Influence of Chloride, Potassium, and Tetraethylammonium on the Early Outward Current of Sheep Cardiac Purkinje Fibers

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ABSTRACT In voltage clamp studies of cardiac Purkinje fibers, a large early outward current is consistently observed during depolarizations to voltages more positive than −20 mV. After the outward peak of the current, the total membrane current declines slowly. Dudel et al. (1967. *Pfluegers Arch. Eur. J. Physiol.* **294**:197–212) reduced the extracellular chloride concentration and found that the outward peak and the decline of the current were abolished. They concluded that the total membrane current at these voltages was largely determined by a time- and voltage-dependent change in the membrane chloride conductance. We reinvestigated the chloride sensitivity of this current, taking care to minimize possible sources of error. When the extracellular chloride concentration was reduced to 8.6% of control, the principal effect was a 20% decrease in the peak amplitude of the outward current. This implies that the membrane chloride conductance is not the major determinant of the total current at these voltages. The reversal potential of current tails obtained after a short conditioning depolarization was not changed by alterations in the extracellular chloride or potassium concentrations. We suspect that the tail currents contain both inward and outward components, and that the apparent reversal potential of the net tail current largely reflects the kinetics of the outward component, so that this experiment does not rule out potassium as a possible charge carrier. The possibility that potassium carries much of the early outward current was further investigated using tetraethylammonium, which blocks potassium currents in nerve and skeletal muscle. This drug substantially reduced the early outward current, which suggests that much of the early outward current is carried by potassium ions.

INTRODUCTION

The two-microelectrode voltage clamp technique introduced by Deck et al. (1964) opened the way for an analysis of the membrane currents of the cardiac Purkinje fiber. One of the first observations was that a large early outward current occurs when the Purkinje fiber is voltage-clamped to a potential more positive than −20 mV (Deck et al., 1964). The peak of outward current is reached 10–20 ms after depolarization, and the net current then declines slowly. For ease of reference, we shall refer to the net outward current peak seen at voltages above −20 mV as the "early outward current." This may help us

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maintain a clear distinction between the net current, which is subject to direct experimental observation, and components of the ionic current that are thought to produce the net current.

Dudel et al. (1967) tried to determine the ionic basis of the early outward current. When they substituted larger (presumably impermeant) anions for the chloride of the bathing solution, the early outward current was markedly reduced, so that the net current trace was almost flat or even increased slowly with time during strong depolarizations. Steady-state current was also reduced in low chloride solution. These data suggested that a phasic change in the chloride conductance during strong depolarizations causes the early outward current peak, and that the steady-state current includes a significant time-independent or background current carried by chloride. Fozzard and Hiraoka (1973) and Hiraoka and Hiraoka (1975) confirmed the effects of low chloride on the early outward current, but Hiraoka and Hiraoka (1975) found little change in the steady-state current voltage relation in low chloride.

In general, the voltage clamp data correlated well with investigations of the effects of low chloride solutions on action potentials. Carmeliet (1961) and Hutter and Noble (1961) reported that action potentials lengthen in low chloride, which is consistent with the reduced background current that Dudel et al. (1967) saw. Furthermore, Carmeliet (1961), Dudel et al. (1967), and Hiraoka and Hiraoka (1975) each found that low chloride solutions slow the rate of phase 1 repolarization of the Purkinje fiber action potential. These observations, together with the voltage clamp data, firmly established the idea that a rather large transient chloride current causes the rapid phase 1 repolarization of the Purkinje fiber action potential, and that this current is responsible for most of the early outward current seen in voltage clamp experiments.

Several terms have been used to refer to the transient chloride current. In addition to the chloride current (Dudel et al., 1967), it has been called the positive dynamic current (Peper and Trautwein, 1968), and several authors have referred to it as the transient outward current. In their reconstruction of the Purkinje fiber action potential, McAllister et al. (1975) referred to the transient chloride current as I_{qr}, to help them maintain a distinction between this large time-dependent current and the small time-independent or background current also attributed to chloride. We face an unusual problem of terminology in this paper. The results reported here indicate that little of the net early outward current is carried by chloride, so the terms transient outward current or I_{qr}, which do not specify a particular ion, would seem preferable to the others. However, the results in this and the following paper (Kenyon and Gibbons, 1979) also suggest that there is no single current component with the size and kinetics attributed to I_{qr}. We will, therefore, use I_{qr} or the transient outward current only in referring to earlier conclusions about the basis of the early outward current.

We became interested in the ionic basis of the early outward current because of its physiological role in producing rapid phase 1 repolarization, and because the presence of a large outward current makes it very difficult to analyze the slow inward current (I_{si}) at voltages more positive than −20 mV (Gibbons and

Fozzard, 1975). Published results showed that the early outward current was quite small in low chloride, presumably because I_{qr} was virtually eliminated, but it was not clear that the slow inward current was revealed in low chloride solutions. On the contrary, Peper and Trautwein (1968) found that replacement of chloride by propionate reduced both the early outward current and the current tails that they used as a measure of I_{si}.

We thought the failure to separate I_{qr} and I_{si} satisfactorily might have resulted from problems in the design of low chloride experiments (see Kenyon and Gibbons, 1977), so we reexamined the effects of low chloride solutions on the action potential in experiments in which we tried to minimize possible sources of error. Under our experimental conditions, the rate of phase 1 repolarization did not change when the extracellular chloride was reduced to 8% of normal (Kenyon and Gibbons, 1977). Because it seemed entirely reasonable that the fast phase 1 repolarization of the action potential depends on the early outward current, the action potential experiments raised serious doubt about the accepted idea that a change in the membrane chloride conductance is the principal factor that produces the early outward current. In this paper, we report a voltage clamp investigation of the effects of changing the extracellular chloride and potassium concentrations on the membrane currents of the Purkinje fiber, and the effect of tetraethylammonium chloride on the action potential and membrane currents.

MATERIALS AND METHODS

Solutions

Normal Tyrode's solution contained (in mM): NaCl, 137; KCl, 5.4; $MgCl_2$, 1.05; $NaHCO_3$, 13.5; NaH_2PO_4, 2.4; $CaCl_2$, 2.7; glucose, 11.1. Reagent grade chemicals (Mallinckrodt Chemical Co., St. Louis, Mo., or Baker Chemical Co., Phillipsburg, Pa.) and glass redistilled water were used. Dissection of the Purkinje fibers was usually done in Tyrode's solution with $CaCl_2$ added to bring the final concentration to 4.5 mM, to aid the "healing over" process (Déleze, 1970).

The low chloride solutions were made as described by Kenyon and Gibbons (1977). The NaCl of the normal Tyrode's solution was replaced by the sodium salt of either methylsulfuric or methanesulfonic acid. Sodium methylsulfate was electronic grade from City Chemical Corp. (New York). Sodium methanesulfonate was made by mixing equimolar amounts of NaOH and methanesulfonic acid (Eastman Organic Chemicals, Rochester, N. Y. or Aldrich Chemical Co., Inc., Milwaukee, Wis.). When sodium methylsulfate was used, the calcium concentration was raised to 1.2 times that of the normal Tyrode's solution by the addition of $CaCl_2$. This extra calcium was needed to keep the calcium activity of the low chloride solution equal to that of the normal Tyrode's solution. Sodium methanesulfonate has a negligible effect on calcium ion activity, so the total calcium concentration in methanesulfonate solution was the same as that in normal Tyrode's solution. (For details of the effects of these ions on calcium ion activity see Kenyon and Gibbons, 1977). Tetraethylammonium chloride (TEA) was added to normal Tyrode's solution by substituting 20 or 40 mM of TEA for an equimolar amount of the NaCl of the normal solution.

All solutions were saturated with a 95% O_2, 5% CO_2 gas mixture. At 36°C the pH of these solutions was between 7.3 and 7.5.

Procedure

Sheep were electrocuted and their hearts were rapidly removed. Purkinje fibers were cut out of the left and occasionally the right ventricles and stored at room temperature in a beaker of oxygenated 4.5 mM calcium Tyrode's solution. The fibers were examined with a dissecting microscope and those that appeared to have a single column of cells were selected. The experimental chamber was similar to that described by Aronson et al. (1973) in that a wire grid was used to crush the fiber in 1.6-mm-long segments suitable for voltage clamping. This technique usually provided three or more segments from a single Purkinje fiber that could be tested. The data reported here are from the first exposure of the fibers to low chloride or TEA solutions.

The experiments were performed at 36 to 37°C; during an experiment the temperature was constant within 0.5°C. The solutions were changed using a valve similar to that described by Gibbons and Fozzard (1971). We calculated that the solution in the chamber should have changed in < 1 min after the valve was turned. The measurements made in different solutions were done at least 15 min after the valve was turned and represent steady-state conditions.

The two-microelectrode voltage clamp arrangement was a modification of that used by Gibbons and Fozzard (1975). The intracellular potential was measured with reference to a flowing KCl-calomel electrode (Fisher 13-639-56, Fisher Scientific Co., Pittsburg, Pa.) positioned close to the downstream end of the fiber segment being clamped. This arrangement was used to minimize changes in tip and junction potentials when the chloride was reduced (Woodbury and Miles, 1973). Other modifications were a low drift differential amplifier to amplify the membrane voltage and the use of an Analog Devices 48K operational amplifier (Analog Devices, Inc., Norwood, Mass.) as a current-to-voltage transducer.

Membrane voltage and current were recorded by a Grass kymograph camera (Grass Instrument Co., Quincy, Mass.), and also by a Lockheed Store 4 instrumentation tape recorder (Lockheed Electronics Co. Inc., Plainfield, N. J.) at a tape speed of 15 in/s. For analysis of the data the tapes were replayed at 1.88 or 3.75 in/s. All of the experimental records shown in the figures are from the kymograph camera (either from original records or replayed from the tape) except for Fig. 7 where the tape was played back at a speed of 1.88 in/s into a chart recorder.

The shortened Purkinje fibers usually recovered a resting potential near -70 mV and fired action potentials when stimulated. Typically, the voltage clamp was set to give a holding voltage (V_h) near the resting potential. In the fibers reported here, V_h ranged from -60 to -80 mV. Clamps to various voltages were normally given at a rate of 2/min to allow time for complete recovery of the early outward current between clamps (Fozzard and Hiraoka, 1973). Clamp duration was chosen to be long enough so that the steady-state current could be approximated. As noted in the results, there was usually a small component of decreasing outward current that could be seen even after several seconds when the membrane was clamped to positive voltages. Because this component was small, the clamp duration was usually set at 2 s as a practical matter, but durations of 1 and 4 s were also used. The holding voltage, clamp frequency, and clamp duration for each experiment are listed with the figures.

Current Measurements

Unless otherwise noted, all currents were measured with respect to zero current, which was determined periodically during the experiment by turning off the voltage clamp. Any holding current will therefore be included in the current measurements, as it should be when current voltage relations are determined. The holding current was always very small compared to the phasic outward current or the steady-state current.

Current tails were measured as the difference between the instantaneous ionic current and the steady state current after a step change in voltage. The term "instantaneous" as used here refers to the first noncapacitative current that we could resolve. In fact, measurements could not be made until several milliseconds after the voltage step due to the long capacity transients in this tissue (Fozzard, 1966). This means that the "instantaneous" currents were actually measured ~ 5 ms after the voltage step.

RESULTS

Membrane Currents in Response to Voltage Clamp Depolarizations

The left hand records of Fig. 1 show typical voltage clamp records obtained in normal Tyrode's solution. In each panel, membrane voltage and current are shown for the first second of a 2-s clamp step to the voltage indicated. At the film speed used the capacity and inward sodium currents were not recorded.

During the clamp from a holding voltage of −73 mV to a clamp voltage of −27 mV, the net current was inward for the first 140 ms and then changed to a steady outward current. The phasic inward deflection has been called the slow inward current or I_{si} (Vitek and Trautwein, 1971, Gibbons and Fozzard, 1975). To estimate the magnitude of the slow inward current, Gibbons and Fozzard (1975) measured the difference between the peak of the inwardly directed transient and the steady outward current. In the second control record from the bottom, the results of a clamp to −14 mV are shown. After the sodium inward current (not visible) was over, the net current was outward. The early outward peak of net current, and the slow decline of the outward current, have been thought to be manifestations of the transient outward current. The superimposition of the slow inward current is thought to cause the dip that can be seen in the outward current. This interpretation is based upon Vitek and Trautwein's (1971) conclusion that the slow inward current and the transient outward current are separate ionic currents which overlap at voltages positive to −20 mV.

With further depolarization, the peak of outward current increases rapidly, and for depolarizations to voltages more positive than −10 mV, the net current usually consists of a rapid peak, the "early outward current," followed by a monotonic decline of outward current. The two upper control records in Fig. 1 show records of clamps to +9 and +15 mV. The early outward current peaked about 15 ms after depolarization and then declined in each record. Most of the decline of outward current was complete in 500–1,000 ms, but frequently, as in these records, a small portion of the current decayed over 2 s or more. Thus, the decline of the total current at positive voltages is not a simple exponential (Fozzard and Hiraoka, 1973). It is not known whether this is due to complex kinetics of the current responsible for the declining outward current or because the total current consists of a mixture of currents. If Vitek and Trautwein's analysis is correct, there is an unknown but probably significant amount of slow inward current included in the total current, even at voltages where only the outward current can be seen. This phasic current should complicate the decline of the total current.

As indicated above, most of the decay of the outward current occurred during the first 500–1,000 ms of depolarizing clamps. After 1,000 ms, the current was either flat or continued a very slow decline. Under our conditions, the slowly increasing I_x currents (Noble and Tsien, 1969; McAllister et al., 1975) were not apparent in clamps to voltages less positive than +10 or +20 mV (see also Isenberg, 1976).

Early Currents in Low Chloride Tyrode's Solution

Fig. 1 illustrates the overall effects of chloride reduction on membrane currents during depolarizing clamp steps. The records on the left were obtained in

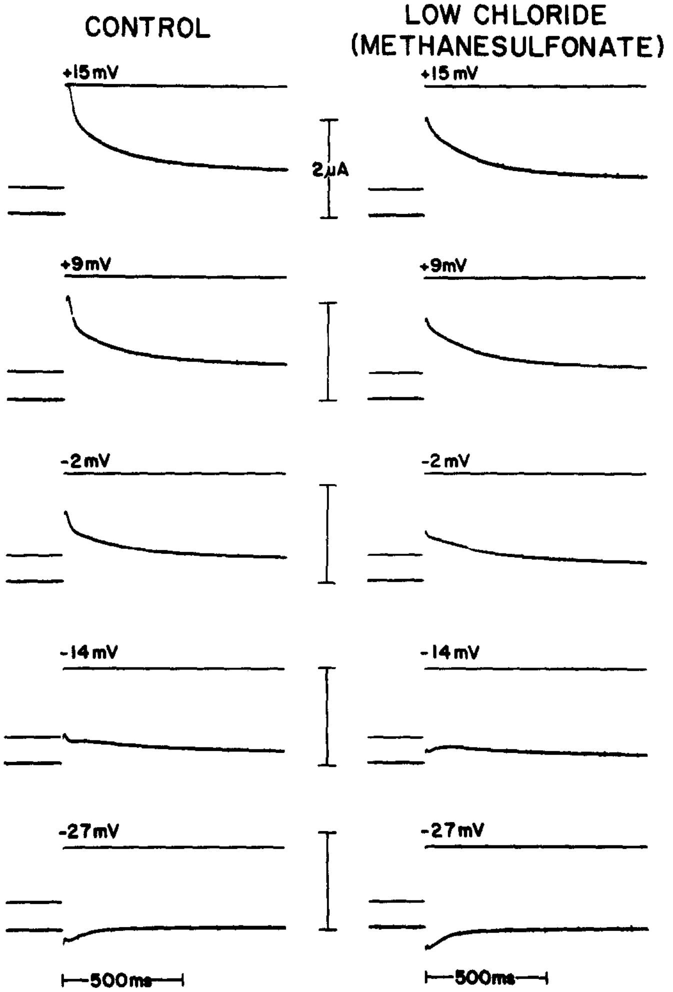

FIGURE 1. Voltage clamp records obtained in (left column) normal and (right column) low-chloride (methanesulfonate) Tyrode's solutions. Only the first part of each voltage clamp step is shown. In each panel, the upper trace is the membrane voltage and the lower trace is the current. See text for a full description of the figure. In all of the voltage clamp records shown in this paper, the bottom of the current calibration is at zero current. Holding voltage, −73 mV; clamp frequency 2/min; clamp duration, 2 s.

normal solution; those on the right were obtained in methane sulfonate solution. Similar results were obtained when methylsulfate was used as the chloride substitute. In low chloride solutions there was a modest but consistent decrease in the peak outward current that flowed in response to strong depolarizing clamps. The difference between the control and low chloride currents was largest during the first 50 ms and then declined to a small constant value after 100–500 ms.

At voltages negative to −25 mV, chloride reduction had very small and variable effects. In Fig. 1, for example, the peak of the phasic inward current at −27 mV is slightly more inward in low chloride, but in other experiments, the inward currents were unchanged in the low chloride solution. This variability was seen in each chloride substitute. Given that a half hour might elapse between a control and the corresponding low chloride measurement, the small variability might have been due to slight changes of some fibers with time. But we cannot rule out the possibility that there is a very small chloride sensitive component of the net current of some fibers at voltages negative to −25 mV.

Whatever the ionic basis of the changes seen in low chloride, one thing seems clear from experiments like the one in Fig. 1: the majority of the declining outward current that has been attributed to I_{qr} remained in solutions that contained only 8.6% of the normal chloride concentration. This result is considerably different from earlier reports showing that the net current is almost flat or even a slowly increasing outward current in low chloride solutions (Dudel et al., 1967; Fozzard and Hiraoka, 1973; Hiraoka and Hiraoka, 1975).

The most straightforward measure of the effect of chloride reduction is to compare the net membrane currents (relative to zero current) in normal and low chloride Tyrode's solutions. Isochronal current voltage relations, in which currents are measured at a specific time after clamps to different voltages, give a useful overview of changes in membrane currents in a preparation where several conductance systems are thought to exist.

Fig. 2 is a plot of the current voltage relation 20 ms after depolarizing and hyperpolarizing clamp steps from a holding voltage of −80 mV to the clamp voltages indicated on the abcissa. This time was chosen because I_{si} and the early outward current each are thought to peak near this time, and because any voltage nonuniformities resulting from the sodium inward current should be over. Most of the inward (negative) current region between −65 and −30 mV may be attributed to the slow inward current (see Fig. 1) and, at 20 ms, chloride reduction had no consistent effect on this region of the isochronal current voltage relation. At voltages more positive than −20 mV, the early outward current should predominate. The outward (positive) current in this voltage range was consistently reduced by low chloride. The changes in this preparation were not very large at 20 ms; a summary of the changes in peak current in this and other preparations will be given below.

For clamps to voltages more negative than the holding potential (−80 mV), the 20-ms current should consist of the pacemaker current, I_{K2}, and background (i.e., time-independent) currents (McAllister et al., 1975). These did not appear different in normal and low chloride solutions.

Steady-State Currents in Low Chloride Solutions

The steady-state currents changed only slightly in low chloride solution, when they changed at all (Figs. 3 and 4). For these measurements, the current at the end of 1,000–4,000-ms clamps was taken as the steady-state current, although in most experiments the current was still changing very slowly at the end of clamps to positive voltages.

In two fibers there was no discernible change in the steady-state current voltage relation when the chloride was reduced; Fig. 3 was obtained from one of these preparations. In four other fibers, the steady current at depolarized voltages was less outward in the low chloride solution, and the control and low chloride curves either converged or crossed at voltages between −70 mV and −90 mV. Fig. 4 shows results from a preparation in which there was a particularly large difference between the steady-state currents in normal and low chloride solution; here the curves converge near −85 mV. The data on the voltage at which the curves intersect or converge do not allow one to infer the original reversal potential for chloride (assuming, of course, that the decreased outward current resulted from a change in background chloride conductance). If it is supposed that chloride redistributes so that the original ratio of external to internal chloride is reestablished in low chloride, then the normal and low

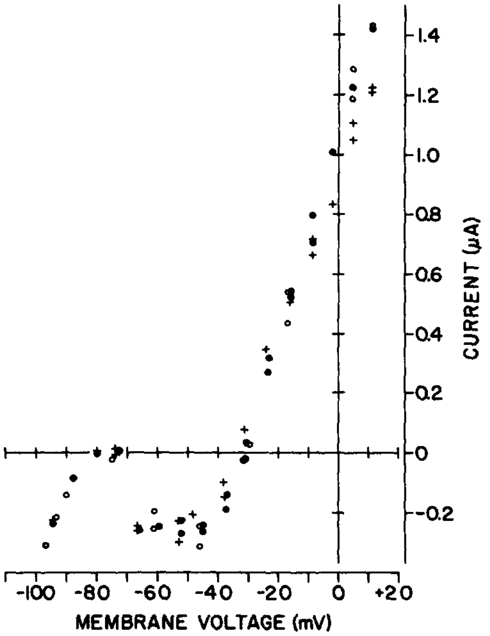

FIGURE 2. 20-ms current voltage relation in (●) normal, (+) low chloride (methylsulfate), and (O) recovery Tyrode's solutions. At each voltage the current was measured 20 ms after the start of the voltage clamp step from the holding voltage. For details see text. Holding voltage, −80 mV; clamp frequency, 2/min; clamp duration, 4 s.

chloride curves should cross at E_{Cl}. But if the original ratio is not reestablished, the curves should converge at a voltage more negative than the original value of E_{Cl}.

Dudel et al. (1967) reported that preparations tended to depolarize in low chloride, and that there were substantial changes in the current necessary to

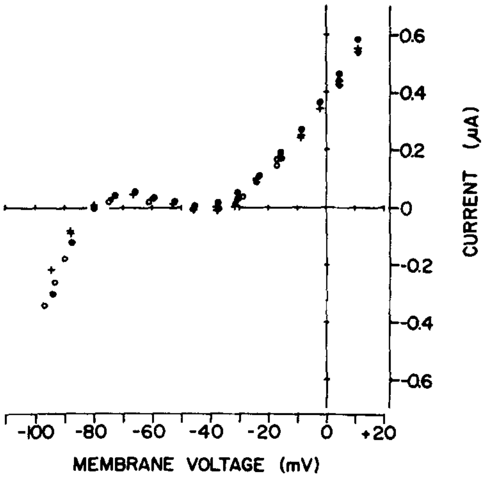

FIGURE 3. Steady-state current voltage relation in (●) normal, (+) low chloride (methylsulfate), and (O) recovery Tyrode's solutions. At each voltage the current was measured at the end of the voltage clamp step. The experiment is the same one shown in Fig. 2. Holding voltage, −80 mV; clamp frequency, 2/min; clamp duration, 4 s.

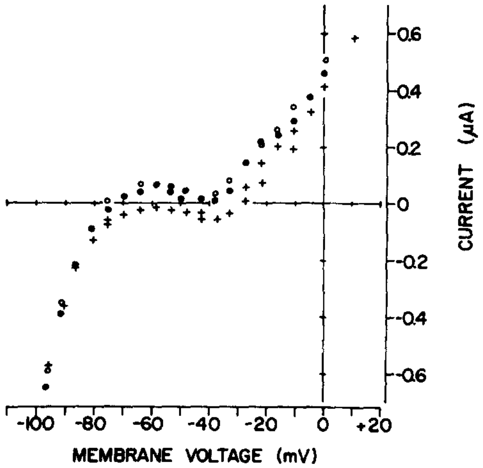

FIGURE 4. Steady-state current voltage relation in (●) normal, (+) low chloride (methanesulfonate), and (O) recovery Tyrode's solutions. At each voltage the current was measured at the end of the voltage clamp step. Holding voltage, −64 mV; clamp frequency, 4/min; clamp duration, 1 s.

maintain a particular holding potential (the holding current). Hiraoka and Hiraoka (1975) also reported an average 7-mV depolarization in low chloride. In three fibers, we saw no consistent change in the holding current when chloride was reduced. In four other fibers, the holding current was more negative (inward) during the low chloride perfusion (corresponding to a depolarization in an unclamped fiber), but the changes were usually small. The largest change in holding current (-0.1 μA) was observed in the experiment presented in Fig. 4.

We did not attempt a detailed analysis of each of the currents thought to exist in the Purkinje fiber in these experiments, but from the time-course of currents obtained in response to depolarizing and hyperpolarizing clamps in normal and low chloride Tyrode's solutions, and from comparisons of 20-ms and steady-state current voltage relations in the two solutions, it would appear that there were no consistent changes in the pacemaker current, I_{K2}, or the slow inward current, I_{si}. Changes in time-independent currents seen in some preparations (e.g. Fig. 4) were consistent with a background chloride conductance. The variability of the change in the steady-state current would suggest that there are substantial differences in the amount that a background chloride conductance contributes to the net steady-state current voltage relations of different fibers.

Changes in Peak Outward Current

Although it is apparent from Fig. 1 that the peak outward current was reduced in low chloride, the current voltage relation at 20 ms (Fig. 2) does not show the full extent of this change which occurred several milliseconds earlier. Fig. 5 shows a representative plot of peak outward current as a function of voltage, for depolarizing clamp steps to voltages more positive than -25 mV, in normal

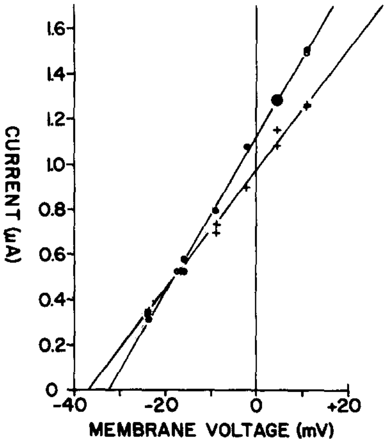

FIGURE 5. The peak outward current as a function of membrane voltage in (●) normal, (+) low chloride (methylsulfate), and (O) recovery Tyrode's solutions. The straight lines are linear regressions fitted to the control and low chloride points by the method of least squares. For details on how the currents were measured and a full description see text. The experiment is the same as that shown in Figs. 2 and 3. Holding voltage, -80 mV; clamp frequency, 2/min; clamp duration 4 s.

and low chloride solutions. The peak outward currents were very nearly linear functions of voltage, as Hiraoka and Hiraoka (1975) observed, and the lines in Fig. 5 are linear regressions fitted to the data by the method of least squares.

Data from four preparations were analyzed in this way, to allow us to compare the effects of chloride reduction in different preparations. Table I summarizes the results by comparing the regression lines at various voltages. Chloride reduction caused an average 20% (±1.7% S.E.M.) decrease in the peak outward current for clamp steps to voltages more positive than −10 mV. The percent decrease was not correlated with the clamp voltage ($r^2 = 0.026$). Because the change was small, a possible explanation was that low chloride reduced the peak of outward current by causing a slight change in its inactivation properties. At the voltages used, a slight negative shift of the curve relating the steady-state inactivation of the transient outward current (Fozzard and Hiraoka, 1973, Fig. 10) could decrease the peak outward current. We tested this possibility by comparing the peak outward current obtained during clamps to +1 mV from holding potentials of −80 and −91 mV in normal and low chloride solutions. The peak outward currents obtained in normal and low chloride when the holding potential was −91 mV were within 3% of those seen in the corresponding solutions where the holding potential was −80 mV, indicating that the current system or systems responsible for the early outward current were fully available at either holding voltage in either solution. The peak outward current was reduced by 20% in low chloride, independent of the holding voltage. This experiment seems to rule out the possibility of a parallel shift of the inactivation curve.

TABLE I

THE EFFECT OF CHLORIDE REDUCTION ON THE PEAK TRANSIENT OUTWARD CURRENT

		Decrease in peak transient outward current		
Preparation	V_R	at −10 mV	at 0 mV	at +10 mV
	mV		%	
1	−64	26	21	19
2	−80	9	13	16
3	−73	20	29	29
4	−67	18	18	18

Effects of Chloride and Potassium on Tail Currents

A valuable means of identifying the ion responsible for a particular current is to show that the reversal potential of the current is related to the equilibrium potential of a particular ion. Peper and Trautwein (1968) and Fozzard and Hiraoka (1973) estimated the reversal potential of the transient outward current by imposing a short depolarizing clamp to activate the current, and then stepping the voltage to various tests potentials. The sudden change in the driving force when the voltage was changed from the conditioning to the test voltage gave rise to "tail" currents. At the beginning of each tail current, the membrane conductance should be that which existed at the end of the conditioning clamp, while the driving force will be determined by the test voltage. In the simplest case, where the current is carried by a single ionic species, the voltage at which the tails change from outward to inward should be the same as the equilibrium potential for that ion. However, if the total current at the end of the conditioning clamp is made up of more than one ionic current, then the voltage at which the tails reverse will not necessarily be the equilibrium potential for any one of the ions involved. In this case, however, a substantial shift in the reversal potential of one of the component currents should produce a proportional shift in the reversal potential of tail currents (Fozzard and Hiraoka, 1973).

Fig. 6 shows the magnitudes of tail currents as a function of test voltage in normal and low chloride Tyrode's solution. In each of the tail current experiments, we measured the magnitude of the tail current as the difference between the earliest ionic current (after the capacity current) and the current at the end of the test clamp. Thus, the tail currents are not measured relative to zero current. Instead they represent the change in current at the test voltage. In the experiment shown in Fig. 6, the membrane voltage was clamped from a holding voltage of −62 mV to 0 mV for 40 ms to activate a large outward current. The voltage was then stepped to various test voltages for 2 s. A 28-s recovery period separated the tests. In this experiment, the voltage at which the tail currents changed direction was about −25 mV in both normal and low chloride Tyrode's solutions.

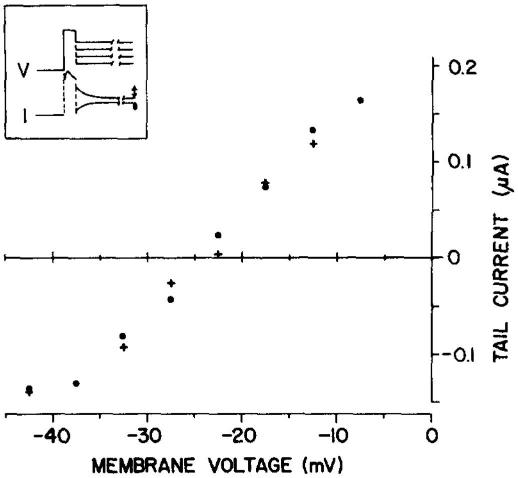

FIGURE 6. Tail currents at different test voltages in (●) normal and (+) low chloride (methanesulfonate), Tyrode's solutions. The membrane voltage was stepped from the holding potential of −62 mV to 0 mV for 40 ms and then stepped to various test voltages for 2 s as shown in the diagram. The procedure for measuring the tail currents is discussed in the text. A 28-s recovery period separated the tests.

The results above indicate that chloride ions do not carry the major portion of the peak outward current. One reasonable alternative is that the current could be largely a potassium current (Kenyon and Gibbons, 1977, 1979), so we checked to see if we could cause a shift in the reversal potential of the tail currents by varying the extracellular potassium concentration. In the experiment shown in Fig. 7, tail currents were recorded in Tyrode's solutions containing 2.7 and 10.8 mM potassium. The potassium equilibrium potential should change by 37 mV in this experiment and one might expect some change in the magnitude and reversal potential of the tail currents if potassium movements were involved. However, Fig. 7 shows that changing the extracellular potassium, at least over this range, has little effect on the tail currents. This insensitivity of the tail currents was also seen in another experiment which failed after tail currents were recorded at test voltages between −30 mV and +30 mV. In a third experiment, in which we compared tail currents in 2.7 and 5.4 mM extracellular potassium, the tail currents were slightly smaller at positive voltages

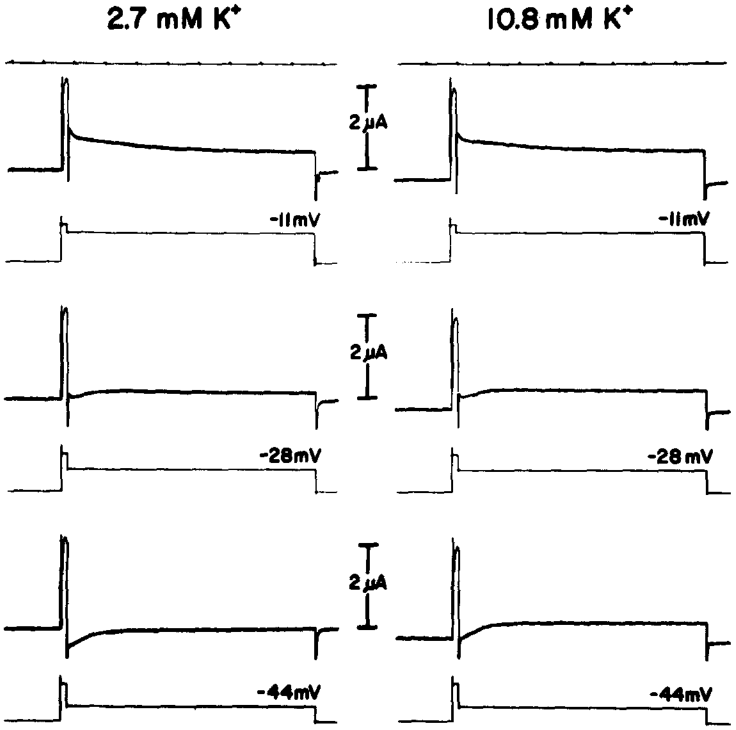

FIGURE 7. Voltage clamp records showing the effect of potassium on the current tails. The membrane voltage was stepped from the holding potential of −76 mV to +7 mV for 25 ms and then stepped to various test voltages for 1 s. The records in this figure were obtained from a chart recorder as described in Methods. The time marks at the top of the figure are 125 ms apart. A 28-s recovery period separated the tests.

in the higher potassium, but the reversal potential was −36 mV in both solutions.

The persistent reversal of the tail currents near −30 mV, in spite of changes in the extracellular chloride or potassium, is quite striking and confirms the similar observations by Peper and Trautwein (1968). Possible explanations are that neither of these ions contributes much to the early outward current, or that the conductance system that causes the early outward current is rather nonspecific (see, for example, Peper and Trautwein, 1968). Still another explanation, which we think is quite possible, is that the current or currents that give rise to the early outward current deactivate quickly when the preparation is clamped to voltages negative to −20 or −30 mV. Such kinetics for the outward component, in a mixed tail current that also contains an inward component, would cause an apparent reversal potential of the net tail current near −30 mV, and this apparent reversal potential would be insensitive to changes of the equilibrium potential of the ion or ions carrying outward current (see Discussion). If we are correct, then the fact that the reversal potential of the tail currents is unaffected by changes in the extracellular potassium ion concentration does not rule out the possibility that potassium ions carry much of the early outward current seen during voltage clamp steps positive to −20 mV.

The Effect of TEA on the Action Potential and Membrane Current

We are not the first to consider the possibility that the early peak of outward current is largely a potassium current. Dudel et al. (1967) also considered this hypothesis and noted that the lack of positive evidence in its favor did not rule it out as a possibility. They abandoned the potassium hypothesis when they found that chloride reduction decreased the early outward current, and they were able to attribute the current to a chloride flux. Since we have shown that chloride reduction does not slow the rate of phase 1 repolarization (Kenyon and Gibbons, 1977), and since we have also shown that a large portion of the outward current remains in low chloride solution, we feel that the possibility that most of the early outward current is a potassium current must be reconsidered. To do this we have tested the effect of tetraethylammonium chloride, which has been shown to block potassium currents in nerve and skeletal muscle (Armstrong, 1975), on the action potential and on membrane currents of the Purkinje fiber.

Haldimann (1963) reported that 20 mM TEA slowed phase 1 repolarization and increased the duration of sheep Purkinje fiber action potentials. We stimulated Purkinje fibers at a basic rate of 4/min, to allow time for full recovery of the early outward current (Fozzard and Hiraoka, 1973). At approximately one-half hour intervals, we evoked trains of action potentials at 40/min, in order to compare our results more directly with Haldimann's (1963) data. When TEA was applied, the first effects were seen within 15 min, and a full effect developed over several hours. At the higher rate of stimulation, TEA increased the action potential duration and shifted the plateau to less negative voltages (Fig. 8, row A, third panel). High sweep speed recordings (Fig. 8, row B) show that TEA slowed phase 1 repolarization. Unlike Haldimann (1963), we found a small but consistent depolarization in TEA at the 40/min rate. The decrease in the maximum rate of rise shown by the differentiated trace was probably caused by this depolarization and by the decrease in extracellular sodium used to keep the solution isotonic.

At the lower 4/min stimulation rate (not illustrated), depolarization of the resting potential was more marked, and two of three fibers became spontaneously active at a rate greater than the driving frequency after about 2 h in TEA. Phase 1 repolarization in TEA was also slower than control during 4/min stimulation, but because of the depolarization and later development of spontaneous activity, we could not be sure the slower phase 1 was a direct effect of the drug. The effects of TEA were not irreversible, but recovery was very slow and variable.

The action potential experiments indicated that TEA might reduce the early outward current seen in voltage clamp experiments. To examine this possibility, we tested the effect of TEA on membrane currents.

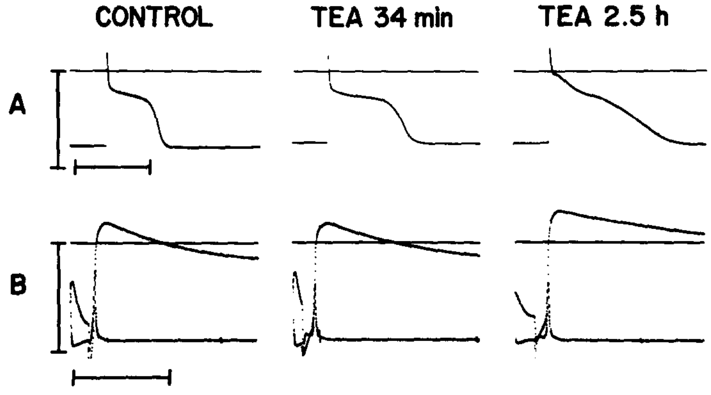

FIGURE 8. Action potentials in 20 mM TEA. Row A: action potentials at a slow sweep speed. Row B: action potentials (upper traces) and d*V*/d*t* (lower traces) at a faster sweep speed. The action potentials were evoked at a rate of 40/min as described in the text. Vertical bars: row A, 100 mV; row B, 100 mV and 426 V/s. Horizontal bars: row A, 500 ms; row B, 10 ms. The horizontal lines across the panels are at 0 mV.

The voltage clamp records in Fig. 9 show that TEA substantially decreased the early outward current. In this experiment, the peak outward current (measured as in Fig. 5) was reduced to 35% of control over the voltage range of −10 to +10 mV. Also, the steady-state currents and the holding current were less outward (or more inward) in TEA solutions. This change in the holding current corresponds to the depolarization seen in the action potential experiments. We recorded membrane currents from three Purkinje fibers in TEA, and obtained results similar to those shown in Fig. 9 from each. In every case a peak of outward current remained in TEA solution. This may mean that the full effect of TEA was not attained in these experiments, or that there is a component of the early outward current that is not sensitive to TEA. The component of the early outward current that remains in TEA is roughly comparable in magnitude and time-course to the chloride sensitive component described earlier, which suggests that there may be two separable components of early outward current. Later work with the drug 4-aminopyridine supports this hypothesis (Kenyon and Gibbons, 1979).

A detailed voltage clamp study of the effect of TEA on membrane currents would have been very difficult or impossible because of the long time between the control recordings and those taken after the drug had had its full effect. The variable reversibility of the effects added to the difficulty. However, we are confident that the effects described above represent changes caused by the drug, since similar changes of the holding current seldom occurred and substantial decreases in the peak early outward current never occurred during prolonged experiments in normal solution.

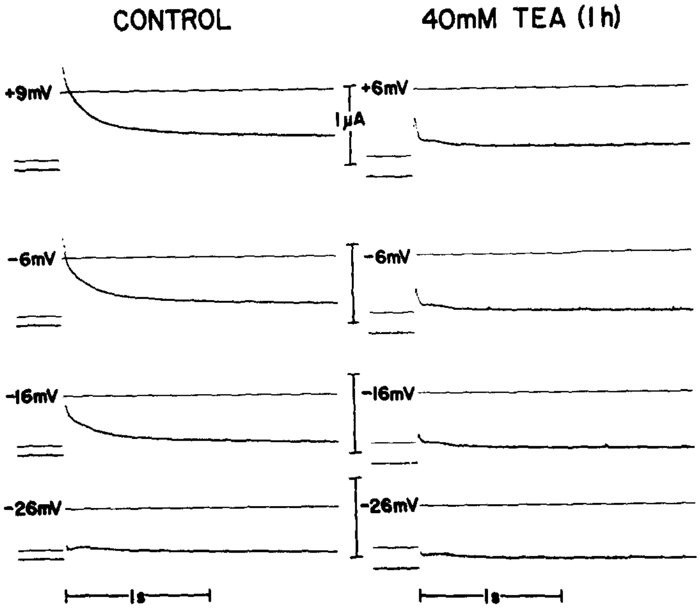

FIGURE 9. Voltage clamp records in (left) normal and (right) 40 mM TEA Tyrode's solutions. Only the first part of each voltage clamp step is shown. In each panel the upper trace is the membrane voltage, and the lower trace is the current. Holding voltage, −80 mV; clamp frequency, 2/min; clamp duration, 4 s.

DISCUSSION

The effects of low chloride solutions reported above are quantitatively very different from the effects seen by Dudel et al. (1967), Fozzard and Hiraoka (1973), and Hiraoka and Hiraoka (1975). In a recent paper (Kenyon and Gibbons, 1977), we mentioned sources of error that may arise in low-chloride experiments. We did not attempt to see how much each of these may affect the results, but each could have contributed to the differences between our results and those previously published.

In the experiments of Dudel et al. (1967), Fozzard and Hiraoka (1973), and Hiraoka and Hiraoka (1975), chloride reduction markedly decreased the early outward current of the Purkinje fiber, so that the current trace during strong depolarizing clamps was nearly flat or even increased with time. These observations led to the conclusion that a transient outward chloride current dominates the total current seen during depolarizations to voltages less negative than −20 mV. In our experiments, low chloride decreased the peak early outward current by only 20% at voltages above −10 mV. Other time-dependent currents, in particular I_{K2} and I_{si}, seemed unaffected by low chloride. Changes in the steady-state currents were about what one would expect if there is a rather small time-independent or background chloride conductance that varies considerably from fiber to fiber. Finally, the potential at which tail currents reversed was not changed by low chloride.

Taken together, these data strongly suggest that a time-dependent chloride conductance is not the major factor that determines the time-course of the net current at potentials above −20 mV. A reasonable alternative hypothesis is that most of the early outward current is caused by the outward movement of potassium ions. The data showing that the potassium-blocking agent TEA substantially decreased the early outward current support this suggestion, and further evidence using the drug 4-aminopyridine is given in the following paper (Kenyon and Gibbons, 1979). However, this proposal leaves unanswered a number of questions which we will address in the remainder of this discussion: (*a*) what role, if any, does chloride play in producing the large outward current; (*b*) how can we reconcile even a 20% reduction of peak outward current with our earlier report that low chloride does not change phase 1 of the action potential; and (*c*) why is it that alterations in extracellular potassium affect neither the peak outward current nor the reversal potential determined in the tail current experiment?

Role of Chloride

A significant time- and voltage-dependent component of the total current was sensitive to chloride removal. This component increased in proportion to the total early outward current, so that it was a relatively constant fraction of the peak outward current at voltages positive to −10 mV. The obvious interpretation is that the substitution of larger anions for chloride reduces chloride conductance without changing the membrane conductance for other ions. In this case, the data would support a time- and voltage-dependent chloride conductance that contributes a small amount to the total outward current (in addition to the background chloride conductance discussed above). However, an alternative explanation is suggested by the recent work of Carmeliet and Verdonck (1977). They reported that chloride reduction decreased the rate of ^{42}K efflux from quiescent Purkinje fibers, and they concluded that changes observed in low-chloride solutions may not be solely due to reduced chloride conductance. Their results may or may not apply to the stimulated preparation, but the work does raise the possibility that low chloride might cause the relatively small changes that we report via effects upon potassium conductances.

Relation between the Outward Current and Phase 1 Repolarization

The early outward current that appears at voltages positive to −20 mV is very likely related to the rapid phase 1 repolarization that is characteristic of the Purkinje fiber action potential (Dudel et al., 1967; McAllister et al., 1975). One would think that a 20% reduction of the peak outward current should produce a detectable change in the rate of phase 1 repolarization, yet we showed that phase 1 is not noticeably affected by the low-chloride solutions used here (Kenyon and Gibbons, 1977). This apparent paradox may be explained by comparing the time-courses of phase 1 and the peak outward current. Phase 1 repolarization is complete 10–20 ms after the beginning of the action potential upstroke. The peak of outward current during a clamp step, on the other hand, is not reached until after 10 or 15 ms of maintained depolarization. In addition, the action of the outward current during an action potential is to repolarize the membrane, and this repolarization should deactivate the current. Thus, the outward current should not be fully activated during an action potential. Apparently, the rising phase of the outward current causes the normal phase 1 repolarization and this early current is large enough in low chloride solution to cause a normal phase 1 repolarization. One possibility is that the chloride-sensitive portion of the early outward current activates more slowly than the system or systems that cause the majority of the early outward current. The very early time-course of the current was not always easy to resolve in our experiments, but an examination of several of our records indicated that low chloride caused little change in the rising phase of the outward current until the current neared its peak. The fact that there is a significant difference between the peak outward current in normal and low-chloride Tyrode's solution during a maintained depolarization leaves open the possibility that chloride removal could have a noticeable effect upon a phase 1 that is slower than the phase 1 we record in normal solutions. This might explain some of the difference between our action potential results (Kenyon and Gibbons, 1977) and those reported by Carmeliet (1961), Dudel et al. (1967), Hiraoka and Hiraoka (1975).

Effects of Altered Extracellular Potassium

The fact that alterations of the extracellular potassium did not change either the peak outward current or the reversal potential determined in the tail current experiments seems inconsistent with the suggestion that most of the early outward current is carried by potassium ions. We think these failures can be explained, and that the experiments do not rule out potassium as the major charge carrier.

The tail current experiment has been used several times in an effort to determine the ion or ions responsible for the early outward current (Peper and Trautwein, 1968; Fozzard and Hiraoka, 1973). Vitek and Trautwein (1971) and Fozzard and Hiraoka (1973) have pointed out that the net current 20–40 ms after depolarization is probably caused by a mixture of currents, so that the reversal potential determined at this time should be a multi-ionic potential. Even if this is correct, it is still surprising that the reversal potential near −30 mV is not affected by changes in the extracellular concentrations of the two ions, chloride and potassium, which are the most likely candidates as carriers of the large outward current. Nor is the reversal potential affected by lowering extracellular bicarbonate (Peper and Trautwein, 1968). We think the persistent reversal at voltages near −30 mV results from the kinetics of the current or currents responsible for the peak of outward current, and from practical limitations on measurements of instantaneous current voltage relations in heart.

If we suppose that a transient potassium current carries most of the early outward current, it seems reasonable to assume the conductance change responsible is one which activates rapidly and inactivates slowly when the muscle is depolarized. I_{qr}, the current which produces most of the early outward current in the McAllister et al. (1975) reconstruction of the Purkinje fiber action potential, behaves in this way, and we will use the kinetics proposed for I_{qr} as concrete examples in our proposal. We must note that I_{qr} was based on conclusions about the chloride sensitivity of the net current which now appear to have been in error, and that the activation kinetics of I_{qr} were not based on experimental data other than the behavior of the net membrane current. Even so, this does not invalidate the use of I_{qr} as a model in describing how a current with seemingly simple kinetics may give confusing results in experiments like those in Figs. 6 and 7.

In the McAllister et al. (1975) formulation, I_{qr} is proportional to the product of an activation variable q and an inactivation variable r, each of which is a function of voltage and time. The steady-state value of q, $q_\infty(V)$, varies between 0.01 and 0.98 over the voltage range −30 mV to +30 mV, and the time constant $\tau_q(V)$ varies between 0.86 ms and 3.9 ms over the same voltage range (τ_q is even shorter than 0.86 ms at voltages negative to −30 mV). The steady-state value of the inactivation variable, $r_\infty(V)$, varies between 0.98 and 0.01 between −110 mV and −40 mV, and $\tau_r(V)$ is much larger than $\tau_q(V)$ at all physiological voltages (see McAllister et al., 1975, for the complete formulation used). The experiments of Fozzard and Hiraoka (1973) suggest that $r_\infty(V)$ should perhaps be a steeper function of voltage than that used by McAllister et al., but such a change would not materially affect our argument.

During a 20–40-ms conditioning clamp from a holding voltage V_h to a clamp voltage V_1, q reaches the steady-state value $q_\infty(V_1)$, while r changes little from the value it had at V_h. Thus, activation of the current is complete, but little inactivation occurs. The purpose of the steps to various voltages V_2 is to determine the instantaneous current voltage relation of the preparation at a time when the conductance system of interest is strongly activated. In practice, however, the tail currents at V_2 cannot be measured immediately after the step to V_2 because they are overlapped by much larger capacity current. Our measurements were made 5 ms after the steps to the various voltages V_2. It is easy to appreciate that part of the tail current may be lost because it is obscured by capacity current; in fact, most of the current may be lost at some voltages. Still using I_{qr} as a model, we can consider the effect of a 40-ms conditioning clamp from $V_h = -80$ mV to $V_1 = +20$ mV, followed by a step to a voltage $V_2 = -10$ mV. In this case, the tail of I_{qr} at V_2 (or more properly, the contribution of I_{qr} to the net current tail at V_2) would decay with two time constants. The faster phase of decay of I_{qr} corresponds to deactivation of I_{qr}, i.e., it is caused by the change of q from $q_\infty(+20\text{mV})$ to $q_\infty(-10\text{mV})$. The slower phase of I_{qr} decay corresponds to inactivation of the current as r decreases from the value it had reached at the end of V_1 to the value $r_\infty(-10\text{mV})$. In this example, roughly three-fourths of the decay of I_{qr} would be due to the fast deactivation process, and more than half of the decay of I_{qr} would take place in the first 5 ms, when measurements cannot be made. Moreover, it would not be possible to recover the initial value of the tail current by plotting the later tail current on a semilog plot and extrapolating back to the time of the step to V_2, because the contribution of I_{qr} to the net current does not decay monoexponentially.

During a trial in which the voltage V_2 was -30 mV, the contribution of I_{qr} to the net current tail would decay to $< 2\%$ of its initial value in 5 ms because of deactivation, leaving a negligible fraction of the net tail current composed of I_{qr} at a time when measurements could be made. For any voltage V_2 more negative than -30 mV, $q_\infty(V_2) = 0$, and there also would not be a significant contribution of I_{qr} to the net tail current at times when ionic current could be distinguished from capacity current.

If the current that generates most of the early outward current behaves anything like the McAllister et al. (1975) formulation of I_{qr}, then it no longer seems so odd that the tail currents show a persistent reversal near -30 mV as one changes the concentrations of the ions that might carry outward current, because such a current would contribute outward current to the tails measured at voltages positive to -30 mV, but make little or no contribution to tails measured at voltages negative to -30 mV. Considerations of this type may also apply to other currents. For example, Siegelbaum et al. (1977) have proposed that I_{si} activation is very rapid in Purkinje fibers. If it is, then problems may arise in attempting to analyze tails of I_{si}.

If the failure to observe a change in the reversal potential of the tail currents in altered extracellular potassium does not rule out the possibility that potassium ions carry a major portion of the early outward current, then another test of the hypothesis might be to see if changing extracellular potassium alters the current recorded during strong depolarizations. Increasing the potassium should decrease the current. However, if the potassium concentration bathing a Purkinje fiber is raised much above 10.8 mM, a very large holding current is needed to maintain a negative holding voltage (McAllister and Noble, 1966). This makes analysis of the early outward current in high extracellular potassium extremely difficult.

Over the 2.7–10.8 mM range that we examined, altered extracellular potassium might not cause readily detectable changes in the early outward current even if the current were a pure potassium current. Dudel et al. (1967) pointed out that, if one assumes that the early outward current shows constant field behavior (Goldman, 1943), alterations of potassium over the range 0–10 mM should result in less than an 8% decrease of outward current at positive voltages. If the early outward current is a mixed current, the percent change should be even less. Dudel et al. (1967) confirmed this prediction using ramp clamps. Our results are similar, in that the data obtained in different potassium concentrations showed no consistent change in the peak of outward current when outside potassium was varied between 2.7 and 10.8 mM.

Thus, the usual ways of illustrating that a current is carried by potassium may not be reliable in the case of the early outward current. If we are correct in our interpretation of the tail current experiments, it obviously also means that the fact that the reversal of the tail currents was not changed by low chloride should be disregarded as evidence against this ions's being the principal carrier of early outward current. However, the other evidence against the accepted role of chloride still seems convincing, and the experiments with TEA, by analogy with studies of nerve and skeletal muscle, suggest rather strongly that potassium ions carry most of the early outward current seen at voltages positive to −20 mV. In the following paper, we will attempt a more complete dissection of the early outward current.

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