hyperpolarization of 10 mV which was readily reversed upon washing out the drug. Neither of these concentrations of acetylcholine caused any change in membrane potential when applied in the presence of 7.2 × 10⁻⁶ M atropine (5 μg/ml); that concentration of atropine did not itself have any effect on the resting potential. The results of Fig. 7 B suggest that acetylcholine may have an action on Purkinje fibers qualitatively similar to its well-known action on atrial cells, namely, to increase potassium permeability.

Lidocaine is an antiarrythmic agent which is thought to have several effects on Purkinje fibers: in addition to its well-known “local anesthetic” effect of reducing the steady-state, voltage-dependent availability of excitable sodium channels (e.g., Davis and Temte, 1969; Strichartz, 1973; Hille, 1977), it may increase time-independent potassium conductance (Wittig et al., 1973; Weld and Bigger, 1976), and diminish both the background inward current and the pacemaker potassium current (Weld and Bigger, 1976). Any or all of the first three of these effects would cause a net decline in inward current and, consequently, a hyperpolarization of the membrane if lidocaine were applied to a fiber with a resting potential at the lower level. Lidocaine was indeed found to cause a rapid and reversible increase in membrane potential in fibers that were partially depolarized, in Cl-free solutions containing 4 mM K. As shown in Fig. 8, the hyperpolarization resulting from the addition of a relatively high concentration of lidocaine (3.5 × 10⁻⁵ M) was often of sufficient magnitude to
carry the membrane potential to the higher resting level. When reapplied to the same fiber at the high level of resting potential, however, this concentration of lidocaine caused no further change in membrane potential (not illustrated).

DISCUSSION

Low Chloride Solutions

Since this study was primarily concerned with resting potentials, preparations in which pacemaker activity was either absent or easily suppressed at both levels of resting potential were selected for study. The majority of preparations exposed to virtually Cl-free solutions qualified in this way; in still others quiescence could be induced, particularly at the lower level of resting potential, by a brief, temporary increase in [K]o. An eventual decline in the frequency of spontaneous activity of Purkinje fibers when Cl ions were replaced by the less permeant methylsulfate ions was observed by Hutter and Noble (1961): their explanation was that, in a spontaneously active fiber, the equilibrium potential for a permeant anion lies positive to the maximum diastolic potential, so that inward anion current contributes to the pacemaker depolarization, and that this current is smaller for less permeant anions. Since the anion equilibrium potential may shift in a negative direction as repetitive activity slows, the resulting further decline in that inward current might eventually lead to quiescence. More recent results suggest that Pk might decline when Cl ions are replaced by larger anions (Carmeliet and Verdonck, 1977). Such a mechanism could provide an alternative explanation for the depolarization and enhanced spontaneity initially observed after such solution changes, but cannot account for the subsequent lowering of the rate of spontaneous discharge to below control values (cf. Hutter and Noble, 1961). The further possibility that anion substitution in some way modifies pacemaker currents cannot at present be excluded. Apart from the absence of spontaneous activity, other advantages in the present context accrue from the replacement of Cl\textsuperscript{−} by larger anions, namely a reduction in the overall membrane conductance, and removal of the slowing or "stabilizing" effect exerted by cellular chloride movements on potential changes initiated by alterations in other components of membrane current (Adrian, 1960; Hodgkin and Horowicz, 1959; Carmeliet, 1961; Wiggins and Cranefield, 1976). Although the demonstration of two levels of resting potential is facilitated by the absence of Cl ions, this is not a necessary condition.
and the membrane potential of some fibers exposed to normal Tyrode’s solution may be switched between the two resting levels with small, 1-2-s current pulses (see Fig. 6 of Cranefield, 1977). Fig. 5A above also illustrates that two levels of resting potential may be obtained in Cl-containing solution.

**The Resting Potential as a Function of \([K]_o\)**

The results presented in this paper confirm the linear dependence of the membrane potential, \(V_m\), on log \([K]_o\) at relatively high potassium concentrations, and the large depolarization at very low concentrations, as previously described by Weidmann (1956). The existence of two separate levels of resting potential at moderately low potassium concentrations will be discussed later. Previous studies showing two levels of resting potential in Purkinje fibers, e.g., those of Chang and Schmidt (1960) and of Carmeliet (1961) were recently reviewed by Wiggins and Cranefield (1976).

As already mentioned, the presence of a small inward sodium current causes the resting potential to become progressively less negative than \(E_K\) as \([K]_o\) is lowered, so that the outward driving force on K ions, \(V_m-E_K\), progressively increases. Since the potassium permeability of cardiac and skeletal muscle fibers is known to decline to a low value when \(V_m-E_K\) becomes large and positive (e.g., Hodgkin and Horowicz, 1959; Noble, 1965; see Hagiwara et al., 1976, for further references), the sodium to potassium permeability ratio, \(\alpha\), may be expected to increase when \([K]_o\) is very low; that increase in \(\alpha\) may provide an explanation for the depolarization which is observed under these conditions. An attempt to reconstruct the dependence of \(V_m\) on \([K]_o\), using empirical equations to take some account of the dependence of \(P_K\) on \(V_m-E_K\), was made by Noble (1965). At low values of \([K]_o\), the resulting curves showed a progressive depolarization as \([K]_o\) declined, the extent of which depended on the assumed magnitude of the constant sodium conductance. In contrast to that calculated behavior, however, the observed depolarization as \([K]_o\) is lowered is quite abrupt, as is the greater part of the repolarization when \([K]_o\) is subsequently raised (Fig. 1). These abrupt potential changes result from the presence of a region of marked negative slope conductance in the net membrane current-voltage relationship (see Fig. 4, and below). It is probably mainly in this respect that the calculated and observed behaviors differ since the net (sodium plus potassium) current-voltage curve used by Noble has a negative slope region only when the sodium conductance is negligibly small (see Fig. 1 of Noble, 1965).

Note that whereas the negative chord conductance in Fig. 4 is readily attributable to some other ion, the negative slope conductance may be a property of the inwardly rectifying potassium ion channel (see, e.g., McAllister and Noble, 1966; Dudel et al., 1967b). Such a negative slope region was previously demonstrated in the current-voltage characteristic of the inward-going rectifier in frog skeletal muscle fibers by use of electrical conductance measurements (Adrian and Freygang, 1962). This negative slope has also been demonstrated by measurements of tracer-potassium flux in both Purkinje fibers (Haas and Kern, 1966) and skeletal muscle fibers (Horowicz et al., 1968).

An additional point concerns the reversibility of the membrane potential.
changes at low [K]₀ illustrated in Fig. 1. On close inspection it can be seen that
after the brief exposure to K-free solution, the membrane potential was about
2 mV more negative shortly after the return to 1 mM [K]₀ than it was some 15 s
later in that solution. In other experiments it has been shown that the
magnitude of this “undershoot” increases with [K]₀ in a low range of concentra-
tions, increases with the duration of the exposure to K-free solution, and is
abolished by cardiac steroids (Gadsby and Cranefield, 1977). The undershoot
probably reflects a transient increase in outward membrane current caused by
the enhanced activity of an electrogenic Na-K pump following its temporary
paralysis in potal-a-free solution.

Two Levels of Resting Potential

Over a limited range of [K]₀ (1–4 mM in these experiments) two sets of values
were obtained for the resting potential. As shown in Fig. 2, the more negative
values are reasonably well approximated by the Goldman, Hodgkin, Katz
equation with a constant permeability ratio, α, of 0.01, whereas to fit the lower
values, at 1 and 2 mM [K]₀, it would be necessary to increase α about 25-fold.
The increase in α probably results partly from a decline in Pₖ and partly from
an increase in Pₙa. Some increase in Pₙa may be expected in partially depolarized
fibers since the present results suggest that, under these conditions, steady-
state inward sodium current flows through TTX-sensitive, “excitable” sodium
channels (see discussion below of the nature of the steady-state inward current).
A reduction in Pₖ is also to be expected: the tracer-potassium efflux data
obtained under voltage-clamp conditions by Hass and Kern (1966) indicate that
Pₖ declines about sevenfold in response to a membrane depolarization of 55
mV from near Eₖ. In the present study, the slope conductance was found to be
smaller about the lower than about the higher resting potential (see Fig. 4). This
finding is consistent with a decline in Pₖ on depolarization if it can be
assumed that there are no large changes in the slope of the steady-state inward
current-voltage curve between the two resting potential levels.

As previously stated, the existence of two possible stable resting potentials at
a given value of [K]₀ requires that the steady-state net current-voltage relation-
ship be “N-shaped,” with two zero-current intercepts in regions of positive
slope conductance. The third, unstable intercept occurs in a region of negative
slope conductance. These features are seen in the graph of applied current
against recorded voltage illustrated in Fig. 4. Conductance measurements were
made on fibers that were short and thin so that, in the steady state, approximate
spatial uniformity of the membrane potential is likely to have been achieved
over the voltage range of interest. Although it is possible that the membrane
potential was not always uniform throughout the fiber (for example, near −100
mV, where the conductance is greatest) the important features, i.e. the two
resting potentials, the greater slope conductance at the more negative of these
potentials, and the negative slope between them, are independent of the small
correction for cable properties which might apply. Such a cable correction
might, however, reduce the peak-to-peak amplitude of the negative slope
conductance region, i.e. the magnitude of the hysteresis. It is in any event clear
from Fig. 4 that relatively small current changes would suffice to displace the current-voltage relationship vertically in either direction so that only a single, high or low, zero-current potential remains. For example, some of the large shifts in resting potential caused by small changes in \([K]_0\) (Figs. 1 and 2) may be explained in this way. Thus it is known that raising \([K]_0\) increases the K current for a given potential displacement, whereas lowering \([K]_0\) reduces the K currents (Hall et al., 1963; Noble, 1965; Dudel et al., 1967b). If the steady-state inward current is little influenced by small changes in \([K]_0\), then the changes in K current just described may be sufficient to account for the loss of the two more positive zero-current potentials at higher values of \([K]_0\) (e.g., Fig. 2, \([K]_0 > 2 \text{ mM}\)), and the loss of the two more negative intercepts at very low \([K]_0\) (e.g., Fig. 2, \([K]_0 < 1 \text{ mM}\)).

The large shifts in resting potential shown in Fig. 7 which resulted from the application of ACh, or outward current pulses, may be explained in similar terms: i.e. a temporary upward shift of the net current-voltage curve secondary to the increase in steady-state outward current. The failure of many previous studies on Purkinje fibers to demonstrate such large hyperpolarizations in response to the application of ACh (cf. Hoffman and Cranefield, 1960; Cranefield, 1975) need not be surprising. As discussed above, a very small change in net membrane current in the outward direction may suffice to shift the resting potential from the lower to the higher level, whereas the same current change may cause only a small hyperpolarization from the higher resting potential where the membrane conductance is greater (cf. Fig. 4). It would also be expected that in Cl-containing solutions, such small potential changes as do occur would be obscured to some extent by the accompanying cellular chloride ion movements. On the other hand, the diminished rate of pacemaker depolarization in Purkinje fibers, reported by Bailey et al. (1972) and by Tse et al. (1976), is consistent with the present results.

The Nature of the Steady-State Inward Current

The net current-voltage relationship may also be shifted in an upward (i.e. outward) direction by a decrease in inward current; the hyperpolarization caused by the replacement of sodium ions by Tris ions, or by the addition of TTX (Fig. 5) and possibly also that caused by the addition of lidocaine (Fig. 8), presumably resulted from such a shift. In cooled Purkinje fibers reduction of \(N_a\) also tended to stabilize the membrane potential at the more negative of the two possible levels, presumably because of a reduction in inward Na current (Chang and Schmidt, 1960).

The hyperpolarization recorded in TTX strongly suggests that at least some of the fast sodium channels undergo only incomplete inactivation in the plateau range of potentials, if it can be assumed that TTX is without effect on other components of membrane current. The existence of a TTX-sensitive steady-state sodium current in this voltage range is implicit in the model of McAllister et al. (1975) and might in part explain the shortening of the action potential and loss of plateau caused by the addition of TTX (Dudel et al., 1967a). The fiber of Fig. 5 C was found to hyperpolarize more, from the −50 mV resting...
potential, when $[Na]_o$ was reduced by 25% than it did when TTX was added. If three simplifying assumptions are made, namely, that inward Na current is proportional to $[Na]_o$, that only sodium current is sensitive to TTX, and that $2 \times 10^{-6}\text{g/ml}$ is a supramaximal concentration of TTX, then this result suggests that in this preparation less than 25% of the steady-state sodium current near $-50\text{mV}$ was sensitive to TTX. It is clear, however, that changes in even such small components of membrane current may have significant effects on plateau potentials (see, e.g., Fig. 5 B).

Another example of the effects of presumably small inward currents is the depolarization in K-free solution shown in Fig. 6, which occurred in a Na$^+$- and Ca$^{++}$-free solution containing sucrose and 2.7 meq/liter Mn$^{++}$ and 0.5 meq/liter Mg$^{++}$. Two features of the time course of those potential changes deserve comment. Thus the membrane potential took more than 30 s to reach a new steady level after $[K]_o$ was changed from 4 mM to 0, but less than 15 s to return to its original level after reversing this change. A similar asymmetry in the time course of the membrane potential changes in response to raising or lowering $[K]_o$ was seen in isolated skeletal muscle fibers by Hodgkin and Horowicz (1960). Their explanation, which is based on a retention of K ions near the membrane in a space from which diffusion occurs only slowly, may also apply in the present case: immediately after the change in $[K]_o$ in Fig. 6 the K ion concentration at the periphery of the fiber falls rapidly towards zero while that deeper in the clefts between adjacent cells is, as yet, essentially unchanged. $E_K$ at the surface of the fiber therefore increases more rapidly than the intracellular potential and, as a result of inward-going rectification, $P_K$ at the surface membrane becomes very small. Under these conditions changes in resting potential are primarily determined by changes in the concentration of K$^+$ in the clefts as these ions slowly diffuse out of the bundle. Immediately after switching back to 4 mM $[K]_o$, however, the situation is reversed so that both the K ion concentration and $P_K$ become greater at the surface of the fiber than in the clefts. The recorded changes in potential thus largely reflect the more rapid increase in K concentration which occurs towards the periphery of the fiber; the slower rise in K concentration in the clefts affects the membrane potential much less since $P_K$ remains smaller there until the potassium concentration becomes uniform throughout the extracellular spaces.

The second point concerns the nature of that depolarization in K-free solution. The decline with time of the K ion concentration in the clefts should be monotonic if the rate is determined primarily by diffusion. The more complicated time course of the resulting potential changes then presumably results from the nonlinearity of the steady-state current-voltage relationships in this range of K concentrations. As already mentioned, a reduction of the external K concentration has two principal effects on the current-voltage curve for that ion: in the first place, the K current for any potential displacement is reduced and, second, there is a shift of the curve to more negative potentials. The latter effect is probably responsible for the initial hyperpolarization seen in Fig. 6. The progressive decline in K current, however, eventually causes a positive shift of the zero net current (i.e. resting) potential because of the
presence of a small inward current component. The resting potential would be expected to fall most rapidly over the voltage range in which the current-voltage relationships are shallowest. The more rapid depolarization from near $-90\, \text{mV}$ to $-50\, \text{mV}$ in Fig. 6 is consistent with this interpretation since inward-going rectification is marked in this region (e.g., Dudel et al., 1967b; cf. current-voltage relationship in Fig. 4, above). It seems possible, then, that the later phase of depolarization, from about $-40\, \text{mV}$ to $-25\, \text{mV}$, indicates the presence of a second shallow (low conductance) region of the current-voltage relationship. One might further speculate that the current responsible for this decline in membrane slope conductance flows via incompletely inactivated slow inward current channels (Gibbons and Fozzard, 1975; Kass et al., 1976). In this context it should be mentioned that Mn ions may carry some slow inward current in cardiac cells (Ochi, 1970, 1976; Delahayes, 1975). Since the depolarization began near $-90\, \text{mV}$, however, there must have been some pathway for steady-state inward current other than through “excitable” channels. Thus Mn$^{++}$ and Mg$^{++}$ may carry current via the channel through which the normal inward leak of sodium ions occurs; the ionic specificity of this channel is not yet known. Other, presumably nonspecific, current paths may exist around the impaling microelectrode and at the cut ends of the fiber bundle.

Carmeliet (1961) found that omission of external K ions did not result in depolarization of Purkinje fibers exposed to a Na-free, Cl-containing solution even in the presence of Ca$^{++}$. In Cl-containing solutions, if the membrane conductance to other ions becomes very small, the membrane potential may be determined for a time by the existing transmembrane distribution of Cl ions (Hodgkin and Horowicz, 1959; Adrian, 1960). The absence of this “stabilizing” effect of Cl ions under the conditions of the present experiments could permit a very small inward current to cause a relatively rapid depolarization of the sort seen in Fig. 6.

**Implications for Excitation and Conduction**

It has been well established that two distinct types of propagated action potential may be recorded, under different conditions, in cardiac Purkinje fibers (see Cranefield, 1975). The more “normal” action potential has a rapid upstroke caused by inward current flowing in “fast” sodium channels and it is most readily elicited in cells showing a high resting potential (e.g., $-90\, \text{mV}$). The upstroke of the “slow response” action potential results from a “slow” inward current presumably carried by sodium and calcium ions and is most readily elicited from a lower resting potential (e.g., $-50\, \text{mV}$), at which level the “fast” channels are largely inactivated. In fibers depolarized to $-50\, \text{mV}$ by elevation of $[K]_o$, however, $P_k$ is high and the membrane resistance is correspondingly low so that slow response activity may usually be seen only if the slow inward current is enhanced by the addition of catecholamines. On the other hand, at moderately low $[K]_o$ values it is relatively easy to initiate slow responses from the lower of the two possible resting potential levels since, under these conditions, $P_k$ is low and the membrane resistance is high. Since fibers showing slow response activity can generate abnormal rhythmic activity or be a
locus of slow conduction which may permit circus movement of excitation, a
fall in membrane potential from the higher to the lower level occurring over a
portion of the cardiac syncytium might be an important step in the generation
of cardiac arrhythmias. In that case the return of the membrane potential to
the higher level would tend to abolish such arrhythmias.

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