4-Aminopyridine and the Early Outward Current of Sheep Cardiac Purkinje Fibers

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ABSTRACT We have studied the effects of the potassium-blocking agent 4-aminopyridine (4-AP) on the action potential and membrane currents of the sheep cardiac Purkinje fiber. 4-AP slowed the rate of phase 1 repolarization and shifted the plateau of the action potential to less negative potentials. In the presence of 4-AP, the substitution of sodium methylsulfate or methanesulfonate for the NaCl of Tyrode's solution further slowed the rate of phase 1 repolarization, even though chloride replacement has no effect on the untreated preparation. In voltage clamp experiments, 4-AP rapidly and reversibly reduced the early peak of outward current that is seen when the Purkinje fiber membrane is voltage-clamped to potentials positive to -20 mV. In addition, 4-AP reduced the steady outward current seen at the end of clamp steps positive to -40 mV. 4-AP did not appear to change the slow inward current observed over the range of -60 to -40 mV, nor did it greatly change the current tails that have been used as a measure of the slow inward conductance at more positive potentials. 4-AP did not block the inward rectifying potassium currents, Is1 and Ik2. A phasic outward current component that was insensitive to 4-AP was reduced by chloride replacement. We conclude that the early outward current has two components: a chloride-sensitive component plus a 4-AP-sensitive component. Since a portion of the steady-state current was sensitive to 4-AP, the early outward current either does not fully inactivate or 4-AP blocks a component of time-independent background current.

INTRODUCTION

When cardiac Purkinje fibers are clamped to voltages positive to -20 mV, the total membrane current (after the capacity and inward sodium transients) shows an outward peak and then declines. This early outward current has been related to the rapid phase 1 repolarization that is characteristic of the Purkinje fiber action potential (Dudel et al., 1967; Fozzard and Hiraoka, 1973; Hiraoka and Hiraoka, 1975; McAllister et al., 1975).

Dudel et al. (1967) and Fozzard and Hiraoka (1973) attributed the peak and decline of the total membrane current to an influx of chloride ions on the basis of chloride replacement experiments. This component of the total membrane current has been called the positive dynamic current (Peper and Trautwein, 1968), the chloride current (Dudel et al., 1967), and Icl (McAllister et al., 1975). Recently, however, we reexamined the effects of chloride substitution on the action potential and membrane currents of the Purkinje fiber in experiments.
designed to minimize possible sources of error. In low-chloride solutions, the rate of phase 1 repolarization was not changed (Kenyon and Gibbons, 1977), and although the peak of the early outward current was reduced by ~20%, a large declining outward current remained (Kenyon and Gibbons, 1979). These data led us to conclude that there was a time-dependent component of the total current that was sensitive to chloride reduction, but that the major portion of the early outward current was carried by some ion other than chloride.

The early outward current that is not sensitive to chloride reduction might be a potassium current. However, the usual electrophysiological methods for determining the ionic nature of a membrane current do not provide positive evidence for this hypothesis (Dubel et al., 1967; Kenyon and Gibbons, 1979).

Indirect evidence suggesting that potassium is involved in the early outward current has been obtained using tetraethylammonium chloride (TEA), a known blocker of potassium currents in nerve and skeletal muscle (Armstrong, 1975). TEA slows the rate of phase 1 repolarization of the Purkinje fiber action potential (Haldimann, 1963; Kenyon and Gibbons, 1979) and markedly reduces the early outward current (Kenyon and Gibbons, 1979). However, externally applied TEA acts over a period of several hours. This is inconvenient in a voltage clamp experiment, and one cannot be sure that the full effect of TEA is being observed. This question becomes critical because we always saw a residual phasic outward current (~30-40% of control) in TEA solutions (for example, see Fig. 9 of Kenyon and Gibbons, 1979). Because of the problems in using TEA, we tried 4-aminopyridine (4-AP) which blocks potassium currents in nerve and skeletal muscle (Pelhate and Pichon, 1974; Gillespie and Hutter, 1975; Wagner and Ulbricht, 1975; Schauf et al., 1976; Yeh et al., 1977a,b; Meves and Pichon, 1977).

**MATERIALS AND METHODS**

**Solutions**

4-AP is not soluble in the Tyrode's solutions that we used in previous experiments because of the high phosphate concentration of that solution. In the first experiments with 4-AP we used Tyrode's solution buffered with 1 mM Hepes (Calbiochem, La Jolla, Calif.) but soon changed to a low-phosphate Tyrode's solution which contained (in mM): NaCl, 137; KCl, 5.4; MgCl₂, 1.05; NaHCO₃, 11.9; NaH₂PO₄, 0.33; CaCl₂, 2.7; glucose, 11.1. The control Tyrode's solution was made with reagent grade chemicals (Mallinckrodt Chemical Co., St. Louis, Mo., or Baker Chemical Co., Phillipsburg, Pa.) and all solutions were made with glass redistilled water. Low-chloride Tyrode's solution was made by substituting either sodium methylsulfate (City Chemical Corp., New York) or sodium methanesulfonate (made by NaOH neutralization of methanesulfonic acid, Aldrich Chemical Co., Inc., Milwaukee, Wis.) for the NaCl of the normal Tyrode's solution. The calcium concentration of sodium methylsulfate solution was raised to 1.2 times that of the normal Tyrode's solution to keep the calcium activity of the low-chloride solution equal to that of the normal Tyrode's solutions. Because sodium methanesulfonate has a negligible effect on calcium ion activity, the total calcium concentration in the methanesulfonate solution was the same as that in the normal solution. (For details on the effects of these ions on calcium ion activity see Kenyon and Gibbons, 1977). 4-AP (Sigma Chemical Co., St. Louis, Mo., or Aldrich Chemical Co.) was added to the normal and
low-chloride Tyrode's solutions without osmotic correction. D600 was generously supplied by Knoll A.G. - Chemische Fabriken, Ludwigshafen am Rhein, Germany.

All solutions were saturated with 95% O₂, 5% CO₂ except for the Hepes-buffered solutions which were saturated with 100% O₂. The experiments were carried out between 36 and 37°C with the temperature held constant to within 0.2°C during an experiment. At 36°C each of the solutions had a pH between 7.3 and 7.5.

Techniques

The details of the experimental techniques have been described elsewhere (Gibbons and Fozzard, 1975; Kenyon and Gibbons, 1977, 1979). Action potentials were recorded from long (>3 mm) sheep cardiac Purkinje fibers using standard microelectrode techniques. A flowing KCl-calomel reference electrode (Fisher 13-639-56, Fisher Scientific Co., Pittsburgh, Pa.) was used as the extracellular reference electrode to minimize changes in tip and junction potentials when solutions were changed (Woodbury and Miles, 1973).

This was most important in the low-chloride experiments but we used this electrode in all of the experiments described in this paper. The fibers were stimulated at a basic rate of 4/min, and all action potential data reported here were obtained at this stimulation rate. The maximum rate of rise of the action potential was obtained by electronic differentiation.

Purkinje fibers were voltage-clamped using the two-microelectrode technique (for details, see Kenyon and Gibbons, 1979). Current-voltage relations were obtained by setting the clamp holding voltage (Vₜₘ) near the normal resting potential and then applying step clamps to various voltages. The current was measured as a function of voltage at the end of the clamp step (the steady-state or "late" current) and 20 ms after the beginning of the clamp step. These current measurements were made relative to zero current which was determined periodically by turning the voltage clamp off. Clamp frequency was 2/min and the duration of the clamp step was usually 2 s. The holding voltage, frequency, and clamp duration are listed with the figures.

RESULTS

The Effects of 4-Aminopyridine on the Action Potential

We recorded action potentials from four preparations exposed to increasing concentrations of 4-AP (0.01, 0.1, 0.5, and 1.0 mM). The fibers were exposed to each concentration for ~15 min. At each concentration there was some effect after 30 s and the steady state was reached in 3–5 minutes. As the concentration of 4-AP was increased, it caused greater changes in the configuration of the action potential up to a concentration of 0.5 mM. Increasing the concentration from 0.5 to 1.0 mM caused no further change in the action potential and we have used the lower concentration as the standard dose. The effects of 4-AP on the action potential were reversed after 30–45 min of perfusion in normal Tyrode's solution.

The effects of 4-AP on the action potential are shown in Fig. 1. The upper panels are action potentials recorded at a slow sweep speed whereas the lower panels are the early portions of the same action potentials (upper trace) and the differentiated signal (lower trace) recorded at a much faster sweep speed. In the fast sweep speed records, only the upstroke, phase 1, and the early portion of phase 2 can be seen. The most striking effects of 4-AP are the slowing of the phase 1 repolarization (shown best in the lower panels) and the elevation of the
plateau to less negative potentials (the upper panels). The dose response data for these parameters are summarized in Table I. 4-AP had a variable effect on the action potential duration. Action potentials longer than 0.5 s (four preparations) were shortened while the duration of action potentials of preparations in which the control duration was shorter than 0.5 s (three preparations) changed little (see Table II).

As shown in Fig. 1, 0.5 mM 4-AP did not appear to have any effect on the resting potential, overshoot, or maximal rate of rise of the upstroke. In order to be certain that there was not a small but significant effect of 4-AP on these parameters we collected the data from seven preparations that were exposed to 0.5 or 1.0 mM 4-AP. These data are tabulated in Table II. Using a paired t-test, there was no significant difference ($P > 0.05$) in these parameters when we compared control vs. 0.5 mM 4-AP (five preparations) or control vs. 1.0 mM 4-AP (six preparations).

**Figure 1.** Action potentials recorded in normal, 0.5 mM 4-AP, and recovery Tyrode's solutions. These data were recorded during one of the dose-response experiments (fiber 7702.01). See text for a full discussion. The horizontal lines are at 0 mV, and the 80 mV calibration bar at the beginning of the lower row corresponds to 1,333 V/s on the differentiated trace.

**Combined Effects of 4-Aminopyridine and Low Chloride on the Action Potential**

Fig. 2 illustrates one of a number of experiments in which we examined the individual and combined effects of chloride replacement and 4-AP. In low chloride (methylsulfate), the action potential duration was slightly increased (compare Fig. 2 b with a). The rate of phase 1 repolarization was not changed by the chloride replacement (compare Fig. 2 b' with a') as we have previously reported (Kenyon and Gibbons, 1977). Addition of 0.5 mM 4-AP to the low-chloride solution drastically reduced the rate of phase 1 repolarization so that only a slight inflection in the repolarization phase remained. In other preparations even this slight inflection was absent.

After the records in Fig. 2 c and c' were obtained, the fiber was returned to
the normal Tyrode’s solution containing 0.5 mM 4-AP. The return of chloride caused in increase in the rate of phase 1 repolarization (compare Fig. 2 d’ with c’) and a distinct notch appeared between phase 1 and phase 2. The action potential configuration shown in Fig. 2 d is typical for Purkinje fibers treated with 4-AP. After 45 min in normal Tyrode’s solution the action potential regained its control configuration (Fig. 2 e and e’).

It was apparent from these experiments (four fibers) that chloride replacement does effect the rate of phase 1 repolarization in the presence of 4-AP even though it has no effect on the untreated preparation. The results were the same when the treatments were done in the reverse order from that illustrated in Fig. 2.

Membrane Current in the Presence of 4-Aminopyridine

Fig. 3 shows a voltage clamp experiment in normal Tyrode’s solution and in 0.5 mM 4-AP. In each panel the upper trace is the membrane voltage and the lower trace is current. The holding voltage was −70 mV. The records in the left hand column show the typical currents obtained in normal solution. When the membrane was stepped from −70 mV to −29 mV, the total membrane current

| Fiber | Treatment | Plateau | Rate of phase 1 repolarization |
|-------|-----------|---------|-------------------------------|
|       | 0.01 mM 4-AP | +15.0 | +15.1 |
|       | 0.5 mM 4-AP   | +15.0 | +15.1 |
|       | 1.0 mM 4-AP   | +15.0 | +15.1 |

* The plateau was measured at its most positive point after the end of the phase 1 repolarization. The rate of phase 1 repolarization was measured from photographs of the action potentials and represents the fastest rate of repolarization.
(after the capacitative and inward sodium transients which were not recorded at the film speed used) was net inward (negative) for 60 ms before becoming a steady outward (positive) current. This negative current has been attributed to an influx of calcium and sodium ions and has been called the slow inward current (Reuter, 1967; Vitek and Trautwein, 1971; Gibbons and Fozzard, 1975). When the membrane was clamped to more positive voltages, the total membrane

**TABLE II**

**EFFECTS OF 4-AMINOPYRIDINE ON THE ACTION POTENTIAL**

| Fiber  | Treatment | Resting potential | Overshoot | Rate of rise | Duration* |
|--------|-----------|-------------------|-----------|--------------|-----------|
|        |           | mV                | mV        | V/s          | s         |
| 7611.23| Control   | -75.8             | +28.7     | 509          | 0.494     |
|        | 1.0 mM 4-AP | -75.8             | +29.4     | 451          | 0.388     |
|        | recovery   | -75.3             | +27.5     | 439          | 0.494     |
| 7611.30| Control   | -89.4             | +13.4     | 393          | 0.871     |
|        | 1.0 mM 4-AP | -75.3             | +21.2     | 490          | 0.555     |
| 7612.14| Control   | -77.6             | +25.9     | 549          | 1.412     |
|        | 0.25 mM 4-AP | -77.6             | +29.4     | 549          | 0.988     |
|        | recovery   | -80.0             | +23.5     | 509          | 1.365     |
|        | 0.5 mM 4-AP | -80.0             | +28.2     | 549          | 0.494     |
|        | 1.0 mM 4-AP | -80.0             | +29.4     | 549          | 0.541     |
| 7701.11| Control   | -74.8             | +25.5     | 470          | 0.259     |
|        | 0.5 mM 4-AP | -78.8             | +21.2     | 470          | 0.271     |
|        | 1.0 mM 4-AP | -77.6             | +20.0     | 431          | 0.251     |
|        | recovery   | -80.0             | +18.8     | 470          | 0.282     |
| 7701.13| Control   | -72.2             | +27.1     | 392          | 0.271     |
|        | 0.5 mM 4-AP | -74.6             | +30.6     | 313          | 0.288     |
|        | recovery   | -75.3             | +20.0     | 314          | 0.282     |
| 7701.27| Control   | -84.7             | +28.2     | 840          | 1.600     |
|        | 0.5 mM 4-AP | -87.1             | +25.9     | 843          | 1.290     |
|        | 1.0 mM 4-AP | -82.4             | +30.6     | 823          | 0.920     |
|        | recovery   | -81.2             | +28.7     | 882          | 3.040     |
| 7702.01| Control   | -75.6             | +30.4     | 648          | 0.344     |
|        | 0.5 mM 4-AP | -75.6             | +31.1     | 648          | 0.359     |
|        | 1.0 mM 4-AP | -74.4             | +30.4     | 648          | 0.356     |
|        | recovery   | -75.6             | +32.2     | 685          | 0.440     |

* Action potential duration was measured from the upstroke to the time when the membrane voltage crossed the original resting potential.

† These fibers were exposed to increasing concentrations (0.01, 0.1, 0.5, and 1.0 mM) 4-AP in dose-response experiments. Data from the higher concentrations only are reported here. Fiber 7612.14 was the first dose-response experiment that we tried. The initial dose of 0.25 mM 4-AP had a larger effect than we expected, so the fiber was returned to normal Tyrode's (recovery) and a complete experiment was started with 0.01 mM 4-AP after recovery. The recovery action potential was used as the control in the statistical analyses described in the text.
current showed an outward (positive) peak and then declined. The complex
time-course of this current during the clamp to -18 mV presumably reflects the
summing of the slow inward current with a phasic outward current. During
stronger depolarizing voltage clamp steps (e.g., to +13 mV) the current peaked
between 10 and 20 ms and then declined smoothly. Most of the decline was over
in ~500 ms, but a small component continued to decrease for the duration of
the 2-s voltage clamp step. Dudel et al. (1967) and Fozzard and Hiraoka (1973)
referred to this time dependent portion of the outward current as the positive
dynamic current.

In the presence of 0.5 mM 4-AP (Fig. 3, right hand column), the current that
flowed at -29 mV was more inward during the first 20-30 ms of depolarization,
but otherwise had a time-course almost identical to the control. However, 4-AP
had striking effects when the membrane was clamped to more positive poten-
tials. The early outward current seen in normal solution at -18 mV was

![Diagram](https://via.placeholder.com/150)

**Figure 2.** Separate and combined effects of 4-AP and low chloride (methylsul-
fate) on the action potential. Action potentials are shown in records a–e. Below
each of the records a–e is a fast sweep record (a’–e’) obtained under the same
conditions at nearly the same time. The upper trace of the fast sweep records
shows the upstroke, phase 1, and the early part of phase 2. The lower trace of the
fast sweep records is the electronically differentiated signal, dV/dt. The calibrations
in record e apply to records a–e: the horizontal line is drawn at 0 mV and represents
200 ms; the vertical line represents 40 mV. The calibrations in record e’ apply to
records a’–e’: the horizontal line is drawn at 0 mV and represents 4 ms; the vertical
line represents 40 mV and 667 V/s.

eliminated, and the total membrane current resembles the slow inward current
seen at more negative voltages. At +13 mV, the early outward current was
reduced to ~30% of control. The remaining phasic outward current peaked
~15 ms after the start of the clamp step then declined to a minimum ~50 ms
later. For the remainder of the clamp step the total membrane current was
increasing outward. These effects are qualitatively similar to those of TEA
(Kenyon and Gibbons, 1979, Fig. 9).

In order to get a more complete view of the effects of 4-AP, we plotted the
current-voltage relations obtained at the end of the clamp step (the “late”
current) and 20 ms after the beginning of the clamp step (see Methods) on three
preparations. The graphs from one of these fibers are shown as Figs. 4 and 5.
Negative to ~40 mV, 4-AP seemed to have little or no effect on the current-
voltage relation of the late current in this and the other experiments (Fig. 4). At
more positive voltages the late currents were less outward in the presence of 4-
AP. Similarly, the 20-ms current-voltage relation was not changed at voltages more negative than -40 mV, but at more positive voltages the early outward current was greatly reduced by 4-AP (Fig. 5).

4-AP and tetraethylammonium each block potassium current in nerve and skeletal muscle, and they produce changes of the net current in Purkinje fibers which are most easily interpreted as resulting from a decrease in outward current. However, we have become very sensitive to the problems that may result from inferring the behavior of individual current components from changes of the net current, and we feel obliged to raise the possibility that the net current change at voltages positive to -40 mV could also be explained by supposing that 4-AP greatly increases an inward current such as the slow inward current, thus masking the early outward current. 4-AP apparently does have a positive inotropic effect (Fastier and McDowall, 1958; Lemeignan et al., 1975), which suggests that the drug might either increase calcium influx or decrease calcium efflux, so this possibility merits consideration.

4-AP does not appear to change \( I_{\text{Na}} \) at voltages negative to -40 mV, where a transient related to \( I_{\text{Na}} \) can be observed directly, but it is much more difficult to say whether or not 4-AP increases \( I_{\text{Na}} \) at more positive potentials. We did several experiments in an effort to rule out this possibility. None could be considered conclusive, but taken together they convinced us that the principal, and perhaps the only, effect of 4-AP was to decrease outward current in our preparations.

One of the experiments performed is illustrated in Fig. 6. The membrane voltage was stepped from -70 mV to +1 mV for decreasing clamp durations (250, 150, 100, 50, 25, and 10 ms). Each panel shows six superimposed voltage clamps. In normal Tyrode's solution (left panel), the early outward current during the depolarization peaked at 2.9 \( \mu \)A which is off the scale of these records. In 0.5 mM 4-AP, the peak of outward current was reduced to -0.25
Repolarization to $-70\, \text{mV}$ elicited inward current tails, the amplitude of which varied with the duration of the conditioning clamp step. In 4-AP, the inward tails were very slightly smaller than the controls. The result is consistent with the view that 4-AP blocks an outward current which is responsible for most of the early outward current, and that the current blocked by 4-AP contributes little to the tail currents at $-70\, \text{mV}$ (see Discussion of Kenyon and Gibbons, 1979). The envelope of inward tail currents at voltages near $-70\, \text{mV}$ has been used as a measure of the slow inward current conductance as a function of time during the conditioning depolarization (Vitek and Trautwein, 1971; Gibbons and Fozzard, 1975). If that conclusion is correct, the experiment in Fig. 6 also demonstrates that 4-AP does not greatly increase the slow inward conductance during the conditioning depolarization, inasmuch as that should have increased the amplitude of the inward tails at $-70\, \text{mV}$. Although this interpretation is attractive, there are problems with it. The evidence that the inward tails obtained in an experiment like this represent pure slow inward current is very weak. Furthermore, Siegelbaum et al. (1977) have argued that $I_{Ih}$ activation in Purkinje fibers may be quite rapid. If it is, then $I_{Ih}$ could represent another case in which much of the tail current may deactivate before one can separate ionic current from capacity current. Thus, even if one could demonstrate that the tails were relatively pure $I_{Ih}$, their amplitudes might not accurately reflect differences in $I_{Ih}$ conductance in the two experimental conditions.

In another set of experiments, we examined the effects of agents (D600 and manganese) which are thought to reduce or block $I_{Ih}$. We reasoned that, if 4-AP
decreased early outward current by greatly increasing $I_h$, some reversal of the effect should be seen when $I_h$ is blocked. Three experiments were done using the rate of phase 1 repolarization of action potentials as an index of outward current changes. In each experiment, 0.5 mM 4-AP was added. After phase 1 repolarization had slowed, 5 mg/liter D600 was added in two experiments and 2 mM manganese was added in the third. Neither of these agents increased the rate of phase 1 repolarization in the presence of 4-AP. A single voltage clamp experiment was done in which 5 mg/liter D600 was added after the 4-AP effect was complete. D600 caused no reversal of the 4-AP effect. These experiments are consistent with the interpretation that 4-AP does not reduce the early outward current by increasing $I_h$. However, they also are not conclusive, because neither D600 nor manganese may be considered specific for $I_h$; in fact, each agent appears to decrease a component of early outward current (Siegelsbaum, et al., 1977). Thus, although we believe the weight of evidence supports our conclusion that 4-AP acts by blocking outward current, evidence other than voltage clamp experiments will probably be necessary to establish the point unequivocally.

The goal of these experiments was to examine the effects of 4-AP on the early outward current and, with the exception of the slow inward current, we have not attempted an analysis of its possible effects on any of the other currents that are thought to exist in the Purkinje fiber (Trautwein, 1973; McAllister et al., 1975). We can say, however, that 4-AP did not appear to have any gross effects on the pacemaker current, $I_{K_2}$, or on the background potassium current $I_{K_1}$, as evidenced by the lack of change in the 20-ms and steady-state current voltage relations at voltages negative to $-40$ mV, and by observation of the pacemaker current in normal and 4-AP containing solutions.

**Figure 5.** 20-ms current-voltage relations in normal and 0.5 mM 4-AP Tyrode's solutions. The current was measured 20 ms after the beginning of voltage clamp steps to the different voltages. These data were obtained from the same experiment as shown in Fig. 4. The fiber used in this experiment had a very small slow inward current. (●) Control measurements; (+) data in 4-AP.
In normal Tyrode's solution the membrane current at the end of a 2-s-long clamp step to voltages positive to \(-20 \text{ mV}\) was always a decreasing outward current. We did not see any sign of the slowly increasing outward $I_X$ currents that are thought to be carried in part by potassium ions (Noble and Tsien, 1969). However, in 0.5 mM 4-AP the current near the end of the clamp steps was a slowly increasing outward current. McAllister et al. (1975) have suggested that the failure to observe the $I_X$ currents in some experiments might be due to the masking of these very small currents by the larger early outward current. It is possible then, that 4-AP revealed the $I_X$ currents by reducing the early outward current. This implies that 4-AP did not block the $I_X$ currents. This conclusion is supported by the time-course of the 4-AP-sensitive current (see below).

**The 4-Aminopyridine-Sensitive Current**

In four experiments we examined the time- and voltage-dependent behavior of the current that is blocked by 4-AP. To do this we subtracted the current remaining in 0.5 mM 4-AP from the control current obtained in matching voltage clamp steps. The very early behavior of this current is not certain because of the limitations of the voltage clamp technique, but for voltage clamp steps to potentials positive to \(-30 \text{ mV}\) the 4-AP-sensitive current appeared to be a rapidly activating outward current (i.e., with a time constant on the order of 5 ms or less). For voltage clamp steps to potentials negative to \(-30 \text{ mV}\) the control and 4-AP currents were very close, and the difference was too small to allow its time-course to be resolved accurately.

The decay of the 4-AP-sensitive current varied somewhat from experiment to experiment. In two experiments, including that which was illustrated in Fig. 3, the current decay could be fitted reasonably by a single exponential in spite of the fact that the control currents and those recorded in 4-AP each followed rather complex time-courses. In the other two experiments analyzed in this way, the decay of current was not monoexponential. The current decay in these experiments could be fitted reasonably well by two exponentials, with the more rapid time constant accounting for most of the current decay. One of these experiments is illustrated in Fig. 7.
When the four experiments were considered as a group, it was not clear that the time constants depended on voltage in any consistent way. In the two experiments fitted by single exponentials, the time constant of decay decreased as voltage increased in the range -30 mV-0 mV. In one of the experiments fitted by two exponentials (the one in Fig. 7), the fast and slow time constants each decreased with greater depolarization, but in the other experiment the fast and slow time constants showed no consistent change with voltage.

![Diagram](image)

**Figure 7.** Time-course of the 4-AP-sensitive current. Measurements of currents in 0.5 mM 4-AP during clamps to -30 mV, -19.5 mV, -9 mV, +1 mV, and +13.5 mV were subtracted from measurements of control records obtained earlier at these same voltages in normal Tyrode's solution. The earliest measurement of each record was 20 ms after depolarization. The points plotted above give the differences as a function of time after depolarization to the various voltages. The solid lines are fitted to each set of data according to the equation $\Delta I = A \exp(-at) + B \exp(-bt) + C$. The constants used to fit each curve to the points in this experiment are given in the table in the inset. The experiment was the same one which provided the data for Figs. 4-6. Holding voltage, -70 mV; clamp frequency, 2/min; clamp duration, 2s.

The 4-AP-sensitive current decreased throughout the 2-s voltage clamps that we used. We saw no sign that the 4-AP-sensitive current contained a component of slowly increasing outward current. In particular, any deviation of the currents from exponential decay was in the opposite direction from that which would be expected if they contained a component of increasing outward current. Thus, there is no reason to suspect that the 4-AP-sensitive current contains any $I_{k2}$ or $I_x$ currents.

**Combined Effects of 4-Aminopyridine and Low Chloride on Membrane Current**

In TEA (Kenyon and Gibbons, 1979) and in 4-AP, a phasic outward current remains. Increasing the concentration of 4-AP to 2 mM in one experiment did not cause any change in the remaining current. Since we have previously shown
a chloride-sensitive component of the early outward current (Kenyon and Gibbons, 1979) and since chloride replacement further slows the rate of phase 1 repolarization of the action potential in the presence of 4-AP (Fig. 2 of this paper), it seemed possible that the 4-AP-insensitive phasic outward current might be chloride-sensitive. Fig. 8 and 9 are taken from one of the experiments in which we recorded membrane currents in normal, 0.5 mM 4-AP, low-chloride plus 0.5 mM 4-AP, low-chloride, and recovery Tyrode's solutions. Representative experimental records are shown in Fig. 8. In each panel, the upper trace is membrane voltage and the lower trace is current. Only the first 750 ms of 2-s clamp steps are shown, and as in other records at this sweep speed, the capacity and fast inward sodium currents are not visible.

Control results at three selected voltages are shown in the left column. Currents in 0.5 mM 4-AP are shown in the center column; these resemble results in 4-AP shown in Fig. 3. As in the -29 mV record of Fig. 3, the net current at -40 mV was slightly more inward than the control for a short period after depolarization; thereafter, the \( I_{in} \) transient closely resembled the control. The early outward currents at -21 and +9 mV were much smaller than the control currents, but there was still a small early outward current that remained in 4-AP solution. This outward current is obvious in the clamp to +9 mV, and it appears in the clamp to -21 mV as a very small outward current preceding the region of net inward current.

When the chloride was replaced by methylsulfate (right column), the early outward current was further reduced. During a clamp to +9 mV in 4-AP and low chloride, the early outward current was never more outward than the
steady-state current, and there was an inward-going deflection that resembled the slow inward current seen at -40 mV. At -21 mV, the early outward current that was just visible in 4-AP was abolished in 4-AP and low chloride. At -40 mV, the current in low chloride and 4-AP was virtually identical to the corresponding record in 4-AP. At voltages positive to -40 mV, the size of the chloride-sensitive current, measured at 20 ms, was between 14 and 18% of the peak early outward current in the absence of 4-AP. This is in good agreement with our earlier finding that chloride replacement reduced the peak outward current by ~20% (Kenyon and Gibbons, 1979) and supports the idea that 4-AP and chloride reduction act independently.

When this fiber was returned to normal solution the early outward current was larger than control (not shown), indicating that the treatment used, rather than a progressive deterioration, caused the effects shown in Figs. 8 and 9. It is not uncommon for the early outward current to increase over the course of a long experiment.

The complete 20-ms current voltage relation that was obtained in this experiment is shown in Fig. 9. At -41 mV, the most negative voltage tested in this experiment, the current at 20 ms presumably represents mostly the slow inward current and was not changed by either of the treatments. At more positive voltages the control current was dominated by the early outward current and the 20-ms current voltage relation shows this large outward (positive) current. On the other hand, the 20-ms currents in 4-AP and in low chloride plus 4-AP are net inward until about -12 mV. The effect of chloride replacement in the presence of 4-AP was not different from the effects that we saw in normal solution. In those experiments, chloride reduction appeared to decrease an outward current at voltages positive to about -20 mV and the size of the chloride-sensitive current was an approximately constant fraction of the total current at more positive voltages (see Fig. 5 of Kenyon and Gibbons, 1979). This is quite similar to the effect of chloride reduction in the presence of 4-AP as shown in Fig. 9.

**Discussion**

4-AP appears to block a component of outward current at voltages more positive than -40 mV. Between -40 and -30 mV, the time-dependent portion of the current blocked by 4-AP is small, and its principal contribution to the net current is a brief outward current at the beginning of the slow inward current transient. Positive to -30 mV, the 4-AP-sensitive current makes up most of the early outward current that is a consistent feature of voltage clamp experiments in Purkinje fibers. In the presence of 4-AP, steady-state current is reduced at voltages positive to -40 mV. The size of the steady current blocked by 4-AP increases with increasing depolarization.

4-AP does not appear to change the slow inward current at voltages where an inward transient can be measured directly, not does it have much effect on the current tails that are often used to estimate the slow inward conductance at more positive potentials. The compound does not change the resting potential or the 20-ms and steady-state current-voltage relations negative to -40 mV, which implies that it does not block the inward-rectifying potassium currents,
$I_{K_1}$ and $I_{K_2}$. In this respect, 4-AP differs from cesium, another blocker of potassium currents, for cesium blocks the inward-rectifying currents without affecting the early outward current (Isenberg, 1976).

Lemeignan et al. (1975) studied the effects of 4-AP on the electrical activity of guinea pig ventricular strips. They found that 4-AP did not change the resting potential or the overshoot of the action potential. However, the action potential duration first increased and then decreased. They also reported that the maximum rate of rise of the action potential was increased and they concluded that 4-AP increased the fast sodium inward current. In the Purkinje fiber, we found no effect of 4-AP on the maximum rate of rise so it does not seem to increase the fast sodium current in this tissue. It is interesting to note the

![Figure 9](image)

Figure 9. The complete 20-ms current-voltage relations obtained in the three solutions from the experiment shown in Fig. 8. (●) Normal Tyrode's solution; (+) 0.5 mM 4-AP; (○) low chloride plus 0.5 mM 4-AP. See text for a complete discussion of the experiment and the figure.

relative lack of effect of 4-AP on the repolarization phase of the ventricular myocardium (Lemeignan et al., 1975, Fig. 1) which does not appear to have an early outward current like that seen in Purkinje fibers (Beeler and Reuter, 1977; Trautwein, 1973).

The effects of 4-AP on membrane current and on the action potential are quite consistent. The decrease in the rate of phase 1 repolarization confirms that phase 1 is dependent on the early outward current as suggested by Dudel et al. (1967), Hiraoka and Hiraoka (1975), and McAllister et al. (1975). In earlier experiments we found that although chloride replacement had no effect on the rate of phase 1 repolarization (Kenyon and Gibbons, 1977), the peak of the early outward current was significantly reduced in low-chloride solutions (Kenyon and Gibbons, 1979). The present experiments in 4-AP and in low chloride plus 4-AP support our earlier suggestion that the chloride-sensitive component of the early outward current is not activated in the normal action potential because
the chloride-insensitive early outward current repolarizes the membrane too quickly. In the presence of 4-AP, phase 1 is slower, and the chloride-sensitive component has a clear effect on the action potential. In their mathematical reconstruction of the Purkinje fiber action potential, McAllister et al. (1975) tested the effect of a reduction of the transient outward current ($I_{to}$) on the action potential configuration. Comparing their Fig. 6 B with Fig. 2 of this paper, one sees that both 4-AP and chloride replacement are necessary to produce the behavior they predicted when $I_{to}$ was eliminated.

The effects of 4-AP on the plateau and action potential duration are similar to the effects of increased extracellular calcium ion concentration described by Kass and Tsien (1976). Although both treatments cause a decrease of the net outward current observed in voltage clamp experiments, increasing the extracellular calcium ion concentration presumably increases an inward current whereas 4-AP decreases outward current. In either case, the result is a shifting of the plateau to more positive voltages. This is thought to cause a faster inactivation of the slow inward current and a faster activation of $I_h$, leading to an earlier repolarization (Kass and Tsien, 1976). 4-AP had little effect on the duration of rather short action potentials. In these cases, the increase of $I_h$ and the decrease of $I_a$ must have just counteracted the reduction of outward current caused by 4-AP.

Yeh et al. (1976 b) noted that 4-AP caused a depolarization of the squid axon that resulted in repetitive firing, whereas TEA had no effect on the resting potential. We have observed the opposite behavior in the Purkinje fiber. TEA caused a depolarization that sometimes led to spontaneous firing (Kenyon and Gibbons, 1979), whereas 4-AP did not affect the resting potential.

The Early Outward Current of the Sheep Cardiac Purkinje Fiber

The early outward peak of the total membrane current that is seen when the Purkinje fiber is voltage clamped to potentials positive to -20 mV appears to be made up of at least two outward current components with qualitatively similar but quantitatively different kinetics. One of these is sensitive to chloride replacement (Kenyon and Gibbons, 1979) and is insensitive to 4-AP. This chloride-sensitive component accounts for ~20% of the normal total early outward current as measured by chloride reduction in the absence and in the presence of 4-AP (see Figs. 8 and 9). This implies that the two components are independent. In addition, the presence of a phasic outward current in TEA solution suggests that the chloride-sensitive component may be less sensitive to TEA as well as 4-AP. The chloride-sensitive component of the total current may represent a time- and voltage-dependent chloride conductance. This interpretation is based on the assumption that chloride replacement specifically reduces the membrane chloride conductance, an assumption which recently has been questioned by Carmeliet and Verdonck (1977).

The other component of the early outward current is sensitive to 4-AP but is insensitive to chloride reduction. As revealed by subtraction of currents in 4-AP from normal currents, the 4-AP-sensitive current is rapidly activated at potentials positive to ~30 mV and decays slowly. We found no evidence that the 4-AP-
sensitive current included any of the increasing outward \( I_{K2} \) or \( I_{X1} \) currents. At voltages positive to \(-40\) mV the 4-AP-sensitive current has a steady-state component. This implies either that the early outward current fails to inactivate completely at voltages positive to \(-40\) mV or that the 4-AP-sensitive current consists of a time-dependent current that fully inactivates plus a time-independent background current. Whatever the nature of this steady component, it presumably is not the same as the background \( I_{X1} \) conductance (McAllister and Noble, 1966), which is thought to show inward-going rectification as the fiber is depolarized.

These experiments do not provide any direct evidence as to what ion carries the 4-AP-sensitive current. However, the fact that the current is blocked by two different compounds that have been shown to block potassium currents in nerve and skeletal muscle, and the fact that the current is not carried by chloride, suggest very strongly that the current is a potassium current. The sequence of activation followed by inactivation is not unusual for potassium conductances. The potassium conductance of squid axon slowly inactivates (Ehrenstein and Gilbert, 1966). Furthermore, the inactivation of the delayed rectifier of frog skeletal muscle (Nakajima et al., 1962; Adrian et al., 1966), and the \( I_x \) current of gastropod neurons (Connor and Stevens, 1971) are qualitatively similar to the 4-AP-sensitive current. We would emphasize that the evidence that the 4-AP-sensitive current is a potassium current is indirect, and we therefore cannot rule out the possible involvement of other ions (with the exception of chloride).

Peper and Trautwein (1968) suggested that the slow inward current (negative dynamic current) and the early outward current (positive dynamic current) were manifestations of a mixed current with a reversal potential of \(-30\) mV. Vitek and Trautwein (1971), however, showed that the currents were separable, and they concluded that these two current components each contributed to the total membrane current. The observation that 4-AP reduces the early outward current while not changing the inward current strongly supports the idea that the slow inward current and the early outward current are determined by different conductances.

The results that we obtained in low-chloride solutions and in the presence of 4-AP imply that one is not justified in measuring the difference between the peak of the net outward current and the steady-state net current as a specific component of the total membrane current. It was this measurement which has been referred to as the positive dynamic current, the chloride current, and \( I_{or} \) (Dubel et al., 1967; Peper and Trautwein, 1968; Fozzard and Hiraoka, 1973; McAllister et al., 1975; Hiraoka and Hiraoka, 1975). We have found that the outward current during strongly depolarizing voltage clamp steps is qualitatively as well as quantitatively different from what has been previously thought. The difference goes beyond a simple substitution of potassium for chloride as the charge carrier of the “positive dynamic current”.

When we started this work we hoped that we could find a way to eliminate the early outward current and reveal the slow inward current at voltages positive to \(-20\) mV. The current that remains in low-chloride, 4-AP solution (Fig. 8, third column) is very similar to the slow inward current that is seen at more negative
voltages in normal solutions, perhaps complicated by $I_X$ and $I_{K2}$. Further experiments are necessary before the nature of the remaining current can be stated with any certainty.

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REFERENCES

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1966. Voltage clamp experiments in skeletal muscle fibres. J. Physiol. (Lond.). 186:51P-52P.

ARMSTRONG, C. M. 1975. Ionic pores, gates, and gating currents. Q. Rev. Biophys. 7:179-210.

BEELER, G. W., and H. REUTER. 1977. Reconstruction of the action potential of ventricular myocardial fibres. J. Physiol. (Lond.). 268:177-211.

CARMELIET, E., and F. VERDONCK. 1977. Reduction of potassium permeability by chloride substitution in cardiac cells. J. Physiol. (Lond.). 255:193-206.

CONNOR, J. A., and C. F. STEVENS. 1971. Voltage clamp studies of a transient outward membrane current in gastropod neural somata. J. Physiol. (Lond.). 213:21-30.

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

EHRENSTEIN, G., and D. L. GILBERT. 1966. Slow changes of potassium permeability in the squid giant axon. Biophys. J. 6:553-566.

FASTIER, F. N., and M. A. McDOWALL. 1958. A comparison of the pharmacological properties of the three isomeric aminopyridines. Aust. J. Exp. Biol. Med. Sci. 36:365-372.

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.

GIBBONS, W. R., and H. A. FOZZARD. 1975. Relationships between voltage and tension in sheep cardiac Purkinje fibers. J. Gen. Physiol. 65:345-365.

GILLESPIE, J. I., and O. F. HUTTER. 1975. The actions of 4-aminopyridine on the delayed potassium current in skeletal muscle fibres. J. Physiol. (Lond.). 252:70P-71P.

HALDIMANN, C. 1963. Effet du tétrathylammonium sur les potentiels de repos et d'action du coeur de mouton. Arch. Int. Pharmacodyn. Ther. 146:1-9.

HIRAOKA, M., and M. HIRAOKA. 1975. The role of the positive dynamic current on the action potential of cardiac Purkinje fibers. Jpn. J. Physiol. 25:705-717.

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

KASS, R. S., and R. W. TSIEH. 1976. Control of action potential duration by calcium ions in cardiac Purkinje fibers. J. Gen. Physiol. 67:599-617.

KENYON, J. L., and W. R. GIBBONS. 1977. Effects of low chloride solutions on action potentials of sheep cardiac Purkinje fibers. J. Gen. Physiol. 70:635-660.

KENYON, J. L., and W. R. GIBBONS. 1979. Influence of chloride, potassium, and tetraethylammonium on the early outward current of sheep cardiac Purkinje fibers. J. Gen. Physiol. 75:117-138.

LEMEIGNAN, M., M. C. AUCLAIR, A. RODALLEC, and P. LECHAT. 1975. Analyse électro-
physiologique des effets de l'amiol-4 pyridine sur le lambeau ventriculaire isolé de coeur de cobaye. Arch. Int. Pharmacodyn. Ther. 216:165–176.

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.

McAllister, R. E., D. Noble, and R. W. Tsien. 1975. Reconstruction of the electrical activity of cardiac Purkinje fibres. J. Physiol. (Lond.). 251:1–59.

Meves, H., and Y. Picelon. 1977. The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. J. Physiol. (Lond.). 268:511–532.

Nakajima, S., S. Iwasaki, and K. Obata. 1962. Delayed rectification and anomalous rectification in frog's skeletal muscle membranes. J. Gen. Physiol. 46:97–115.

Noble, D., and R. W. Tsien. 1969. Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. J. Physiol. (Lond.). 200:205–231.

Pelhate, M., and Y. Picelon. 1974. Selective inhibition of potassium current in the giant axon of the cockroach. J. Physiol. (Lond.). 242:60P–62P.

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.

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

Schauf, C. L., C. A. Colton, J. S. Colton, and F. A. Davis. 1976. Aminopyridines and sparteine as inhibitors of membrane potassium conductance: effect on Maxicola giant axons and the lobster neuromuscular junction. J. Pharmacol. Exp. Ther. 197:414–425.

Siegelbaum, S. A., R. W. Tsien, and R. S. Kass. 1977. Role of intracellular calcium in the transient outward current of calf Purkinje fibres. Nature (Lond.). 269:611–613.

Trautwein, W. 1973. Membrane currents in cardiac muscle fibers. Physiol. Rev. 53:798–835.

Vitek, M., and W. Trautwein. 1971. Slow inward current and action potential in cardiac Purkinje fibers. Pfluegers Arch. Eur. J. Physiol. 323:204–218.

Wagner, H. H., and W. Ulbricht. 1975. 4-aminopyridine block of K channels and its partial relief on depolarization. Abstr. 5th Int. Biophys. Congr., Copenhagen I.U.P.A.B. 138.

Woodbury, J. W., and P. R. Miles. 1973. Anion conductance of frog muscle membranes: one channel, two kinds of pH dependence. J. Gen. Physiol. 62:324–353.

Yeh, J. Z., G. S. Oxford, C. H. Wu, and T. Narahashi. 1976a. Interactions of aminopyridines with potassium channels of squid axon membranes. Biophys. J. 16:77–81.

Yeh, J. Z., G. S. Oxford, C. H. Wu, and T. Narahashi. 1976b. Dynamics of aminopyridine block of potassium channels in squid axon membrane. J. Gen. Physiol. 68:519–535.