Depletion and Accumulation of Potassium in the Extracellular Clefts of Cardiac Purkinje Fibers during Voltage Clamp Hyperpolarization and Depolarization

Experiments in Sodium-Free Bathing Media

CLIVE MARC BAUMGARTEN, GERRIT ISENBERG, TERENCE F. McDONALD, and ROBERT E. TEN EICK

From the II. Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg (Saar), Federal Republic of Germany. Dr. Baumgarten's present address is the Department of Medicine, University of Chicago, Chicago, Illinois 60637; Dr. Ten Eick's present address is the Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611; and Dr. McDonald's present address is the Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

ABSTRACT Voltage clamp hyperpolarization and depolarization result in currents consistent with depletion and accumulation of potassium in the extracellular clefts of cardiac Purkinje fibers exposed to sodium-free solutions. Upon hyperpolarization, an inward current that decreased with time (i_d) was observed. The time course of tail currents could not be explained by a conductance exhibiting voltage-dependent kinetics. The effect of exposure to cesium, changes in bathing media potassium concentration and osmolarity, and the behavior of membrane potential after hyperpolarizing pulses are all consistent with depletion of potassium upon hyperpolarization. A declining outward current was observed upon depolarization. Increasing the bathing media potassium concentration reduced the magnitude of this current. After voltage clamp depolarizations, membrane potential transiently became more positive. These findings suggest that accumulation of potassium occurs upon depolarization. The results indicate that changes in ionic driving force may be easily and rapidly induced. Consequently, conclusions based on the assumption that driving force remains constant during the course of a voltage step may be in error.

INTRODUCTION

The voltage clamp technique has contributed to characterization of voltage- and time-dependent membrane conductances. A membrane ionic current is formally considered to equal the product of the conductance of that ion and driving force, the membrane potential minus the electrochemical equilibrium potential for the ion carrying the current. Therefore, time-dependent membrane cur-

rents can be attributed solely to time-dependent conductance changes when it is assumed that driving force is not altered by an imposed voltage change. However, recent analyses of sheep Purkinje fiber membrane currents suggest that the assumption of a constant driving force is not strictly valid, at least for potassium (Baumgarten et al., 1976; Baumgarten and Isenberg, 1977; Cohen et al., 1976a). We have observed that driving force was altered by even 5-mV polarizations (cf. Cohen et al., 1976a). This was attributed to depletion or accumulation of potassium in the narrow convoluted extracellular clefts between cells of the Purkinje fiber bundle (Sommer and Johnson, 1968; Page et al., 1969; Mobley and Page, 1972; Hellam and Studt, 1974). Consequently, analysis utilizing the assumption of constant driving force for potassium will result in inaccurate estimates for the time course and magnitude of conductance changes (Baumgarten and Isenberg, 1977).

Most studies of accumulation and depletion of potassium have been made in sodium-containing solutions, and analysis of the currents is complicated by the superimposition of several time-dependent currents. As a result, the time-dependent current due to accumulation or depletion of potassium cannot be easily measured. In an attempt to reduce the complexity of the currents elicited, Purkinje fibers were studied in sodium-free media. This experimental arrangement abolished the rapid and unclampable inward sodium current elicited by depolarization (Deck and Trautwein, 1964; Dudel et al., 1966), shifted the threshold for delayed rectification to more positive potentials (McAllister and Noble, 1966), made time-dependent changes in the pacemaker current i_{K_2} undetectable (Deck and Trautwein, 1964; McAllister and Noble, 1966), and markedly reduced the amplitude of the slow inward current, i_{si} (Reuter, 1968; Vitek and Trautwein, 1971). Therefore, in sodium-free bathing media, membrane currents could be studied in a voltage range around the resting membrane potential with less interference from several of the time-dependent currents that ordinarily flow in sodium-containing media.

Time-dependent membrane currents that could not be explained solely by a conductance change were observed. These currents were attributed to a change in driving force on potassium. Thus, it appears that depletion and accumulation of potassium in the extracellular clefts of Purkinje fibers may also be rapidly and easily induced under sodium-free conditions. Characterization of the resultant current and of some interventions that modify it was begun. Preliminary reports on these findings have been made (Baumgarten, 1975; Baumgarten et al., 1975).

MATERIALS AND METHODS

Fine unbranched Purkinje fibers obtained from the hearts of freshly slaughtered sheep were cut to a length of approximately 1.5 mm and pinned in a chamber. The chamber was perfused with Tyrode's solution which contained (in mM): NaCl 150; KCl 5.4; CaCl$_2$ 3.6; MgCl$_2$ 1.0; glucose 5.0. The solutions were buffered at pH 7.4 with 5 mM Trismaleate, saturated with oxygen, and warmed to 37°C before entering the chamber. Only fibers that rapidly regained normal resting and action potentials were retained for study. To insure full recovery from the trauma of manipulation, an "equilibration" period of at

least 30 min was used. During this time stimuli were applied at a rate of 12–15/min. Then, the fibers were exposed to sodium-free Tyrode's solution. This was prepared by substituting an equimolar amount of tetramethyl ammonium chloride for NaCl. Where indicated, Tris (tris [hydroxymethyl] aminomethane) and choline were also utilized as sodium substitutes. Except where shorter times are indicated, fibers were exposed to sodium-free solution for at least 30 min before the experimental protocol was begun.

A two-microelectrode voltage clamp technique employing conventional circuitry was used (Deck et al., 1964). In addition, the bath was clamped to ground potential (Julian et al., 1962). Glass microelectrodes filled with 2 M potassium citrate and having resistances of 8–12 $M\Omega$ were used for both current passing and voltage detection. Current and voltage were displayed on an oscilloscope from which photographic records were made. Records were also made with a Brush model 220 pen writer (frequency response, 3 dB down at 125 Hz).

RESULTS

Exposure to sodium-free media rapidly (within 2 min) resulted in several changes in the electrical activity of Purkinje fibers. As has been reported by others (e.g., Draper and Weidmann, 1951), the duration of the action potential became shorter and the potential of the plateau more negative. Concurrently, the slope of the pacemaker depolarization decreased. Finally, the fibers failed to generate action potentials in response to stimulation. Membrane potential remained slightly negative (3–7 mV) to the maximum diastolic potential attained in sodium-containing solution.

Currents Elicited by Hyperpolarization

Membrane currents elicited by hyperpolarization 10 min after admitting sodium-free media are illustrated in Fig. 1. The holding potential was set equal to resting potential. Upon hyperpolarization, an inward current was recorded that decreased with time. As the magnitude of the hyperpolarization was increased, both the time-dependent portion, here termed i_d, and the late current (i.e. that at the end of the hyperpolarization step) increased in amplitude. With appropriate amplification of the current signal, i_d was detectable even during a 2-mV hyperpolarization.

The currents elicited at this time were similar to those recorded within 1–2 min after cessation of action potential generation and to those recorded after 1 h in sodium-free media. Similar currents were also recorded from fibers exposed to media containing either Tris or choline rather than tetramethyl ammonium as the sodium substitute. Thus, i_d could not be attributed to the effects of a particular sodium substitute, nor was it a transitory event related to the establishment of new ionic gradients after substitution for sodium.

The time course of i_d is analyzed in Fig. 2. The current elicited by hyperpolarizing from −72 mV, the resting potential of that fiber, to −90 mV for 8 s was well fitted by three exponential functions with time constants of: τ_1, 145 ms; τ_2, 723 ms; and τ_3, 3,410 ms. These results were typical, although interfiber variation in both the time course and amplitude was substantial. The ranges of the time course were: τ_1, 125–250 ms; τ_2, 700–1,000 ms; and τ_3, 2,750–4,000 ms.

Consideration of Conductance Changes that May Explain i_d

Currents similar to those in Fig. 1 have been reported previously (Deck and Trautwein, 1964; Vassalle, 1966) and attributed to deactivation of the pacemaker current, i_{K_2}, at a potential negative to its reversal potential (Vassalle, 1966). Since
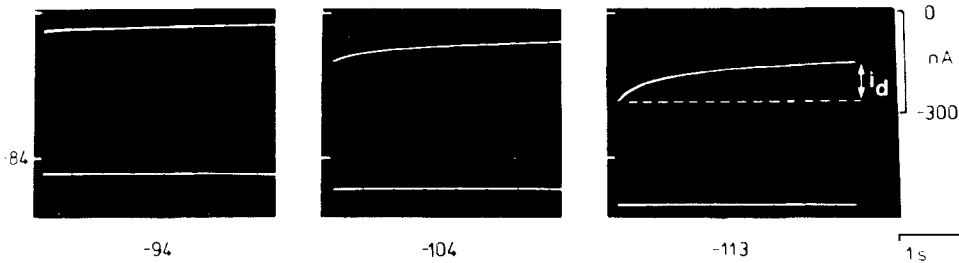

FIGURE 1. Membrane currents (upper traces) elicited by hyperpolarizing for 4 s from holding potential, −84 mV, to various potentials (values appear below each record). The amplitude of the time-dependent portion, labeled i_d, and the late current increased as the magnitude of the hyperpolarization was increased.
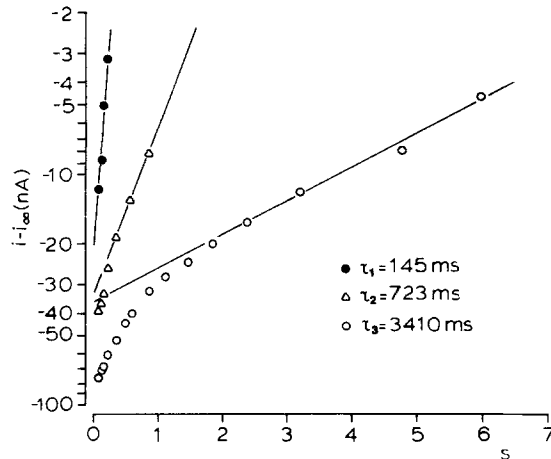

FIGURE 2. The time course of i_d (open circles) elicited by hyperpolarizing from −72 to −90 mV is plotted on a semilogarithmic scale. i_d was taken as the current (i) at a given time minus the "steady-state" current $(i_\infty;$ 8 s). An exponential function was fitted by eye and subtracted from the original data (open circles) yielding the values depicted by the open triangles. The process was then repeated, yielding a second exponential function and the values depicted by the filled circles. These were well described by a third exponential function.

exposure to sodium-free media abolishes the pacemaker potential (Draper and Weidmann, 1951), and the time course of i_d was not simple, it is unlikely that the underlying mechanism involves i_{K_2}. Nevertheless, a potassium conductance that activated with time upon depolarization and deactivated with time upon hyperpolarization could result in records similar to those in Fig. 1.

The predicted contribution of the potassium current resulting from such a conductance to the total membrane current is depicted in Fig. 3, panel A. In panel B, the currents elicited by hyperpolarizing to −100 mV and then depolarizing back to the resting membrane potential of −80 mV are shown. Independent of the duration of the hyperpolarization, a tail current was seen that became less outward directed with time. Its amplitude was increased with hyperpolariza-

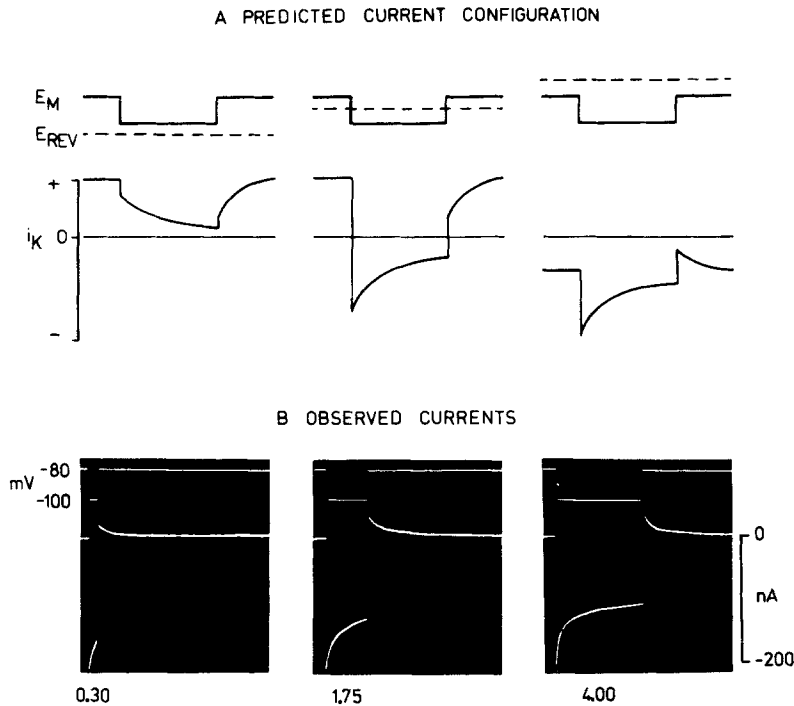A PREDICTED CURRENT CONFIGURATION

B OBSERVED CURRENTS

Figure 3. A, The predicted contribution to membrane current of a potassium current which flows through a conductance that exhibits both a time-dependent increase on depolarization and a decrease on hyperpolarization. The three cases considered are when the equilibrium potential (E_{REV}) is: (a) negative to both holding and clamp potential; (b) between them; and (c) positive to both holding and clamp potential (left to right). B, The currents elicited experimentally upon hyperpolarization to −100 mV from holding potential which was set equal to the resting potential, −80 mV, and then depolarization back to holding potential. Independent of the duration of the hyperpolarization (given in seconds below each record), the configuration of i_d and its tail current matches case (c).

tions of increasing duration. This is the configuration of currents predicted by panel A when the reversal potential E_K was assumed to be positive to both the holding and clamp potentials. However, in this experiment, holding potential was set equal to the resting membrane potential. Since E_K is not believed to be positive to the testing membrane potential, this finding suggests that a pure potassium current such as i_{K_2} (Noble and Tsien, 1968) cannot be responsible for i_d and its tail current.

A mixed current with a reversal potential positive to the resting membrane potential and an appropriate voltage dependence also could produce the pattern of currents illustrated in panel B and therefore might be responsible for i_d. i_{x_1} is an unlikely candidate since under sodium-free conditions it appears to activate over a range of potentials far positive to those utilized here (McAllister and Noble, 1966). Hyperpolarizations from about −80 mV should not alter its state of activation and a time-dependent current should not flow through this channel.

The tail currents are analyzed further, as illustrated in Fig. 4. If the mechanism underlying the tails is solely a conductance change with voltage-dependent kinetics similar to those described by Hodgkin and Huxley (1952), the time course of the tail current elicited at a fixed potential should be totally independent of the parameters of the preceding hyperpolarization. The tail current recorded at −83 mV after hyperpolarizing to −107 mV was best fitted by two exponential functions with time constants of 260 and 4,650 ms. In contrast, the tail current recorded at the same potential, but after hyperpolarizing to −98 mV, was best fitted by two exponential functions with time constants of 390 and 3,500 ms. Similarly, the time constants of the tail current depended on the duration of the preceding hyperpolarization. The difference between the two time constants in a given tail current decreased as either the amplitude or the duration of the preceding hyperpolarization was decreased. In the fiber used to obtain the data contained in Fig. 4, a 5-mV, 2-s hyperpolarization was followed by a tail current apparently well described by a single exponential function with a time-constant of 1,800 ms. Thus, a voltage-dependent conductance cannot be the sole explanation for i_d and its tail current.

Alterations in Extracellular Cleft Potassium Concentration Can Explain i_d and Its Tail Current

A change in extracellular cleft potassium concentration (K_c) during a voltage clamp pulse can explain these currents. K_c is constant when the transmembrane flux of potassium between the cells and the clefts, an unstirred region, is zero or is exactly balanced by diffusion of potassium between the spaces adjacent to the cell membrane and the bulk phase of the bathing media. As passive transmembrane flux varies with membrane potential, K_c will change, the magnitude of the change depending on the magnitude and duration of the imbalance. Therefore, upon hyperpolarization, depletion of potassium is expected to result from the influx of potassium. Similarly, depolarization, by increasing the passive efflux of potassium, will result in an accumulation (or re-equilibration) of potassium. Since transmembrane current is the product of conductance and driving force, the proposed change in K_c over time would produce a time-dependent potassium current even if the conductance of the potassium channel through which the current flows were time-independent, i.e. similar to G_{K_1} (Noble, 1962). The magnitude of the time-dependent current will reflect the magnitude of the change in driving force that has occurred as well as the K_c dependence of G_K (Carmeliet, 1961; Hall et al., 1963; Dudel et al., 1967b). Thus, a hyperpolarization of greater magnitude or longer duration will result in a time-dependent current of greater magnitude.

The configuration of the time-dependent potassium current that would flow through a time-independent potassium conductance if K_c changed can be predicted (e.g., Baumgarten and Isenberg, 1977). Hyperpolarization to a potential negative to E_K will result in an inward current. As driving force decreases with
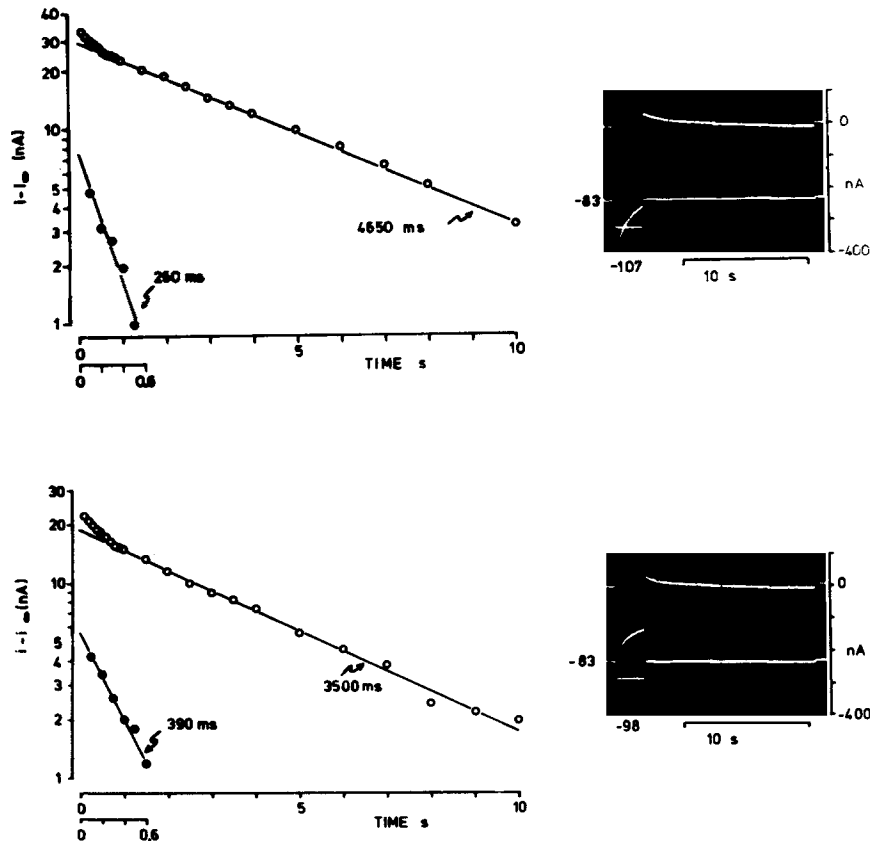

FIGURE 4. The time course of the tail current elicited by depolarization to -83 mV after hyperpolarization for 2.5 s to either -107 mV (upper graph and record) or -98 mV (lower graph and record) is different. In each case, the current (i) at a given time after depolarization minus the "steady-state" current (i_∞; 14 s) was plotted on a semilogarithmic scale. An exponential function was fitted by eye to the original data (open circles) and subtracted, yielding the values depicted by the closed circles. These are plotted on an expanded time scale and are well described by a second exponential function. The time constants of the functions are indicated. Current was recorded on magnetic tape and measurements made later from the output of a Biomation waveform recorder (Biomation, Capertino, Calif.).

time due to the depletion of potassium from the extracellular clefts, E_K becomes closer to the imposed potential and the current decreases with time. Similarly, upon depolarization back to resting membrane potential, re-equilibration of potassium will result in a decrease in driving force and an outward-directed tail current that decreases with time.

The characteristics of the time-dependent current that would flow through a channel with a time-independent potassium conductance if the postulated changes in K_c occurred upon hyperpolarization and depolarization are the same as those of i_d and its tail current (Fig. 3, panel B, and Fig. 4). The magnitude of the inward current i_d increased as the magnitude of the hyperpolarization and corresponding flux imbalance was increased (Figs. 1 and 4). Its magnitude also increased as the duration of the hyperpolarization was increased (Fig. 3, panel B). Similarly, the amplitude of the tail current increased as either the magnitude (Fig. 4) or duration (Fig. 3, panel B) of the preceding hyperpolarization was increased.

The magnitude of depletion and the time course of re-equilibration can also be followed by observing the behavior of membrane potential after termination of voltage control (Fig. 5). A preparation was hyperpolarized by varying amounts (5–24 mV) for 1 s. Then, after a clamp back to holding potential for 40 ms to allow for filling of the membrane capacitance, the voltage clamp was turned off. Immediately, the preparation hyperpolarized and then, with a much slower time course, depolarized to the original preclamp steady-state potential. After strong 1-s hyperpolarizations membrane potential did not return to its preclamp value for more than 10 s. The magnitude of the postclamp hyperpolarization increased as both the magnitude of the voltage clamp-induced hyperpolarization was increased and its duration was increased. A similar postclamp hyperpolarization has been observed in frog ventricle and attributed to depletion of potassium (Cleeman and Morad, 1976).

If potassium depletion is responsible for i_d and its tail current, increasing the concentration of potassium in the extracellular clefts should make the depletion induced by hyperpolarization less severe because a given potassium current will then produce a smaller change in driving force (Frankenhaeuser and Hodgkin, 1956; McAllister and Noble, 1966). In attempting to confirm this prediction, the instantaneous current was used as a measure of the potassium current and i_d was used as a measure of the change in driving force during the pulse. The instantaneous current is offset from the true value of the potassium current by contributions of other membrane currents to the instantaneous current. However, these contributions are probably not significantly altered by changes in K_c (Cohen et al., 1976b).

Fig. 6 suggests that the severity of the depletion process is dependent on K_c. As the bathing media potassium concentration was varied between 2.0, 5.4, and 10 mM, the slope of the instantaneous current-i_d relationship decreased. The result shown here was independent of the order of exposure and was reversible.

Consideration of factors controlling K_c suggests that increasing the width of the extracellular clefts should lessen the severity of depletion induced by a given potassium current both by making diffusion more rapid and by increasing the number of moles of potassium in the clefts. To increase the width of the clefts, preparations were exposed to bathing media made hypertonic by addition of 100 mM mannitol (Page, 1962; Freygang et al., 1964b; Jones et al., 1973). Fig. 7 shows that the slope of the instantaneous current-i_d relationship decreased during exposure to mannitol. Thus, as with increasing the extracellular potassium concentration, a larger potassium current was required to induce a given

change in driving force. The magnitude of the change in slope was related to the duration of exposure to mannitol. The alteration in the slope could be reversed by returning to Tyrode's solution of normal osmolarity.

The increase in extracellular cleft volume induced by mannitol is expected to slow the time course of i_d while the increase in the rate of diffusion between the clefts and bulk phase should quicken the time course (Freygang et al., 1964 a). Fig. 8 shows semilogarithmic plots of the current elicited by hyperpolarization from -72 mV to -95 mV for 4 s both before (control) and 6 min after exposure
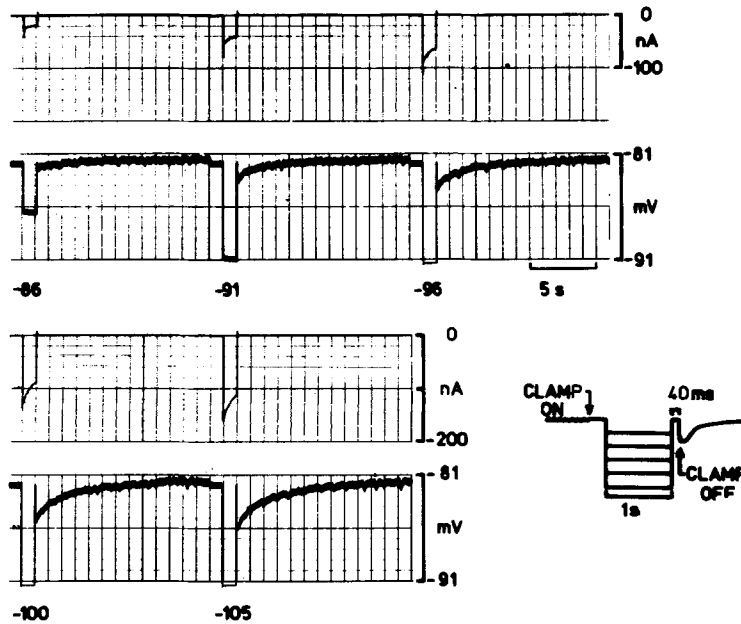

FIGURE 5. The effect of hyperpolarization on membrane potential immediately after the termination of voltage control. The preparation was hyperpolarized for 1 s by varying amounts (command potential is given below each voltage record; -96, -100, and -105 mV are off scale) from the holding potential which was set equal to the resting membrane potential, -81 mV, depolarized back to holding potential for 40 ms, and then the voltage clamp was turned off (see *inset* for schematic plan of voltage record). As the magnitude of the voltage clamp hyperpolarization was increased, the magnitude of both postclamp hyperpolarization and i_d also increased.

to hypertonic bathing media. The time constants were markedly increased by exposure to 100 mM mannitol—τ_1 increased from 216 to 273 ms and τ_2 from 886 to 1,977 ms—suggesting that the effect of volume predominates.

The potassium depletion hypothesis is also supported by pharmacological evidence. In Purkinje fibers, cesium is thought to specifically block potassium currents exhibiting inward-going rectification (Isenberg, 1976). Thus, a reduction of the potassium conductance by exposure to cesium should result in a smaller amplitude potassium current and less depletion. Fig. 9 shows that 2 min after 5 mM cesium chloride was added to the superfusate, a substantial reduc-
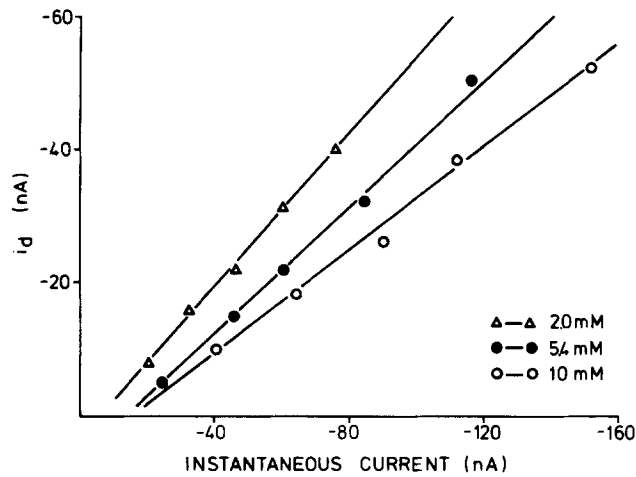
FIGURE 6. The relationship between instantaneous current and i_d is dependent on the potassium concentration of the bathing media. The slope decreased as the potassium concentration of the bathing media was increased. The relationships were obtained by measuring the current elicited by 4-s hyperpolarizations (5-25 mV) from resting potential. The order of exposure was: 5.4 mM, filled circles; 10 mM, open circles; 2.0 mM, open triangles. 10 min were allowed for equilibration.
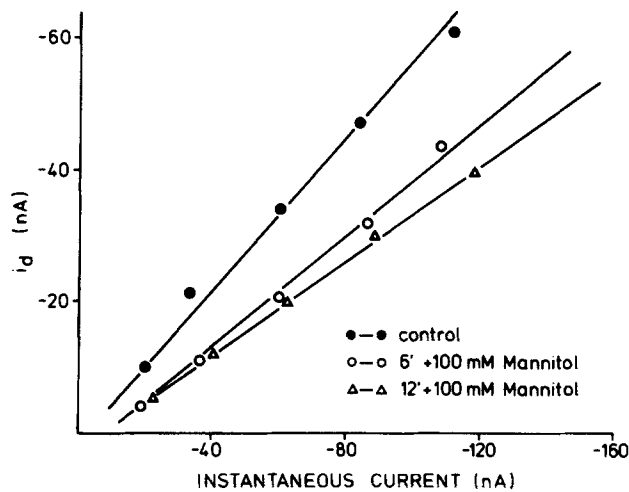

FIGURE 7. The relationship between instantaneous current and i_d is dependent on the osmolarity of the bathing media. After exposure to bathing media made hypertonic by addition of 100 mM mannitol, the slope of the relationship decreased (control, filled circles; 6 min, open circles; 12 min, open triangles). The relationships were obtained as in Fig. 6.

tion occurred in i_d, the instantaneous current and the late current. Another important question clarified by these records is the direction of the potassium current flow during hyperpolarization. After exposure to cesium the net membrane current at all potentials was less inward directed. This suggests that at
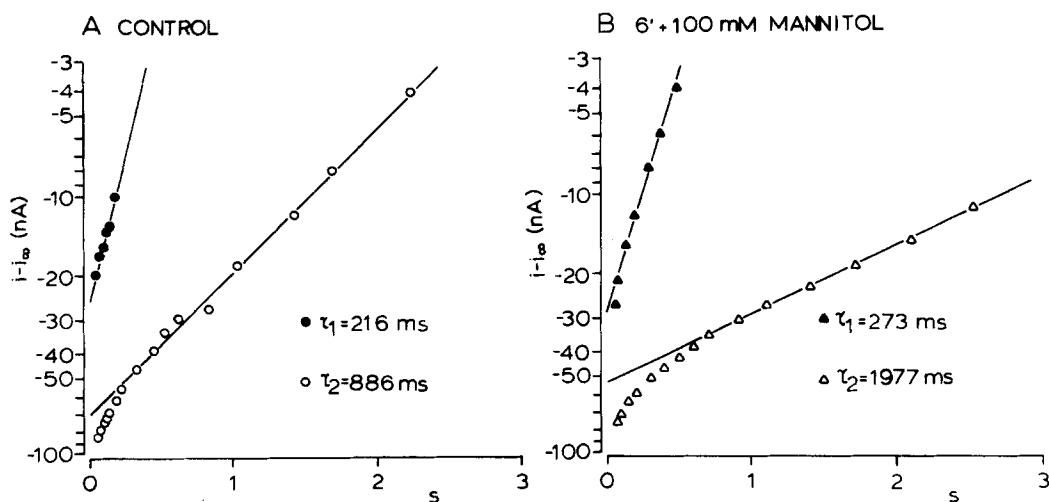
A CONTROL

B 6' + 100 mM MANNITOL

FIGURE 8. Exposure to bathing media made hypertonic by addition of 100 mM mannitol slowed the time course of i_d: A, control; B, 6-min exposure. The current (i) at a given time after hyperpolarization minus the "steady-state" current (i_∞; 4 s) was plotted on a semilogarithmic scale (open symbols). An exponential function was fitted by eye and subtracted, yielding the values depicted by the filled symbols. These were well described by another exponential function.
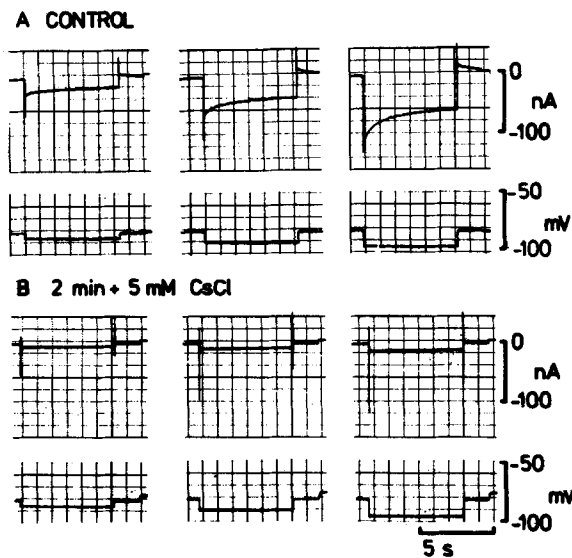
A CONTROL

B 2 min + 5 mM CsCl

FIGURE 9. The effect of cesium on membrane currents. A, control currents elicited by hyperpolarization. B, currents elicited by hyperpolarization 2 min after addition of 5 mM cesium chloride. The amplitude of i_d and both the instantaneous and late currents were reduced. A small time-dependent current remains. The shift of net current in the outward direction implies that the cesium-sensitive current was inward directed.

these potentials the potassium current was inward directed. The same result was obtained with 2-mV hyperpolarizations.

Accumulation of Potassium upon Depolarization

The same process that results in depletion of potassium upon hyperpolarization also would be expected to cause accumulation in the extracellular clefts on depolarization. Accumulation has been seen during brief (<1 s) depolarizations in Purkinje fibers exposed to sodium-containing solutions (Baumgarten and Isenberg, 1977) and after longer depolarizations in sodium-free media (McAllister and Noble, 1966). Here evidence is presented that suggests that in fibers exposed to sodium-free media, accumulation of potassium may be induced by depolarizations of small magnitude and short duration.

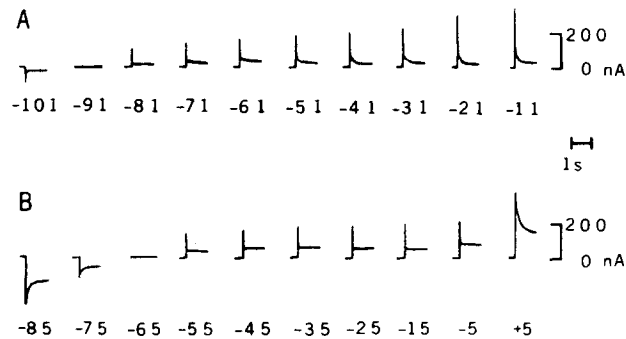Fig. 10 depicts the currents elicited by depolarization of a Purkinje fiber

FIGURE 10. The effect of the potassium concentration of bathing media on outward currents. A, Bathing media potassium concentration was 2.0 mM. Currents were elicited by clamping from holding potential, −91 mV, to various potentials for 1 s. The command potential is given below each record. A time-dependent current can be seen upon hyperpolarization and upon depolarization to potentials positive to −71 mV. B, Bathing media potassium concentration was raised to 10 mM. Currents were elicited by clamping from holding potential, −65 mV, to various potentials for 1 s. A time-dependent current can be seen upon hyperpolarization and upon depolarization to potentials positive to −15 mV.

preparation bathed in sodium-free media. An outward current that decreased with time was recorded upon depolarization from holding potential (−91 mV) to potentials positive to −71 mV (panel A, K_o = 2 mM). This result was typical. However, the potential at which a time-dependent decrease in current was noted was variable. A current with similar characteristics was observed by Reuter (1968).

Accumulation of potassium in extracellular clefts upon depolarization could produce an outward current that decreased with time. This would result if the effect of accumulation-induced decrease in driving force was greater than that of any potassium-dependent change in conductance that might occur. If this were true, increasing the potassium concentration of the bathing solution would tend to reduce the extent of accumulation and result in smaller time-dependent currents (Frankenhaeuser and Hodgkin, 1956; McAllister and Noble, 1966).

Panel B of Fig. 10 illustrates the currents elicited after the bathing media potassium concentration was raised to 10 mM. In this case, although outward currents of greater magnitude flowed upon depolarization, a time-dependent decrease in the outward current was not observed until the preparation was depolarized to −15 mV.

If potassium accumulation is the mechanism underlying these time-dependent currents, after termination of voltage control the membrane potential of the preparation should reflect any alteration in K_c that has occurred during depolarization. A preparation was depolarized for 1 s from holding potential, −78 mV, by varying amounts (6–35 mV). Then, after clamping back to holding potential, the voltage clamp was turned off. The preparation rapidly depolarized and then slowly repolarized to the previous steady-state membrane potential. Fig. 11 depicts the relationships between the magnitude of the voltage clamp
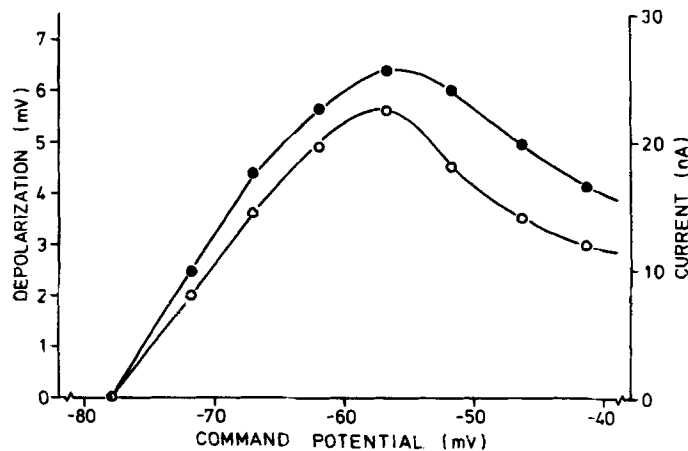

FIGURE 11. The relationships between the magnitude of a voltage clamp depolarization and both the postclamp depolarization observed on termination of voltage control (open circles) and the instantaneous current elicited by the voltage clamp depolarization (filled circles) have similar configurations. The preparation was depolarized from holding potential which was set equal to resting potential, −78 mV, to the potentials indicated for 1 s. Then after repolarizing to holding potential for 40 ms, the clamp was turned off. The most positive membrane potential attained postclamp was plotted.

depolarization and both the instantaneous current elicited upon depolarization (filled circles) and the postclamp depolarization (open circles). Both relationships have similar configurations and, therefore, the magnitude of the postclamp depolarization is roughly linearly related to the magnitude of the instantaneous current. The magnitude of the postclamp depolarization was also related to the duration of the voltage clamp depolarization. A greater postclamp depolarization was seen after voltage clamp depolarizations of longer duration. Similar postclamp depolarizations have been observed in frog ventricle (Cleeman and Morad, 1976).

If accumulation occurs, the amplitude of the inward tail current elicited by hyperpolarization should increase as the change in driving force induced by the

depolarization increases. Thus, the tail current amplitude should increase with depolarizations of longer duration. Fig. 12 illustrates the envelope of tails elicited by depolarizing from −90 to +15 mV for varying periods of time and then clamping back to −120 mV to elicit a tail current. As the duration of the depolarization was increased, the amplitude of the tail current increased. Over the voltage ranges studied, neither the strength of the depolarization (−20 to +15 mV) nor that of the hyperpolarization (−60 to −120 mV) qualitatively
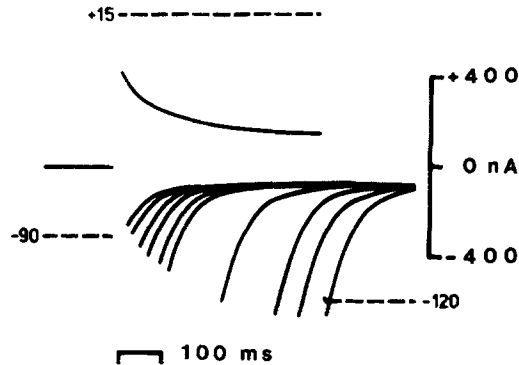

FIGURE 12. The envelope of tails was elicited by depolarizing from holding potential, −90 mV, to +15 mV for various periods of time, and then hyperpolarizing to −120 mV. The amplitude of the tails increased as the duration of the hyperpolarization increased. Voltage record (dashed line) is traced from the depolarization of longest duration (475 ms). Current calibration appears at right, time calibrations below. Potassium concentration of bathing media was 2.0 mM. Enlarged projected images were traced to achieve superimposition of the current records.

altered the behavior of the envelope of tails. However, both the amplitude and time course of the tail current were voltage dependent.

DISCUSSION

Depletion of Potassium from the Extracellular Clefts upon Hyperpolarization

A time-dependent transmembrane current i_d elicited upon hyperpolarization could not be explained solely by a conductance change, but it is consistent with a change in the equilibrium potential for a permeant ionic species. The configuration, time, and magnitude of hyperpolarization dependence of i_d and its tail current are consistent with this hypothesis. The sensitivity to cesium, bathing media potassium concentration, and osmolarity suggest that a change in the potassium equilibrium potential is responsible. As intracellular potassium concentration is far greater than that extracellular, a given flux of potassium should have a greater effect on the extracellular concentration K_c than on the intracellular concentration. Therefore, it seems likely that a change in K_c begins to occur immediately upon hyperpolarization.

Extracellular depletion of potassium is made more likely by the presence of restricted extracellular clefts, i.e. the clefts between cells, invaginations of the cell membrane, and the intercalated disks (Sommer and Johnson, 1968; Page et

al., 1969; Mobley and Page, 1972; Hellam and Studt, 1974). This view is supported by the fact that exposure to hypertonic bathing media, presumably by increasing the width and volume of the clefts, made depletion less severe and slowed its time course. These restricted clefts may be viewed as an unstirred compartment(s) extending from the surface to deep within the preparation through which potassium must diffuse.

E_K under Sodium-Free Conditions

The present results suggest that $E_K[1]$ is very near or equal to the resting membrane potential in fibers exposed to sodium-free bathing media. Under sodium-free conditions, depletion of extracellular cleft potassium can only be induced by hyperpolarizing to potentials negative to the electrochemical equilibrium potential for potassium. This is because after prolonged exposure to sodium-free bathing media and washout of intracellular sodium, Na^+-K^+ pump activity is probably negligible (Mullins and Brinley, 1969). Therefore, since hyperpolarizations of even 2 mV appear to result in depletion of potassium, the resting membrane potential must be near or equal to E_K. This is further supported by the experiment depicted in Fig. 9. Cesium reduced the amplitude of the net inward current (measured from 0 current) elicited by even a 2-mV hyperpolarization. This implies that the contribution of potassium to membrane current was inward directed and, therefore, that the hyperpolarization was to a potential negative to E_K. Thus, when bathing media potassium concentration is 5.4 mM, under sodium-free conditions the Purkinje fiber membrane potential may nearly equal the equilibrium potential for potassium.

In contrast to the above conclusion, in Purkinje fibers exposed to sodium-containing solutions it is generally agreed that E_K is negative to the resting membrane potential. This is based on several observations. The reversal potential for i_{K_2}, a pure potassium current, is 15–20 mV negative to the observed resting potential (Noble and Tsien, 1968; Peper and Trautwein, 1969; Cohen et al., 1976a). However, difficulties in interpreting the apparent reversal of i_{K_2} owing to hyperpolarization-induced depletion have been brought to light (Baumgarten et al., 1976; Baumgarten and Isenberg, 1977). Measurements of the intracellular potassium ion activity in canine and sheep Purkinje fibers have been made (Miura et al., 1977; Lee and Fozzard, personal communication). Calculations based on these measurements and the bulk phase activity of potassium also suggest that E_K is negative to the resting membrane potential.

The apparent sodium-dependence of the difference between E_K and E_m can be partially explained by considering that in sodium-free solutions inward currents, which tend to depolarize the membrane, are reduced (Dudel et al., 1966; Reuter, 1968; Vitek and Trautwein, 1971). Therefore, under sodium-free conditions the membrane is expected to hyperpolarize and more closely approach an ideal potassium electrode. This does not provide a complete explanation, however. A fiber in a sodium-containing bathing medium with 5.4 mM potassium will usually have a resting potential of -75 to -80 mV and an E_K based on either the reversal potential of i_{K_2} (Noble and Tsien, 1968; Peper and

---

[1] The apparent E_K depends upon the potassium concentration at both surface and cleft membranes and the ratio of their surface areas. As well, potassium gradients may exist within the clefts.

Trautwein, 1969; Cohen et al., 1976a) or potassium activity measurements (Miura et al., 1977) of near -95 mV. Exposure to sodium-free media results in hyperpolarization to approximately -80 to -85 mV, which according to the present results is the value of E_K. Thus, not only has resting potential increased but also E_K apparently has decreased as a result of sodium-free conditions. It is possible that the estimates of E_K are incorrect or that intracellular potassium is lost (Miura et al., 1976). An alternative explanation is that in the quiescent fiber exposed to sodium-containing media active uptake of potassium by the Na^+-K^+ pump causes in the steady state a depletion of extracellular cleft potassium with respect to the concentration in the bulk phase (Baumgarten and Isenberg, 1977; Cohen et al., 1976b). Stopping pump activity allows re-equilibration with the bulk phase and a more positive E_K. Until more accurate estimates of intracellular and extracellular cleft potassium activity are made, this matter remains speculative.

An Estimate of the Magnitude of Depletion upon Hyperpolarization

Knowledge of the extent of change in K_c would be useful as an indicator of the significance of the process and of the magnitude of the error introduced into the analysis of voltage clamp results. In order to simplify calculations of K_c from the data, it has been assumed that: (a) the extracellular clefts are composed of a single homogeneous compartment; and (b) all of the membrane that generates current is in contact with this compartment. The latter assumption is reasonable because approximately 80% of the cell membrane faces extracellular clefts (Mobley and Page, 1972). However, the former assumption is less valid because the severity of the depletion process should vary with the distance from the well-stirred bulk phase (with diameter of the fiber) and result in a concentration gradient in the clefts even if there is only one compartment. Thus, the calculations present an "averaged" response to hyperpolarization.

If the cell membrane is considered to be a perfect potassium electrode and therefore membrane potential E_m equals E_K, the equilibrium potential for potassium, K_c, may be calculated from the Nernst equation. Table I lists the calculated K_c for each of the 1-s hyperpolarizations in Fig. 5. However, a far larger change in K_c is expected if the hyperpolarization is maintained. For example, the 19-mV, 1-s hyperpolarization caused a reduction in K_c from 5.4 to 4.7 mM. Yet Fig. 2 shows that during an 18-mV hyperpolarization, i_d declines to only approximately 50% of its steady-state (8 s) value in 1 s. Thus, if the time course of the 18-mV hyperpolarization applies and depletion is the sole mechanism underlying i_d, a 19-mV hyperpolarization will lower K_c to approximately 3.4 mM in the steady state. These calculations suggest that significant errors will be introduced into the analysis of voltage clamp records by ignoring K_c changes induced by hyperpolarization.

Accumulation of Potassium upon Depolarization

Potassium accumulation would appear to be responsible, at least in part, for time-dependent currents elicited by depolarization (Fig. 10). This conclusion is supported by several observations predictable from the potassium accumulation hypothesis. First, the magnitude of the time-dependent current is less in fibers

exposed to high potassium even though far more outward current is required to maintain the depolarization. This potassium dependence of the membrane current is presumably equivalent to the potassium dependence of the postclamp depolarization (McAllister and Noble, 1966). As well, the IV relationship of the time-dependent current exhibits marked inward-going rectification at potentials negative to -40 mV, a characteristic of the i_{K_1} and i_{K_2} channels (McAllister and Noble, 1966; Noble and Tsien, 1968, 1969). This is expected from the accumulation hypothesis because the magnitude of the change in K_c and that of the resultant current should be dependent on the potassium efflux. Table II lists the calculated change in K_c required to elicit the postclamp depolarization depicted in Fig. 11. It should be noted, however, that the time-dependent currents (Fig.

TABLE I

CALCULATION OF K_c AFTER VOLTAGE CLAMP
HYPERPOLARIZATION

Voltage clamp hyperpolarization	Postclamp hyperpolarization	Calculated K_c
mV*	mV	mM‡
0	0	5.40§
5	0.7	5.26
10	1.8	5.05
15	2.7	4.88
19	3.6	4.71
24	4.5	4.56

* Holding potential was set equal to the resting potential, -81 mV.

‡ K_c was calculated by using concentration rather than activity. K_c assumed to be constant throughout clefts. This may be incorrect (McAllister and Noble, 1966; Baumgarten and Isenberg, 1977).

§ K_c assumed to equal bathing media potassium concentration. This may be incorrect (Baumgarten and Isenberg, 1977; Cohen et al., 1976b).

TABLE II

CALCULATION OF K_c AFTER VOLTAGE CLAMP
DEPOLARIZATION

Voltage clamp depolarization	Postclamp depolarization	Calculated K_c
mV*	mV	mM‡
0	0	5.40§
6	2.0	5.82
11	3.6	6.19
16	5.0	6.52
21	5.7	6.70
26	4.6	6.42
31	3.6	6.19
36	3.1	6.07

* Holding potential was set equal to the resting potential, -78 mV.

‡ K_c was calculated using concentration rather than activity. K_c assumed to be constant throughout clefts. This may be incorrect (McAllister and Noble, 1966; Baumgarten and Isenberg, 1977).

§ K_c assumed to equal bathing media potassium concentration. This may be incorrect (Baumgarten and Isenberg, 1977; Cohen et al., 1976b).

10) may be more complex than is assumed above. The possibility that a change in E_{Cl} contributed to their genesis was not excluded. As well, the apparent time independence of membrane current at more negative potentials conceivably may also result from the summation of several time-dependent components rather than from the lack of accumulation.

Potassium accumulation upon prolonged depolarization has been suggested previously on the basis of current clamp experiments utilizing Purkinje fibers exposed to sodium-free media (McAllister and Noble, 1966). The present results deviate from the earlier findings in several regards. First, we observed a time-dependent current immediately upon modest depolarization. This suggests that accumulation is easily and rapidly induced by depolarization. Thus, it may play a more important role in determining the configuration of the action potential than has previously been believed. Second, the time-dependent outward current elicited by depolarization in sodium-free media decreased with time (Fig. 10; Reuter, 1968) rather than exhibiting a time-dependent increase, as might be expected on the basis of behavior of membrane potential during current clamp (Hall et al., 1963; McAllister and Noble, 1966) and on the empirical formulations for the inward-going potassium rectifiers (Noble, 1965; Noble and Tsien, 1968). The reason for this difference is unknown. In contrast to the present findings in sodium-free media, accumulation-induced delayed rectification was observed in fibers bathed in sodium-containing media (Baumgarten and Isenberg, 1977).

Tail Currents Elicited after Strong Depolarizations: Implications Concerning the Positive Dynamic Current

As the duration or magnitude of a strong depolarization was increased, the amplitude of the tail current elicited upon a subsequent hyperpolarization increased. This observation was somewhat unexpected. The positive dynamic current i_{pd} is attributed to a time- and voltage-dependent activation and inactivation of a chloride conductance (Dudel et al., 1967 a; Peper and Trautwein, 1968; Fozzard and Hiraoka, 1973). Acceptance of this hypothesis is based in part on the critical observation that the envelope of tails recorded in sodium-containing media mirrors i_{pd}, the tail current amplitude decreased with depolarizations of increasing duration (Peper and Trautwein, 1968). The envelope of tails recorded in sodium-free media (Fig. 12) shows the opposite behavior and is incompatible with the hypothesis that inactivation of a chloride conductance underlies the "inactivation" of i_{pd}. As the time course of inactivation of the slow inward current (i_{si}) is similar to that of i_{pd} (Reuter, 1967; Reuter, 1968; Peper and Trautwein, 1968), it is likely that i_{si} contributed to the tail currents recorded in sodium-containing media and previously attributed to i_{pd} (Vitek and Trautwein, 1971).

On the basis of our inability to identify a tail current component compatible with a change in chloride conductance and in view of the behavior of the tail currents, it seems likely that a change in driving force must at least contribute to the time-dependent current termed i_{pd}. Accumulation of either potassium in extracellular clefts or chloride inside the cell could result in the currents illustrated in both Figs. 10 and 12. Potassium accumulation is expected to be greater than in sodium-containing media because of the inactivity of the Na^+-K^+ pump (Mullins and Bainley, 1969). It should also be noted that an E_{Cl} change might ex-

plain the anion-dependence of i_{pd} and is made more likely by the increase in chloride permeability on depolarization (Carmeliet, 1961; Hutter and Noble, 1961). Both Peper and Trautwein (1968) and Fozzard and Hiraoka (1973) alluded to the possibility that i_{pd} results from more than just a time- and voltage-dependent chloride conductance.

In summary, evidence has been presented supporting the hypothesis that extracellular potassium depletion and accumulation occur upon hyperpolarization and depolarization, respectively, in Purkinje fibers exposed to sodium-free media. Upon strong depolarization, evidence was obtained compatible with the notion that a change in E_{Cl}, E_K, or both rather than in chloride conductance underlies the "inactivation" of i_{pd}.

It has been assumed previously that the driving force on an ionic species crossing the membrane remains constant during a voltage clamp pulse. The data presented here suggest that this assumption is not strictly valid and, as a consequence, quantitative and in some instances qualitative conclusions based on this assumption must be interpreted with caution.

REFERENCES

BAUMGARTEN, C. M. 1975. Does voltage clamp hyperpolarization deplete extracellular potassium? *Pfluegers Arch. Eur. J. Physiol.* **359:**R130.

BAUMGARTEN, C. M., AND G. ISENBERG. 1977. Depletion and accumulation of potassium in the extracellular clefts of cardiac Purkinje fibers during voltage clamp hyperpolarization and depolarization. *Pfluegers Arch. Eur. J. Physiol.* **368:**19-31.

BAUMGARTEN, C. M., G. ISENBERG, and R. E. TEN EICK. 1975. Hyperpolarization by voltage clamp may deplete extracellular potassium ions. *Circulation.* **52:**II-157.

BAUMGARTEN, C. M., G. ISENBERG, and R. E. TEN EICK. 1976. Depletion of K$^+$ on voltage clamp hyperpolarization alters the reversal potential of the pacemaker current in Purkinje fibers. *Fed. Proc.* **35:**451.

CARMELIET, E. E. 1961. Chloride and potassium permeability in Purkinje fibers. *In* The Specialized Tissues of the Heart. A. Paes de Carvalho, W. C. DeMello and B. F. Hoffman, editors. Elsevier, Amsterdam.

CLEEMAN, L., and M. MORAD. 1976. Extracellular potassium accumulation and inward-going potassium rectification in voltage clamped ventricular muscle. *Science (Wash. D. C.).* **191:**90-92.

COHEN, I., J. DAUT, and D. NOBLE. 1976a. The effects of potassium and temperature on the pacemaker current, I_{K_2}, in Purkinje fibres. *J. Physiol. (Lond.).* **260:**55-74.

COHEN, I., J. DAUT, and D. NOBLE. 1976b. An analysis of the action of low concentrations of ouabain on membrane currents in Purkinje fibres. J. Physiol. (Lond.). 260:75-104.

DECK, K. A., R. KERN, and W. TRAUTWEIN. 1964. Voltage clamp technique in mammalian cardiac fibers. Pfluegers Arch. Eur. J. Physiol. 280:50-62.

DECK, K. A., and W. TRAUTWEIN. 1964. Ionic currents in cardiac excitation. Pfluegers Arch. Eur. J. Physiol. 280:63-80.

DRAPER, M. H., and S. WEIDMANN. 1951. Cardiac resting and action potentials recorded with an intracellular electrode. J. Physiol. (Lond.). 115:74-94.

DUDEL, J., K. PEPER, R. RÜDEL, and W. TRAUTWEIN. 1966. Excitatory membrane current in heart muscle (Purkinje fibers). Pfluegers Arch. Eur. J. Physiol. 292:255-273.

DUDEL, J., K. PEPER, R. RÜDEL, and W. TRAUTWEIN. 1967a. The dynamic chloride component of membrane current in Purkinje fibers. Pfluegers Arch. Eur. J. Physiol. 295:197-212.

DUDEL, J., K. PEPER, R. RÜDEL, and W. TRAUTWEIN. 1967b. The potassium component of membrane current in Purkinje fibers. Pfluegers Arch. Eur. J. Physiol. 296:308-327.

FOZZARD, H. A., and M. HIRAOKA. 1973. The positive dynamic current and its inactivation properties in cardiac Purkinje fibres. J. Physiol. (Lond.). 234:569-586.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1956. The after effect of impulses in the giant nerve fibres of Loligo. J. Physiol. (Lond.). 131:341-376.

FREYGANG, W. H., JR., D. A. GOLDSTEIN, and D. C. HELLAM. 1964a. The after-potential that follows trains of impulses in frog muscle fibers. J. Gen. Physiol. 47:929-952.

FREYGANG, W. H., JR., D. A. GOLDSTEIN, D. C. HELLAM, and L. D. PEACHEY. 1964b. The relationships between the late afterpotential and the size of the transverse tubular system of frog muscle. J. Gen. Physiol. 48:235-263.

HALL, A. E., O. F. HUTTER, and D. NOBLE. 1963. Current-voltage relations of Purkinje fibres in sodium-deficient solutions. J. Physiol. (Lond.). 166:225-240.

HELLAM, D. C., and J. W. STUDT. 1974. Linear analysis of membrane conductance and capacitance in cardiac Purkinje fibres. J. Physiol. (Lond.). 243:661-694.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.). 117:500-544.

HUTTER, O. F., and D. NOBLE. 1961. Anion conductance of cardiac muscle. J. Physiol. (Lond.). 157:335-350.

ISENBERG, G. 1976. Cardiac Purkinje fibers: cesium as a tool block inward rectifying potassium currents. Pfluegers Arch. Eur. J. Physiol. 365:99-106.

JONES, A. W., A. P. SOMLYO, and A. V. SOMLYO. 1973. Potassium accumulation in smooth muscle and associated ultrastructural changes. J. Physiol. (Lond.). 232:247-273.

JULIAN, F. J., J. W. MOORE, and D. GOLDMAN. 1962. Current-voltage relations in lobster giant axon membrane under voltage clamp conditions. J. Gen. Physiol. 45:1217-1238.

McALLISTER, R. E., and D. NOBLE. 1966. The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. J. Physiol. (Lond.). 186:632-662.

MIURA, D. S., B. F. HOFFMAN, and M. R. ROSEN. 1976. Effect of ouabain on intracellular potassium activity and transmembrane potential of canine cardiac Purkinje fibers. Fed. Proc. 35:320.

MIURA, D. S., B. F. HOFFMAN, and M. R. ROSEN. 1977. The effect of extracellular potassium on the intracellular potassium activity and transmembrane potentials of beating canine cardiac Purkinje fibers. J. Gen. Physiol. 69:463-474.

MOBLEY, B. A., and E. PAGE. 1972. The surface area of sheep cardiac Purkinje fibres. *J. Physiol. (Lond.)*. **220**:547-563.

MULLINS, B. A., and F. J. BRINLEY. 1969. Potassium fluxes in dialyzed squid axon. *J. Gen. Physiol.* **53**:704-740.

NOBLE, D. 1962. A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pacemaker potentials. *J. Physiol. (Lond.)*. **160**:317-352.

NOBLE, D. 1965. Electrical properties of cardiac muscle attributable to inward-going (anomalous) rectification. *J. Cell. Comp. Physiol.* **66**(Suppl. 2):127-136.

NOBLE, D., and R. W. TSIEN. 1968. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol. (Lond.)*. **195**:185-214.

NOBLE, D., and R. W. TSIEN. 1969. The outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol. (Lond.)*. **200**:205-231.

PAGE, E. 1962. Cat heart muscle *in vitro*. III. The extracellular space. *J. Gen. Physiol.* **46**:201-213.

PAGE, E., B. POWER, H. A. FOZZARD, and D. A. MEDDOFF. 1969. Sarcolemmal evaginations with knob-like or stalk projections in Purkinje fibers of sheep's heart. *J. Ultrastruct. Res.* **28**:288-300.

PEPER, K., and W. TRAUTWEIN. 1968. A membrane current related to the plateau of the action potential of Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* **303**:108-123.

PEPER, K., and W. TRAUTWEIN. 1969. A note on the pacemaker current in Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* **309**:356-361.

REUTER, H. 1967. The dependence of slow inward current in Purkinje fibres on the extracellular calcium concentration. *J. Physiol. (Lond.)*. **192**:479-492.

REUTER, H. 1968. Slow inactivation of currents in cardiac Purkinje fibres. *J. Physiol. (Lond.)*. **197**:233-253.

SOMMER, J. R., and E. A. JOHNSON. 1968. Cardiac muscle: a comparative study of Purkinje fibers and ventricular fibers. *J. Cell Biol.* **36**:497-526.

VASSALLE, M. 1966. Analysis of the cardiac pacemaker potential using a "voltage clamp" technique. *Am. J. Physiol.* **210**:1335-1341.

VITEK, M., and W. TRAUTWEIN. 1971. Slow inward current and action potential in cardiac Purkinje fibers. The effect of Mn^{++} ions. *Pfluegers Arch. Eur. J. Physiol.* **323**:204-218.