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MEASUREMENT AND SIGNIFICANCE OF THE REVERSAL POTENTIAL FOR THE PACE-MAKER CURRENT (i_{K_2}) IN SHEEP PURKINJE FIBRES

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SUMMARY

1. The apparent reversal potential (E_{rev}) of the pace-maker current (i_{K_2}) is found to depend on the experimental protocol used for its measurement. Evidence is presented showing that depolarizing (hyperpolarizing) pulses given before a test hyperpolarization used to determine E_{rev}, shift E_{rev} to more negative (positive) values. These shifts are opposite to those expected if the only effect of pre-pulses were to change the concentration of potassium in extracellular clefts ($[K]_c$) via accumulation and depletion processes.

2. This effect is shown to be due to the fact that E_{rev} is dependent on s_0, the degree of activation of i_{K_2} at the start of the test hyperpolarization.

3. When a suitable protocol is used, depletion of cleft K can be demonstrated to take place during a large hyperpolarization. Changes in the level of $[K]_c$ induced by pre-pulses must therefore also affect the E_{rev} determination.

4. A simplified three-compartment model has been used to investigate how K accumulation and depletion can affect the time course of i_{K_2}, with particular reference to the problem of E_{rev} determination. Computed examples show that the model is able to reproduce the main features of the time course of i_{K_2} recorded near its reversal potential and the changes induced by pre-pulses on E_{rev} measurement. By contrast, simulation on a linear cable model rules out the possibility that such results are due to voltage non-uniformity.

5. The three-compartment model predicts that the measured value of E_{rev} differs from E_{K_2} for two reasons: (1) when the recorded current trace is flat i_{K_2} is still outward and decaying, and (2) the K equilibrium potential shifts to more negative values while the test hyperpolarization is applied.

6. The finding that E_{rev} is directly affected by changes in s at the beginning of the test pulse is discussed in relation to the action of agents (such as Ca^{2+}, H^+, salicylate, adrenaline and ouabain) which are found to shift both the s_{∞} curve and E_{rev}.

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INTRODUCTION

The 'pace-maker' current (i_{K_2}) has been described in the Purkinje fibre as a pure potassium current which obeys first-order Hodgkin-Huxley kinetics (Noble & Tsien, 1968). The behaviour of i_{K_2} in the vicinity of its reversal potential is, however, apparently inconsistent with the above description in at least two respects: first, the current recorded during a voltage clamp near E_{K_2} does not usually show the expected single-exponential time course (see for example Figs. 1, 2 and 3); secondly, the measured reversal potential is more negative than the expected K equilibrium potential (Cohen, Daut & Noble, 1976*a*). According to the latter authors the presence in the Purkinje fibre of restricted extracellular spaces can explain the highly negative value of E_{K_2} if the steady level of K in the clefts is kept low by pump activity. This assumption implies that any shifts observed in the measured reversal potential can be attributed to cleft K concentration ($[K]_c$) variations. However, in view of the fact that a large hyperpolarization decreases the K outflow thus inducing a decrease in $[K]_c$, it is important to determine the extent to which the time course of i_{K_2} decay is affected by changes in $[K]_c$ *during* the pulse used to measure E_{K_2}. In this paper we investigate the problem of interference of K accumulation and depletion processes on the measurement of E_{rev} in two stages. First we shall describe experimental observations that show that E_{rev} changes according to the protocol used in its determination and so is not a unique function of the ratio of external to internal K concentration. We find that E_{rev} depends on the degree of activation of i_{K_2} at the start of the hyperpolarization used in the E_{rev} measurement and at the same time that K depletion takes place during this hyperpolarization. The second stage consists in developing a three-compartment model for the description of the time course of i_{K_2}, where accumulation and depletion phenomena are taken into account, and in comparing the predictions of this model with the experimental data. The model gives a satisfactory description of the results obtained, and resolves the two apparent inconsistencies mentioned above, i.e. the non-monotonic time course of the current recorded at potentials near E_{rev}, and the fact that E_{rev} appears to be more negative than it should be. Furthermore, the finding that E_{rev} depends on the degree of activation of i_{K_2} also provides a possible interpretation for at least part of the shift observed in E_{rev} when the concentration of a surface charge agent is changed, as this is known to produce a shift in the position of the s_∞ activation curve along the voltage axis. Some of this work has appeared in abstract form (DiFrancesco & Ohba, 1978).

METHODS

A conventional two-micro-electrode voltage-clamp technique has been used in these experiments. Fine unbranched Purkinje fibres from sheep hearts, of lengths ranging between 1·5 and 2·2 mm were placed in a bath and perfused with oxygenated Tyrode solution at a constant temperature of 35 °C. The Tyrode solution composition was the following: NaCl, 128 mM; KCl, 4 mM; $NaHCO_3$, 12 mM; NaH_2PO_4, 0·4 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM; glucose, 5 g/l.

The normal K concentration was changed by substituting NaCl in equimolar quantities. 4 M-K acetate micro-electrodes with resistances from 6 to 15 MΩ were used for passing current and measuring membrane potential. The current was continuously recorded at two different gains on a Devices pen recorder together with membrane potential and temperature. A storage oscilloscope (Tektronix 7313) was also used occasionally for enlarged displays.

The current and voltage micro-electrodes were usually positioned at about $X = 0{\cdot}5\,L$ and $X = 0{\cdot}7\,L$ respectively from one end of a fibre of total length L, in order to minimize errors coming from spatial non-uniformity during a voltage clamp pulse (see DiFrancesco & McNaughton, 1979, Fig. 1.).

RESULTS

Symbols

i_{K_1}	Time-independent K current, as described in McAllister & Noble (1966)
i_{K_2}	'Pace-maker' K current, as described in Noble & Tsien (1968)
i_p	Electrogenic pump current (always outward directed)
i_l	Over-all non-K-dependent transmembrane current
E	Membrane potential
E_{K_2}	Reversal potential of i_{K_2}. If i_{K_2} is a pure K current E_{K_2} coincides with the K equilibrium potential
E_{rev}	Apparent reversal potential of i_{K_2} as derived from the experimental results
K_i, K_c, K_b	Internal, cleft and external (bulk) potassium concentration respectively
V	Total cleft volume
P	Permeability coefficient for the K exchange between cleft and external solution.
F	Faraday's constant
t	Time
s	Degree of activation of i_{K_2}
s_0, $[K]_{c,0}$	Values of s and K_c at the beginning of a hyperpolarization to (or near) E_{K_2}

The total current crossing the membrane is described by

$$i_t = i_{K_1} + i_{K_2} + i_p + i_l$$

where

$$i = i_{K_1} + i_{K_2} + i_p$$

is the $[K]_c$-dependent part of it.

The total K flux across the membrane is described by

$$j_K = (1/F)\,(i_{K_1} + i_{K_2} + r i_p)$$

where r is the K fraction of the pump current. For a 3 : 2 Na – K exchange, $r = -2$.

I. *Experimental determination of the reversal potential of* i_{K_2}

In this section we will investigate the effects of altering the voltage-clamp protocol on the determination of the reversal potential of i_{K_2}. The purpose of the experiments is to see whether or not changes in E_{rev} are directly correlated with changes in the cleft K concentration, which we can vary by applying conditioning pre-pulses before measuring E_{rev}.

Given that the time course of i_{K_2} decay at (or near) its reversal potential is non-monotonic (see Introduction), we will adopt the convention that in a series of voltage-clamp hyperpolarizations of increasing amplitude, the measured reversal potential E_{rev} corresponds to the potential at which the current trace starts to have no region of *negative* slope. This convention is commonly used (see for example Cohen, Daut & Noble, 1976*a*). As will become clear later, the results presented in this paper and their interpretation are independent of the convention used to define E_{rev}.

(a) *Changes in* E_{rev} *induced by pre-pulses*

A depolarizing pulse by increasing the K outflow across the membrane, should increase the $[K]_c$ through accumulation. Therefore, applying a positive (negative) pre-pulse before the hyperpolarization used to measure E_{rev} should give rise to a positive (negative) shift in E_{rev}, reflecting a shift in the K equilibrium potential. However, from the experiment shown in Fig. 1 it is apparent that the opposite occurs. In the reference run, when no conditioning pulse is applied and the holding potential is chosen near the half-activation point of the s_∞ curve (see insert), E_{rev} appears to be about -105 mV. A depolarizing pre-pulse to -56 mV for 5 sec moves E_{rev} *negative* (a value of about -125 mV, not shown in the picture, was recorded on the oscilloscope). Hyperpolarizing to -94 mV for the same length of time causes a positive shift of E_{rev} to about -96 mV.

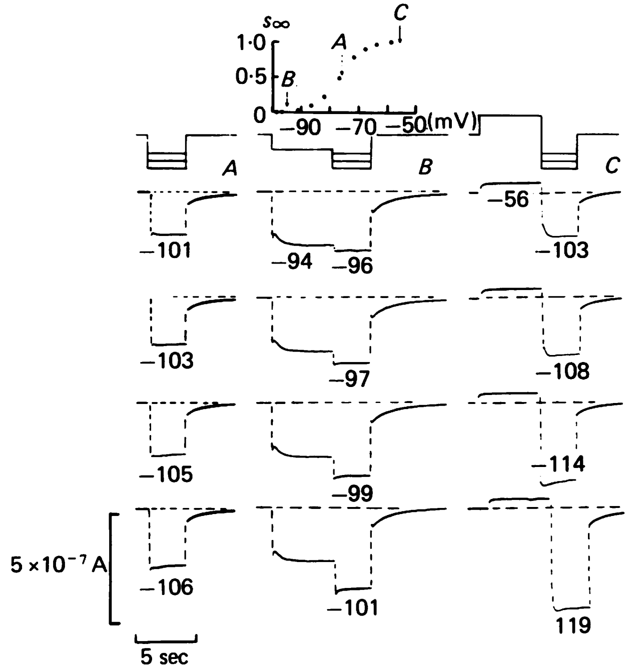

Fig. 1. Effect on E_{rev} of 5 sec pre-pulses to the bottom and the top of the s_∞ activation curve, as shown on top of the Figure. E_{rev} shifts from the control value of -105 mV (*A*), obtained holding at the $\frac{1}{2}$ activation point of the s_∞ curve (-76 mV) to -96 mV (*B*) after a pre-hyperpolarization to -94 mV, and to -125 mV (*C*) after a pre-depolarization to -56 mV. The hyperpolarizations negative to -119 mV were recorded only on the oscilloscope. Voltage protocols are shown above current traces. Voltages (in mV) indicated near the correspondent current records.

These results clearly indicate that E_{rev} does not only depend on the level of $[K]_c$ set by the pre-pulse. This does not exclude the possibility that a change in $[K]_c$ will affect the position of E_{rev} but does rule out the idea that accumulation or depletion processes preceding the test pulse play a unique role in determining E_{rev}.

Fig. 2 shows another experiment confirming the results of Fig. 1. E_{rev} measured from a holding potential of −80 mV with the usual protocol (top left) is about −100 mV. Pre-hyperpolarizing to −85 mV for 1 sec shifts E_{rev} slightly in the positive direction (−99 mV, top middle) and pre-depolarizing to −51 mV for 1 sec gives rise to a large negative shift of E_{rev} (−110 mV, top right). In this experiment the pre-pulse duration has also been changed, in order to see whether this can affect the observed shift of E_{rev}. In fact, prolonging the length of pre-pulses to 7 sec reinforces their effect on E_{rev}, which moves to −97 mV following the pre-hyperpolarization and to −112 mV following the pre-depolarization.

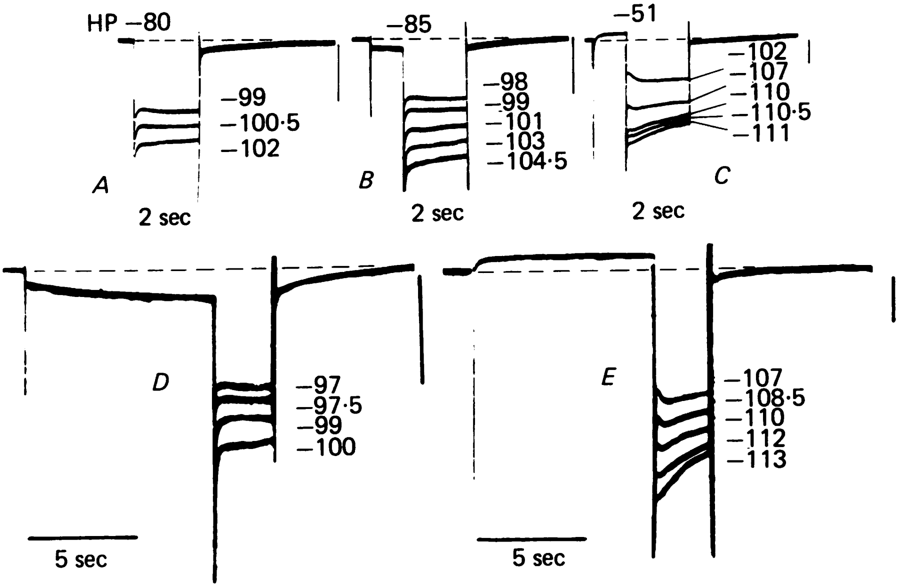

Fig. 2. Effects of hyperpolarizing (HP) and depolarizing pre-pulses on E_{rev}. *A*: control run from a holding potential of −80 mV gives an E_{rev} of about −100 mV. *B*, *C*: a 5 mV pre-hyperpolarization 1 sec long shifts E_{rev} to about −99 mV, while a 29 mV pre-depolarization of the same duration gives rise to a larger negative shift (E_{rev} = −110·5 mV). *D*, *E*: prolonging the duration of pre-pulses to 7 sec increases the shifts of E_{rev} to −97·5 and −112 mV respectively. For every set the vertical bar is 5×10^{-8} A.

The dependence of the shift of E_{rev} on the duration of pre-pulse has been examined in more detail in the experiment shown in Fig. 3.

A 30 mV pre-depolarization from the holding potential of −68 mV was applied for different durations (0–10 sec) before the test hyperpolarization to −101 mV. At this potential the current trace is nearly flat when no conditionong pre-pulse is applied (trace *a*), but as soon as the 30 mV step is given for a brief time (0·2 sec) an outward deflection becomes apparent (trace *b*) and this progressively increases with the pre-pulse duration (*c*–*g*). The steady value of this outward deflection, which will obviously be associated with a negative shift of E_{rev} (not shown) is reached only between traces *d* and *e*, i.e. after a pre-pulse duration of 1 sec.

The experiments shown in Figs. 1, 2 and 3 demonstrate that E_{rev} is not simply a parameter fixed by internal and external K concentrations, but depends on the protocol used for the measurement. More precisely, E_{rev} depends on a time- and voltage-dependent process which is activated by pre-pulses of different amplitudes and durations, applied before the test hyperpolarization. This process cannot be K accumulation or depletion, as if it were the shifts in E_{rev} would be opposite to those observed experimentally.

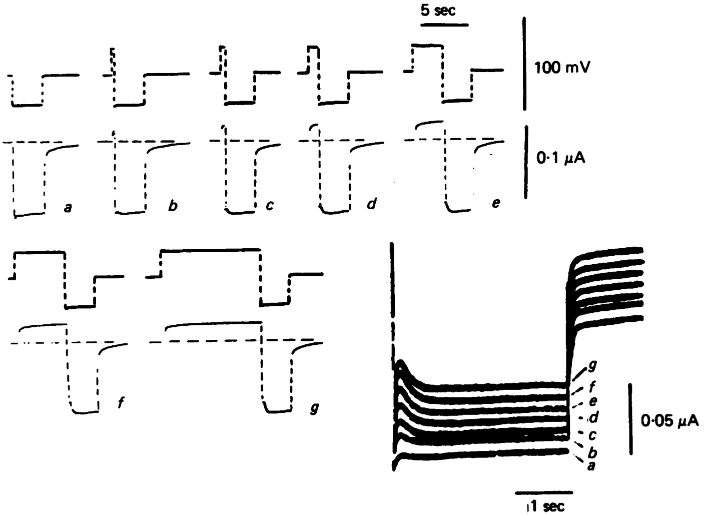

Fig. 3. Time course of the current recorded on hyperpolarization to −101 mV is modified by the duration of a pre-depolarization. A +30 mV pre-pulse was superimposed on the holding potential of −68 mV and its duration was varied between 0 and 10 sec. Voltage-clamp pulses (above) and current traces (below) are shown for the following pre-pulse durations (in sec): trace *a*, 0; *b*, 0·2; *c*, 0·5; *d*, 1; *e*, 3; *f*, 5; *g*, 10. The current is flat in the control case (*a*), but when the pre-pulse is applied for 0·2 sec (*b*) an outward decay appears, which increases with the duration of the pre-pulse itself (*c*–*g*). In the enlargement taken from the oscilloscope (bottom right) the current traces have been displaced vertically by arbitrary amounts for clarity.

(b) *The time- and voltage-dependent variable which affects the measurement of E_{rev} is the degree of activation of i_{K_2} (s)*

From Fig. 3 it is apparent that the outward deflexion activated by pre-pulsing to −38 mV reaches its half-amplitude somewhere between traces *b* and *c*, which follow pre-depolarizations lasting 0·2 and 0·5 sec respectively. A process known to have a time constant of comparable magnitude at −38 mV is the activation of i_{K_2} (a value of 200 msec is given for τ_s at −50 mV by Noble & Tsien, 1968). It is therefore worth investigating whether the time- and voltage-dependent process found to affect the position of E_{rev} after pre-pulses is indeed the activation of i_{K_2}. This was done in the experiment shown in Fig. 4. Following a protocol similar to that of Fig. 3, pre-pulses of 35 mV amplitude and variable durations (0 to 5 sec) were applied from the holding potential of −84 mV before the test hyperpolarization to −102 mV (Fig. 4*B*). As seen in Fig. 3, the current trace is flat during the test hyperpolarization given from the holding potential, but when the positive pre-pulse is applied an outward tail appears which increases with the duration of the pre-pulse. If this outward tail reflects the decay of extra current (i_{K_2}) activated by the pre-pulse, the dependence

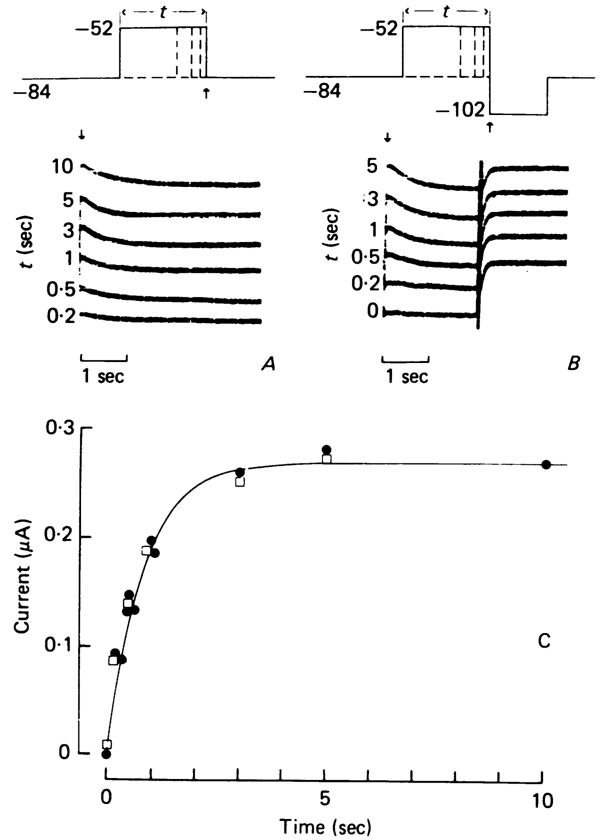

Fig. 4. Comparison between the dependence of tail amplitude on pulse duration in an envelope test and the dependence of the amplitude of current change measured at E_{rev} on pre-pulse duration. The voltage protocols are shown on top. In both cases, current traces are recorded after depolarizations to −52 mV (time marked by an arrow) and superimposed on a store oscilloscope. Traces displaced on the vertical axis for clarity. The duration of depolarization is indicated near each trace. *A*: envelope test at −84 mV. *B*: change in the current time course at −102 mV induced by pre-depolarization to −52 mV. Note that the trace is flat when no pre-pulse is applied ($t = 0$). *C*: amplitudes of tails from envelope test (*A*) and of current changes at −102 mV (*B*), these latter scaled up by multiplication by 1·25, plotted against duration of pulse to −52 mV. The full-line curve is given by the equation $0{\cdot}135\,(1-e^{-t/0{\cdot}8})$.

of its amplitude on the pre-pulse duration should be similar to that obtained in an envelope test at the holding potential.

To test this the amplitude of the i_{K_2} tail on return to the holding potential after a pulse to −52 mV was changed by varying the pulse duration (Fig. 4*A*). Fig. 4*C* shows results from these two different protocols. Tail amplitudes from the envelope test are plotted against pulse duration, and those coming from the protocol of Fig.

4*B* are scaled up to compare the two curves and plotted against pre-pulse duration. The similarity between the two time courses is evident, and implies that the time- and voltage-dependent process which causes E_{rev} to shift when pre-depolarizations are applied before a test hyperpolarization, is the change in the degree of activation (s).

(c) *Contribution of a depletion process to the time course of* i_{K_2} *near its reversal potential*

The results presented so far can be explained by assuming that the reversal potential of i_{K_2} is itself a function of s before the application of the test pulse. This

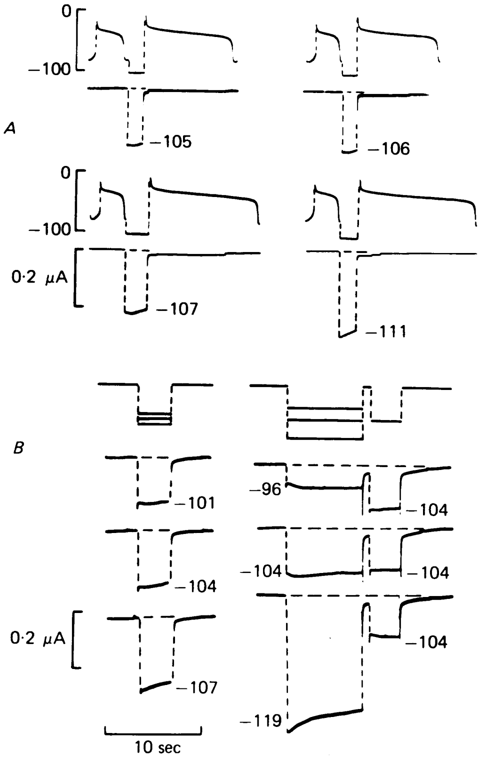

Fig. 5. Determination of E_{rev} in a spontaneously beating fibre in different conditions. *A* : measurement made by clamping at the start of the diastolic depolarization (E_{rev} = $-106/-107$ mV); notice the lengthening of the action potential after clamp release. *B* (left); conventional protocol from a holding potential of -76 mV (E_{rev} = -104 mV); *B* (right): 8 sec long pre-hyperpolarizations to various levels (indicated on the left of the current traces) followed by a 1 sec return to -76 mV are given before the test pulse to -104 mV. Notice that the current trace is nearly flat after pre-hyperpolarizations to -96 and -104 mV, but after the prepulse to -119 mV an outward decay is apparent.

does not exclude the possibility that a pre-pulse also induces a change in $[\mathrm{K}]_{\mathrm{c}}$ and that the effect of this on the time course of i_{K_2} during the test hyperpolarization is masked by the overwhelming effect of the change in s. Changes in E_{rev} due to K depletion occurring as a result of large hyperpolarizations have already been observed (Baumgarten & Isbenberg, 1977). Fig. 5 shows an experiment designed to establish the presence of accumulation and depletion phenomena associated with large voltage-clamp pulses and their effect on E_{rev}. Clamping at the beginning of the diastolic depolarization in a spontaneously beating fibre (Vassalle, 1965) gives an E_{rev} value of -106 mV (Fig. 5*A*). The action potential duration is greatly increased when the clamp is released at the end of the hyperpolarization, which suggests that a depletion of $[K]_c$ has taken place during the clamp at -105 mV. In the plateau range of potentials, where inward-going rectification of the K current occurs, a decrease of outward current will give a longer action potential as a result of a reduction in external K concentration ($[K]_c$). As shown in Fig. 5*B*, a similar value of -104 mV is obtained for E_{rev} when a conventional protocol is used by holding at the zero-current potential (-76 mV in this case). A third protocol on the same fibre (*B*, right) gives further confirmation that the measure of E_{rev} can be influenced by the level of $[K]_c$ set by the previous history of clamp pulses applied. The membrane is hyperpolarized for 8 sec to different levels and then clamped back for 1 sec to the holding potential of -76 mV before a fixed clamp to -104 mV is applied. The rationale behind this experiment is that the 1 sec return to the holding potential of -76 mV lasts long enough to reset s to the value s_∞ (-76), but is not long enough to reset $[K]_c$ to its steady value at -76 mV, after the depletion induced by pre-hyperpolarizing to various levels. Thus, at the start of the test pulse, s_0 will be the same in all cases, while $[K]_{c,0}$ will depend almost entirely on the level of the previous conditioning hyperpolarization, and any modifications of the current time course at -104 mV should be produced only by the changes in $[K]_c$. In fact, E_{rev} is seen to shift to more negative values after pre-hyperpolarizations, i.e. in the *opposite* direction to that observed in the experiments of Figs. 1, 2 and 3. After pre-pulsing to -87, -96 and (less markedly) -104 mV, the subsequent clamps to -104 mV each produced a nearly flat current trace. However, when the conditioning pulse was to -119 mV, clamping to -104 mV resulted in a decaying outward current. This can be explained if the K depletion caused by the hyperpolarization to -119 mV had been sufficiently large to cause a detectable shift of E_{rev} towards more negative potentials. We therefore conclude that a substantial depletion of cleft K takes place during a hyperpolarization in the range of potentials near E_{rev}.

II. *Theory and computed results*

The results presented in section I demonstrate that depletion of K_c occurs during large hyperpolarizing pulses and therefore a negative shift of E_{rev} is expected when it is measured during or after such pulses. However, E_{rev} also depends on the degree of activation of i_{K_2} (see Figs. 3 and 4).

In this section we shall show that these results can be entirely explained using a simplified three-compartment model for the current flow across the membrane, in which accumulation and depletion of K in the intercellular spaces (clefts) is taken into account. The model predicts that if a depletion process contributes together with the decay of i_{K_2} to the time course of the current during a hyperpolarizing clamp, then the apparent reversal potential will not coincide with the true reversal potential of i_{K_2} (considered to be a pure K current). In fact, during a hyperpolarizing voltage-clamp pulse a flat current trace will be obtained as a balance between two interacting processes: a decay of i_{K_2} (outward current decreasing) and the current change due to the depletion process (outward current increasing). It is found that the model predicts that changes in s as well as changes in $[\mathrm{K}]_c$ will affect the measurement of E_{rev}, in agreement with the experimental results.

(a) *The three-compartment model*

The model, as illustrated in Fig. 6, is similar to the one used by Eisner, Cohen & Attwell (1980) and assumes that the restricted extracellular spaces (clefts) behave like a compartment of total volume V in which $[\mathrm{K}]_c$ is uniform and no electrical gradient exists. At any time t the recorded total transmembrane current, positive when outward, is given by:

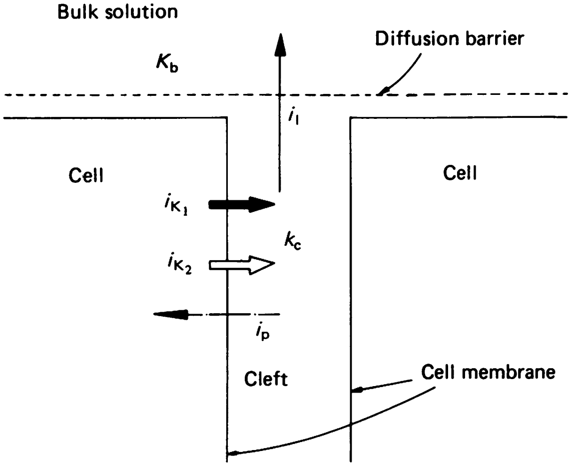

Fig. 6. Schematic illustration of the three-compartment model. The arrows point in the direction of the K movements in the resting state. Symbols are defined in the list of symbols.

$$i_t(E,[\mathrm{K}]_c,t) = i(E,[\mathrm{K}]_c,t)+i_l(E,t) \tag{1}$$

where

$$i(E,[\mathrm{K}]_c,t) = i_{\mathrm{K}_2}(E,[\mathrm{K}]_c,t)+i_{\mathrm{K}_1}(E,[\mathrm{K}]_c)+i_{\mathrm{p}}([\mathrm{K}]_c) \tag{2}$$

The pace-maker current i_{K_2} can be described by the product

$$i_{\mathrm{K}_2}(E,[\mathrm{K}]_c,t) = s(E,t).\bar{i}_{\mathrm{K}_2}(E,[\mathrm{K}]_c)$$

where s is the Hodgkin-Huxley type activation variable and $\bar{i}_{\mathrm{K}_2}$ the fully activated current (Noble & Tsien, 1968). At a fixed potential, the time derivative of the current i is

$$\frac{\mathrm{d}i}{\mathrm{d}t} = \frac{\mathrm{d}i_{\mathrm{K}_2}}{\mathrm{d}t} + \frac{\partial}{\partial[\mathrm{K}]_c}(i_{\mathrm{K}_1}+i_{\mathrm{p}}).\frac{\mathrm{d}[\mathrm{K}]_c}{\mathrm{d}t} \tag{3}$$

When voltage-clamp steps are given in a potential range where the inward component i_l has no time dependence, as is the case when hyperpolarizing to the vicinity of E_{K_2}, then the time course of i will differ from that of the recorded current i_t only by a constant. Furthermore, if the total range of $[\mathrm{K}]_c$ changes is small enough, the first order partial derivatives of $\bar{i}_{K_2}$, i_{K_1} and i_p with respect to $[K]_c$ can be regarded as constant (see Appendix A). In this case $\bar{i}_{K_2}$ is described by

$$\bar{i}_{K_2}(E,[K]_c(t)) = i_{K_2,0} + \frac{\partial \bar{i}_{K_2}}{\partial [K]_c}([K]_c(t) - [K]_{c,0}) \tag{4}$$

where $[K]_{c,0}$ and $i_{K_2,0} = \bar{i}_{K_2}(E,[K]_{c,0})$ are the values of $[K]_c$ and $\bar{i}_{K_2}$ at the start of the clamp pulse to E. Eqn. (3) can thus be written as

$$\frac{di}{dt} = \bar{i}_{K_2,0}\frac{ds}{dt} + \lambda\frac{d}{dt}(s\Delta[K]_c) + \nu\frac{d\Delta[K]_c}{dt} \tag{5}$$

where

$$\Delta[K]_c(t) = [K]_c(t) - K_{c,0} \tag{6a}$$

$$\lambda = \lambda(E) = \partial \bar{i}_{K_2}/\partial [K]_c \tag{6b}$$

$$\nu = \nu(E) = \partial(i_{K_1} + i_p)/\partial [K]_c \tag{6c}$$

(b) *Prediction of the influence of K depletion on the time course of i_{K_2} during a voltage clamp*

The reversal potential of i_{K_2} is usually defined as the potential at which di_t/dt changes sign, during a test hyperpolarization from a holding potential where the current is substantially activated (Noble & Tsien, 1968; Peper & Trautwein, 1969; Cohen *et al.* 1976*a*). However, increasing the pulse amplitude is often accompanied by a change of the current trace from a simple outward decay to a biphasic time course (see for example Figs. 2 and 3). As we have mentioned in Section I, this complicates the exact determination of the reversal potential, and raises the question of how the 'pure' gated current decay is distorted by accumulation–depletion processes. This problem can be qualitatively approached making use of eqn. (3) and analysing the effects of the changes in $[K]_c$ occurring during a hyperpolarization on the time course of i. It is clear in fact from eqn. (5) that only in the absence of any depletion ($\Delta[K]_c = 0$, $d\Delta[K]_c/dt = 0$) will the time course of i be determined by the decay of the Hodgkin-Huxley gating variable alone (i.e. the term $\bar{i}_{K_2,0}\,ds/dt$), and the observed reversal potential therefore correspond to E_{K_2}. When depletion is present, on the other hand, the current time course (and therefore E_{rev}) will vary. Changes occurring in $i_{K_1} + i_p$ (term $\nu d\Delta[K]_c/dt$) as well as in $\bar{i}_{K_2}$ (cross term $\lambda d(s\Delta[K]_c)/dt$) will in fact contribute to the total membrane current change. In Appendix A it is shown that (with the assumption of linearity between currents and $[K]_c$) the coefficients ν and λ are negative for potentials near E_{K_2}. Thus during a hyperpolarization (as depletion occurs) $d\Delta[K]_c/dt < 0$, and the term $\nu d\Delta[K]_c/dt$ will be positive. Supposing for simplicity that $\bar{i}_{K_2}$ were constant (as for $\lambda = 0$), the effect of the term $\nu d\Delta[K]_c/dt$ would then be to shift the apparent reversal potential E_{rev} *positive* to E_{K_2}: in fact at $E = E_{K_2}$, $i_{K_2,0} = 0$, so that $di/dt = \nu d\Delta[K]_c/dt$ would be positive and consequently the current would appear as inward decaying. However, when the changes of $\bar{i}_{K_2}$ are also taken into account, the description of how they affect the current development, and therefore of how they affect E_{rev}, is complicated by the fact that the sign of the cross-term $\lambda \mathrm{d}(s\Delta[\mathrm{K}]_{\mathrm{c}})/\mathrm{d}t$ changes with time.

In fact, for hyperpolarizations in the vicinity of E_{K_2} $\lambda s\ \Delta[\mathrm{K}]_{\mathrm{c}} = 0$ at $t = 0$ (as $[\mathrm{K}]_{\mathrm{c}}\ (0) = [\mathrm{K}]_{\mathrm{c},0}$) and at $t = \infty$ (as $s_\infty = 0$). As for $0 < t < \infty$, the product $\lambda s\ \Delta[\mathrm{K}]_{\mathrm{c}}$ is positive, its time derivative is positive at $t = 0$, has at least one zero point and therefore one interval must exist in which it is negative.

In this case, a description of $i(t)$ as predicted by eqn. (5) can be given graphically. Fig. 7 shows the reconstruction of the current time course expected with the simplifying assumption that $[\mathrm{K}]_{\mathrm{c}}$ decreases exponentially during a hyperpolarization with time constant $\tau_{\mathrm{K_c}}$. Two cases are illustrated in which the decay of $[\mathrm{K}]_{\mathrm{c}}$ is three times faster than the decay of s (Fig. 7*A*) and three times slower (Fig. 7*B*). The need to investigate a large range of $\tau_{\mathrm{K_c}}/\tau_s$ ratios will become clear later (see Discussion). The lower traces of each panel have been obtained by integrating the term $\bar{\imath}_{\mathrm{K}_2,0}\mathrm{d}s/\mathrm{d}t$ of eqn. (5) and therefore represent the decay of i_{K_2} unperturbed by depletion. The integral of the term $\lambda \mathrm{d}(s\Delta[\mathrm{K}]_{\mathrm{c}})/\mathrm{d}t$, which accounts for the changes induced by depletion on $\bar{\imath}_{\mathrm{K}_2}$ only, has then been added (middle traces). The further addition of the integral of the term $\nu \mathrm{d}\Delta[\mathrm{K}]_{\mathrm{c}}/\mathrm{d}t$ (current due to the effect of depletion on $i_{\mathrm{K}_1}+i_{\mathrm{p}}$) yields the total current (upper traces). The examples of Fig. 7 serve to illustrate how the presence of a depletion process *during* a hyperpolarizing voltage-clamp pulse modifies the current time course, distorting a single exponential decay into a biphasic (or even triphasic) change. Non-monotonic time courses like those shown in Fig. 7 are often recorded during hyperpolarizations near E_{rev} (see for example Baumgarten & Isenberg, 1977, Fig. 2; DiFrancesco & McNaughton, 1979, Figs. 3 and 6; this paper, Figs. 1 and 2) and as we have mentioned in the Introduction they do not conform with the expected behaviour of a simple deactivation process of the Hodgkin-Huxley type. Here we observe that if K depletion is taken into account a non-monotonic time course can be reproduced. Also, the effect on the apparent reversal potential can be very large. If we assume, as we have done for the experimental data, that in a series of increasing voltage-clamp hyperpolarizations E_{rev} corresponds to the potential at which the current trace starts to have no region of negative slope, then from Fig. 7 it is apparent that E_{rev} is shifted in the negative direction when a fast depletion process is present (Fig. 7*A*). However, from the traces of Fig. 7 it is clear that the over-all effect of depletion on E_{rev} does not only depend on the ratio $\tau_{\mathrm{K_c}}/\tau_s$, but also on the balance between the different contributions of the depletion-induced changes in $\bar{\imath}_{\mathrm{K}_2}$ and $i_{\mathrm{K}_1}+i_{\mathrm{p}}$.

(c) *Prediction of the effect of changing* $[\mathrm{K}]_{\mathrm{c}}$ *and* s *on the measurement of* E_{rev}

A direct consequence of the finding that, according to the model, E_{rev} depends on the balance of the terms contributing in eqn. (5) to the total current derivative is that any change of the holding potential in a protocol for measuring E_{rev} will itself affect the measured value of E_{rev}.

In fact, a variation in the holding potential has two actions: (1) it sets $[\mathrm{K}]_{\mathrm{c},0}$ at a different level, and (2) it modifies s_0, the value of s at the start of the test pulse. Fig. 8 illustrates the effects of changing s_0 or $[\mathrm{K}]_{\mathrm{c},0}$ on E_{rev} as predicted by eqn. (5). The traces have been calculated as in Fig. 7 for two values of the ratio $\tau_{\mathrm{K_c}}/\tau_s$ (slow

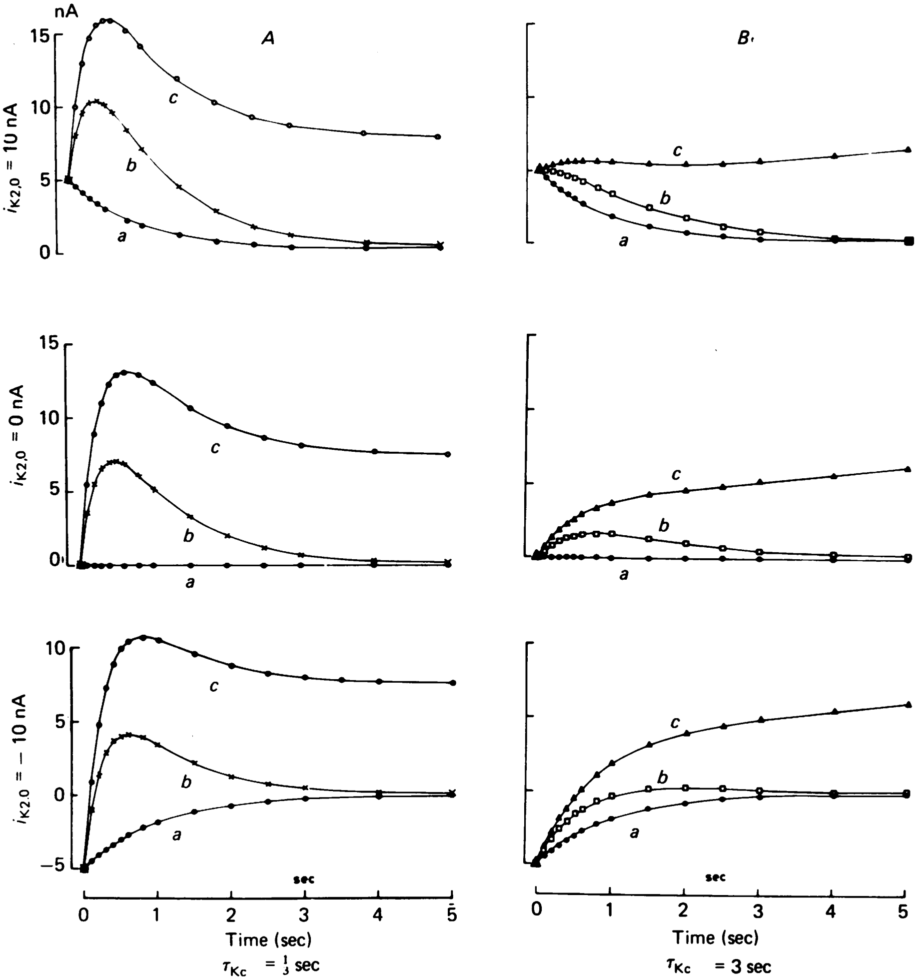

Fig. 7. Theoretical time course of current during hyperpolarizing pulses as predicted by eqn. (5) in the simple case of an exponential K_c depletion process. The total current has been calculated with the expression

$$i = \bar{i}_{K_{2}0} s + \lambda s \Delta[\mathrm{K}]_c + \nu \Delta[\mathrm{K}]_c$$

where

$$s = s(t) = s_0 \exp(-t/\tau_s)$$

$$\Delta[\mathrm{K}]_c = \Delta[\mathrm{K}]_c(t) = ([\mathrm{K}]_{c,0} - [\mathrm{K}]_{c,\infty})(\exp(-t/\tau[\mathrm{K}]_c) - 1)$$

The coefficients λ and ν have values of −10 and 2·5 nA/mM respectively. While s changes from $s_0 = 0{\cdot}5$ to $s_\infty = 0$ with a time constant of 1 sec, $[\mathrm{K}]_c$ has been chosen to vary from $[\mathrm{K}]_{c,0} = 4$ mM to $[\mathrm{K}]_{c,\infty} = 1$ mM with time constant $\tau_{Kc} = 1/3$ sec (*A*) or $\tau_{Kc} = 3$ sec (*B*). In each frame the lower traces represent the term $\bar{i}_{K_2,0}s$ (a) the middle traces $\bar{i}_{K_2,0}s + \lambda s\ \Delta[\mathrm{K}]_c$ (*b*) and the upper traces the total current $i(c)$. From top to bottom each pair of panels simulate clamp pulses in which $\bar{i}_{K_2,0} = 10$, 0 and −10 nA respectively. Note that for $\tau_{K_c} = 3$ sec the total current is nearly flat when $\bar{i}_{K_2,0} = 10$ nA (top *B*) while for $\tau_{K_c} = 1/3$ sec when $\bar{i}_{K_2,0} = -10$ nA an outward decay is still apparent at late times (bottom *A*). The absolute values of currents have been arbitrarily referred to the initial value of $\bar{i}_{K_2}$.

depletion process at the right, fast at the left). Even though these cover only a limited number of all the possible cases, the examples of Fig. 8 illustrate the expected behaviour of the current time course as affected by changes in s_0 or $[K]_{c,0}$. The computations of Fig. 8 (top) show that the model predicts a negative shift of E_{rev} when s_0 is increased.

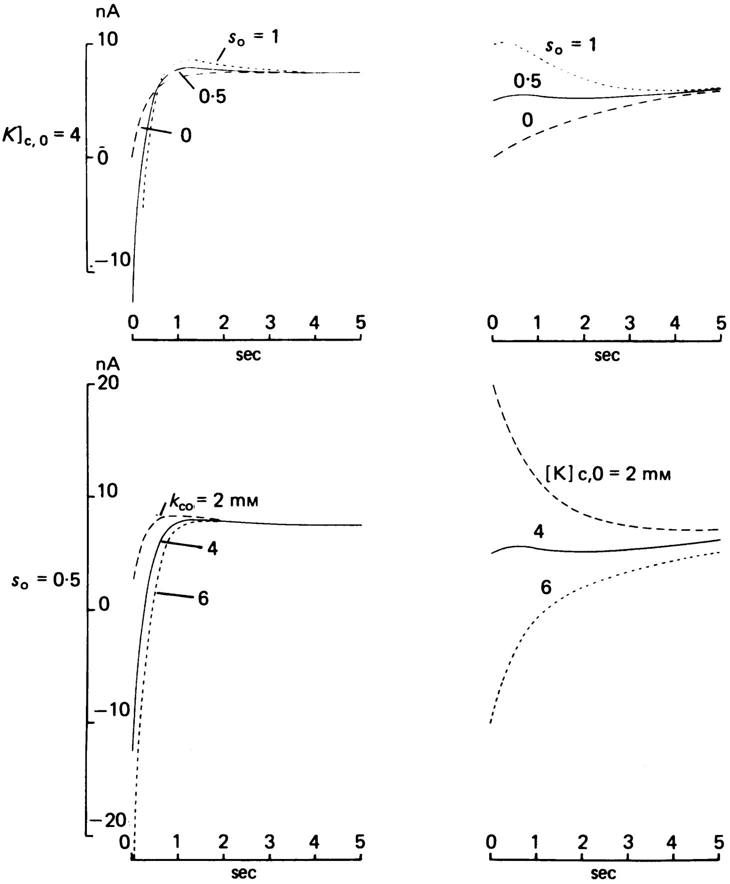

Fig. 8. Effect of changing s_0 and $K_{c,0}$ on the current development during a hyperpolarization near E_{rev} as described by eqn. (5). The currents have been calculated using the same expressions given in the example of Fig. 7 with a fixed $\tau_s = 1$ sec and $\tau_{K_c} = 1/3$ sec (left) or 3 sec (right); also $\lambda = -10$ nA/mM, $\nu = -2·5$ nA/mM. Top: The following coefficients have been used: $\bar{\imath}_{K_2,0} = 10$ nA, $[K]_{c,0} = 4$ mM, $[K]_{c,\infty} =$ 1 mM. s_0 has then been changed from 1 (- - -) to 0·5 (——) and 0 (— — —) in both cases. Bottom: For these sets $s_0 = 0·5$ and $[K]_{c,\infty} = 1$ mM. $[K]_{c,0}$ has the values of 2 mM (- - - -), 4 mM (———) and 6 mM (····). $\bar{\imath}_{K_2,0} = -2·5$ nA for $[K]_{c,0} = 4$ mM, and in the other cases it has been changed by the value $\lambda([K]_{c,0}-4)$. The traces have also been shifted by $\nu([K]_{c,0}-4)$ in agreement with the proper border conditions.

This should be compared with the experimental results of Figs. 1, 2 and 3 in Section I, where we have shown that the process underlying shifts in E_{rev} caused by pre-pulses (apart from large hyperpolarizing pre-pulses) applied before the measure of E_{rev}, is the modification of s_0 (see Fig. 4).

In Fig. 8 (bottom) the effects of changing $[K]_{c,0}$ are to shift E_{rev} negative (positive) when $[K]_{c,0}$ is decreased (increased). They should be compared with the experimental results of Fig. 5, where the use of a three-pulse protocol allowed us to fix s_0 at a constant value, and thus to observe the effects on E_{rev} of changes in $[K]_{c,0}$ only. In both cases the comparison demonstrates that, at least qualitatively, the model reproduces the experimental data.

Specificity of i_{K_2} for potassium

The evidence that the pace-maker current is K-specific comes from the fact that the Nernst plot of E_{rev} versus $[K]_b$ is the one expected for a K electrode, its slope being 61 mV/decade (Noble & Tsien, 1968; Peper & Trautwein, 1969; Cohen *et al.* 1976*a*; Isenberg, 1977). However, as we have shown that E_{rev} depends on the experimental protocol, it is interesting to know whether the slope of the Nernst plot of E_{rev} versus $[K]_b$ is also dependent on the way E_{rev} is measured. The experiment of Fig. 9 illustrates that this is the case.

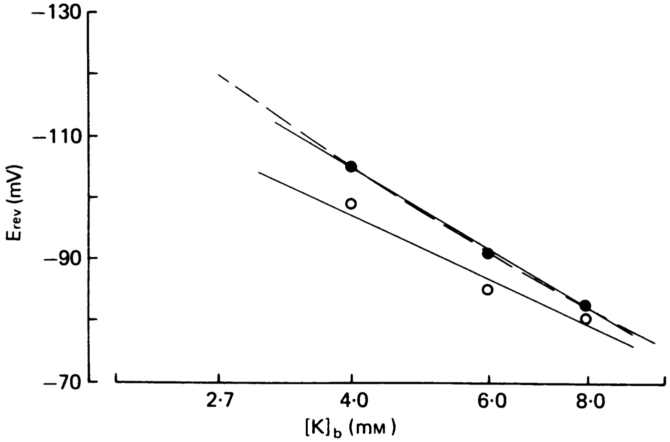

Fig. 9. Dependence of E_{rev} upon external K concentration ($[K]_b$) (Nernst plot). The measurements were made on the same preparation from a holding potential of −58, −59 and −56 mV and $[K]_b$ of 4, 6 and 8 mM respectively (open circles), or from a holding potential of −38 mV for all concentrations (filled circles). The straight lines are obtained with a least-squares regression: $E_{rev} = 62{\cdot}64 \log ([K]_b/147{\cdot}44)$ for open circles and $E_{rev} = 75{\cdot}06 \log ([K]_b/99{\cdot}53)$ for filled circles. Dashed line given by $E_{rev} = 62{\cdot}64 \log ((K_b - 0{\cdot}9)/147{\cdot}44)$.

The reversal potential has been measured from the holding potential of −58, −59 and −56 mV in 4, 6, 8 mM-K_b (open circles) and after a pre-depolarization of 10 sec to −38 mV (filled circles). It can be seen that, as in previous work, the slope of the best fitting line for the open circles is close to the value expected for a pure K current (−62·64 mV/decade). However, the filled circles, which should be closer to the real value of E_{K_2}, require about −75 mV/decade. If we assume that i_{K_2} is very K-specific, the results can be fitted by:

$$E_{rev} = -62.64 \log [K]_c/[K]_i$$

with $[K]_i$ = 147·4 mM, $[K]_c = [K]_b - 0{\cdot}9$ mM, as shown in Fig. 9 (interrupted line) (D. Noble, personal communication).

However, a constant difference between cleft and bulk potassium concentration is not predicted by the three compartment model (see Appendix A, eqn. (A6)). The result of Fig. 9 therefore raises the question of whether $\bar{i}_{K_2}$ is in fact less K-specific than has been assumed. This, by itself, would reduce the slope but, as we have seen, postulating the existence of a difference between $[K]_b$ and $[K]_c$ steepens the relationship. If we assumed i_{K_2} to be less K-specific, we could still fit the results by assuming a larger value of $[K]_b$–$[K]_c$. Clearly, knowledge of the slope alone does not allow an unambiguous conclusion concerning the K specificity. This is true even if a 61 mV/decade slope is obtained. Our results suggest that this value is, in part, coincidental and that a different slope is obtained when the time course of the current during the hyperpolarizing pulses more closely approaches the time course of the gating variable s.

DISCUSSION

The presence of K accumulation–depletion processes in extracellular spaces has been extensively demonstrated on a variety of excitable tissues (Frankenhauser & Hodgkin, 1956; Maughan, 1973; Kline & Morad, 1976; Dubois & Bergman, 1975). In the heart, the extracellular spaces located between adjacent membranes form a tortuous system of pathways where ionic diffusion into and out of the bulk solution is restricted (Hellam & Studt, 1974). An obvious problem arising from the evidence that accumulation–depletion processes are present, is how these processes interfere with the properties of K-dependent currents as measured during voltage-clamp pulses (Almers, 1972*a*, *b*; S. J. Noble, 1976; Dubois, 1980). In the Purkinje fibres, accumulation–depletion-induced changes in the cleft K concentration, if large enough, are likely to influence the determination of the reversal potential of the pace-maker current i_{K_2} (Baumgarten & Isenberg, 1977; Baumgarten, Isenberg, McDonald & Ten Eick, 1977). It is particularly important to understand how the reversal potential of i_{K_2} is affected by accumulation–depletion phenomena, in view of the fact that the dependence of its reversal potential on external K leads to its identification as a pure K channel. Thus it has been shown that the slope of the Nernst plot of E_{rev} versus $[K]_b$ is that expected for a K-specific current (Noble & Tsien, 1968; Peper & Trautwein, 1969).

The effects of K accumulation–depletion on E_{rev} have already received attention. According to Cohen, Daut & Noble (1976*a*), during a test hyperpolarization used to measure E_{K_2} a depletion process takes place but, being a slow process, this interferes with the current time course only at late times, and thus the early part of the current trace reflects the true decay of i_{K_2}. This view implies that the true reversal potential, and hence the K equilibrium potential, can be measured with hyperpolarizing voltage-clamp pulses if only the first part of the current trace is considered. On the other hand, according to Baumgarten & Isenberg (1977) a fast depletion process interferes with the early part of the time course of i_{K_2} during a hyperpolarization. They suggest that the duration of the overlap of i_{K_2} decay with depletion current depends on the time constants of these two processes and varies from fibre to fibre.

However, the depletion process which takes place during the hyperpolarizing voltage-clamp pulse used to determine E_{rev} will affect not only the background component, but also i_{K_2} itself. The two processes (depletion and i_{K_2} decay) cannot therefore be regarded as independent.

In order to give a quantitative description of the way K depletion affects the measurement of E_{rev}, a three-compartment model has been developed. According to this model, the events leading to the recorded current time course during a hyperpolarizing voltage-clamp pulse can be summarized in eqn. (5), which gives the total current time derivative:

(1) i_{K_2} decays. If its decay were unperturbed by depletion, it would be described by the term $\bar{i}_{K_2,0}\, ds/dt$.

(2) i_{K_2} is modified by depletion during its decay. The term expressing this modification is $\lambda\, d(s\Delta[K]_c)/dt$.

(3) The background K component is modified by depletion. The apparent time-dependence of this contribution is described by the term $\nu d\Delta[K]_c/dt$.

Characterization of the current time course

We have mentioned in the Introduction that the current i_{K_2} does not satisfy the requirements of a specific current governed by a single Hodgkin-Huxley kinetic variable in two respects: first, its time course during a voltage-clamp pulse is not always exponential. This is particularly evident when large hyperpolarizations are applied and a biphasic or even triphasic time course can be obtained; secondly, the potential at which the current reverses is more negative than expected. However, the use of a model where accumulation–depletion processes are allowed for during a voltage-clamp pulse shows that these apparent contradictions can be explained. The computed traces of Figs. 7 and 8 show that a non-monotonic time course is readily reproduced by the model for a large range of values of the ratio speed of depletion/time constant of i_{K_2} during a hyperpolarization. The mechanism which makes E_{rev} appear more negative than the true reversal potential in the resting state ($E_{K_2,0}$) is also clear from Fig. 7. If a fast depletion is present (Fig. 7*A*) a high negative value for E_{rev} is obtained because of the rapid negative shift of E_K during the pulse. However, even if the depletion process is slow (Fig. 7*B*) a negative shift of E_{rev} relative to $E_{K_2,0}$ can occur. Suppose that the effect of depletion on $i_{K_1}+i_p$ were negligible in comparison with that on $\bar{i}_{K_2}$, then the recorded current would be the one given by the middle traces of Fig. 7*B* where $E_{rev} < E_{K_2,0}$.

To study the time course of the changes in the background current ($i_{K_1}+i_p$) caused by depletion, hyperpolarizations can be applied from a holding potential where i_{K_2} is fully inactivated. This was done in the experiment shown in Fig. 1 (middle). From the model, when i_{K_2} is completely inactivated by a conditioning hyperpolarization, the time course of i will be determined only by the term $\nu d\Delta[K]_c/dt$ (eqn. (5)). In Appendix A a solution for $i(t)$ and $[K]_c(t)$ is given in this case, assuming that all the K-dependent currents are linearly related to $[K]_c$ in the range of interest. The time course predicted for $[K]_c(t)$ and $i(t)$ is a single exponential, whose amplitude decreases with decreasing test pulses (see Fig. 10 in Appendix A). Indeed, the traces shown in Fig. 1 (middle) become flat at $E > -97$ mV, but nevertheless for $E < -97$ mV the semi-log plots of the current reveal a more complex time-dependence than a simple exponential (not shown). Current records in Na-free solution, when i_{K_2} is absent, have been fitted by two exponentials (Baumgarten *et al.*, 1977), but in the few cases we have analysed, we find that the two-exponential fit is also only an approximation. This fact is not surprising, though, because for large enough variations of $[K]_c$ the hypothesis of linearity between currents and K made in this model may not hold. If the time course of $[K]_c$ is not a single exponential when i_{K_2} is changing and fast components are present, they will obviously intervene at the beginning of the test clamp, while slower components will become evident only after longer clamp durations when s has reached zero. Thus the recorded current will appear as a combination of the examples shown in Figs. 7 and 8 for separate values of τ_{K_c}. This shows why it is important to investigate the predictions of the model for a large range of the ratio τ_{K_c}/τ_s as is done in Figs. 7 and 8.

In this connection it is worth noting that the first phase of the current trace (labelled 'i_d' by Baumgarten & Isenberg, 1977) is not simply determined by the changes induced in $i_{K_1}+i_p$ by depletion, since $\bar{i}_{K_2}$ also contributes to the total derivative as expressed in eqn. (5). In other words, even assuming that only $\bar{i}_{K_2}$ is dependent on $[K]_c$, an initial outward directed current can develop in the range $E > E_{K_2}$ as apparent from the middle traces of Fig. 7.

Voltage non-uniformity during a clamp pulse

During a hyperpolarizing voltage-clamp pulse used to measure E_{rev}, a large current is passed which can give rise to non-uniform distribution of potential along and across the fibre. If the non-uniformity is large enough, a distortion of the time course of i_{K_2} might arise and overlap with the distortion caused by depletion of $[K]_c$. To estimate the effects of non-uniformity on the determination of E_{rev}, a computation on a model of the Purkinje fibre is necessary (see Brown, Noble & Noble, 1976). In Appendix B such a computation has been carried out for various lengths of a cable-like structure, using the dependence of i_{K_2} kinetics and of currents on voltage as given in McAllister, Noble & Tsien (1975). For simplicity only longitudinal non-uniformity has been considered, but, as discussed in Appendix B, the properties of the time-dependent current change during a pulse should not be qualitatively altered by introducing radial non-uniformity also. The results are shown in Fig. 11. The presence of non-uniformity causes E_{rev} to shift in the negative direction with respect to E_{K_2} (Fig. 11*C*). This effect is very large with large non-uniformities, but for the normal length of fibres used in our experiments ($l = 0{\cdot}5\,\lambda$ if $\lambda = 2000\,\mu$m) the predicted displacement is only about -2 mV. Also, the displacements in E_{rev} shown in Fig. 11*C* (filled circles) correspond to the case where voltage electrode and current electrode are both positioned in the middle of the fibre. If we move the voltage electrode away from the middle point, however, $E_{K_2}-E_{rev}$ is reduced until, when the voltage electrode is $(1/3)l$ away from the centre (open circles) the displacements become very small for all lengths of fibre analysed. DiFrancesco & McNaughton (1979) have found a similar result for a linear cable. They show that the true current passed during a voltage-clamp pulse equals the current theoretically needed to space-clamp the fibre perfectly, when current and voltage electrodes are about $(1/2{\cdot}5)l$ apart, and this distance is largely independent of the fibre's length. We conclude that non-uniformity can contribute to the negative deviation of E_{rev} from the expected K equilibrium potential, but it cannot be the cause of this deviation, which is therefore likely to be mainly attributable to fast depletion of K_c during the test hyperpolarization. As for the simulation of the current time course by non-uniformity, we have noticed that when large non-uniformities are allowed for, an initial *inward* deflexion can appear in the current trace (not shown). Also in this case, however, this deflexion simply reflects the capacity discharge. In addition, non-uniformity is not able to reproduce the slow creep seen in the late part of the recorded traces (see for example Figs. 1, 2 and 3) even when fibres are used as long as 2λ (which corresponds to a total length of 8 mm for $\lambda = 2000$ μm). As for the effects of changing the value of s at the start of the hyperpolarizing clamp pulse on the current time course, here also non-uniformity fails to reproduce the experimental data observed in Figs. 1–4, as no appreciable shift in E_{rev} is observed on passing from $s_0 = 1$ to $s_0 = 0{\cdot}5$ (not shown). Hence, we can exclude the possibility that in the normal case non-uniformity does more than slightly shift E_{rev} negative to E_{K_2}, and therefore it cannot explain the experimental findings, either in relation to the time course of i_{K_2} during a hyperpolarizing voltage-clamp pulse at (or near) E_{K_2} or to its dependence on the potential preceding the test pulse.

Changes in E_{rev} *induced by pre-pulses*

Pre-pulsing before a test hyperpolarization induces changes in E_{rev} which are incompatible with the view that they simply act on the level of $[K]_{c,0}$ (Figs. 1–4). In the experiment of Fig. 4 we have shown that the changes in E_{rev} induced by single pre-depolarizations can be explained as due to changes in the degree of activation of i_{K_2}, which the pre-pulses set to a new value before the measurement of E_{rev} is made. This result is not surprising. In fact, if the total [K]–*IV* relationship shows pronounced inward-going rectification in the region of the holding potential (Noble, 1965; Deck & Trautwein, 1964; Cohen, Noble, Ohba & Ojeda, 1979*a*; DiFrancesco & McNaughton 1979) a depolarization will not cause a large increase in the K outflow and therefore a large amount of accumulation will not be expected. In the case of a pre-hyperpolarization, on the other hand, the decrease in the K outflow will be proportionately larger, and a significant depletion can occur. In the experiment of Fig. 1, for example, an 18 mV pre-hyperpolarization causes a much larger background current jump than a pre-depolarization of the same amplitude, despite the fact that these pulses change s_∞ by the same amount ($\pm 0{\cdot}5$ units: see inset in Fig. 1). Therefore the effect of changing $[K]_{c,0}$ on E_{rev} is evident for the large current decrease triggered by the negative pre-pulse, but not as evident for the positive pre-pulse, which causes a much smaller background current change. This interpretation also applies to the experiment of Fig. 4, where the changes induced in the current time course at the apparent reversal potential are entirely attributable to the extra i_{K_2} activated by pre-depolarizations.

The results that can be interpreted as due to the effect of s on E_{rev} are reproduced by the model in Fig. 8*A*. Those of Fig. 8*B* refer to the opposite situation, i.e. when only a change in $[K]_{c,0}$ is responsible for varying the time course of i_{K_2} and E_{rev} (experimental protocol used in Fig. 5*B*).

In view of the lack of detailed information about the precise voltage and K dependence of $\bar{i}_{K_2}$, i_{K_1} and i_p, and therefore of most of the partial derivatives of eqn. (5), we have concentrated our attention on simple cases, without attempting to reconstruct the changes induced in i_{K_2} time course by both s_0 and $[K]_{c,0}$ variations, as one expects to be the case when large pre-hyperpolarizations are applied. However, the model gives a satisfactory description of all the experimental data available even if only these simple cases are treated.

The main conclusions we can draw from the theoretical treatment of the interference of accumulation-depletion with the measurement of E_{rev} are the following: (1) when the total current derivative is zero, i_{K_2} is still outward and decaying, and (2) E_{K_2} itself shifts in the negative direction with time during a hyperpolarizing pulse. The assumption that the apparent reversal potential E_{rev} equals E_{K_2} is therefore incorrect. E_{K_2} must in general be even more negative than E_{rev}.

Effects on E_{rev} of agents which shift the s_∞ curve

Changes in pH, Ca^{2+}, salicylate and adrenaline are known to shift the activation curve s_∞ (E_m) (Hauswirth, Noble & Tsien, 1968; Tsien, 1974; Brown & Noble, 1978; Cohen, Noble, Ohba & Ojeda, 1979*b*). Since these agents have also been shown to change the reversal potential for i_{K_2} (Cohen, Eisner & Noble, 1978; Brown, Cohen & Noble, 1978; DiFrancesco & McNaughton, 1979; Cohen *et al.* 1979*b*) it is important to assess whether these changes in E_{rev} may have been produced by the changes in s_∞ rather than by genuine changes in E_{K_2}. We will discuss each of these cases in turn.

Adrenaline. This is the simplest case to deal with since the shifts in E_{rev} and in s_∞ (E) are always in *opposite* directions. s_∞ shifts in a depolarizing direction while E_{rev} shifts in a hyperpolarizing direction. Any artifactual shift in E_{rev} due to changes in s_∞ in this case would be in the depolarizing direction. It is likely therefore that the observed change in E_{rev} does reflect a change in E_{K_2}. This is consistent with the results of Rogus, Cheng and Zierlier (1977) which support the evidence for a stimulating action of adrenaline on the Na-K transport in rat skeletal muscle.

pH. In this case an increase in external proton concentration produces the expected positive (depolarizing) shifts in the Na current threshold, but consistent results for the shifts in the s_∞ curve are not found. As it has been reported by Brown & Noble (1978) sometimes the s_∞ curve shifts in the depolarizing direction, but more often unexpected hyperpolarizing shifts are observed. The shift in E_{rev} is always in the depolarizing direction (Brown *et al.* 1978) and therefore it is difficult to assess how important is in this case the role played by changes in the position of s_∞ on E_{rev}.

Calcium and salicylate. These cases are more complex since the changes in s_∞ and in E_{rev} are always in the *same* direction. Calcium induces depolarizing shifts in both variables, while salicylate produces hyperpolarizing shifts in both variables. An artifactual shift of E_{rev}, dependent on the change in s_∞, is therefore a possibility in these cases. The change in E_{rev} *alone* cannot be conclusive evidence for a change in E_{K_2}. However, surface charge agents have also been shown to produce changes in the steady-state *I–V* relationship which can be accounted for by variations in the steady level of $[K]_c$ (Brown *et al.* 1978; DiFrancesco & McNaughton, 1979). These changes cannot be explained only in terms of shifts of the s_∞ curve, and therefore we cannot conclude that the action of surface charge agents on i_{K_2} is solely due to s_∞ shifts. Nevertheless these shifts are certainly contributing part of the changes in E_{rev} observed on changing the Ca^{2+} (or salicylate) concentration.

Ouabain. A detailed study of the effects of ouabain on the position of s_∞ is not available. Some data however suggest that ouabain can exert such an action on the s_∞ curve (Aronson & Gelles, 1977; Cohen, Daut & Noble, 1976*b*, Fig. 12). If this is the case, at least part of the effect of ouabain on E_{rev} can be attributed to shifts in s_∞. As in the case of Ca^{2+} and salicylate, the changes induced by low doses of ouabain on the steady-state $I-V$ relation, however, cannot be explained in terms of shifts in s_∞ only (Cohen *et al.* 1976*b*).

To summarize, it cannot be concluded that the changes in E_{rev} so far reported in the literature may be solely attributed to changes in s_∞. Nevertheless if shifts in s_∞ are present, then it is clearly important to check on the possibility that the associated shifts of E_{rev} may be significant, or to exclude this possibility experimentally by always using a holding potential at which $s_\infty = 1$. Although even in this case E_{rev} does not equal E_{K_2} changes in E_{rev} should then follow changes in E_{K_2}.

Conclusion

The results presented in this paper are explained on the basis of a three-compartment model according to which K depletion in the extracellular spaces during a hyperpolarizing clamp can distort the measurement of the reversal potential of i_{K_2}. Even though the three-compartment model discussed here is likely to be only a rough approximation to the real situation created by the restricted diffusion of K along the clefts, it can nevertheless account for the observed behaviour of the current i_{K_2} during large hyperpolarizations. The reversal potential is strongly affected by the degree of activation of i_{K_2} at the beginning of the test pulse, but even when s is fully activated the measured E_{rev} will be different in general from the value of E_{K_2} at the holding potential. On the basis of these findings the degree of selectivity of i_{K_2} for K has to be reconsidered, as well as the action of all the agents shown to affect the kinetics or the apparent reversal potential of i_{K_2}. Unless methods are developed to measure E_{K_2} without the interference of a depletion process, or alternatively to measure accurately how $[K]_c$ changes during a hyperpolarization, a distortion of the fully-activated $\bar{i}_{K_2}$ relationship near E_{K_2}, as measured with conventional voltage-clamp protocols, seems unavoidable.

APPENDIX A

According to the three-compartment model, as illustrated in Fig. 6, the change of the K concentration $[K]_c$ in the clefts is given by the continuity equation:

$$\begin{aligned} d[K]_c/dt &= (i_{K_2}+i_{K_1}+ri_p-FP([K_c-[K]_b))/VF \\ &= (i-(1-r)i_p-FP([K]_c-[K]_b))/VF \qquad \text{(A1)} \end{aligned}$$

(the symbols are defined in the list of symbols).

The exchange between clefts and external solution is described by a simple diffusion process. During a voltage clamp applied from the holding potential E_H at which the system has reached its steady-state, eqn. (A1) can also be written:

$$d\Delta[K]_c/dt = (\Delta i-(1-r)\,\Delta i_p-FP\,\Delta[K]_c)/VF \qquad \text{(A2)}$$

where Δ represents the deviation from the steady-state value at E_{H}. If the total excursion of $[\mathrm{K}]_{\mathrm{c}}$ during a clamp to the test potential E_{T} is small enough the dependence of i_{K_1}, $\bar{i}_{\mathrm{K}_2}$ and i_{p} on $[\mathrm{K}]_{\mathrm{c}}$ can be considered linear.

Under this assumption eqn. (5) in the text and (A2) can be written:

$$\frac{\mathrm{d}\,\Delta i}{\mathrm{d}t} = \bar{i}_{\mathrm{K}_2,0}\frac{\mathrm{d}s}{\mathrm{d}t} + \lambda\frac{\mathrm{d}}{\mathrm{d}t}(s\Delta[\mathrm{K}]_{\mathrm{c}}) + \nu\frac{\mathrm{d}\,\Delta[\mathrm{K}]_{\mathrm{c}}}{\mathrm{d}t} \tag{A3a}$$

$$\frac{\mathrm{d}\,\Delta[\mathrm{K}]_{\mathrm{c}}}{\mathrm{d}t}\quad \mathrm{d}t = (\Delta i - \xi\Delta[\mathrm{K}]_{\mathrm{c}})/VF \tag{A3b}$$

where

$$\lambda(E) = \partial\bar{i}_{\mathrm{K}_2}/\partial[\mathrm{K}]_{\mathrm{c}} \tag{A4a}$$

$$\nu(E) = \partial(i_{\mathrm{K}_1} + i_{\mathrm{p}})/\partial[\mathrm{K}]_{\mathrm{c}} \tag{A4b}$$

$$\xi = FP + (1-r)\,\mathrm{d}i_{\mathrm{p}}/\mathrm{d}[\mathrm{K}]_{\mathrm{c}} \tag{A4c}$$

For every potential E, the eqns. (A3a and b) can be solved knowing the $s(t)$ kinetics and the dependence of the currents on E, $[\mathrm{K}]_{\mathrm{c}}$. The steady-state relation between $[\mathrm{K}]_{\mathrm{c}}$ and $[\mathrm{K}]_{\mathrm{b}}$ at a fixed potential E is derived from eqn. (A1), setting $\mathrm{d}[\mathrm{K}]_{\mathrm{c}}/\mathrm{d}t = 0$, so that

$$s_{\infty}(E)\bar{i}_{\mathrm{K}_2}(E, [\mathrm{K}]_{\mathrm{c}}) + i_{\mathrm{K}_1}(E, [\mathrm{K}]_{\mathrm{c}}) + ri_{\mathrm{p}}([\mathrm{K}]_{\mathrm{c}}) - FP([\mathrm{K}]_{\mathrm{c}} - [\mathrm{K}]_{\mathrm{b}}) = 0$$

or, defining

$$j_{\mathrm{K},\infty} = (s_{\infty}\bar{i}_{\mathrm{K}_2} + i_{\mathrm{K}_1} + ri_{\mathrm{p}})/F,$$

$$j_{\mathrm{K},\infty}(E, [\mathrm{K}]_{\mathrm{c}}) = P([\mathrm{K}]_{\mathrm{c}} - [\mathrm{K}]_{\mathrm{b}}) \tag{A5}$$

The first order approximation of eqn. (A5) is obtained developing $j_{\mathrm{K},\infty}$ in the vicinity of $[\mathrm{K}]_{\mathrm{c}} = [\mathrm{K}]_{\mathrm{b}}$:

$$[\mathrm{K}]_{\mathrm{c}} - [\mathrm{K}]_{\mathrm{b}} = (j_{\mathrm{K},\infty}(E, [\mathrm{K}]_{\mathrm{c}} = [\mathrm{K}]_{\mathrm{b}}))/(P - (\partial j_{\mathrm{K},\infty}/\partial[\mathrm{K}]_{\mathrm{c}})_E) \tag{A6}$$

Even when $(\partial j_{\mathrm{K},\infty}/\partial[\mathrm{K}]_{\mathrm{c}})_E$ is independent of $[\mathrm{K}]_{\mathrm{c}}$, which is a consequence of extending the assumption that every current is linear in $[\mathrm{K}]_{\mathrm{c}}$ up to the point $[\mathrm{K}]_{\mathrm{c}} = [\mathrm{K}]_{\mathrm{b}}$, eqn. (A6) shows that the steady-state relation $[\mathrm{K}]_{\mathrm{c}}([\mathrm{K}]_{\mathrm{b}})$ at the fixed potential E is neither a constant ratio (unless $j_{\mathrm{K},\infty}(E, [\mathrm{K}]_{\mathrm{c}} = [\mathrm{K}]_{\mathrm{b}})/[\mathrm{K}]_{\mathrm{b}}$ is independent of $[\mathrm{K}]_{\mathrm{b}}$) nor a constant difference (unless $j_{\mathrm{K},\infty}(E, [\mathrm{K}]_{\mathrm{c}} = [\mathrm{K}]_{\mathrm{b}})$ is independent of $[\mathrm{K}]_{\mathrm{b}}$). Also, in general, $[\mathrm{K}]_{\mathrm{c}}([\mathrm{K}]_{\mathrm{b}})$ is a function of the membrane potential. It may be noted that if the normal fibre resting potential falls into the voltage range in which $\partial j_{\mathrm{K},\infty}/\partial[\mathrm{K}]_{\mathrm{c}} < 0$, then $P - (\partial j_{\mathrm{K},\infty}/\partial[\mathrm{K}]_{\mathrm{c}})_{E\mathrm{H}} > 0$ and therefore $[\mathrm{K}]_{\mathrm{c}} - [\mathrm{K}]_{\mathrm{b}}$ has the same sign as $j_{\mathrm{K},\infty}$ according to eqn. (A6). For $[\mathrm{K}]_{\mathrm{c}}$ being less than $[\mathrm{K}]_{\mathrm{b}}$, $s_{\infty}\bar{i}_{\mathrm{K}_2} + i_{\mathrm{K}_1}$ should be less than the absolute value of ri_{p}. Even if no data are available about the precise ratio between i_{p} and i_{K_1}, it seems that i_{p} is rather small ($\sim$ 20 nA in the resting state of a 2 mm long Purkinje fibre, see for example Isenberg & Trautwein, 1974). In this case $j_{\mathrm{K},\infty} > 0$, which implies that in the normal resting state $[\mathrm{K}]_{\mathrm{c}} > [\mathrm{K}]_{\mathrm{b}}$. High negative values of E_{rev} (see Fig. 8 of text) can be predicted by the model without the assumption that a steady depletion exists in the clefts.

At the potential E, an infinitesimal change in $[\mathrm{K}]_{\mathrm{b}}$ determines an infinitesimal change in the stationary value of $[\mathrm{K}]_{\mathrm{c}}$ given by

$$(\mathrm{d}[\mathrm{K}]_{\mathrm{c}}/\mathrm{d}[\mathrm{K}]_{\mathrm{b}}) = P/(P - (\partial j_{\mathrm{K},\infty}/\partial[\mathrm{K}]_{\mathrm{c}})_E \tag{A7}$$

as obtained by derivation of eqn. (A6). $(d[K]_c/d[K]_b)_E$ is constant and its sign can be determined when E is in the range near E_{K_2}. In this range

$$(\partial \bar{i}_{K_2}/\partial[K]_c)_E < 0 \tag{A8a}$$

$$(\partial i_{K_1}/\partial[K]_c)_E < 0 \tag{A8b}$$

which corresponds to saying that $\bar{i}_{K_2}$ and i_{K_1} increase in the inward direction when K_c increases. For a 3:2 Na:K electrogenic pump we also have $r = -2$, and therefore

$$r\,di_p/d[K]_c < 0 \tag{A9}$$

as i_p increases in the outward direction for an increase in $[K]_c$. We then have

$$F(\partial j_{K,\infty}/\partial[K]_c)_E = s_\infty(E)\,(\partial \bar{i}_{K_2}/\partial[K]_c)_E + (\partial i_{K_1}/\partial[K]_c)_E + r\,di_p/d[K]_c < 0 \tag{A10}$$

as all the three terms are negative.

Therefore we conclude that, according to eqn. (A7):

$$0 < (d[K]_c/d[K]_b)_E < 1 \tag{A11}$$

(cf. Eisner *et al.* 1979). The limits set by eqn. (A11) show that increasing K_b leads to a smaller but *positive* increase in $[K]_c$, whatever the difference between $[K]_c$ and $[K]_b$ is. About the signs of the coefficients given in eqn. (A4), it is a straight consequence of eqns. (A8) and (A9) that $\lambda < 0$ and $\xi > 0$. The sign of ν can also be predicted as follows: from eqns. (1) and (2) of text, the steady-state total current i_t at a fixed potential E changes with $[K]_c$ according to

$$(\partial i_t/\partial[K]_c)_E = (\partial i/\partial[K]_c)_E = s_\infty(E)\,\lambda(E) + \nu(E) = \nu(E) \tag{A12}$$

where λ and ν are assumed not to depend on $[K]_c$ at negative enough potentials. The last equality holds for E below the activation of s. In this range the total I–V relationship is well known to be affected by $[K]_b$ in such a way that $(\partial i_t/\partial[K]_b)_E < 0$ (Hall, Hutter & Noble, 1963; Dudel *et al.* 1968; DiFrancesco & McNaughton, 1979). It is thus possible to argue that also $(\partial i_t/\partial[K]_c)_E$ is negative as far as the relation (A11) holds and consequently $\nu(E) < 0$ from eqn. (A12).

A simple analytical solution of the system (A3) can be derived in the case where the test hyperpolarization starts from a holding potential at which $s = 0$. In this case $ds/dt = 0$, so that

$$d\Delta i/dt = \nu d\Delta[K]_c/dt \tag{A13}$$

$$d\Delta[K]_c/dt = (\Delta i - \xi\Delta[K]_c)/VF \tag{A14}$$

Substituting $d\Delta[K]_c/dt$ in the first equation,

$$d\Delta i/dt = (\nu/VF)\,\Delta i - (\nu\xi/VF)\,\Delta[K]_c \tag{A15a}$$

$$d\Delta[K]_c/dt = (1/VF)\,\Delta i - (\xi/VF)\,\Delta[K]_c \tag{A15b}$$

When the following boundary conditions are used

$$\Delta i(E, t = 0) = i_j(E) \tag{A16a}$$

$$\Delta[K]_c(E, t = 0) = 0 \tag{A16b}$$

the solutions of eqns. (A15*a* and *b*) are

$$\Delta i(t) = i_j\left(\frac{-\nu}{\xi-\nu}\exp\left(-t\bigg/\frac{VF}{\xi-\nu}\right)+\frac{\xi}{\xi-\nu}\right) \qquad \text{(A17}a\text{)}$$

$$\Delta[\mathrm{K}]_\mathrm{c}(t) = \frac{i_j}{\xi-\nu}\left(1-\exp\left(-t\bigg/\frac{VF}{\xi-\nu}\right)\right) \qquad \text{(A17}b\text{)}$$

(See also Eisner *et al.* 1980). The time course of Δi and $\Delta[\mathrm{K}]_\mathrm{c}$ is a single exponential with a time constant equal to $VF/(\xi-\nu)$. It is worth noting that $-\nu$ and ξ are both positive, as shown above. Hence the time constant $VF/(\xi-\nu)$ is positive, as are the coefficients $-\nu/(\xi-\nu)$ and $\xi/(\xi-\nu)$ which appear in eqn. (A17*a*). An example of the time dependence given in (A17*a* and *b*) is shown in Fig. 10 for a linear dependence $i_j(E)$.

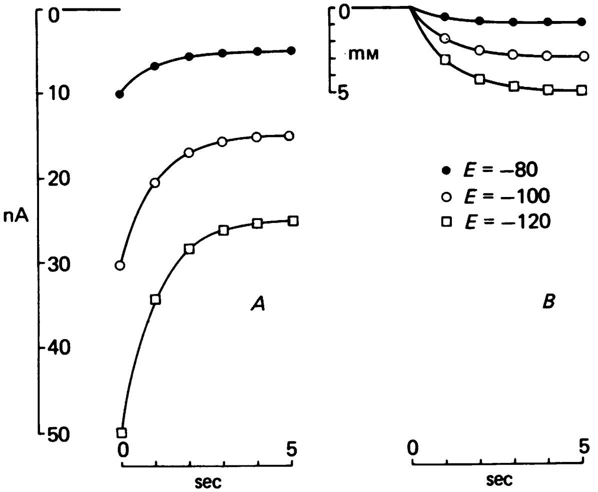

Fig. 10. Exponential time course for current (*A*) and cleft K concentration (*B*) predicted by the model in the case $s_0 = 0$ (eqns. (A17*a* and *b*)). The relationship $i_j = E+70$ nA has been used. The coefficients have the following values: $-\nu = 5$ nA/m-mole, $\xi = 5$ nA/m-mole, $VF = 10^{-8}$ C/m-mole (corresponding to $V \simeq 10^{-10}$ *lt*).

APPENDIX B

BY D. DiFRANCESCO AND D. NOBLE

The influence of voltage non-uniformity on the determination. of E_rev for i_{K_2}

To assess the effects of voltage non-uniformity on the reversal potential we used the cable equation

$$(\partial^2 E/\partial x^2)/r_\mathrm{a} = (\partial E/\partial t)\,c_\mathrm{m}+i_\mathrm{B}+i_{\mathrm{K}_2} \qquad \text{(B1)}$$

where i_B is the background current (including $i_{\mathrm{K}_1}+i_\mathrm{P}+i_\mathrm{l}$ and other non-time-dependent currents). This current, in nA, was represented by the polynomial expression:

$$i_\mathrm{B} = 1250\ ((E/100)-3(E/100)^2+2{\cdot}5(E/100)^3) \qquad \text{(B2)}$$

which gives a curve strongly resembling that found experimentally in Purkinje fibres (cf. Jack, Noble & Tsien, 1975, chapter 12). E is expressed as a deflexion from a membrane potential of -80 mV.

For i_{K_2} we used the s equations given by McAllister *et al.* (1975):

$$\alpha_s = 0{\cdot}001(E-28)/(1-\exp(-0{\cdot}2(E-28))) \tag{B3}$$

$$\beta_s = 0{\cdot}00005\exp(-0{\cdot}067(E-28)) \tag{B4}$$

The maximum activated current $\bar{i}_{K_2}$ was represented by the equation

$$\bar{i}_{K_2} = 12{\cdot}5\,(E-E_{K_2}) \tag{B5}$$

where E_{K_2} is the true reversal potential which was set to -30 mV. This equation gives a maximum conductance for G_{K_2} which is equal to that for i_B at the value $E = 0$, i.e. 50% of the total conductance in the region of the resting potential is attributable to i_{K_2}. $E_{K_2} = -30$ mV corresponds to an absolute value of -110 mV which is similar to that found experimentally when $[K]_b = 2{\cdot}7$ mV.

The analytical method used for the computation was the same as that used by Noble & Stein (1966).

To reproduce the experimental situation we injected the current at the same end of the cable as the voltage-recording electrode. This arrangement corresponds to that with the two micro-electrodes placed together at the centre of a short fibre. We will also consider the effect of placing the voltage electrode one-third of the distance towards the end of the preparation.

Fig. 11 shows the currents computed for various hyperpolarizations with the value of s at $t = 0$ (s_0) set equal to 1. Fig. 11*A* shows the currents for a cable length of 1 mm (which is 0·5 λ at the resting potential). The apparent reversal potential was -32 mV instead of -30 mV, with a negative displacement of 2 mV. Note, however, that the results do not reproduce either the initial inward current surge or the slow upward drift that, in the model discussed in the main part of this paper, are attributed to the process of K depletion. Fig. 11*B* shows the results when the cable length is 2 mm (i.e. 1 λ at rest). The apparent reversal potential is now -38 mV instead of -30 mV. Once again, however, apart from capacity surges which are too fast to be plotted, all the tails are simple monotonic functions of time. Diphasic patterns could only be obtained at much longer cable lengths and the slow upward creep was never found at any cable length.

We may therefore conclude that voltage non-uniformity, *per se*, will not account for current patterns of the kind observed experimentally. Although we have computed the results for a short cable corresponding to the fibre as a whole, it is clear that a similar result would apply to non-uniformity generated radially in the clefts of a preparation since, to a first approximation, these will also behave as a short cable when polarized from the outside surface (see Jack *et al.* 1975, chapter 6).

However, the results do show that some displacement of E_{rev} in a negative direction is produced by voltage non-uniformity. This displacement was not, however, sensitive to the initial value of s. Using $s_0 = 0{\cdot}5$ instead of 1 did not give significantly different results.

The question does arise however as to whether such negative displacements could be of importance in the relatively negative experimental values of E_{rev} measured by

Noble & Tsien (1968), Peper & Trautwein (1969) and Cohen *et al.* (1976*a*). This seems unlikely to be quantitatively important for two reasons.

First, the displacement at $l = 0{\cdot}5\,\lambda$, which is a typical cable length for experimental work, is only 2 mV. Secondly, even this displacement would not occur in most experimental results since the micro-electrodes are not usually placed in the same position. In fact, as Fig. 11*C* shows, if we place the voltage electrode one-third of

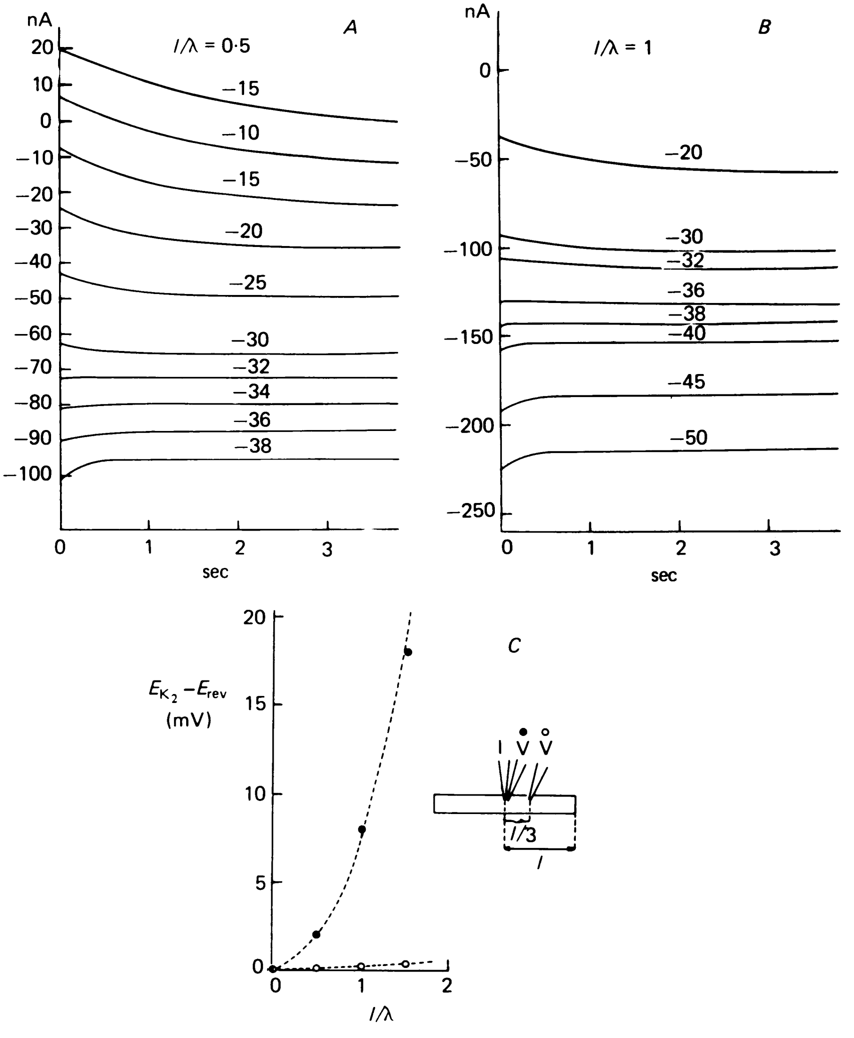

Fig. 11. *A* and *B*: time course of current in a non-uniform cable as computed from the equations given in Appendix B, for various hyperpolarizing voltage-clamp pulses. Current and voltage electrodes are both placed in the centre of the fibre (filled circles in *C*). Pulse amplitudes are indicated near each trace as displacements (in mV) from the resting potential. E_{K_2} was fixed at −30 mV in both cases. Half-length of fibre l is $0{\cdot}5\,\lambda$ and λ respectively in *A* and *B*. Note that E_{rev} is about −32 mV in *A*, and −38 mV in *B*. *C*: displacements of E_{rev} from E_{K_2} are plotted against l/λ for the case where voltage electrode is placed in the centre of the fibre (full circles) or at 1/3 from the centre (open circles) as shown in inset.

the distance towards the end of the preparation (DiFrancesco & McNaughton, 1979) the displacement of E_{rev} is very small even for fairly long cable lengths. In fact the displacement diminishes rapidly as the voltage electrode is moved away from the current electrode. This is because the region of steepest non-uniformity lies near the current electrode. Even a distance of $(1/3)l$ (about 150 μm) reduces the displacement of E_{rev} to very small values. We conclude therefore that in Purkinje fibres, voltage non-uniformity would contribute only a small error in the estimate of E_{rev} for i_{K_2}.

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