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THE KINETICS AND RECTIFIER PROPERTIES OF THE SLOW POTASSIUM CURRENT IN CARDIAC PURKINJE FIBRES

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SUMMARY

1. The reversal potential of the slow outward current in Purkinje fibres varies with $[K]_o$ in accordance with the expected potassium equilibrium potential. It is concluded that virtually all of this current is carried by potassium ions.

2. The magnitude of the current is determined by two separable factors. The first factor is directly proportional to a variable obeying first-order voltage-dependent kinetics of the Hodgkin–Huxley type but with extremely long time constants. The time constants of this variable are extremely sensitive to temperature and the Q_{10} over the range 26–38° C is 6.

3. The second factor shows inward-going rectification with a marked negative slope in the current–voltage relation beyond about 25 mV positive to the K equilibrium potential. The current–voltage relations measured at different values of $[K]_o$ cross each other on the outward current side of the equilibrium potential.

4. The changes in slow potassium current during pace-maker activity have been calculated. It is shown that the mechanism of the pace-maker potential differs in several important respects from that described by Noble's (1962) model. The negative slope in the current–voltage relation appears to be an important factor in generating the last phase of pace-maker depolarization.

5. The role of the slow potassium current during the action potential and the consequences of the high temperature dependence of the kinetics are discussed.

INTRODUCTION

The form of the membrane currents recorded in Purkinje fibres from mammalian hearts under voltage clamp conditions depends on the range of voltages applied to the membrane. At voltages negative to the plateau

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potential (approximately -20 mV) the conductance changes may be analysed in terms of three components: a fast sodium conductance, g_{Na}, similar to that observed in nerve cells (Weidmann, 1955; Deck & Trautwein, 1964; Dudel, Peper, Rüdel & Trautwein, 1967*b*); a time-independent potassium conductance, g_{K_1}, which rectifies in the inward-going direction (Hutter & Noble, 1960; Carmeliet, 1961; Hall, Hutter & Noble, 1963; Noble, 1965); and a slow time-dependent potassium conductance, g_{K_2} (Hall *et al.* 1963; Vassalle, 1966; McAllister & Noble, 1966, 1967). These components were assumed by Noble (1962) in order to reproduce the essential features of the action potential and pace-maker activity in Purkinje fibres. However, it is now evident that the behaviour of g_{K_2} differs from that assumed by Noble (1962) in several important respects (McAllister & Noble, 1966, 1967). In the work described in this paper we have therefore studied the kinetics and rectifier properties of g_{K_2} in sufficient detail to allow a reformulation of the potassium current equations.

At voltages more positive than about -20 mV the current records become more complicated and additional components appear. These components have been attributed to calcium ions (Reuter, 1966, 1967) and to chloride ions (Dudel, Peper, Rüdel & Trautwein, 1967*a*). However, since most of the important kinetic features of g_{K_2} may be studied with only moderate levels of depolarization, we shall not be concerned with these currents in the present paper.

METHODS

Short (1–2 mm) Purkinje strands taken from fresh sheep hearts obtained from the Oxford Co-operative Society Slaughterhouse were used. The voltage clamp technique was identical with that used by McAllister & Noble (1966) and is similar to that used by Deck & Trautwein (1964). A Devices Digitimer was used to obtain more accurate control over the voltage step sequences applied to the membrane. Since only very slow time course phenomena were studied, a pen recorder (4 channel, Devices) was routinely used for recording, an oscilloscope being used only to monitor the records and to check that fast time course components were normal. The pen recorder had a fairly flat frequency response up to about 50 c/s but, on many occasions, the frequency response was deliberately reduced by an RC filter to minimize noise. Checks were made to ensure that the current components being measured were not influenced by the filter. Current was continuously recorded at low and high amplification (as shown, for example, in the top records in Fig. 6) in order to obtain both the total current record and sufficiently amplified records of the important components to make accurate measurements for analysis. Temperature was continuously monitored with a copper-constantan thermocouple lying near the preparation and could be controlled over the range between room temperature and 40° C.

In order to obtain sufficient information for analysis it was usually necessary to record for several hours from the same preparation. It is difficult to eliminate completely small drifts in current and voltage recording systems over such periods of time and the absolute potential levels in these experiments are probably not accurate to within less than about 5 mV since the final voltage calibration was sometimes made a few hours after a particular experiment was performed. However, more reliance may be placed on comparisons between

potentials during individual experiments since the records in one solution were usually obtained about half an hour after the records in the previous solution. Wherever possible, solution changes were reversed and repeated to check the results. All solutions contained 140 mM-Na^+, 144·6 mM-Cl^-, 0·5 mM-Mg^{2+}, 1·8 mM-Ca^{2+} and 1 g/l. glucose and were bubbled with oxygen. The K concentration was varied by adding various quantities of K phosphate buffer. Thus, the 4 mM-K solution contained 4 mM-K^+, 1·65 mM-HPO_4^{2-} and 0·7 mM-$H_2PO_4^-$. Solutions with other K concentrations contained the same ratio of K_2HPO_4 to KH_2PO_4 but different total quantities to give total K concentrations of 2 mM, 2·7 mM or 6 mM. In all cases the buffer capacity was quite adequate to keep the pH at about 7·4 since the preparations were very small and were bathed in a large volume of solution which usually flowed over the preparation continuously.

In the region of the normal resting potential, the current changes occur with very long time constants and it was found necessary to allow up to 30 sec, or sometimes even longer, between clamp pulses in order to ensure that a steady state had been restored. In a typical experiment only about 60–100 pulses could be applied per hour. It proved very difficult therefore to obtain all the information for a full and accurate analysis of both the kinetics and rectifier properties from a single preparation. This was nearly achieved in the experiments on which the illustrations in this paper are based. In many other experiments, only partial results could be obtained. However, these results were consistent with those obtained from more complete experiments. Another difficulty arises from the fact that over some ranges of potential the slow current changes are very small and it is usually necessary to measure currents less than 10^{-8} A in order to specify the reversal potential to within a few mV. Moreover, small background current changes sometimes obscure the reversal point. However, more accurate estimates were sometimes obtained by interpolation using measurements of currents on both sides of the reversal potential (as shown, for example, in Fig. 9, bottom).

THEORY

The results and analysis to be described in this paper require fairly substantial changes in the equivalent circuit model for the K current equations and it may be helpful, therefore, to describe first the theoretical basis of the analysis.

Noble's (1962) model assumed that the potassium conductance can be represented by two parallel rectifiers (Fig. 1*a*). One, g_{K_1}, rectifies instantaneously in the inward-going direction, whereas the other, g_{K_2}, rectifies very slowly in the outward-going direction. g_{K_1} was described empirically as the sum of two exponentials. g_{K_2} was described by the Hodgkin–Huxley n equations (Hodgkin & Huxley, 1952) with the rate constants α_n and β_n reduced by a factor of 100. The absolute magnitude of the conductance was also greatly reduced.

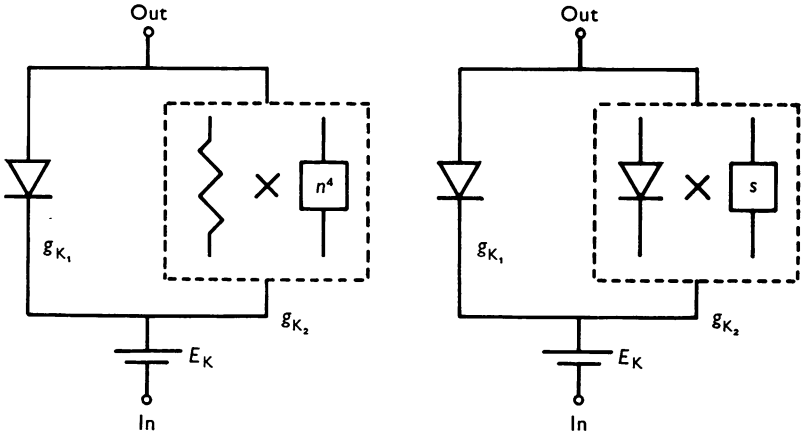
The model required to account for the results described in the present paper is shown in Fig. 1*b*. The major differences are that g_{K_2} also rectifies in the inward-going direction for instantaneous changes in the potential (cf. Armstrong & Binstock, 1965) and that at any particular potential g_{K_2} is directly proportional to a first-order variable:

$$g_{K_2} \propto s^\gamma \quad \text{where} \quad \gamma = 1, \tag{1}$$

whereas Noble used

$$g_{K_2} \propto n^{\gamma} \quad \text{where} \quad \gamma = 4. \tag{2}$$

The reasons for making these changes are that i_{K_2} is not a linear function of the driving force during sudden changes in membrane potential (McAllister & Noble, 1966) and that γ is already known to be less than 4 (the change in nomenclature from n to s for the variable obeying first-order kinetics is not important in the present context and will be justified



$g_{K_1} = f_1(E_m, E_K)$
$g_{K_2} \propto n^4$
$i_K = (g_{K_1} + g_{K_2})(E_m - E_K)$

(a)

$g_{K_1} = f_1(E_m, E_K)$
$g_{K_2} = sf_2(E_m, E_K)$
$i_K = (g_{K_1} + g_{K_2})(E_m - E_K)$
$f_1 \propto f_2$?

(b)

Fig. 1. Equivalent circuit diagrams for (*a*) Noble's (1962) K current equations and (*b*) the model obtained from the analysis of the results described in the present paper. The major changes made in the new model are that g_{K_2} also shows inward-going rectification and that the exponent on the kinetic variable is reduced to 1.

later—see Discussion). McAllister & Noble (1966, 1967) assumed $\gamma = 2$ in their analysis. However, most of their results could also be fitted fairly well with $\gamma = 1$, and the evidence against $\gamma \neq 1$ is strong enough (see Results) to conclude that McAllister & Noble's results were subject to a small systematic error in the indirect method which they used for measuring the time course of change in current. If the behaviour of Purkinje fibres is described by the model shown in Fig. 1*b* then they should show the following properties:

1. The instantaneous current–voltage relations for g_{K_2} should show certain resemblances to those for g_{K_1}. These relations may have a negative slope region, and relations obtained at different external K concentrations

should cross each other when depolarizing currents are applied to the membrane, as has already been shown for g_{K_1} (Hall & Noble, 1963; McAllister & Noble, 1966). It is usually assumed that inward-going rectification occurs virtually instantaneously but the possibility of a small activation time cannot be excluded since the capacitance currents in Purkinje fibres last too long to determine the behaviour of the ionic currents at times shorter than a few msec (Fozzard, 1966). However, the changes due to inward-going rectification may be treated as instantaneous compared to the duration of the action potential.

2. *Changes* in the magnitude of g_{K_2} at any given potential should be determined entirely by changes in s. Thus, if s increases by a given factor then g_{K_2} should increase by the same factor at all potentials. Another way of expressing this property is to say that the *shape* of the $i_{K_2}(E_m)$ relation (where E_m is membrane potential) should be independent of the degree of activation of s.

3. The time constant, τ_s, for changes in s should be a unique function of E_m. Moreover, if $\gamma = 1$, the time course of current change should always be exponential and the time courses of onset and decay at a particular potential should be symmetric. This will not be true for any other value of γ.

4. The reversal potential for i_{K_2} should change as E_K changes.

The notation used in Fig. 1 differs both from Noble's notation and from that used by Armstrong & Binstock (1965). The reasons for adopting the new notation are twofold. First, it does not seem appropriate to represent a channel controlled by a Hodgkin–Huxley variable by an outward-going rectifier (as in Noble's notation), since these variables are voltage dependent rather than current dependent. Thus, the direction of current flow is determined by the appropriate ionic equilibrium potential but the voltage dependence of the permeability variable is not. Secondly, Armstrong & Binstock's use of symbols in series can be misleading since the property which needs to be represented at the macroscopic level is that the conductance factors should be multiplied, not that the resistance factors should be added. It seems more appropriate therefore to represent the Hodgkin–Huxley variable by a labelled element and to make the mathematical relation between the variables explicit in the diagram. However, the intended meaning of the 'in series' notation may still be valid since, at the single pore level, the processes of rectification and kinetics may well take place serially (Armstrong, 1966).

RESULTS

Kinetics. We have confirmed McAllister & Noble's (1967) observation that most of the slow potassium current may be activated by potentials which are too negative to activate the fast sodium conductance, and their suggestion that the steady-state value of this current at a holding potential around -80 mV is not zero. In fact, as shown in Fig. 4 below, it is sometimes possible to activate virtually all the slow current by subthreshold

depolarizations. This enables the kinetics of this current to be studied directly over an important range of membrane potentials, including the whole range of the pace-maker potential, so that any errors which may arise using indirect methods may be avoided.

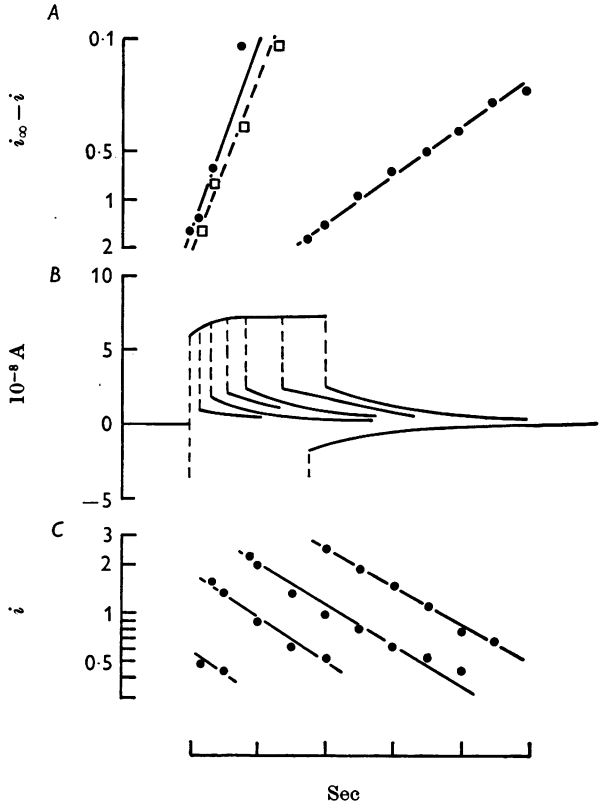


Fig. 2. Typical set of current changes in response to depolarizing and hyperpolarizing clamp pulses. Holding potential −75 mV. Preparation 40–2. $[K]_o = 4$ mM.

A. Left: continuous-line (●) shows time course of slow current change during depolarization to −56 mV plotted on a logarithmic scale as deviation $(i_\infty - i)$ from steady-state current. Interrupted line (□) shows envelope of peak currents following restoration of holding potential after depolarizations of various durations. The lines have virtually equal slopes so that the time constants are equal. *Right*: time course of recovery of current following a hyperpolarization to −85 mV. Note that the time constant is equal to that of the changes plotted in *C*.

B. Superimposed tracings of currents on linear scale.

C. Decay of currents following depolarizations of various durations plotted on logarithmic scale (for the sake of clarity only the first, second, fourth and sixth records are plotted). Note that time constant of decay is independent of degree of activation and is equal to the time constant of recovery of current at same potential (see right-hand plot in *A*).

Figure 2*B* shows a typical set of superimposed current records in response to subthreshold rectangular depolarizations and part of a single current record in response to a rectangular hyperpolarization. In Figs. 2*A* and *C* the current changes have been plotted on logarithmic scales. It can be seen from these results that the time course of the current change is a simple exponential and is not sigmoid. The time constant of the exponential is a unique function of the membrane potential, i.e. the time constant does not depend, for example, on previous values of membrane potential, and the time constant of decay of current following a depolarizing pulse is identical with the time constant of recovery of current following a hyperpolarizing pulse. It can also be seen from Fig. 2*A* that the slow change in current following a step change in potential and the envelope of the peak currents following return to the holding potential after various intervals of time have the same time course. This means that the proportionate change in the time-dependent component of current does not depend on the potential at which it is measured. It follows from this observation that the shape of the instantaneous current–voltage relation must be independent of the degree of activation of the system (see Theory). A result similar to this was also described by McAllister & Noble (1966, Fig. 4). However, they used depolarizations which also activated the fast sodium conductance and their analysis therefore required an arbitrary choice of the position of the current scale during depolarization, since the initial part of the potassium current change is then partly obscured by the declining sodium current. This difficulty is avoided in the present experiments.

The time constants of the current change on either side of the holding potential are shorter than that at the holding potential itself. The time constant must therefore be at a maximum (τ_s^{-1} at a minimum) in the region of the holding potential (see Fig. 4).

A comparison between responses to depolarizing and hyperpolarizing pulses shows that less initial current is required to depolarize than to hyperpolarize. In the records shown in Fig. 7, for example, similar initial current changes occur in response to a 16 mV depolarization and a 8 mV hyperpolarization. This result would be expected if inward-going rectification is present. However, since g_{K_1} and g_{K_2} both contribute to these initial changes (the steady state g_{K_2} is not zero at the holding potential) it is not possible to obtain the rectifier properties of either system directly from these results. The rectifier properties must therefore be obtained by indirect means (see *Rectifier Properties* below).

These results have been repeated at a number of different values of membrane potentials and they show fairly conclusively that the kinetics of i_{K_2} are first order and that γ is 1.

The question therefore arises why McAllister & Noble (1966, 1967) sometimes observed sigmoid time courses consistent with the view that $\gamma = 2$. The most likely explanation is that the currents following clamps of small duration are underestimated by the indirect method. Two possible factors might account for such an error. First, the capacity currents take a few msec to subside following a step change in potential since a substantial fraction of the membrane capacity is in series with a resistance (Fozzard, 1966). If current is measured in terms of the peak current following repolarizations, then for similar amplitudes of clamp pulse the smaller currents (i.e. those following clamps of short duration) would be reduced by the slower capacity currents to a greater proportionate extent than the larger currents following longer lasting pulses. However, it is very unlikely that this is the major factor involved since, as shown in Fig. 3, the fast transients following repolarizations are sometimes considerably longer than those following depolarizations. It is more likely, therefore,

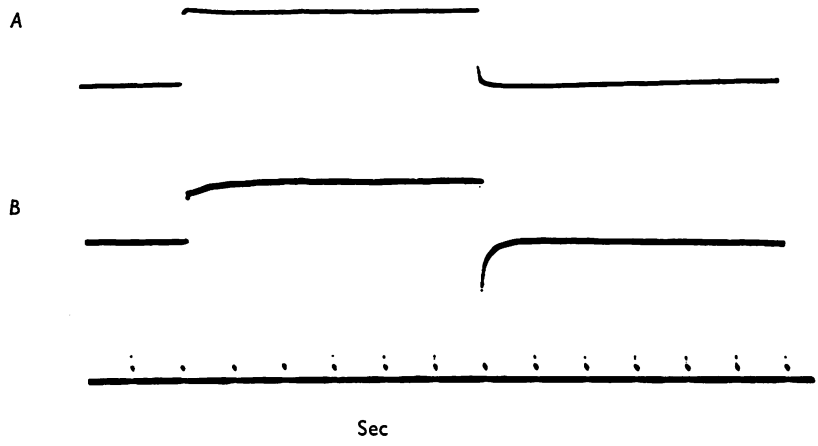


Fig. 3. Example of current record showing a much larger fast transient on repolarization than on depolarization.

A. Membrane potential. A step change 20 mV in amplitude and 6 sec in duration was applied. This depolarization is below the sodium threshold in this preparation.

B. Current record. Note that although the capacitive transients are too fast to be recorded accurately by the recording instrument, the time constant of the apparatus is short enough to record a characteristic capacity transient on depolarization. The longer-lasting fast transient on repolarization cannot therefore be due to long-lasting capacitive currents. This degree of asymmetry in the fast transients is an extreme example. Many current records (see e.g. Fig. 7) show little or no asymmetry of this kind.

that some other, relatively fast, conductance change is involved on repolarization. This phenomenon may also be responsible for the fast transients observed by Vassalle (1966), Fig. 7) on clamping back to the resting potential at various stages during the action potential plateau. Vassalle suggests that these transients might be attributable to sodium inactivation. If this interpretation is correct, the sodium conductance involved is probably not the one responsible for the spike of the action potential since, as in the record shown in Fig. 3, this transient can occur following depolarizations which are too small to activate the fast sodium conductance. Moreover, the transient is not greatly changed when pulses strong enough to activate the fast sodium conductance are applied. At present, therefore, we cannot identify

the origin of the fast repolarizing transients. However, the slowness of the g_{K_2} changes in the region of the holding potential ensures that only very small errors can arise from this factor. Moreover, only very small errors are required to account for the deviation from exponential time courses sometimes observed with the indirect method. We have therefore continued to use this method for obtaining estimates of the time constants at potentials beyond the sodium current threshold.

In order to determine the variation in τ_s and s_∞ (the steady-state fraction of activation) with E_m, several sequences of potential change were applied to the membrane:

1. Simple rectangular steps of various durations and magnitudes from the holding potential (usually about -80 mV).

2. Repolarization to various membrane potentials following a sufficiently large depolarization to fully activate the slow current system. This enabled the time constants to be measured for a finer gradation of potentials in the vicinity of the holding potential.

3. Repeat of (1.) using different holding potentials. As shown below (see *Rectifier Properties*) this enabled more information on the rectifier properties to be obtained.

In this way a sufficient number of measurements was made to check that s_∞ and τ_s are both unique functions of E_m. Although some degree of variation in the results was obtained between different preparations, the important features were found to be regular and are illustrated by the results obtained from a single preparation shown in Fig. 4. It can be seen that the $s_\infty(E_m)$ curve is very similar to the curves obtained for conductance variables in other excitable cells (Hodgkin & Huxley, 1952; Frankenhaeuser, 1962; see Noble, 1966, for further references). The shape is sigmoid and the steepest part of the curve has a slope of 0·43/10 mV change in potential. The only other permeability variable for which this curve has been obtained in cardiac fibres is the sodium inactivation variable, h, and in this case a maximum slope of 0·5/10 mV was obtained (Weidmann, 1955).

The slow potassium current changes are extremely slow over a wide range of potentials, as indicated by the wide valley in the $\tau_s^{-1}(E_m)$ curve. Using the equation

$$ds/dt = \alpha_s(1-s) - \beta_s s \tag{3}$$

and the continuous lines for s_∞ ($= \alpha_s/(\alpha_s+\beta_s)$) and τ_s^{-1} ($= \alpha_s+\beta_s$), α_s and β_s were calculated and are shown as interrupted lines. It can be seen that both rate coefficients are monotonic functions of E_m. McAllister & Noble (1967) found that the β rate coefficient is sometimes non-monotonic when $\gamma = 2$ is used in the analysis. This irregularity disappears when $\gamma = 1$.

Variation of reversal potential with $[K]_o$. The slow outward current has previously been attributed to an outward movement of K ions. The best

evidence for this view has come from voltage clamp experiments showing that the current tails on repolarization reverse when the likely normal value for E_K is exceeded (Deck & Trautwein, 1964; Vassalle, 1966; McAllister & Noble, 1966). A typical result of this kind obtained in the present series of experiments is shown in Fig. 5. In this case the records

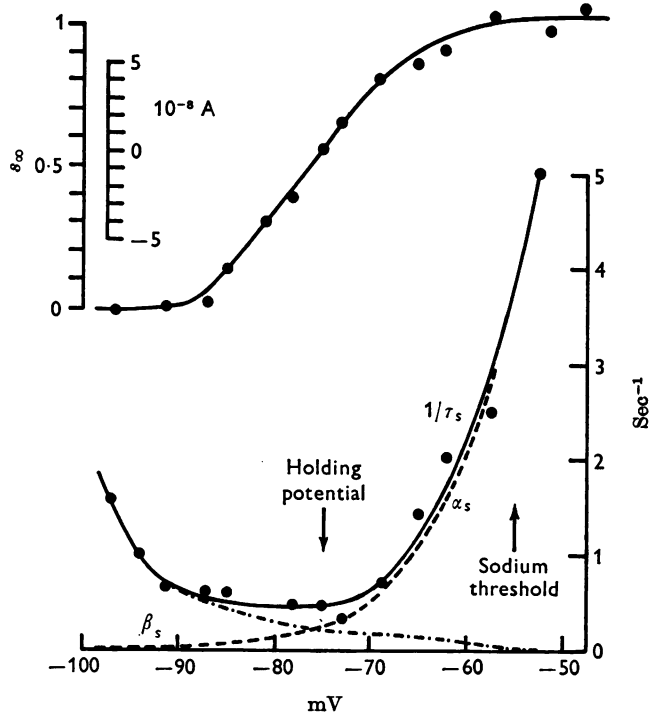


Fig. 4. Voltage dependence of kinectics of i_{K_2}.

Top: voltage dependence of fractional activation (s_∞) in the steady state measured as the peak current change from background on return to holding potential (−75 mV). Ordinates: peak current and s. Abscissa: membrane potential (as in bottom curves).

Bottom: points show measured values of τ_s^{-1}. Interrupted lines show α_s(----) and β_s (-·-·-) calculated from $\tau_s^{-1} = \alpha_s + \beta_s$ and $s_\infty = \alpha_s/(\alpha_s+\beta_s)$, using continuous curves for s_∞ and τ_s^{-1}, drawn by eye through points. Arrows show position of sodium threshold and of holding potential. Temperature 36° C. $[K]_o$ = 4 mM.

show currents in response to various hyperpolarizations from the holding potential (−65 mV). Since an appreciable steady-state slow current is present at this potential, the records show a slow change in current. At −113 mV (the reversal potential in this experiment) the current change is zero and at −129 mV the current change reverses sign.

However, it is conceivable that some other mechanism may be respon-

sible for this result and more conclusive evidence for identifying this current with a movement of K ions would therefore be obtained by observing a shift in the reversal potential as the external K concentration is varied. We have found that the reversal potential follows the predicted shifts in E_K over a range of $[K]_o$ between 2·6 mM and 6 mM, corresponding to a 20 mV range for E_K. Below about 2·6 mM $[K]_o$, the reversal potential change appears to be smaller than expected (a change from 4 to 2 mM,

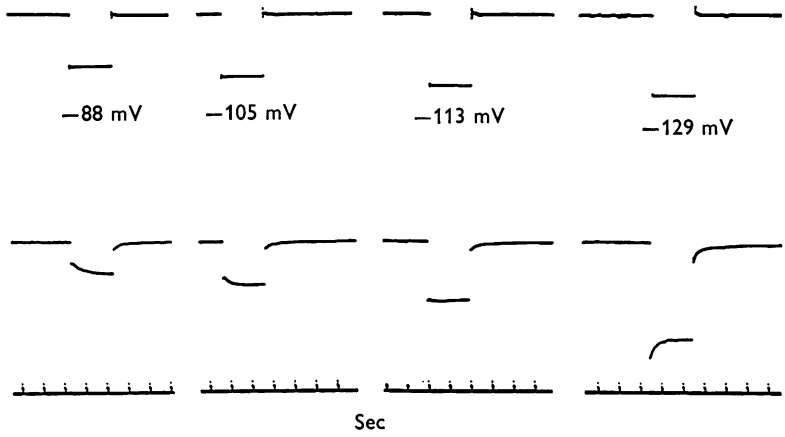


Fig. 5. Voltage clamp current records showing reversal potential determination. Preparation 46–8. $[K]_o$ = 2 mM. Holding potential = −65 mV. The hyperpolarizing pulses deflected the membrane potential to −88 mV, −105 mV, −113 mV (reversal potential) and −129 mV.

for example, produces only half the expected change in reversal potential). This may be due to the fact that at very low values of $[K]_o$ a net loss of K from the cells will prevent $[K]_o$ immediately outside the cell membrane from falling to the value of [K] in the bathing solution.

Figure 6 shows samples from three series of currents recorded during changes in K concentration. Constant hyperpolarizing clamp pulses were used as tests to show the gradual onset of the effects of changing $[K]_o$:

1. There is a marked increase in the amount of steady-state current required to achieve the same hyperpolarization when $[K]_o$ is increased. Since s_∞ is virtually zero at the hyperpolarized potential, this current increase must be attributed to changes in the current–voltage relations for g_{K_1} and any other currents. These changes are in the direction expected from the 'cross-over' effect observed previously (Hall & Noble, 1963; McAllister & Noble, 1966).

2. Changes in the amplitude of the slow current change during the hyperpolarization reflect the shifts in the reversal potential. Measuring

the reversal potentials with various hyperpolarizations (as in Fig. 5) gives -92 mV in 6 mM $[K]_o$, -99 mV in 4 mM $[K]_o$ and -111 mV in 2·7 mM $[K]_o$. These changes are reasonably close to the 10·5 mV shifts in E_K predicted by the Nernst equation (a few mV differences in the absolute potential in these experiments is not very significant—see Methods):

$$E_K = 61 \log([K]_o/[K]_i). \quad (4)$$

with $[K]_i = 151$ mM (Robertson & Dunihue, 1954), the calculated values for E_K are -86 mV (6 mM), $-96{\cdot}5$ mV (4 mM) and -107 mV (2·7 mM).

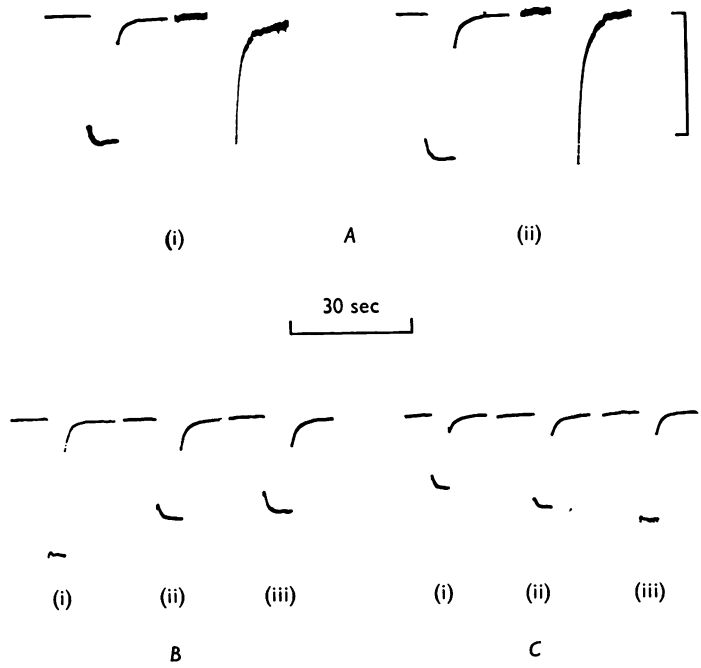


Fig. 6. Current records in response to hyperpolarizing pulses during changes in $[K]_o$. Holding potential -68 mV. Same preparation as in Fig. 2.

A. Records at low (left) and high (right) amplification (i) before and (ii) during change from 4 mM $[K]_o$ to 6 mM $[K]_o$. Pulses were -28 mV in amplitude and 7 sec in duration. Note increase in total amplitude and in the current tail on return to holding potential when $[K]_o$ is increased. This increase occurs despite a decrease in driving force on K ions.

B. Records at low amplification (i) before, (ii) and (iii) during, change from 6 mM to 2·7 mM. Pulses -22 mV. In 6 mM $[K]_o$ this pulse was sufficient to deflect potential to reversal potential. Note growth of current change during pulse as $[K]_o$ falls. This change in $[K]_o$ corresponds to a 20 mV change in E_K.

C. Records at low amplification (i) before, (ii) during and (iii) after change from 2·7 mM to 4 mM. Pulses -32 mV. Time scale: 30 sec; current scale: $2{\cdot}5 \times 10^{-7}$ A (low) and 5×10^{-8} A (high).

3. With increasing $[K]_o$, there is an increase in the magnitude of the slow current that is reactivated at the termination of the hyperpolarization. This change was recorded more accurately at higher gain on a second channel (see top records in Fig. 6). Provided that the same fraction of s is reactivated by the return to the holding potential in each case, as is shown below (see Fig. 10), this increase in current suggests that the instantaneous current–voltage relations, $i_{K_2}(E_m, E_K)$ obtained, in different $[K]_o$ must cross each other since the increase in current occurs despite a decrease in driving force. Further analysis of the rectifier properties of g_{K_2} confirms this suggestion (see Fig. 11).

Rectifier properties. The usual method for measuring the rectifier properties of conductance variables is to activate a certain constant fraction of the system and to plot the current–voltage relation obtained by applying step changes to various membrane potentials. This method depends on being able to establish conditions in which the conductance variable concerned controls virtually all the membrane current. It is not possible to use this method to obtain the rectifier properties of g_{K_2} in Purkinje fibres since we have found no conditions in which g_{K_2} contributes more than a fraction of the membrane conductance. This is largely due to the presence of g_{K_1} (whose absolute value is usually about twice as large as g_{K_2}), but other conductances (e.g. a resting sodium conductance) may also contribute. In this case, therefore, the shape of the instantaneous current–voltage relation for g_{K_2} must be obtained indirectly. According to the model (see Theory), the magnitude of the slow current change is given by the product of the change in s, Δs, and the rectifier function, $i_{K_2}(E, E_K)$, where E is the value of the membrane potential during the clamp pulse. In order to illustrate the analysis, two clamp records are shown in Fig. 7. Here

$$\begin{aligned} i_A &= i(E, E_K, s_H + \Delta s) - i(E, E_K, s_H) \\ &= \Delta s . f_2(E, E_K) . (E - E_K), \end{aligned} \tag{5}$$

where i_A is the total slow current change during the pulse and s_H is the steady state value of s at the holding potential, E_H. The middle quantity in this equation expresses i_A in terms of what is actually measured, i.e. total current change during a certain change, Δs, in the degree of activation. The right hand quantity expresses i_A in terms of the model discussed in the Theory section. Now, during the slow change of current following return to the holding potential, s must return to its original value, s_H. Δs is therefore the same as during the clamp pulse. Any difference in the amplitude of the current change, i_B, must be attributed to the shape of the instantaneous current–voltage relation. Thus, in the case of the top record in Fig. 7, i_A is actually less than i_B which means that the instan-

taneous current–voltage relation must have a negative slope. The shape of the current–voltage relation may be obtained as follows.

$$\begin{aligned} i_B &= i(E_H, E_K, s_H + \Delta s) - i(E_H, E_K, s_H) \\ &= \Delta s . f_2(E_H, E_K) . (E_H - E_K). \end{aligned} \tag{6}$$

The ratio of current changes is therefore

$$\frac{i_A}{i_B} = \frac{f_2(E, E_K) . (E - E_K)}{f_2(E_H, E_K) . (E_H - E_K)} = \frac{i_{K_2}(E, E_K)}{i_{K_2}(E_H, E_K)} \tag{7}$$

Since s is now eliminated, the *shape* of the instantaneous current–voltage relation will be given by plotting the ratio i_A/i_B against E. This ratio (which we shall call the rectifier ratio) has been plotted for various values of $[K]_o$ in Fig. 8. Note that, by definition, the rectifier ratio must be equal

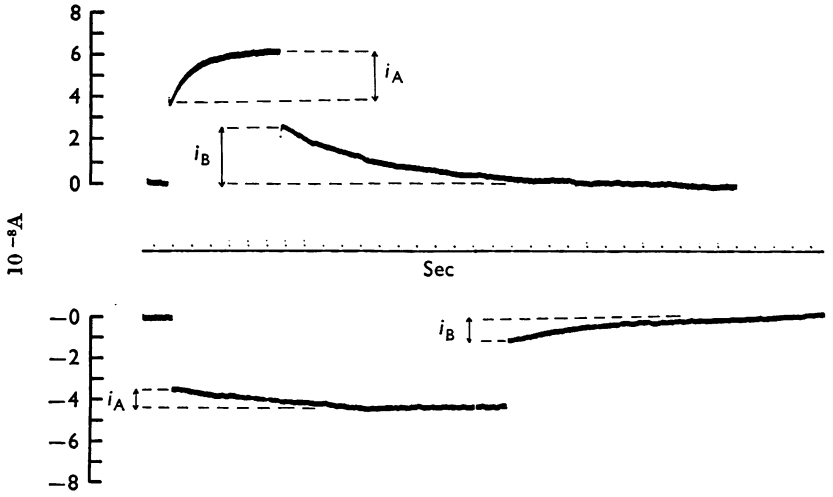


Fig. 7. Current records illustrating measurements made to obtain rectifier functions. Same preparation as Fig. 2. Holding potential −75 mV. $[K]_o$ = 4 mM.
Top: current in response to depolarization to −59 mV.
Bottom: current in response to hyperpolarization to −83 mV. Time scale: sec.
Note that current change (i_A) during depolarization is actually smaller than that (i_B) following repolarization.

to 1 at E_H for all values of $[K]_o$. These plots do not enable us, therefore, to compare the absolute magnitudes of the relations at different values of $[K]_o$. However, some of the important features of the current–voltage relations are already evident. At 2·7 mM $[K]_o$ the ratio passes through a maximum near E_m = −80 V, so that in this range the ratio is greater than unity. Beyond this potential the relation has a negative slope, and this property may be of considerable importance in the mechanism of the

pace-maker potential (see Discussion). The appearance of a negative slope may also be seen in the curve at 4 mM $[K]_o$. Its absence in the curve at 6 mM $[K]_o$ is probably due to the fact that an inadequate range of potentials was explored. It would, of course, be of great interest to determine the rectifier ratio for potentials more positive than the range explored in Fig. 8. Unfortunately, the current records for more positive potentials are complicated by the presence of other time-dependent currents so that further investigation of the rectifier function for g_{K_2} will require the elimination or determination of other currents.

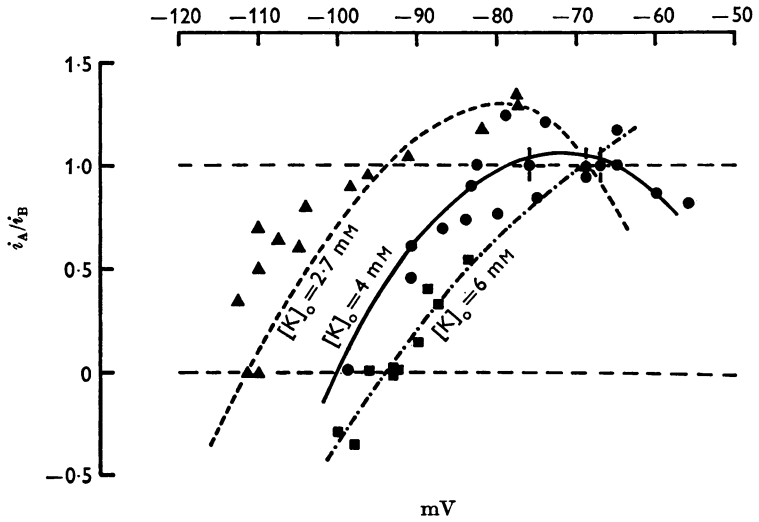


Fig. 8. Variation in rectifier ratio, i_A/i_B, with E_m at 2·7 mM $[K]_o$ (▲– – –), 4 mM $[K]_o$ (●——) and 6 mM [K] (■ –·–·–). Same preparation as Fig. 2. The holding potentials are indicated by crosses (+) through the symbols. The results for 4 mM $[K]_o$ were obtained at two different holding potentials but no scaling was required to match the results. The curves have been drawn by eye to give an indication of the typical shape of the rectifier curves based on this and other experiments (see also Fig. 9 bottom). Note also that in these plots, the rectifier ratio has not been corrected for changes in absolute amplitude of current with changes in $[K]_o$, and that the ratio is unity at the holding potential by definition. The corrected ratios are plotted in Fig. 11.

The degree of scatter of some of the points plotted in Fig. 8 may raise the question how much reliance may be placed on our curves indicating negative slopes. In fact, the evidence for the presence of a negative slope is stronger than it might appear to be. The reason is that the relatively large scatter of points around the holding potentials at, e.g. 4 mM $[K]_o$, arises from the fact that the small pulses required to deflect the potential from the holding potential produce only small current changes. The ratios are therefore obtained by dividing one small quantity by another. The errors involved must therefore be fairly large. However, this is not true for the case of the points on which the evidence for a negative slope is based. The most positive points in the case of the 4 mM $[K]_o$ results were obtained by clamping from the most negative of the two holding potentials. These points are therefore obtained from the ratios

of fairly large quantities (cf. Fig. 7, top record). The fact that these points give a ratio less than unity (in fact about 0·8) is significant since the errors in these measurements must have been considerably less than 20%. Even stronger evidence for the negative slope region is provided by the results of the experiment illustrated in Fig. 9.

Some comment may be in order here on the choice of axes for the current–voltage relations in this paper. The usual convention in voltage clamp work is to plot voltage on the abscissa. Although previous work in cardiac electrophysiology has frequently used the ordinate for voltage it may now be more appropriate to use the abscissa.

The fact that other time-dependent currents appear at more positive potentials suggests an alternative explanation for the negative slopes shown in Fig. 8. For example, a slow conductance change with a reversal potential around −50 mV (D. Noble & R. W. Tsien, unpublished) would give rise to a negative current if activated in the range of voltages studied in the present experiments. If the kinetics of this current were similar enough in time course to be included in the estimate of i_A, i_A would be reduced at more positive potentials and the effect would be to produce a negative slope in the relation obtained from the rectifier ratio, even if no negative slope exists in the true $i_{K_2}(E_m)$ relation. This possibility can be tested by determining steady-state s curves at different holding potentials. The results of such an experiment are shown in Fig. 9 (top). There are two important features of the results to which we want to draw attention:

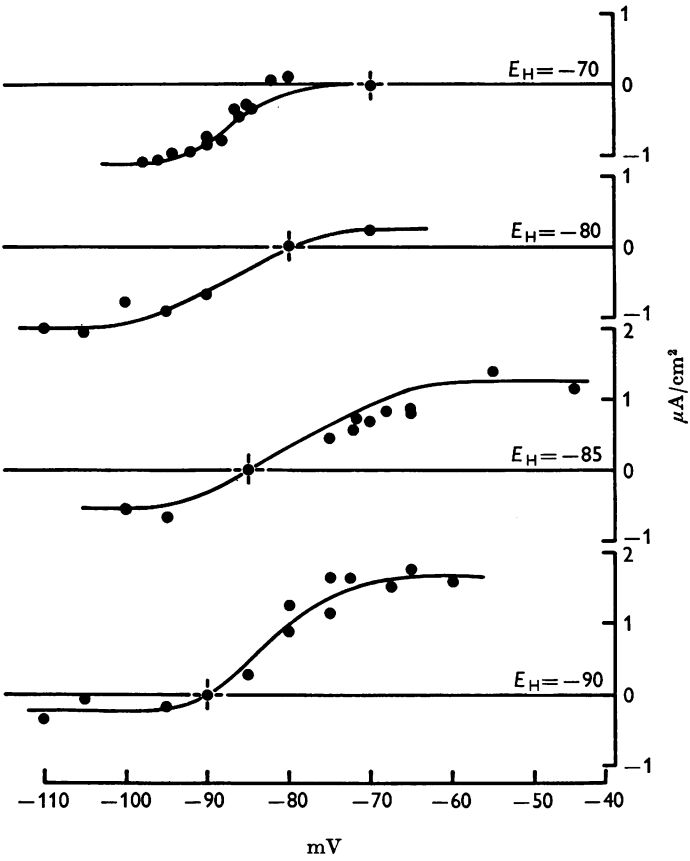
1. The *position* of the $s_\infty(E_m)$ curve on the voltage axis is not significantly dependent on the value of the holding potential, E_H. Thus the values of E_m at which $s_\infty = 0{\cdot}5$ are −83 mV (E_H = 90 mV), −80 mV (E_H = −85 mV), −86 mV (E_H = −80 mV) and −87 mV (E_H = −70 mV). This result would not be obtained either if the value of the holding potential influenced the g_{K_2} kinetics (in which case the rectifier and kinetic factors would not be strictly separable) or if another current component were being included in the estimate of i_{K_2} at more positive potentials. In the latter case the s_∞ curve would be uninfluenced only if the kinetics of

Legend for Fig. 9

Fig. 9. Evidence for negative slope in $i_{K_2}(E_m)$ relation based on measuring currents at different holding potentials. Preparation 47–2. $[K]_o$ = 2 mM.

Top: steady-state variation in degree of activation measured in terms of peak current deflexions on return to various holding potentials. Note that the amplitude of the curves is strongly dependent on E_H but that position on voltage axis is virtually independent of E_H.

Bottom: points show rectifier function obtained at E_H = −70 mV as described in text using equations (5)–(8). Vertical lines show total amplitude of steady state curve, i.e. $i(s = 1) - i(s = 0)$, as a function of E_H. Curve drawn by eye through points is a typical rectifier curve. Note that total amplitudes are also a good fit to the rectifier function. As explained in text, this result provides stronger evidence for the existence of a negative slope region than that given by the results in Fig. 8.



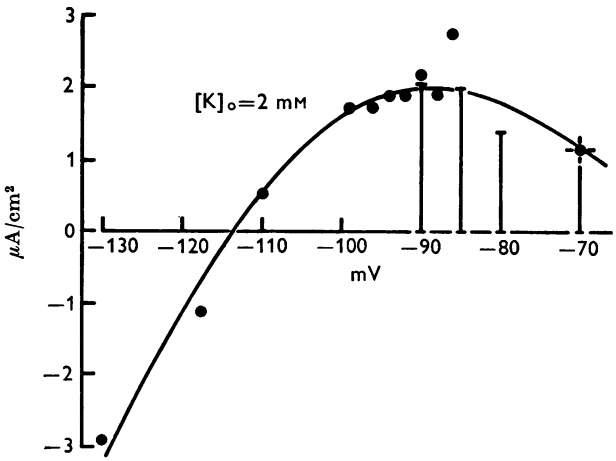


Fig. 9. For legend see opposite page.

the other current were identical with those for i_{K_2}. But in this case the reversal potential of the current controlled by s would not equal the potassium equilibrium potential.

2. The *amplitude* of the steady-state curve is strongly dependent on E_H and decreases at the most positive values of E_H. Hence $i_{K_2}(E, E_K, s = 1)$ must have a negative slope at potentials beyond about 25 mV positive to E_K (see Fig. 9, bottom). Moreover, since the shape of the current–voltage relation is independent of the value of s (see above), this result must hold for all values of s.

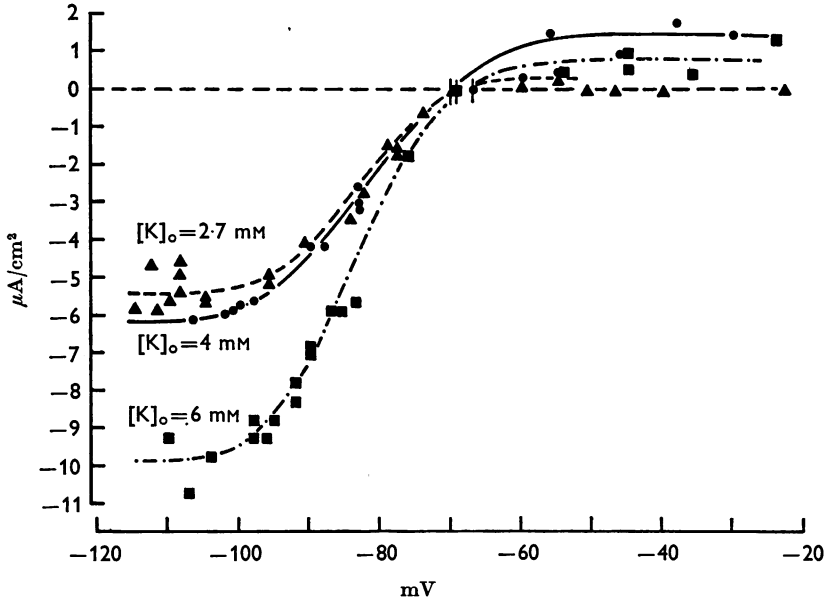


Fig. 10. Variation in peak current deflexion at the holding potential following prolonged step depolarizations. Same preparation as Fig. 2. The relations were obtained at three different K concentrations: 2·7 mM (----), 4 mM (——) and 6 mM (-·-·-). Note that position of curve on voltage axis is virtually independent of $[K]_o$ but that total amplitude increases as $[K]_o$ increases.

It is now possible to obtain the relative magnitudes of the instantaneous current–voltage relations at different values of $[K]_o$ (note that the relations shown in Fig. 8 do not give this information since they are arbitrarily scaled to give a value of 1 at E_H). The information required in order to scale the rectifier ratios to give relations in terms of K current is the value of $i(E_H, s = 1) - i(E_H, s = 0)$ at the holding potential for each value of $[K]_o$. These values may be obtained from the total amplitudes of the steady state activation curves measured (as in Fig. 4, top) in terms of the peak current on return to the holding potential. The results of an experiment in

which these curves are determined are shown in Fig. 10. It can be seen that the position of the activation curve on the voltage axis does not depend appreciably on the value of $[K]_o$ (the small changes which occurred are no greater than the background drifts expected during experiments of this kind—see Methods). This result is further evidence for the separability of the kinetic and rectifier functions. On the other hand, the total amplitude of the curve, which gives $i(E_H, s = 1) - i(E_H, s = 0)$, changes quite substantially as $[K]_o$ is varied.

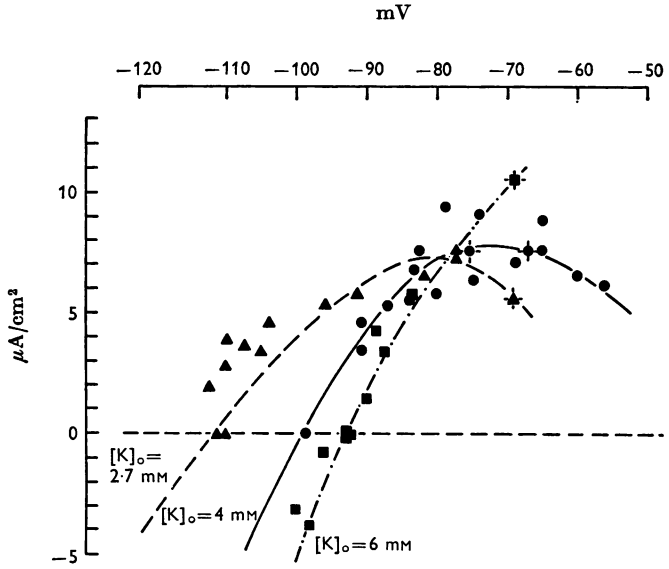


Fig. 11. Rectifier functions, $i_{K_2}(E_m, E_K, s = 1)$, at 2·7 mM $[K]_o$ (----), 4 mM $[K]_o$ (——) and 6 mM $[K]_o$ (–·–·–). The functions were calculated by multiplying ratios plotted in Fig. 8 by total amplitude of steady-state curves in Fig. 10 (see equation (8)). The relations cross each other on the positive side of the reversal potentials and show negative slopes at about 25 mV positive to the reversal potential. As in Figs. 8 and 9, the curves have been drawn by eye through points to indicate typical shape of relations.

If the rectifier ratios plotted in Fig. 8 are multiplied by the appropriate value of $i(E_H, s = 1) - i(E_H, s = 0)$ from Fig. 10, we obtain the instantaneous current–voltage relations for i_{K_2} when $s = 1$, i.e.

$$i_{K_2}(E_m, E_K, s = 1) = \frac{i_A}{i_B}[i(E_H, E_K, s = 1) - i(E_H, E_K, s = 0)]. \quad (8)$$

These relations (which we shall call the rectifier functions) have been plotted in Fig. 11. It can now be seen that the relations show a marked cross-over effect on the positive side of the reversal potentials which is similar to that already described for g_{K_1} (Hall & Noble, 1963; McAllister

& Noble, 1966). This effect is expected when the conductance is a function of the driving force and not simply of the membrane potential (see Noble, 1965, Fig. 1).

In applying equations (5)–(8) to obtain the rectifier functions it is assumed that E_K remains unchanged during the passage of current across the membrane. Since McAllister & Noble (1966) have shown that E_K probably does change in a positive direction during depolarizing currents, this assumption requires justification. The justification is based on the fact that much larger depolarizations were used by McAllister & Noble (1966) than those used in the experiments described in the present paper. They showed that long-lasting depolarizations of the order of 100 mV may temporarily displace the quiescent membrane potential by about 10 mV, probably as a consequence of K accumulation in a space immediately outside the cell membrane. The changes in the present experiments, however, must be considerably smaller than this. Thus, a depolarization of the magnitude and duration shown in the top record of Fig. 7 would be expected to change the quiescent potential by less than 2 mV. Moreover, the major part of the slow current change during depolarization occurs within the first 2 sec during which time the change in quiescent potential is probably less than 1 mV. Although we cannot accurately estimate the effects which would result from such small changes, there are several reasons for thinking that the effects must be negligibly small. First, as McAllister & Noble (1966, Fig. 11) have shown, the changes in quiescent potential become very much smaller when $[K]_o$ is increased, whereas the slow current changes become much larger when $[K]_o$ is increased (see Fig. 10). Secondly, McAllister & Noble (1966) showed that the kinetics of the accumulation and depletion process are not even approximately first order, whereas the kinetics of the slow current changes are first order (see *Kinetics* above). Thirdly, the quiescent membrane potential changes observed by McAllister & Noble (1966) continue to increase with the magnitude of the preceding depolarization, the important parameter being the total charge transferred across the membrane. By contrast, the slow current change recorded on repolarization is virtually independent of the potential during the previous depolarization when potentials positive to -60 mV are applied (see Figs. 4, 10).

Temperature dependence of kinetics. The temperature dependence of the kinetics was determined by measuring the values of τ_s at various potentials as the temperature was slowly varied between 26 and 38° C. Figure 12 shows the variation of log τ_s with T at two different membrane potentials. The Q_{10} in each case is about 6. The value of $i(E_H, s = 1) - i(E_H, s = 0)$ was also found to be temperature dependent. An increase in temperature increases the total amplitude of the activation curve. However, this effect could not be expressed simply in terms of an over-all Q_{10} and more experiments will be required to establish the functional dependence of the magnitude of i_{K_2} on temperature.

DISCUSSION

Separability of kinetic and rectifier variables. The time and voltage dependence of the slow component, g_{K_2}, of the potassium conductance in Purkinje fibres is well described by a model that represents the conductance as the product of two distinct factors. The kinetic factor, s,

represents the fraction of the total g_{K_2} which is activated at any potential. s depends on the past history of the membrane potential through the first-order equation (3) but it cannot change instantaneously (i.e. it is always continuous) even following step changes in membrane potential. The second factor, represented by the rectifier function, $i_{K_2}(E_m, E_K, s = s_0)$, describes the instantaneous current–voltage relation for any constant value of s, s_0. The *shape* of this relation is independent of the previous

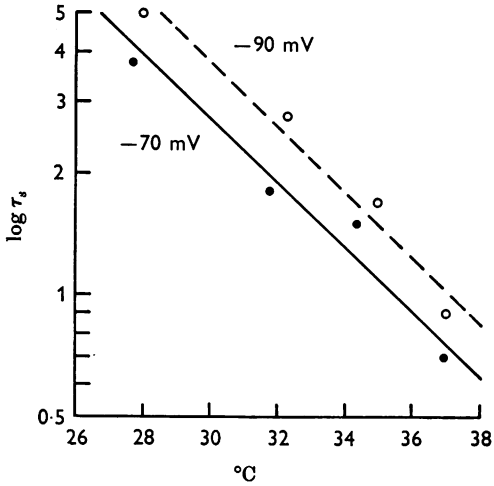


Fig. 12. Influence of temperature on rate of change of i_{K_2}. Preparation 46–5. Ordinate: log τ_s. Abscissa: temperature ° C. Open symbols show values measured at −90 mV. Filled symbols show values obtained at −70 mV. The straight lines have a slope corresponding to a sixfold change in rate for a 10° C change in temperature.

values of membrane potential and of the value of s. However, the shape does depend on the value of E_K, whereas s is independent of E_K. The shape of the relation may be constant if the current is plotted as a function of the driving force, $E_m - E_K$, but the present results are not extensive enough to justify this conclusion.

The results and analysis described in this paper show that the two factors are indeed separable (see *Rectifier properties*). Any interaction between them must be smaller than the experimental errors (see Methods). The kinetic factor is similar to the Hodgkin–Huxley permeability variables, and the $s_\infty(E_m)$, $\alpha_s(E_m)$ and $\beta_s(E_m)$ relations strongly resemble those found for other variables of this kind. The $i_{K_2}(E_m, E_K, s_0)$ function is an inward-going rectifier showing a region of negative slope conductance beyond about 25 mV positive to E_K. These results have a number of important implica-

tions which may be divided into two classes. First, although the previous analysis of the Purkinje fibre action potential and pace-maker activity involving a slow potassium conductance (Noble, 1962) is confirmed in principle, a radical revision of the equations will be required in order to reproduce the details of the potassium current described in this paper and previously (McAllister & Noble, 1966, 1967). Secondly, any explanation of the mechanism of inward-going rectification and of the Hodgkin–Huxley permeability variables must account for the possibility that these phenomena can occur together in the sense that they can control the same ionic current although the two factors themselves may behave independently. In addition to Purkinje fibres, this conjunction has also been found in TEA-treated squid nerve (Armstrong & Binstock, 1965; Armstrong, 1966) and in skeletal muscle (R. H. Adrian, W. K. Chandler & A. L. Hodgkin, personal communication). The clear separability of the two factors suggests that the K channels involved may be controlled by two physically separate gating mechanisms (cf. Armstrong, 1966).

Role of the slow K current in pace-maker activity. The spontaneous depolarization occurring during pace-maker activity in Purkinje fibres extends over the range of potentials over which s_∞ varies from nearly 0 to nearly 1. The position of the $s_\infty(E_m)$ relation is therefore considerably more negative than the slow g_{K_2} curve used by Noble (1962) to compute the time-dependent current. As a result of this, the behaviour of Purkinje fibres will differ from that of Noble's model in several important respects. These may best be indicated by calculating how s and i_{K_2} will vary during pace-maker activity. A full quantitative description of this must await a reconstruction of the action potential using the new model. However, the information on the kinetics and rectifier properties which we have obtained is sufficient to allow a preliminary calculation to be made. In order to do this we need to know how E_m varies during spontaneous activity in a cell which is known to obey the kinetics we have described. Vassalle (1966) has recorded the pace-maker potential in a cell in which he also recorded the time constant of decay of the slow current during a clamp to the maximum diastolic potential (see Fig. 1 in Vassalle's paper). His value for the time constant fits our data and it seems reasonable therefore to use his $E_m(t)$ curve. This has been traced and replotted as the upper diagram in Fig. 13. In order to calculate the variation in s, s_∞ and i_{K_2} we have used the $s_\infty(E_m)$ and $\tau_s^{-1}(E_m)$ relations shown in Fig. 4 and the rectifier function, $i_{K_2}(E_m, E_K, s = 1)$, at 2·7 mM $[K]_o$ (which corresponds to Vassalle's value) obtained from Fig. 11. s was integrated using the numerical approximation

$$\delta s_{t \text{ to } t+\delta t} = \delta t \langle \tau_s^{-1} \rangle (\langle s_\infty \rangle - \langle s \rangle), \tag{9}$$

where $\langle s_\infty \rangle$ and $\langle \tau_s^{-1} \rangle$ are average values over the step t to $t+\delta t$ and $\langle s \rangle$ is estimated from

$$\langle s \rangle = s_t + \tfrac{1}{2}\delta s_{(t-\delta t \text{ to } t)}. \tag{10}$$

A step length of 0·1 sec was used from 0 to 1·5 sec. This was reduced to 0·05 sec thereafter. This procedure gives a sufficiently accurate approximation for s as a function of time. i_{K_2} was then obtained from

$$i_{K_2} = s\, i_{K_2}(E_m, E_K, s = 1). \tag{11}$$

It was assumed that s is virtually equal to 1 at the end of repolarization (this assumption will be justified below).

At the beginning of the pace-maker potential, E_m is very negative so

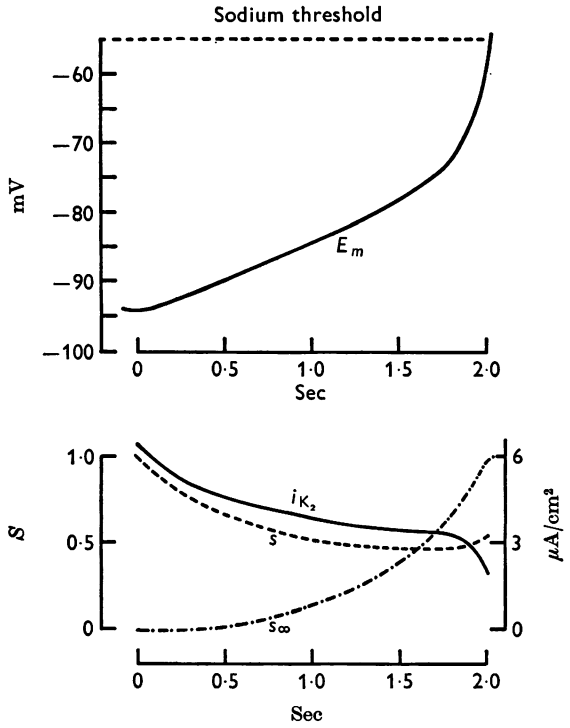


Fig. 13. Mechanism of pace-maker potential based on new model for K current.

Top: variation in membrane potential during pace-maker activity, replotted from Vassalle (1966, Fig. 1).

Bottom: s_∞ (t) relation obtained from $s_\infty(E_m)$ relation shown in Fig. 4. s and i_{K_2} were calculated from equations (9)–(11) using the rectifier function for 2·7 mM $[K]_o$ shown in Fig. 11. Note that, although s does not fall below a certain value and actually increases towards the end of the pace-maker potential, i_{K_2} falls continuously. This is a consequence of the negative slope in the rectifier function.

that s_∞ will be nearly zero. s will therefore decline slowly (the time constants for s are longest in this range of potentials—see Fig. 4). In a full computation it would, of course, be this decline in s which is responsible for generating the pace-maker depolarization. It must be remembered that in these calculations E_m is 'forced' to follow Vassalle's recorded potential. However, the $i_{K_2}(t)$ curve obtained from the calculation is consistent with view that it may have generated this pace-maker potential. In the absence of a full reconstruction, therefore, these calculations give a useful description of the detailed mechanism of the pace-maker potential.

As the membrane depolarizes, s_∞ increases. At some point in time, therefore, s must equal s_∞. This occurs at 1·6 sec in Fig. 13. Thus, s cannot fall below a certain value during the pace-maker potential. Moreover, unless excitation occurs fairly quickly following this point, spontaneous excitation may fail to occur since any further depolarization will cause s to rise again (as in Fig. 13) so that the point at which $s = s_\infty$ could become a stable point. Whether this will happen or not may depend critically on the presence of the negative slope which we have observed in the rectifier function for i_{K_2} and possibly also on the current–voltage relation for i_{K1}. In the case of Fig. 13 the negative slope becomes apparent in the region of potential at which $s = s_\infty$. In this case, therefore, further spontaneous depolarization can occur since i_{K_2} will continue to fall even when s is rising, provided that s does not rise too quickly and that excitation does not occur too slowly. Thus, i_{K_2} declines very sharply towards the end of the pace-maker potential. In fact, most of the rapid increase in the rate of depolarization at this time is attributable to the negative slope in the instantaneous current–voltage relation rather than to activation of g_{Na} (as in Noble's model), since the Na threshold is not reached until the membrane depolarizes to about -55 mV (see Figs. 4 and 13).

The detailed mechanism of the pace-maker potential is therefore strikingly different from that in Noble's model. In this model, a point at which $n = n_\infty$ during the spontaneous depolarization following an action potential must become a stable point since the instantaneous current–voltage relation for i_{K_2} was assumed to be linear. In a spontaneously active system, therefore, n must continue to fall throughout the duration of the pacemaker potential. Other detailed differences arising from the position of the s_∞ curve have been discussed by McAllister & Noble (1967).

Role of the slow K current during the action potential. It was assumed in the previous section that s is nearly equal to 1 at the end of the action potential. This assumption is justified since the time constant, τ_s, in the region of the plateau is sufficiently short (McAllister & Noble, 1966) that s should approximate to 1 within about 200 msec. In this respect the system is similar to the behaviour of g_{K_2} in Noble's model. However, since

the K current controlled by the s system also shows inward-going rectification, and since the rectifier function is known to have a low value in the region of the plateau (about one third of its value at -80 mV—see McAllister & Noble, 1966, Fig. 4), the total amount of repolarizing current supplied by K ions must be smaller than that given by Noble's model. Even when s is fully activated, therefore, repolarization may still occur relatively slowly. Since $s = 1$ and will remain constant between this time and the beginning of the pace-maker potential, this later phase of repolarization may be referred to as the s-independent phase. Unless other slow time-dependent currents also significantly contribute to the plateau mechanism, this phase of repolarization will be determined simply by the recharging of the membrane capacity through the non-linear resistance given by the membrane current–voltage relation when $s = 1$.

The duration of the s-dependent phase of repolarization will, of course, depend on the initial value of s and since s_∞ is very dependent on E_m in the region of the resting or pace-maker potential the action potential duration should be dependent on the initial value of E_m. A dependence of this kind has been shown in solutions containing relatively high concentrations of K ions by Weidmann (1956) who showed that, in these solutions, the plateau is abolished if the action potential is initiated from the depolarized level of membrane potential but that, if the membrane is previously hyperpolarized back to its value in normal K solutions, a plateau could be restored. Since an increased K concentration increases i_{K_1} as well as i_{K_2} the abolition of the plateau cannot be attributed solely to effects on i_{K_2}. A clearer demonstration of the role of i_{K_2} in the plateau would therefore be obtained by varying the initial membrane potential in a fibre bathed in normal Tyrode solution. This is shown in Fig. 14. The action potentials were initiated (*a*) from a membrane potential at which s_∞ is nearly 1 and (*b*) from a membrane potential at which s_∞ is nearly zero. The latter action potential is considerably longer than the first. Van der Walt & Carmeliet (1967) and Carmeliet & van der Walt (1968) have recently investigated this effect more extensively and have shown that the duration of the action potential is related to the magnitude of the initial membrane potential according to an S-shaped relation, which would be expected since the steady state dependence of s on membrane potential is also S-shaped (see Fig. 4). They have also shown that the magnitude of the change in duration of the action potential depends on the duration of the preceding change in membrane potential and lasts for some time after the end of a hyperpolarizing pulse. Both of these effects may be attributable to the time taken for s to change from one steady-state value to another following a sudden change in potential. These results indicate that some part of the plateau phase of repolarization is s-dependent since our results suggest

that s is the only time-dependent activation variable whose steady-state value varies appreciably over the range of potentials between -70 and -100 mV. However, it is becoming evident that the slow K current has a less important role in the mechanism of the plateau than it has in the generation of the pace-maker potential. In an action potential lasting 400 msec only about half the duration of the repolarization can be s-dependent. Moreover, the activation of the slow K current is too fast in the plateau region to be responsible for the extremely slow repolarization observed in certain conditions, e.g. low $[K]_o$ (see Noble, 1965, Fig. 4) and sometimes in Cl-free solutions (see Hutter & Noble, 1961, Fig. 5), or following strong hyperpolarization (Carmeliet & van der Walt, 1968; D. Noble & R. W. Tsien, unpublished). In these cases much slower changes must be involved.

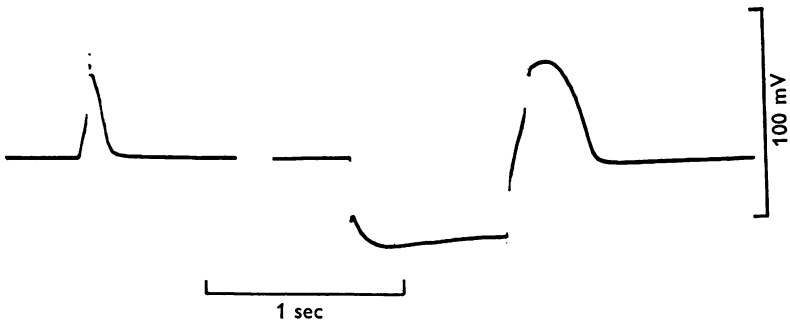


Fig. 14. Influence of initial membrane potential on duration of action potential plateau. Preparation 47–4.

Left: action potential initiated by 1×10^{-7} A depolarizing current applied for 40 msec from a potential (about -70 mV) at which s_∞ is nearly 1.

Right: action potential initiated by the break of a 8×10^{-7} A hyperpolarizing current applied for 630 msec which deflected the potential to a value (about -110 mV) at which s_∞ is nearly 0. The fast components of the action potentials, including the initial spike, are not shown. Note also that the absolute membrane potentials are uncertain for reasons explained in Methods section.

Comparison with other conductance mechanisms. The kinetics of the slow K current show some strong resemblances to the delayed K current in nerve cells. However, there are some important differences.

The s kinetics are generally two orders of magnitude slower than the n kinetics of nerve cells. It is for this reason that we have chosen the variable s rather than continuing to use the variable n as in previous work (Noble, 1962; McAllister & Noble, 1966, 1967). At present, the only other permeability variable of this kind which has been analysed and found to have kinetics as slow as those for s is the K inactivation variable, k, in nerve cells (Frankenhaeuser, 1962; Ehrenstein & Gilbert, 1966). A similar

slow K inactivation has also been observed in skeletal muscle (Adrian, Chandler & Hodgkin, 1966). Over the voltage range and periods of time (up to tens of seconds) investigated in the present paper, the s system does not appear to be inactivated.

The second reason for using a different variable is that the presence of inward-going rectification in the same system which is controlled by the s gates makes it less likely that the s system is simply a slowed-down version of the n system found in nerve cells. This argument is not conclusive since Armstrong & Binstock (1965) interpret their results on squid-nerve to indicate that the presence of TEA introduces inward-going rectification in the n system. However, TEA does not greatly slow the n kinetics (Armstrong, 1966). Moreover, it is still possible that a much faster potassium conductance is also present in Purkinje fibres and it would, perhaps, be more consistent to use the variable n for this system than for g_{K_2}. Further experiments are required to test this possibility.

Another important difference is that the maximum current carried by g_{K_2} is very small. The maximum current around -70 mV (where $s_\infty \to 1$) is only about 10 μA/cm^2 and, at more positive potentials, the maximum current is even smaller. The s kinetics are also more temperature dependent than any other permeability variable so far described in the literature. Between 26 and 38° C the Q_{10} is 6, compared to 2–3 for the K current in nerve cells (Hodgkin, Huxley & Katz, 1952; Frankenhaeuser & Moore, 1963). Finally, g_{K_2} is determined by the first power of s, whereas a higher power (2–4, or even higher) is required for the delayed K current in nerve cells. In fact, the s system is the first known example of a system showing only simple exponential current changes; of course, the inactivation variables (h and k) in other systems also require an exponent of 1.

Influence of temperature on the electrical activity of Purkinje fibres. The extreme slowness and very high temperature dependence of the s kinetics both have important consequences for the over-all electrical activity of Purkinje fibres. Coraboeuf & Weidmann (1954) found a strikingly high temperature dependence of the rate of change of voltage during the pace-maker potential ($Q_{10} = 6{\cdot}2$) and the plateau phase of repolarization ($Q_{10} = 4{\cdot}5$). These results were obtained on spontaneously beating fibres and, although this means that the frequency of beating varied with temperature, it is reasonable to assume that the s system would be in equivalent states at comparable phases of the records obtained at different temperatures. This follows from the fact that the plateau and pace-maker potential account for by far the largest fraction of time during each cycle so that the total duration of the cycle changes by much the same factor as the duration of each phase. These durations are in turn to a large extent determined by the s kinetics.

On the other hand, Trautwein, Gottstein & Federschmidt (1953) using preparations stimulated at a constant frequency obtained lower values for the Q_{10}s than did Coraboeuf & Weidmann (1954). The qualitative reason for this difference is fairly clear. As the temperature is lowered, s will change more slowly and, if the frequency of stimulation is constant, the over-all variation in s during each cycle must be less than at higher temperatures. This means that on average s will deviate more from s_∞, particularly during the spontaneous phase of depolarization following the action potential, and since the rate of change of s, and hence, of membrane potential, depends on how far s deviates from s_∞, the average rate of change of s would be expected to be higher than in situations where s is allowed time to approach its steady-state value. As a consequence, the rate of change of voltage may be less temperature dependent when the preparation is stimulated at a constant frequency than when it is allowed to beat spontaneously. Whether this argument can account quantitatively for the different Q_{10}s obtained by the two methods remains to be seen.

Note added after submission. Dudel, Peper, Rüdel & Trautwein (1967*c*) have recently studied the potassium conductance of Purkinje fibres using the ramp voltage clamp technique. They interpret their results as evidence for the absence of significant time-dependent conductance changes and prefer to attribute changes in the potassium current–voltage relations to changes in E_K. On the basis of some experiments using the rectangular clamp technique, they estimate that less than 10% of the outward current is time-dependent. These results may seem less surprising, however, when we consider the conditions required for observing the slow changes in i_{K_2}:

1. Even when fully activated, the time-dependent component of i_K is normally one third or less of the total potassium current, i.e. at any potential i_{K_1} is usually twice as large as the maximum value of i_{K_2}.

2. The magnitude of the slow current changes observed with rectangular clamps depends critically on the clamp potentials used. Records showing a large degree of time dependence (see Fig. 7) are obtained using depolarizations from a potential which is negative enough so that nearly all of i_{K_2} is yet to be activated (s nearly 0), and yet not so near E_K as to reduce the size of the current change on repolarization. The depolarization must also be large enough to activate a large fraction of i_{K_2}, but not so large as to allow the slow current change to be reduced as a consequence of the negative slope in the current–voltage relation.

Dudel *et al.* (1967*c*) also show that current–voltage relations obtained with different speeds of repolarizing ramp clamps do not intersect each other in the region of -95 mV, which they estimate as the value of E_K.

On the basis of our results, however, we would expect that, at $[K]_0 = 2{\cdot}7$ mM, the relations should intersect at a reversal potential near -110 mV (see Fig. 11) which is beyond the range of potentials studied by Dudel *et al.* (1967*c*).

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