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OUTWARD MEMBRANE CURRENTS ACTIVATED IN THE PLATEAU RANGE OF POTENTIALS IN CARDIAC PURKINJE FIBRES

BY D. NOBLE AND R. W. TSIEN*
From the University Laboratory of Physiology, Oxford

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SUMMARY

1. The membrane currents in Purkinje fibres under voltage clamp conditions have been investigated in the range of potentials at which the action potential plateau occurs. The results show that in this range slow outward current changes occur which are quite distinct from the potassium current activated in the pace-maker range of potentials.

2. The time course of current change in response to step voltage changes is non-exponential. At each potential the current changes may be analysed in terms of the sum of two exponential changes and this property has been used to dissect the currents into two components, i_{x_1} and i_{x_2}, both of which have been found to obey kinetics of the Hodgkin–Huxley type.

3. The first component, i_{x_1}, is activated with a time constant of about 0·5 sec at the plateau. At more positive and more negative potentials the time constants are shorter. The steady-state degree of activation varies from 0 at about -50 mV to about 1 at $+20$ mV. The instantaneous current–voltage relation is an inward-going rectifier but shows no detectable negative slope. In normal Tyrode solution ($[K]_0 = 4$ mM) the reversal potential is about -85 mV.

4. The second component, i_{x_2}, is activated extremely slowly and the time constant at the plateau is about 4 sec. The steady-state activation curve varies from 0 at about -40 mV to 1 at about $+20$ mV. The instantaneous current–voltage relation is nearly linear. The reversal potential occurs between -50 and -20 mV in different preparations.

5. It is suggested that these currents are carried largely by K ions, but that some other ions (e.g. Na) also contribute so that the reversal potentials are positive to E_K.

6. The relation of these results to previous work on delayed rectification in cardiac muscle is discussed.

* Rhodes Scholar.

INTRODUCTION

Recent experiments on Purkinje fibres have shown that depolarizations to potentials within the range of the action potential plateau initiate a sequence of time and voltage dependent permeability changes which involve most of the naturally occurring ions. The first, and most rapid, changes are a substantial fall in potassium conductance (Hutter & Noble, 1960; Carmeliet, 1961; Hall, Hutter & Noble, 1963; Deck & Trautwein, 1964; Noble, 1965) and a large increase in sodium conductance (Deck & Trautwein, 1964; Dudel, Peper, Rüdel & Trautwein, 1967*b*) which is mostly inactivated within about 10 msec, although a small fraction of sodium inactivation requires about 100 msec to occur (Reuter, 1968). It is not yet certain whether this inactivation is complete (see Dudel *et al.* 1967*b*), but there are theoretical (Brady & Woodbury, 1960; Noble, 1962*a*) and experimental (Vassalle, 1966; Reuter, 1968; see also Rougier, Vassort & Stämpfli, 1968) reasons for supposing that incomplete inactivation contributes, together with the low potassium conductance, to maintaining depolarization during the plateau. A slow increase in permeability to calcium ions may also contribute a significant depolarizing current (Reuter, 1967).

In the present paper, and in the paper which follows it (Noble & Tsien, 1969), we shall be concerned with the question how the plateau is terminated and the membrane repolarized. Noble's (1962*a*) model attributed this process to activation of a potassium conductance whose subsequent decline following repolarization is responsible for the pace-maker potential. Such a potassium conductance is activated at plateau potentials (McAllister & Noble, 1966), although its kinetic and rectifier properties are substantially different from those of Noble's model (Noble & Tsien, 1968). Moreover, the time constant of activation of this conductance is very voltage-dependent. At -80 mV the time constant is of the order of a second, which is adequate to account for the pace-maker potential (Noble & Tsien, 1968). However, at -20 mV the time constant is of the order of only 50 msec (McAllister & Noble, 1966), which is too fast to account for the initiation of repolarization, except in the case of action potentials whose duration is not much longer than about 200 msec. Purkinje fibre action potentials frequently last longer than this, particularly in low K (see Noble, 1965) and Cl-free (see Hutter & Noble, 1961) solutions, which suggests that an additional mechanism may be involved in the repolarization process. We have, therefore, investigated the voltage clamp currents during long-lasting depolarizations in the region of the action potential plateau.

METHODS

The methods used have been described in detail by McAllister & Noble (1966) and Noble & Tsien (1968). The voltage clamp technique is similar to that described by Deck, Kern & Trautwein (1964). The solutions contained 140 mM-Na^+, 4 mM-K^+ 145 mM-Cl^-, 0·5 mM-Mg^{2+}, 1·8 mM-Ca^{2+}, 1·65 mM-HPO_4^{2-}, 0·7 mM-$H_2PO_4^-$ and 1 g/l. glucose. Most of the experiments were done in Cl-free solution containing 140 mM-$CH_3SO_4^-$ in place of Cl^-. About 5 mM-Cl^- remained in this solution. The solutions were saturated with oxygen and kept at a constant temperature near 35° C.

The major difficulty encountered in the experiments arises from the fact that very small current changes have to be recorded over a period of several hours in order to obtain sufficient information for a detailed analysis of the kinetics and rectifier properties. A large number of experiments were done in which a partial set of records was obtained before the preparation deteriorated or the electrodes became dislodged. These results were consistent with those of more complete experiments, although there was a significant degree of variation between fibres in the relative voltage dependency of the current components. The membrane current was continuously recorded on a pen recorder (Devices) at a constant low amplification and simultaneously at a high amplification which was varied between voltage clamp pulses to give the optimum amplification for each current record. In a typical experiment the amplification varied by a factor of 10 between the smallest and largest current records. This was essential since the current changes in the plateau region are extremely small. Noise was reduced by using a low pass filter so that only the slower current components which were being analysed were accurately recorded. An oscilloscope was used to monitor the fast components, such as the inward sodium current.

RESULTS

General characteristics of membrane currents

When long-lasting voltage clamp steps are applied to the membrane in the plateau range of voltages (0 to -40 mV) slow time and voltage dependent changes in the outward current are observed. These changes are quite distinct from the slow potassium current changes activated in the pace-maker range of potentials that have been described previously (McAllister & Noble, 1967; Noble & Tsien, 1968). This may be demonstrated in the following way. Noble & Tsien (1968) showed that the slow potassium current, i_{K_2}, whose time-dependent changes are responsible for generating the pace-maker potential, is controlled by a variable, s, which obeys first-order kinetics. The steady-state value of s, s_∞, varies from nearly 0 at -90 mV to nearly 1 at about -65 mV. Positive to -65 mV s_∞ is nearly constant. This means that when depolarizations are applied from about -65 mV (or from any potential positive to this potential) no time-dependent changes which may occur may be attributed to i_{K_2} (although some of the instantaneous variation of the current with voltage may be).

Figure 1 shows the result of an experiment in which the membrane potential was initially clamped at -60 mV. The potential was then

changed in a positive direction in 10 mV steps. The first and second steps (to −40 mV) produce only sudden changes in membrane current and it is evident that there is a negative slope in the steady-state current–voltage relation between −60 and −40 mV. Further depolarization steps produce slowly increasing outward currents. Note that these currents require several seconds to be fully activated. Their kinetics are therefore considerably slower than those of i_{K_2} in this range of potentials (see McAllister &

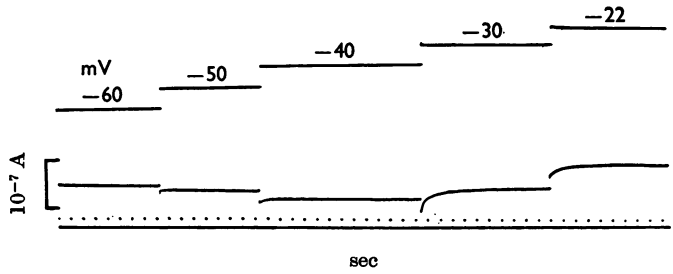

Fig. 1. Response of Purkinje fibre to 10 mV step depolarizations from −60 mV. Top: membrane potential. Bottom: membrane current. Note that little or no slow current change occurs on depolarization to −50 mV. A very small change occurs on depolarization to −40 mV. Further depolarizations produce large slow current changes in outward (upward) direction. The preparation was bathed in virtually Cl-free Tyrode solution containing 4 mM-K and maintained at a constant temperature around 35° C.

Noble, 1966). It is also clear that in this preparation the range of potentials which activates these slow currents is separated from the range which activates i_{K_2} by about 20 mV so that when long-lasting potential steps are applied there is a range within which no slow current changes occur. This means that the slow current changes activated by the plateau range of potentials may be investigated without interference due to changes in i_{K_2} simply by using potentials which are positive to −60 mV. In the experiments to be described below we have in fact chosen to use −30 mV as the potential at which the membrane is held between pulses. We shall refer to this potential as the holding potential. In addition to ensuring that there is no interference from changes in i_{K_2}, holding the membrane potential at about −30 mV also reduces interference from i_{Na} which is largely inactivated and contributes only to the steady-state current–voltage relations. The rapidly inactivated outward transient, identified by Dudel, Peper, Rüdel & Trautwein (1967*a*) as a chloride current, is also inactivated at −30 mV. Of course, it is not possible to completely eliminate interference from these current components when large hyperpolarizations from the holding potential are applied, particularly at the

termination of such hyperpolarizations. The way in which this problem may be resolved will be described later.

Figure 2 shows currents recorded in response to various depolarizations and hyperpolarizations from the holding potential. It can be seen that the magnitude of the slow increase in current greatly increases as the depolarization increases so that the steady-state current–voltage relation rectifies strongly in the outward-going direction (see Fig. 10). The magni-

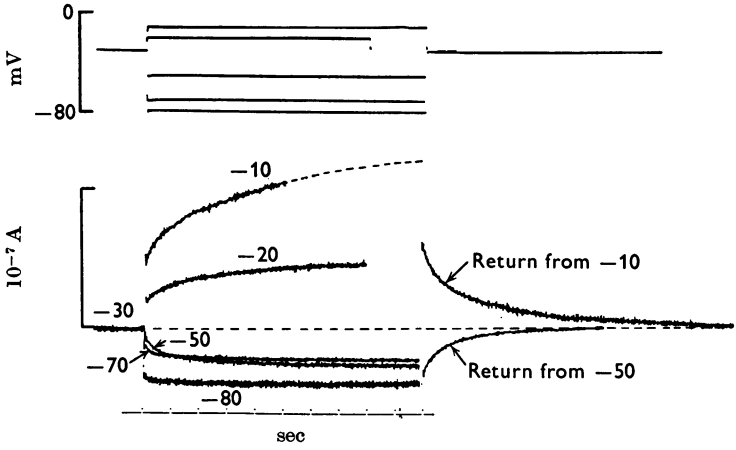

Fig. 2. Membrane currents in response to step potential changes from a holding potential (−30 mV) in the plateau range. The currents in response to steps to −10, −20, −50, −70 and −80 mV are shown. The response to −10 mV was too large to be fully recorded at the amplification used and the interrupted line showing the continuation of this response was obtained from a lower amplification record (see Fig. 3). Large slow current changes occur in response to positive steps. The current changes in response to negative steps are smaller and at −80 mV there is virtually no slow current change (Note: the slow current changes normally recorded in the pace-maker range were very small in this preparation). The records of recovery of current following returns from −10 and −50 mV are also shown. Note that the time courses are not symmetric and that time course of current following return from −10 mV contains a slow component which is almost absent in the case of the recovery from −50 mV.

tude of the current decay on repolarizing to −30 mV also increases as the magnitude of the preceding depolarization increases (see Fig. 3). The current tails therefore probably reflect the decay of the outward current activated during the depolarization. A rigorous test of this view will be described later (see Fig. 5).

Careful inspection of the records shown in Fig. 2 reveals that at least some of the current changes are not simply exponential. Both the onset of current during depolarization and the return to the steady state following repolarization show an initial relatively fast phase of current change followed by a much slower phase. This is best shown by plotting the

currents on logarithmic scales (see Fig. 4). Note also that the decay of current following depolarization is not symmetric with the recovery of current following hyperpolarization. The decay following depolarization to -10 mV shows a slow phase which is almost negligible in the recovery of current following hyperpolarization to -50 mV. The kinetics of the slow current changes in the plateau range therefore differ from those of the current changes in the pace-maker range since the latter follow simple exponential time courses and the current decays following depolarizations are symmetric in time course with the recovery of current following hyperpolarizations (Noble & Tsien, 1968, Fig. 2).

A possible hypothesis to describe current changes

Non-exponential time courses have been observed in other current systems in excitable cells. However, in those cases, the deviation from an exponential time course is in the opposite direction to that observed in the present results. Thus, in nerve cells, the sodium and potassium currents are activated slowly initially (Hodgkin & Huxley, 1952*b*), and this behaviour is explained in the Hodgkin–Huxley theory by making the current change proportional to a power of an exponential variable, the power being greater than 1. The physical interpretation of this behaviour is that more than one event obeying first-order kinetics must occur at each membrane site in order for it to conduct current. The power required to fit the current onsets in Fig. 2 would be less than 1, which does not appear to have any simple physical interpretation. Moreover, the current decays could not be fitted in this way. It is necessary therefore to use some other formulation.

Another possible hypothesis is that there are two or more kinetic processes occurring in parallel and controlling separate currents. In this case the time course of current change should be a sum of simple exponentials. By itself, this would not be particularly convincing evidence for the hypothesis since any decay curve can be fitted by a sum of exponentials if a sufficient number of terms are included in the sum. However, provided that sufficient experimental information is available, it is possible to test the hypothesis more rigorously in several ways. For simplicity (and because the assumption fits the experimental results over most of the range of potentials studied) we will assume that the time courses may be fitted by the sum of two exponentials. Let the slow time-dependent current in the plateau range be i_x and the two components assumed to change exponentially be i_{x_1} and i_{x_2}:

$$i_x = i_{x_1} + i_{x_2}. \tag{1}$$

Then

$$\Delta i_x = \Delta i_{x_1} + \Delta i_{x_2}.$$

To reduce the number of subscripts in some equations we will refer to Δi_{x_1} and Δi_{x_2} as A and B respectively,

$$\Delta i_x = A + B.$$

During each voltage clamp pulse A and B are assumed to change exponentially with time constants τ_1 and τ_2. Hence

$$\Delta i_x = A_\infty[1 - \exp(-t/\tau_1)] + B_\infty[1 - \exp(-t/\tau_2)], \tag{2}$$

where A_∞ and B_∞ are the steady-state values of A and B following a long step change in potential from the holding potential, E_H, to the potential during the pulse, E. On a logarithmic scale, A_∞ and B_∞ will determine the intercepts and τ_1 and τ_2 the slopes of the straight lines required to fit the experimental points (Fig. 4).

On return to the holding potential after a clamp pulse of duration b, the currents will return exponentially to their original values. However, since i_{x_1} and i_{x_2} will also be instantaneous functions of the membrane potential, there will be an initial sudden change during the potential step before A and B return exponentially to the original values. We will assume that these instantaneous changes may be represented separately from the slow kinetic changes (cf. Noble & Tsien, 1968). Let $\overline{i_{x_1}}$ and $\overline{i_{x_2}}$ be the maximum currents which may flow at each potential. Then we define

$$\overline{i_{x_1}} = i_{x_1}(E, x_1 = 1), \tag{3}$$

$$\overline{i_{x_2}} = i_{x_2}(E, x_2 = 1), \tag{4}$$

where x_1 and x_2 are the fractional degrees of activation of each current component. $x_1 = 1$ and $x_2 = 1$ therefore denote the fully activated states. Using this notation, sudden changes in A and B at each potential step may be attributed to changes in $\overline{i_{x_1}}$ or $\overline{i_{x_2}}$, whereas the slow changes may be attributed to changes in the degree of activation, x_1 or x_2. Note that $\overline{i_{x_1}}$ and $\overline{i_{x_2}}$ should be explicit functions of E only, whereas x_1 and x_2 are assumed to be functions of E and t.

The current change following return to the holding potential, E_H, will be given by

$$\Delta i_x = A_{t=b} \exp[(b-t)/\tau_1] + B_{t=b} \exp[(b-t)/\tau_2], \tag{5}$$

where $A_{t=b}$, $B_{t=b}$, τ_1 and τ_2 are now all measured at E_H. $A_{t=b}$ and $B_{t=b}$ are the initial values of the currents immediately following termination of the pulse. Since it is assumed that the activation variables, x_1 and x_2, do not change instantaneously, it follows that

$$A_{E_H,\, t=b} \propto A_{E,\, t=b},$$

$$B_{E_H,\, t=b} \propto B_{E,\, t=b},$$

and the constants of proportionality will be given by the ratios of fully activated currents at each potential:

$$A_{E_H} = A_E \cdot \overline{i}_{x_1, E_H} / \overline{i}_{x_1, E}, \tag{6}$$

$$B_{E_H} = B_E \cdot \overline{i}_{x_2, E_H} / \overline{i}_{x_2, E}. \tag{7}$$

Equations (1)–(7) may be used to analyse the experimental records and the results may then be used to test the hypothesis in the following ways:

1. At any particular potential, τ_1 and τ_2 should be completely independent of the magnitude, direction and duration of the preceding polarization. In practice, the best information on this is given by the very large number of current tails recorded on return to the holding potential. These should all be fitted by exponentials with the same pair of time constants (cf. Fig. 6).

2. The magnitudes of A and B measured immediately following return to the holding potential should be simple exponential functions of the pulse duration, b, and the time constants of these exponentials should be equal to those determined from currents measured during the pulse (cf. Fig. 5).

3. τ_1 and τ_2 should be smooth simple functions of E (cf. Fig. 7) since the hypothesis would be somewhat implausible if the time constants varied with E in too complex a manner. At least it would then be worth while exploring other possible formulations.

4. $A_{b=\infty}$ and $B_{b=\infty}$, measured on return to E_H so as to keep $\overline{i}_{x_1}$ and $\overline{i}_{x_2}$ constant for all measurements, should be smooth functions of E (cf. Fig. 8). These variables will be proportional to the fraction of each component activated in the steady state and should be simple sigmoid functions of E if the variables are of the Hodgkin–Huxley type.

5. If τ_1 is sufficiently small compared to τ_2 then the decay curves for small values of b should be simple exponentials with time constant equal to the value of τ_1 at the holding potential (cf. Fig. 6).

Separation of current changes into fast and slow components

The results required to test the hypothesis described above and to obtain the important variables A_∞, B_∞, $\overline{i}_{x_1}$, $\overline{i}_{x_2}$, τ_1 and τ_2 are the membrane currents during and following polarizations of different amplitudes and various durations. In order for the tests to be fully applied and the variables calculated over a sufficiently wide range of potentials, a large number of voltage clamp pulses must be analysed. We shall therefore describe the detailed analysis of our most complete experiment. Other experiments gave similar (although usually only partial) results. Some variability between preparations was observed in the voltages at which the currents

were activated and the voltages at which the currents reverse. These differences were sometimes of the order of 20 mV between preparations but it is not yet clear what factors may be responsible for this variation.

Figure 3 shows superimposed current records in response to depolarizations and hyperpolarizations of various durations from the holding potential (-30 mV) to -80, -50, -20, -10, 0, 10, 20 and 30 mV. Hyperpolarizations to other potentials were also used but are not shown in the figure since the currents are completely inactivated by a 20 mV hyperpolarization. The important general point to note about these records is that the magnitudes of the tails of current following return to the holding potential are smooth functions of the pulse duration, b. If only one kinetic process were involved, the time course of the envelope (i.e. the maximum values of the current tails plotted as functions of b) should be identical in shape with the time course of current change during the polarization (cf. McAllister & Noble, 1966, Fig. 4; Noble & Tsien, 1968, Fig. 2). When more than one process is present, however, this will be true only if the current channels obey the same current–voltage relations. As will be shown later (Fig. 9) this is not the case for the present results and further analysis is required before comparisons may be made between the time course of current during a polarization and the time course of the envelope of the current tails.

Before discussing the analysis of the results shown in Fig. 3, it is important to explain how the current measurements were made. As will become evident later, much of the analysis depends on measurements of the peak currents immediately following return to -30 mV and it is important to ensure that these currents are measured at a time when the membrane potential is accurately controlled. This may be achieved by increasing the amplification of the feed-back circuit used in clamping the membrane voltage. Unfortunately, this also increases the tendency of the circuit to oscillate and it can be seen that some of the current records shown in Fig. 3 show rapidly damped oscillations at the beginning of each tail. When tracing the current records for analysis it is relatively easy to extrapolate the current record back through the oscillation to the time at which the clamp pulse was terminated. This extrapolation gives a better measure of the true time course of the current than would be obtained using current records at lower feed-back amplification since this would introduce unknown errors due to inadequate control of the membrane potential. In the logarithmic plots shown in Figs. 4–6 only the first point depends greatly on the accuracy of the extrapolation. The general validity of the analysis does not therefore depend very much on the extrapolation, but this factor must be taken into account when assessing the accuracy of the analysis.

Figures 4, 5 and 6 show some of the currents in response to depolarization to -10 mV plotted on logarithmic scales. The points and curves shown in Fig. 4 illustrate the way in which the analysis was done. Figure 5 shows more detailed analysis of the onset of current. The current during the depolarization was subtracted from the steady-state current to give the deviation of the current from its steady-state value, i.e.

$$[\Delta i_x (E, b = \infty) - \Delta i_x (E, t)].$$

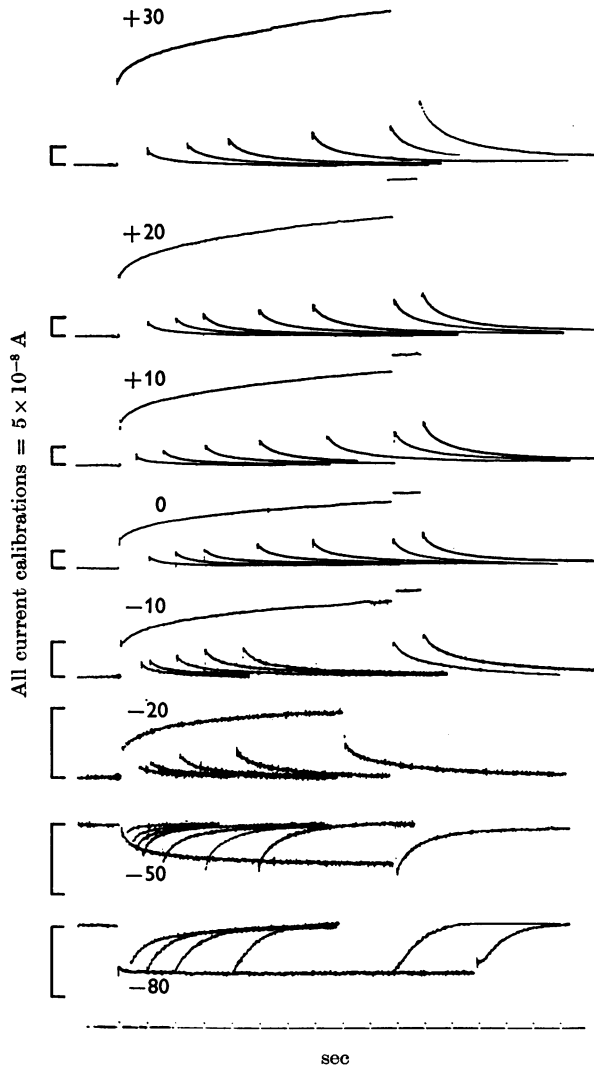

Fig. 3. Superimposed records of membrane currents in response to step depolarizations and hyperpolarizations of various magnitudes and durations from a holding potential of −30 mV. Each set of records shows a complete response to a pulse lasting 10 sec (8 sec in the case of −20 mV, 12 sec in the case of −80 mV). The tails of recovery of current following shorter pulses are shown and, in the case of depolarizations to and beyond −10 mV, the steady levels of current and tails of recovery of current following much longer pulses (20 sec in the case of −10 mV, 0 mV, +10 mV and +20 mV; 40 sec in the case of +30 mV) are also shown. These records allowed the steady-state current–voltage relations and activation curves to be obtained (see Figs. 8, 10 and 11). All current calibrations are 5×10^{-8} A. Note that current amplification was varied to give optimal amplification in each case. The steady-state current at +30 mV was too large to be shown but is plotted in Fig. 10.

The results are plotted as the filled circles in Fig. 5. The later points were fitted by eye with a straight line whose time constant, τ_2, is 5·25 sec and whose intercept, B_∞, with the current axis is $6{\cdot}6 \times 10^{-8}$ A. This line was then subtracted from the filled circles to give the remaining current change which should be attributable to the fast component, A. The results are

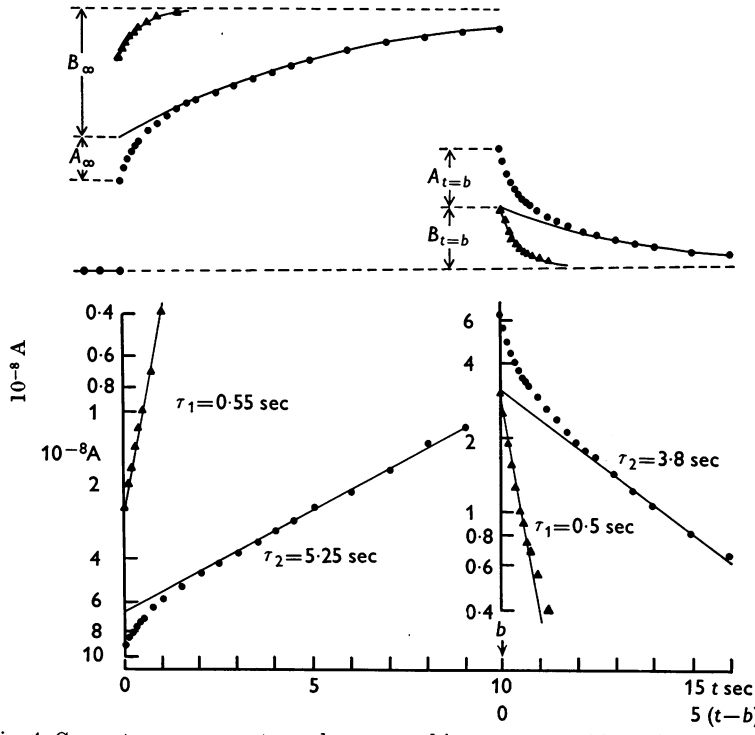

Fig. 4. Current measurements made on record in response to 10 sec depolarization to −10 mV illustrating method of analysis. The filled circles plotted on linear scale (top) show measurements of membrane current made on a tracing of original record which is shown in Fig. 2. These results were then plotted on logarithmic scales (bottom). The continuous lines were obtained by fitting logarithmic plots with straight lines through later points and the exponential lines on the linear plot were obtained from these lines. The filled triangles were obtained by subtracting slow exponential component to give fast component. $A_{t=b}$, $B_{t=b}$, A_∞, B_∞, τ_1 and τ_2 refer to quantities used in equations (1)–(7).

plotted as the filled triangles in Fig. 5. Note that the points are a good fit to a line whose time constant, τ_1, is 0·55 sec and whose intercept, A_∞, is $2{\cdot}3 \times 10^{-8}$ A. This result shows that the onset of time-dependent current during depolarization to −10 mV is given fairly accurately by the sum of two exponentials. Now if $A_{E_H} \propto A_E$ and $B_{E_H} \propto B_E$ (equations (6) and (7)) then the envelope of tails of current on return to the holding potential should be fitted by exponentials with the same time constants, though not

necessarily the same intercepts. The envelope of the tails has been plotted as the open circles in Fig. 5. As can be seen the points may be fitted by the same pair of time constants (the open triangles are obtained in the same way as the filled triangles). The value of B_{∞,E_H} is $4{\cdot}4 \times 10^{-8}$ A which is considerably smaller than $B_{\infty,E}$. This indicates that the current carried by

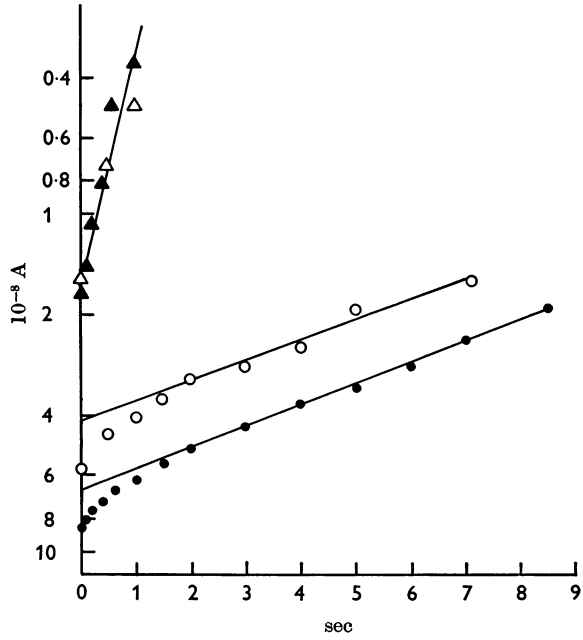

Fig. 5. Analysis of time course of current activated by depolarization to -10 mV. The slow current change during the depolarization is measured as the deviation of the current from the steady-state value and is plotted as filled circles (●). The slow component was fitted by a straight line with intercept $6{\cdot}6 \times 10^{-8}$ A and time constant 5·25 sec. This line was then subtracted from the points to give the fast component (▲). This component was then fitted with a straight line with intercept $2{\cdot}3 \times 10^{-8}$ A and time constant 0·55 sec. The open circles were obtained by measuring the peaks of the tails of current following return to the holding potential. The slow component in this case has the same time constant as the slow component during the depolarization but the intercept is smaller ($4{\cdot}4 \times 10^{-8}$ A). The open triangles (△) were obtained by subtraction and are fitted by the same line as the filled triangles. The coincidence of the filled and open triangles is not significant for the analysis of the time course of current but it does illustrate the non-linearity of the fast current component (see Fig. 9).

the slow component is larger at -10 mV than at -30 mV for a given degree of activation of the system. By contrast, the values of A_{∞,E_H} and $A_{\infty,E}$ are virtually equal which means that the influence of sudden voltage changes on the fast component of current is very small between these two voltages (see also Fig. 9).

The tails of current on return to −30 mV after 0·2, 3·5 and 17 sec at −10 mV are plotted in Fig. 6 as filled circles. At 0·2 sec, the points are fitted by one fast exponential with a time constant equal to 0·5 sec. This is to be expected since by this time only negligible activation of the slow component will have occurred (Fig. 5). After 17 sec, by contrast, two exponentials are required. The slow component was obtained by fitting

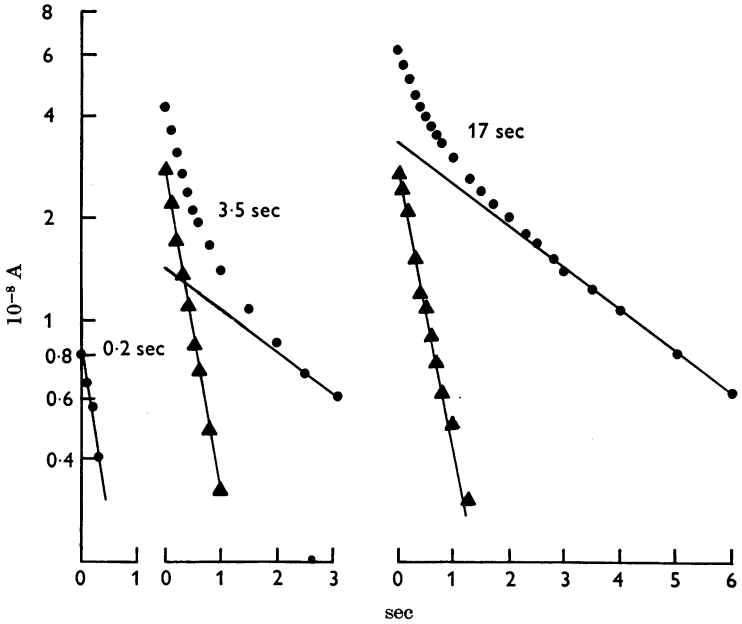

Fig. 6. Analysis of current tails following depolarizations to −10 mV lasting 0·2, 3·5 and 17 sec. The measured currents are plotted as filled circles (●). In the case of the tail following 17 sec depolarization the slow component was fitted by a straight line with intercept $3{\cdot}3 \times 10^{-8}$ A and time constant 3·8 sec. The fast component (▲) was then obtained by subtraction and fitted by a line with intercept $2{\cdot}7 \times 10^{-8}$ A and time constant 0·5 sec. The tail following 3·5 sec depolarization was fitted in a similar way using a slow component with the same slope as for the 17 sec tail. The 0·2 sec tail shows no slow component and has the same slope as the fast components of the 3·5 and 17 sec tails.

the later points, when the fast component may be assumed to be fully deactivated. The fast component (filled triangles) was then obtained by subtraction and is fitted by an exponential with the same time constant as that required to fit the tail following 0·2 sec. The same two exponentials fit the 3·5 sec tail. Note that the intercept of the fast component in this case is about equal to that following the 17 sec pulse. This result is expected since the fast component is fully activated by 3·5 sec (Fig. 5). By contrast, the intercept of the slow component is much smaller than that

following 17 sec. The intercepts following the longest pulses give a second pair of estimates of A_∞ and B_∞ at E_H which compared well with the estimates obtained from plotting the envelope of the current peaks. For subsequent analysis the average of these two estimates of A_∞ and B_∞ have been used.

All the current records were analysed in this way and, with two exceptions, the results were entirely consistent with the hypothesis that two current components are present, each one of which is controlled by a first-order variable with voltage-dependent kinetics.

The exceptions occurred in the case of the responses to the largest polarizations. The first exception was noted at +30 mV. Very long-lasting depolarizations to this potential produced tails of current on return to −30 mV which required three exponentials. The third component had a time constant of 0·8 sec. There are three reasons for considering this to be a genuine third component at very strong depolarizations rather than a change in the behaviour of the other two components:

1. The other two exponentials required to fit the current tail following long-lasting depolarization to +30 mV had time constants equal to those of the fast and slow decays following all other polarizations.

2. Short-lasting depolarizations to +30 mV were followed by tails which required only the usual two components. It is evident that the third component is activated extremely slowly, as is indicated by the fact that at least 40 sec were required to achieve a steady-state current at +30 mV.

3. A short-lasting (2 sec) depolarization to +50 mV was also followed by a two-component tail. This shows that for all the potentials and durations of physiological interest, the third component is never activated. It has, therefore, been ignored in the analysis.

The second exception occurred after long-lasting hyperpolarizations beyond −70 mV. In this case, the currents on return to −30 mV differed qualitatively from all the other records. First, they showed an initial inward sodium transient. However, this component was not recorded on the pen tracings since it was too fast. It is, therefore, not shown in Fig. 3. Secondly, it can be seen that, in the case of the hyperpolarization to −80 mV, the current tails increase in amplitude up to about 5 sec but decrease slightly thereafter. This is attributable to an outward transient which reduces the size of the inward current tail at its beginning. The transient decays completely by 200 msec since after this time the current tails have the same time course as those following shorter duration hyperpolarizations. This current transient is even larger when the membrane potential is returned to potentials positive to −30 mV and has been described previously (Deck & Trautwein, 1964; Hecht, Hutter & Lywood, 1964). Dudel *et al.* (1967*a*) attribute this current to chloride ions. In the present case, very little (< 5 mM) chloride was present which suggests that not all of this current is carried by chloride ions. However, this particular problem need not be resolved in order to take account of the transient in the present analysis. It has already been shown (Reuter, 1968; D. Noble & R. W. Tsien, unpublished) that at the resting potential this current recovers from inactivation only very slowly, the time constant of recovery being about 5 sec. Hence hyperpolarizations from the plateau range which do not last longer than about 2 sec should not activate very much of this current at the termination of the pulse. Since the time constants of i_x are quite short at hyperpolarized potentials, it is possible to fully analyse the behaviour of i_x without interference from the outward transient. However, it is of some importance to include this transient in the total repolarizing current when calculating the repolarization process on the basis of voltage clamp data (Noble & Tsien, 1969).

Kinetics

The results described above suggest that the slow outward current changes in the plateau range depend on two first-order processes obeying the differential equations

$$dx_1/dt = \alpha_{x_1}(1-x_1)-\beta_{x_1}x_1, \tag{8}$$

$$dx_2/dt = \alpha_{x_2}(1-x_2)-\beta_{x_2}x_2. \tag{9}$$

In the steady state at each potential

$$(x_1)_\infty = \alpha_{x_1}/(\alpha_{x_1}+\beta_{x_1}), \tag{10}$$

$$(x_2)_\infty = \alpha_{x_2}/(\alpha_{x_2}+\beta_{x_2}) \tag{11}$$

and, following a step change in potential, x_1 and x_2 should change exponentially with time constants given by

$$\tau_1 = 1/(\alpha_{x_1}+B_{x_1}), \tag{12}$$

$$\tau_2 = 1/(\alpha_{x_2}+\beta_{x_2}), \tag{13}$$

τ_1 and τ_2 have been determined experimentally over a range of potentials from -120 mV to $+30$ mV and their reciprocals are plotted in Fig. 7. τ_1^{-1} is a U-shaped function of membrane potential with a minimum rate at about the level of the plateau and much faster rates at hyperpolarized potentials. The rate also increases somewhat at positive potentials. Hence, x_1 should be activated fairly slowly at the plateau but should decline more quickly in the region of the resting potential. τ_2^{-1} is also at a minimum in the plateau range and becomes larger on hyperpolarization. However, we could find no evidence for an increase of τ_2^{-1} at positive potentials. We doubt whether our results are accurate enough to exclude the possibility of any increase but they probably are accurate enough to exclude an increase of the magnitude shown in the curve for τ_1^{-1}.

In order to extract the rate coefficients, the α's and β's, from these results we must also know the steady-state degree of activation of each component as a function of potential. These values are proportional to the values of A_∞ and B_∞ at E_H obtained in the analysis described in the previous section since these variables give the steady-state degree of activation of each component in terms of the current recorded immediately after return to the holding potential. The advantage of measuring the degree of activation in this way is that the results depend on current measurements which are always made at the same potential. Hence, provided that the shapes of the instantaneous current–voltage relations are constant, non-linearities in these relations will not interfere with the analysis. The values of A_∞ and B_∞ are plotted as functions of the membrane potential in Fig. 8. It

can be seen that both variables are simple sigmoid functions of the membrane potential. The fast component, A_∞, is half-activated in the steady state at about -20 mV. The slow component requires more positive potentials. The curves have also been normalized and the normalized values were used as estimates of $(x_1)_\infty$ and $(x_2)_\infty$. It is now possible to use equations (10)–(13) to calculate the values of the α's and β's and the results are shown in Fig. 7 of the following paper (Noble & Tsien, 1969).

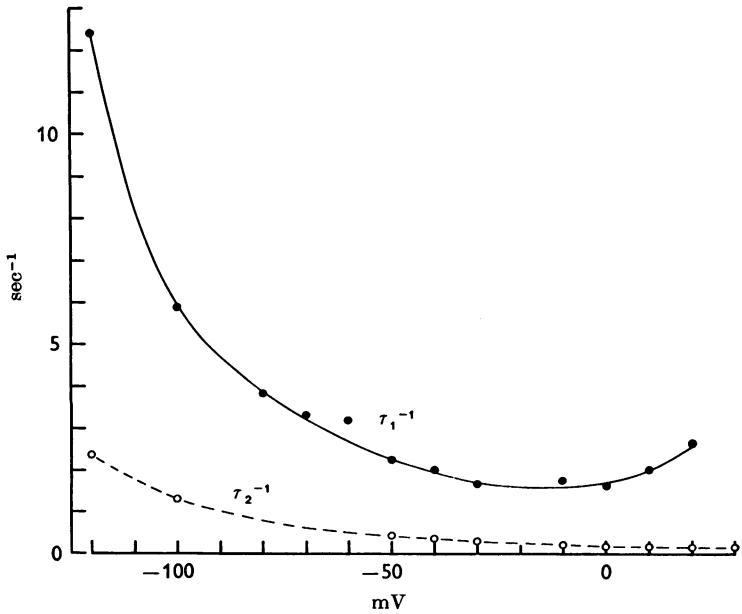

Fig. 7. Variation of rates of slow current changes, measured as reciprocal time constants (τ_1^{-1} and τ_2^{-1}), with membrane potential. The points were obtained from measurements of the slopes of the lines determined by the method explained in Figs. 4, 5 and 6. The continuous curves were drawn to fit the points by eye.

It should be emphasized that, although the curves shown in Fig. 8 are fairly typical, there is considerable variation between preparations, particularly in the position of the x_2 activation curve on the voltage axis. In some preparations the activation curve was at considerably more negative potentials and, since the reversal potential for i_{x_2} is at a fairly depolarized potential (see Fig. 9), in these preparations it was possible to activate x_2 at potentials at which i_{x_2} is negative. The result was a very slow change in current in an inward direction on depolarization of the membrane.

Current–voltage relations

In addition to determining the kinetics of the current changes, the membrane potential also has a more direct influence on the membrane current. This direct influence may be conveniently represented in terms of the current–voltage relation for a constant degree of activation of the

kinetic variable. Since it is not possible to study the current components in isolation it is necessary to use indirect methods. The information required for reconstructing the current–voltage relations is contained in the ratios A_E/A_{E_H} and B_E/B_{E_H} since these ratios give an estimate of the currents at E and E_H for the same degree of activation. If these ratios are multiplied by the total current amplitudes of the steady-state activation

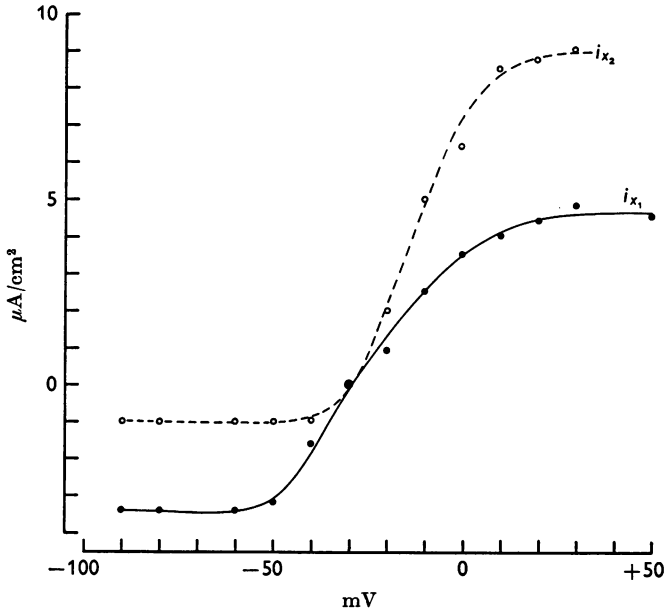

Fig. 8. Steady-state variation in the degree of activation of x_1 and x_2 measured in terms of the currents (A_∞ and B_∞) following long-lasting voltage clamp steps. All measurements are therefore made at the same potential (−30 mV) so that the properties of the fully activated current–voltage relation are eliminated. Continuous curves were fitted by eye to points.

curves (Fig. 8) we obtain the values of i_{x_1} and i_{x_2} as functions of E when x_1 and x_2 equal 1, i.e. rearranging equations (6) and (7):

$$\overline{i}_{x_1,E} = \overline{i}_{x_1,E_H} \frac{A_{E,t=b}}{A_{E_H,t=b}}, \tag{14}$$

$$\overline{i}_{x_2,E} = \overline{i}_{x_2\ E_H} \frac{B_{E,t=b}}{B_{E_H,t=b}}, \tag{15}$$

where $\overline{i}_{x_1,E_H}$ and $\overline{i}_{x_2,E_H}$ are given by the total amplitudes of the curves plotted in Fig. 8. Since these equations give the instantaneous current–voltage relations when the kinetic variable, x_1 or x_2, is fully activated, we shall refer to the resulting current–voltage relations as the 'fully activated

current–voltage relations'. We have previously used the term 'rectifier function' for this relation (Noble & Tsien, 1968, Fig. 11) but this term seems inappropriate when it is possible for the current–voltage relation to show virtually no rectification (as is the case for i_{x_2}—see below). Moreover, the new term indicates more clearly the condition (i.e. maximum activation of the conducting channels at each voltage) to which the relation applies.

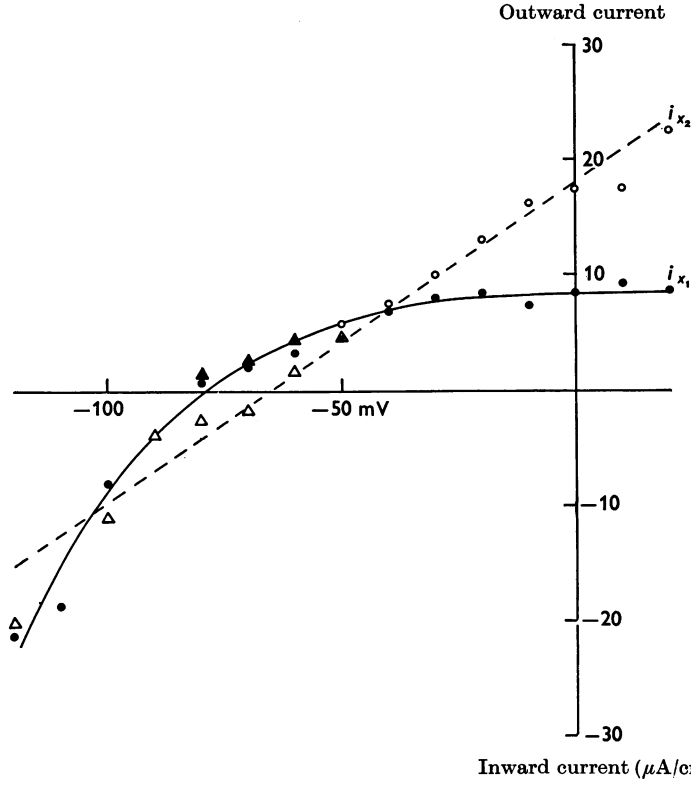

Fig. 9. Fully activated current–voltage relations for i_{x_1} (● ▲) and i_{x_2} (○ △) obtained from the experimental results using equations (14) and (15). The circles were obtained from results using −30 mV as the holding potential. The triangles were obtained by using −20 mV as the holding potential. Curves fitted to points by eye.

The fully activated current–voltage relations obtained using equations (14) and (15) are shown in Fig. 9. $\overline{i_{x_1}}$ shows inward-going rectification although, unlike i_{K_2} (see Noble & Tsien, 1968, Fig. 9), the current–voltage relation does not show a negative slope. The reversal potential in this preparation is about −85 mV. This does not correspond to any known equilibrium potential, particularly since chloride ions are virtually absent from the solutions. Moreover, the reversal potential varies to some extent

between fibres so that the more likely explanation is that the pathways conduct mainly K ions (the K equilibrium potential is about -100 mV) with some leakage to other ions so as to give a reversal potential positive to E_K. Further experiments are required to test this hypothesis, although some preliminary experiments have shown that the reversal potential does vary with $[K]_0$ (O. Hauswirth, D. Noble & R. W. Tsien, unpublished).

$\overline{i_{x_2}}$ is fitted quite well by a straight line at all but the most negative potentials, where there is some suggestion of non-linearity. The reversal potential in this case is about -65 mV which is 20 mV positive to the reversal potential for i_{x_1}. This means that voltage pulses which deflect the membrane potential from the plateau range to a potential between the two reversal potentials (e.g. -75 mV) should produce a positive i_{x_1} component followed by a negative i_{x_2} component. This was found to be the case although it is not easy to obtain records of this kind since the currents are very small at potentials close to the reversal potentials. The reversal potential for i_{x_2} also fails to correspond to any known equilibrium potential and it seems likely that this current is also relatively non-specific.

The current–voltage relations shown in Fig. 9 were determined over a very wide range of potentials, including the range negative to -70 mV. It was unusual to obtain the relations for i_x over such a wide range since i_{K_2} normally dominates the time-dependent changes which occur negative to -70 mV. However, in some preparations (including the one used to obtain Fig. 9) i_{K_2} is relatively small and the tendency to pace-maker activity nearly absent. Since the time constants for i_x are fairly short at negative potentials, it is possible in these preparations to obtain measurements of i_x from the negative tails of current which occur before appreciable changes in i_{K_2} (which produce positive tails at potentials positive to -100 mV) occur. In preparations with a strong tendency to pace-maker activity and large changes in i_{K_2}, i_x may not be estimated accurately at very negative potentials but it is usual to observe a negative tail which is relatively fast compared to the positive tail attributable to the decline in i_{K_2}. Reuter (1968) has described some of the properties of the negative tails on repolarization and it is likely that i_x is mainly responsible for the tails which he found were not abolished by sodium removal.

The results shown in Figs. 7, 8 and 9 completely specify the behaviour of i_x. Each component may be calculated from the equations

$$i_{x_1} = x_1 \overline{i_{x_1}}, \tag{16}$$

$$i_{x_2} = x_2 \overline{i_{x_2}}, \tag{17}$$

where x_1 and x_2 obey equations (8) and (9). These equations will be used in the following paper to reconstruct the behaviour of i_x during the action potential.

Components of the steady-state current–voltage relation

In order to determine the contribution of i_x to the electrical activity of the Purkinje fibre membrane, it is also necessary to know the electrical properties of the membrane in the absence of i_x. As yet, we have no means

of abolishing i_x and the only method for determining the electrical properties in the absence of i_x is to subtract i_x from the total recorded current. This was done by plotting the total steady-state current–voltage relation and then subtracting the calculated steady-state values of i_{x1} and i_{x2} given by the equations

$$(i_{x_1})_\infty = (x_1)_\infty \, \overline{i}_{x_1}, \tag{18}$$

$$(i_{x_2})_\infty = (x_2)_\infty \, \overline{i}_{x_2}. \tag{19}$$

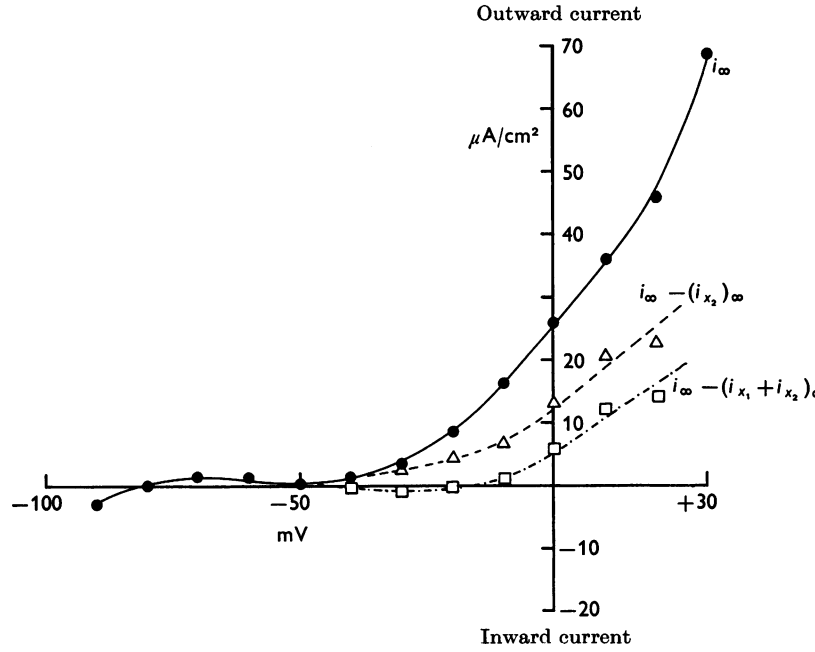

Fig. 10. Current–voltage relations illustrating the contributions of i_{x_1} and i_{x_2} to the steady-state membrane current. The filled circles (●) show the total steady-state current–voltage relation (i_∞). The open triangles (△) show the relation after subtraction of $(i_{x_2})_\infty$, using the experimental results and eqn. (19). The open squares (□) were then obtained by also subtracting $(i_{x_1})_\infty$, using the experimental results and eqn. (18).

The results are shown in Fig. 10. The filled circles and continuous curve show the total steady-state current–voltage relation obtained from the experimental results. As shown previously (Deck & Trautwein, 1964) this relation is N-shaped with a negative slope conductance between −50 and −70 mV. Note, however, that the chord conductance is always positive. The open triangles and interrupted line show the current–voltage relation after subtraction of $(i_{x_2})_\infty$. The open squares and dot-dash line show the current–voltage relation after subtraction of $(i_{x_1})_\infty$ and $(i_{x_2})_\infty$. The latter

relation has a much more extensive negative slope conductance region and the chord conductance also becomes negative between -15 and -50 mV. These features are shown more clearly in Fig. 11 which shows the same curves plotted on a tenfold larger current scale (note that a tenfold range of current amplification was used, and found necessary, in these experiments). Some general conclusions concerning the role of i_x may be drawn immediately from Fig. 11. First, in this preparation, i_{x_2} is not required for repolarization since the subtraction of i_{x_2} does not produce a region of net

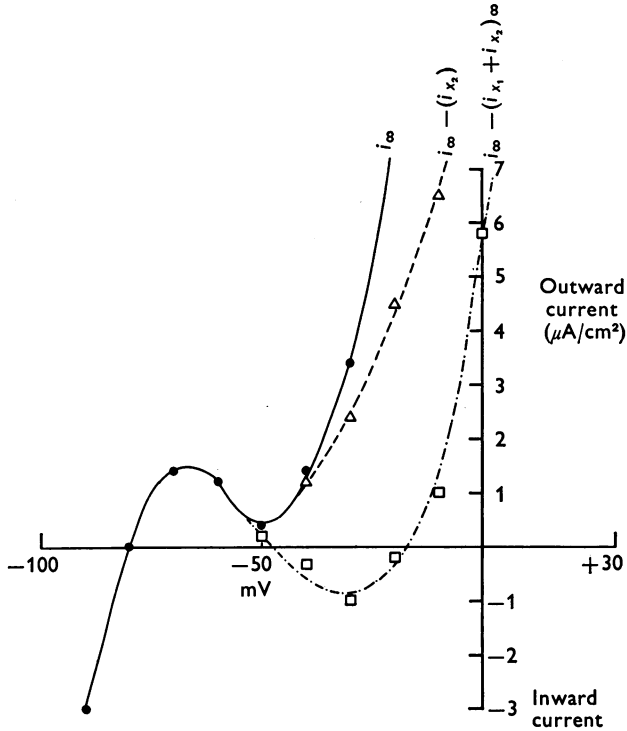

Fig. 11. Current–voltage relations on ten times large current scale. Same symbols as Fig. 10. Note that there is a region of net inward current in the current–voltage relation (dot-dash curves) when the contributions of i_{x_1} and i_{x_2} have been subtracted from the total steady-state current.

inward current. Moreover, as shown above (see *Kinetics*) the time constants of i_{x_2} are so long that it is activated extremely slowly. However, i_{x_1} is required for repolarization in this case since the current–voltage relation does not lose its region of net inward current unless i_{x_1} is at least partially activated. The question which now arises therefore is whether the properties of i_{x_1} allow the repolarization phase of the Purkinje fibre action potential to be reproduced. This question will be discussed in the following paper (Noble & Tsien, 1969).

The current–voltage relation obtained after subtraction of i_x is that for all other currents (i_{Na}, i_{K_1}, i_{K_2}, i_{Ca}) in the steady state. Chloride current is not involved since the chloride concentration was extremely low. The presence of a negative current region in this relation may be accounted for if at least one of the inward currents (i_{Na}, i_{Ca}) is incompletely inactivated in the steady state. Further analysis of this current–voltage relation will be presented in another paper (R. E. McAllister, D. Noble & R. W. Tsien, in preparation).

DISCUSSION

The major conclusion of this paper is that the slow current changes in the plateau range of potentials can be accounted for quantitatively by two separate current components whose kinetics may be described by the first-order variables we have labelled x_1 and x_2. The role of these currents in electrical activity in Purkinje fibres will be discussed in later papers (Noble & Tsien, 1969; Hauswirth, Noble & Tsien, 1969). We shall therefore restrict the present discussion to the relation of our results to previous work.

Relation of results to previous work on delayed rectification

Delayed rectification was first observed in Purkinje fibres by Hutter & Noble (1960). Hall *et al.* (1963) and McAllister & Noble (1966) showed that in many preparations in Na-free solutions a delayed increase in conductance occurs when the membrane is depolarized beyond about -30 mV. Up to -30 mV the only change observed is a sudden decrease in conductance on depolarization which is similar to the inward-going (anomalous) rectification observed in skeletal muscle (Katz, 1949; Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962). These properties of the membrane were both attributed to changes in potassium conductance. The fast changes due to inward-going rectification were described by assuming a non-time-dependent K current, i_{K_1}. The slow current changes responsible for delayed rectification at strong depolarizations were separately described by a time-dependent component, i_{K_2}. Together with a component of excitatory sodium current, a model incorporating these two K currents (Noble, 1962*a*) was adequate to reproduce Purkinje fibre action potential and pace-maker activity. This model explains repolarization in terms of the activation of i_{K_2} and the pace-maker potential by its deactivation. In addition to providing the large initial excitatory current, the sodium component provides a residual inward current that nearly balances the outward K current during the plateau.

Subsequent experiments showed that there are difficulties with this model. The application of the voltage clamp technique (Deck & Trautwein, 1964; Hecht *et al.* 1964; McAllister & Noble, 1966) confirmed the presence

of inward-going rectification and also revealed a region of negative slope in the current–voltage relation in sodium-free solutions. However, there has been considerable disagreement about the existence of delayed rectification and confusion concerning the voltages at which it is activated. Our results suggest a way in which these disagreements may be resolved and it will be convenient to discuss the previous results in the presence and absence of sodium ions separately.

Delayed rectification in sodium containing solutions. It is now clear that in the presence of Na ions there are *two* voltage ranges at which delayed rectification may be observed. In the pace-maker range (-90 to -60 mV) large outward current changes may be observed in response to quite small voltage steps. These currents are attributable almost entirely to K ions since the reversal potential occurs at the K equilibrium potential (Deck & Trautwein, 1964; Vassalle, 1966) and shifts with E_K when $[K]_0$ is varied (Noble & Tsien, 1968). The major differences between this current and the i_{K_2} component assumed by Noble (1962*a*) are that it is activated by small depolarizations (McAllister & Noble, 1967) and that the instantaneous current–voltage relation rectifies strongly in the inward-going direction (Noble & Tsien, 1968, Fig. 9). However, since this was the first time-dependent outward current to be identified in voltage clamp experiments, it was labelled i_{K_2}.

The present results show that delayed rectification also occurs at a voltage range (positive to -40 mV) which closely corresponds to the range over which Noble assumed i_{K_2} to be activated. This delayed rectification is attributable to the two components we have labelled i_{x_1} and i_{x_2}, both of which have reversal potentials which suggest that they are carried largely but not exclusively by K ions. It would clearly be confusing to change the notation yet again and we suggest that i_{K_2} be retained for the specific K current activated in the pace-maker range and that the less specific currents should be called i_{x_1} and i_{x_2}. This notation clearly distinguishes them from i_{K_2} and emphasizes the fact that they are not pure K currents.

Using the ramp voltage clamp technique, Dudel, Peper, Rüdel & Trautwein (1967*c*) have described results which they interpret as showing that delayed rectification is completely absent in Purkinje fibres. Their results may, however, be given a different interpretation which will be explained in the following paper (Noble & Tsien, 1969) where the role of the time-dependent outward currents in repolarization is discussed.

Delayed rectification in sodium-free solutions. Deck & Trautwein (1964) and McAllister & Noble (1966) found that in Na-free solutions the outward current changes in the pace-maker range are greatly reduced or even absent. McAllister & Noble (1966) suggested that this may be due to a

large shift in the threshold for activation of i_{K_2} on removing Na ions. This explanation seemed reasonable at that time since they observed a large degree of time-dependent outward current at strong depolarizations and it was not known at that time that this current is also present and is activated in the same voltage range in Na solutions. The present results show that this hypothesis is wrong and that the delayed rectification in Na-free solutions observed by Hutter & Noble (1960), Hall *et al.* (1963) and McAllister & Noble (1966) must be attributed to i_{x_1} and possibly also to i_{x_2}. This interpretation is strengthened by the observation that sodium removal does not greatly influence the x kinetics (D. Noble & R. W. Tsien, unpublished).

This interpretation of previous work leaves two major problems unresolved. First, delayed rectification in Na-free solutions was not observed by some workers (Deck & Trautwein, 1964; Hecht & Hutter, 1965), and Hall *et al.* (1963) and McAllister & Noble (1966) did not observe it in all preparations. Hall *et al.* (1963) attributed this fact to cable complications since they were working on long fibres. However, the results obtained since 1964 have been obtained on short fibres in which cable complications are negligible (Deck *et al.* 1964). A more likely reason for failing to observe delayed rectification is that it is easily masked by the large initial outward transient identified by Dudel *et al.* (1967*a*) as a chloride current. When this current is inactivated by steady depolarization (as in Fig. 1) it is relatively easy to observe delayed rectification. The outward transient is also largely inactivated when depolarizations from the resting potential are applied at a frequency of 1 Hz or above (Reuter, 1968; D. Noble & R. W. Tsien, unpublished). This suggests that it would be easier to observe delayed rectification in preparations that are regularly pulsed at a rate more closely corresponding to the normal frequency of Purkinje fibre activity.

The second problem which remains unresolved is the effect of Na removal on i_{K_2}. Preliminary experiments (O. Hauswirth, D. Noble & R. W. Tsien, unpublished) indicate that Na removal does not greatly influence the s kinetics but simply reduces the amplitude of the slow K current changes attributable to i_{K_2}. The nature of this effect is not yet clear and more experiments will be required to resolve this problem.

Rougier *et al.* (1968) have recently succeeded in voltage-clamping frog auricular muscle and have demonstrated the presence of delayed rectification in normal and in sodium-free solutions. As yet, there is insufficient information available to allow quantitative comparison, although H. F. Brown & S. J. Noble (personal communication), using a technique similar to that of Rougier *et al.* (1968), have analysed a current component which strongly resembles the component we have called i_{x_2}.

Possible significance of instantaneous current–voltage relations

Perhaps the most surprising outcome of the present work is that there are not one but at least three separate time-dependent outward currents in Purkinje fibres. There may even be a fourth component activated at positive potentials outside the normal range (see Results). All of these currents are proportional to first-order variables of the Hodgkin–Huxley type. Apart from quantitative differences in the kinetics, which will be discussed in the following paper (Noble & Tsien, 1969, Fig. 7), the major difference between these currents is that they obey different instantaneous current–voltage relations. The expression i_{K_2} $(E, s = 1)$, which, using the notation of the present paper, is $\overline{i_{K_2}}$, is a strong inward-going rectifier with a marked negative slope (Noble & Tsien, 1968, Fig. 9). Similarly $\overline{i_{x_1}}$ is a less marked inward-going rectifier with no negative slope and $\overline{i_{x_2}}$ is nearly linear (see Fig. 9 of present paper). i_{K_2} is a pure K current; i_{x_1} and i_{x_2} are mixed currents, i_{x_1} having a reversal potential closer to E_K than i_{x_2}. It seems, therefore, that the degree of rectification becomes weaker as the specificity of the systems for K ions becomes smaller. It is tempting to speculate that each non-specific current component may be a mixture of currents passing through two kinds of channel: one highly specific for K ions and having rectification properties similar to those for i_{K_2}, and another which is a linear non-specific shunt. The only common feature of the two channels (and the only reason why they are considered together as one current component) would be that they are controlled by gating mechanisms obeying the same kinetics. This view has no concrete evidence in its favour but it is in line with the view, suggested by the separability of the kinetic and rectifier properties (Armstrong & Binstock, 1965; Armstrong, 1966; Noble & Tsien, 1968), that these properties correspond to separate molecular mechanisms since it suggests that the basic molecular mechanisms may be combined in different ways to produce a whole range of electrical characteristics.

The analysis of conductance mechanisms in terms of separable kinetic and rectifier properties is not, of course, new. It is implicit in Hodgkin & Huxley's (1952*b*) original notation, which uses $\bar{g}\ (E - E_{rev})$ to specify the fully activated current–voltage relation, since it was evident that the latter could not be strictly linear at all ionic concentrations (Hodgkin & Huxley, 1952*a*, Fig. 7). Frankenhaeuser (1963) carried this approach a stage further by using the constant field equations to describe the instantaneous current–voltage relations in myelinated nerve. These equations describe outward-going rectification. The more recent results (Armstrong, 1966; Noble & Tsien, 1968; present paper) show that Hodgkin–Huxley kinetics may also control channels showing inward-going rectification.

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REFERENCES

ADRIAN, R. H. & FREYGANG, W. H. (1962). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

ARMSTRONG, C. M. (1966). Time course of TEA^+-induced anomalous rectifications in squid giant axons. *J. gen. Physiol.* **50**, 491–503.

ARMSTRONG, C. M. & BINSTOCK, L. (1965). Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. gen. Physiol.* **46**, 859–872.

BRADY, A. J. & WOODBURY, J. W. (1960). The sodium–potassium hypothesis as the basis of electrical activity in frog ventricle. *J. Physiol.* **154**, 385–407.

CARMELIET, E. E. (1961). Chloride ions and the membrane potential of Purkinje fibres. *J. Physiol.* **156**, 375–388.

DECK, K. A. & TRAUTWEIN, W. (1964). Ionic currents in cardiac excitation. *Pflügers Arch. ges. Physiol.* **280**, 63–80.

DECK, K. A., KERN, R. & TRAUTWEIN, W. (1964). Voltage clamp technique in mammalian cardiac fibres. *Pflügers Arch. ges. Physiol.* **280**, 50–62.

DUDEL, J., PEPER, K., RÜDEL, R. & TRAUTWEIN, W. (1967*a*). The dynamic chloride component of membrane current in Purkinje fibres. *Pflügers Arch. ges. Physiol.* **295**, 197-212.

DUDEL, J., PEPER, K., RÜDEL, R. & TRAUTWEIN, W. (1967*b*). The effect of tetrodotoxin on the membrane current in cardiac muscle (Purkinje fibres). *Pflügers Arch. ges. Physiol.* **295**, 213–226.

DUDEL, J., PEPER, K., RÜDEL, R. & TRAUTWEIN, W. (1967*c*). The potassium component of membrane current in Purkinje fibres. *Pflügers Arch. ges. Physiol.* **296**, 308–327.

FRANKENHAEUSER, B. (1963). A quantitative description of potassium current in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol.* **169**, 424–430.

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

HAUSWIRTH, O., NOBLE, D. & TSIEN, R. W. (1969). The mechanism of oscillatory activity at low membrane potentials in cardiac Purkinje fibres. *J. Physiol.* **200**, 255-265.

HECHT, H. H. & HUTTER, O. F. (1965). The action of pH on cardiac muscle. In *Electrophysiology of the Heart*, ed. TACCARDI, B. & MARCHETTI, G., pp. 105–123. Oxford: Pergamon Press.

HECHT, H. H., HUTTER, O. F. & LYWOOD, D. W. (1964). Voltage–current relation of short Purkinje fibres in sodium-deficient solution. *J. Physiol.* **170**, 5*P*.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of frog muscle. *J. Physiol.* **148**, 127–160.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol.* **118**, 473–496.

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

HUTTER, O. F. & NOBLE, D. (1960). Rectifying properties of cardiac muscle. *Nature, Lond.* **188**, 495.

HUTTER, O. F. & NOBLE, D. (1961). Anion conductance of cardiac muscle. *J. Physiol.* **157**, 335–350.

KATZ, B. (1949). Les constantes électriques de la membrane du muscle. *Archs Sci. physiol.* **3**, 285–299.

MCALLISTER, R. E. & NOBLE, D. (1966). The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. *J. Physiol.* **186**, 632–662.

MCALLISTER, R. E. & NOBLE, D. (1967). The effect of subthreshold potentials on the membrane current in cardiac Purkinje fibres. *J. Physiol.* **190**, 381-387.

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

NOBLE, D. (1962*b*). The voltage dependence of the cardiac membrane conductance. *Biophys. J.* **2**, 381-393.

NOBLE, D. (1965). Electrical properties of cardiac muscle attributable to inward-going rectification. *J. cell. comp. Physiol.* **66**, suppl. 2, 127–136.

NOBLE, D. (1966). Applications of Hodgkin–Huxley equations to excitable tissues. *Physiol. Rev.* **46**, 1–50.

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

NOBLE, D. & TSIEN, R. W. (1969). Reconstruction of the repolarization process in cardiac Purkinje fibres based on voltage clamp measurements of membrane current. *J. Physiol.* **200**, 233–254.

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

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

ROUGIER, O., VASSORT, G. & STÄMPFLI, R. (1968). Voltage clamp experiments on frog atrial heart muscle fibres with the sucrose-gap technique. *Pflügers Arch. ges. Physiol.* **301**, 91–108.

VASSALLE, M. (1966). An analysis of cardiac pace-maker potential by means of a 'voltage-clamp' technique. *Am. J. Physiol.* **210**, 1335–1341.

WEIDMANN, S. (1955). The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *J. Physiol.* **127**, 213–224.