THE TIME COURSE OF POTASSIUM CURRENT FOLLOWING POTASSIUM ACCUMULATION IN FROG ATRIUM: ANALYTICAL SOLUTIONS USING A LINEAR APPROXIMATION

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SUMMARY

1. Regular perturbation theory was used to obtain analytical solutions for the time course of membrane current decay following voltage-clamp depolarizing pulses when both time-dependent K conductance mechanisms and the process of K accumulation in extracellular spaces are present. These solutions apply when the current and K concentration changes are small enough for linear relations to be assumed between current and K concentration.

2. In the case of a single Hodgkin–Huxley type conductance variable with time constant τ_x the presence of an accumulation process which, by itself, would produce a current decay with time constant τ_a, induces the appearance of two infinite sets of components with decreasing time constants ($1/(n+1/\tau_x)$ and $1/(1/\tau_a+n/\tau_x)$, where n is integer), and decreasing magnitudes.

3. The analytical solutions are used to investigate the range of conditions over which semi-exponential (curve-stripping) analysis of current decay tails may give useful information on the kinetics of current change. It is shown that, except at very large decay tail amplitudes, the method may give a good estimate of the true time constants of conductance decay even when the currents are assumed to be strongly dependent on external K concentration.

4. The method introduces error in current amplitude, but over the range in which curve-stripping gives useful results, the direct distortion of activation curves by variations in external K concentration is fairly small. However, as the current decay becomes grossly distorted in its time course by accumulation, so does the activation curve. The effects are very similar both to those obtained using numerical computation without linearization, and to those obtained experimentally.

5. Even with a large dependence of current on external K concentration the linear model does not reproduce $i_{x,\text{fast}}$ as a perturbation of $i_{x,\text{slow}}$ by K accumulation.

INTRODUCTION

The preceding paper (Brown, DiFrancesco, Noble & Noble, 1980) showed that semi-exponential curve-stripping analysis of voltage-clamp current tails can give accurate estimates of the time constants of decay of conductance mechanisms. This

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was demonstrated both using experimental results obtained from frog atrial and sinus venosus preparations and also using a numerical model designed to reproduce the major features of the K current and of K accumulation in these tissues.

It is, however, important to give a more detailed analysis of the conditions under which such methods may be applied (compare Attwell, Eisner & Cohen, 1979) and to do this it is desirable to obtain analytical solutions for the current time course. This paper presents such solutions for conditions in which there is a linear dependence of ionic current on changes in extracellular K concentration (as may be expected when the concentration changes are sufficiently small). These solutions are then used to give a more general analysis of the validity of the method of semi-exponential splitting for small current changes and of the possible errors involved.

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THEORY AND RESULTS

List of symbols

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F	Faraday's constant (96 493 C/g-equiv)
V	cleft total volume (10^{-6} l.)
t	time (sec)
E	membrane potential (mV)
K_i, K_c, K_b	internal, cleft, bulk K concentrations (mM)
$i_x(E, K_c, t) = x(E, t) \cdot \bar{i}_x(E, K_c)$	gated K current (nA)
$x(E, t)$	activation variable for i_x, assumed to change with time according to a first-order kinetics
$\bar{i}_x(E, K_c)$	fully-activated current for i_x (nA)
$i_{K_1}(E, K_c)$	non-gated K current (nA)
$i_p(K_c)$	pump current (nA)
$i_1(K_c) = FP(K_c - K_b)$	current exchanged between clefts and bulk (nA)
$i_{in}(E)$	total non-K-dependent current (nA)
$i(E, K_c, t) = i_x + i_{K_1} + i_p$	total K-dependent current (nA)
$i_1 = i_x + i_{K_1} + i_p + i_{in}$	total current (nA)
P	permeability constant for cleft–bulk exchange (10^{-6} l./sec)
$\lambda = \partial \bar{i}_x / \partial K_c$	(nA/mM)
$\nu = \partial(i_{K_1} + i_p)/\partial K_c$	(nA/mM)
r	K fraction of the pump current (for a 3:2 Na:K exchange $r = -2$)
$\sigma = \partial i_{K_1}/\partial K_c + r \, di_p/dK_c - FP$	(nA/mM)
τ_x	time constant of x change (sec)
$\tau_a = -VF/\sigma$	(sec)
E_h	holding potential (mV)
$K_{c,h}, i_h, i_{x,h}, i_{K_1,h}, i_{p,h}$	cleft K concentration and currents at the steady state corresponding to E_h
$\Delta(\ )$	deviation of the variable ( ) from its steady-state value at E_h
$x_0, \Delta K_{c,0}$	values of x and ΔK_c at the beginning of repolarization to E_h

$a = -1/\tau_a = \sigma/VF$ $\qquad$ (sec^{-1})

$b = \lambda x_0/VF$ $\qquad$ (sec^{-1})

$c = x_0 \bar{i}_{x,\,\mathrm{h}}/VF$ $\qquad$ (mM/sec)

$v = -1/\tau_x$ $\qquad$ (sec^{-1})

q_n, u_n $\qquad$ coefficients in series defined by eqn. (12)

Ω $\qquad$ value of series defined by eqn. (A 9)

$\dot{i}_{\mathrm{split}}$ $\qquad$ current decay after subtracting component with time constant τ_a

1. *General equations for the time course of current and cleft K concentration in the linear case*

The model for K diffusion across the extracellular spaces used here is the same as in the previous paper (Brown *et al.* 1980). To obtain an analytical solution for the time course of the recorded current, we will assume that the changes occurring in the cleft K concentration (K_c) are small enough to allow any K-dependent current to be linearly dependent on K_c concentration. The total ionic current crossing the membrane at any time is

$$i_\mathrm{l}(E, K_c, t) = i_x(E, K_c, t) + i_{K_1}(E, K_c) + i_\mathrm{p}(K_c) + i_\mathrm{in}(E, t)$$
$$= i(E, K_c, t) + i_\mathrm{in}(E, t). \qquad (1)$$

As discussed earlier (see Brown *et al.* 1980) an experimental procedure can be used such that the K-dependent component i_in does not greatly contribute to the time dependence of the recorded current during a voltage clamp. In this case $di_\mathrm{l}/dt = di/dt$ and the time course of i_l is coincident with the time course of i, apart from a constant. The current $i(t)$ can be referred to the steady state at a potential E_h at which no current i_x is activated ($x_\infty(E_\mathrm{h}) = 0$; for the definition of symbols, see list of symbols):

$$\Delta i(t) = i(t) - i_\mathrm{h} = i_x(t) + \Delta i_{K_1}(t) + \Delta i_\mathrm{p}(t)$$
$$= x(t)\,\bar{i}_x(t) + \Delta i_{K_1}(t) + \Delta i_\mathrm{p}(t). \qquad (2)$$

During a voltage-clamp pulse to E_h the time-dependent changes of $\bar{i}_x$, i_{K_1} and i_p are only due to changes in the concentration K_c. Therefore, in the limit of linearity between the currents and K_c concentration, we can write

$$\Delta i(t) = x(t)\,\bar{i}_{x,\,\mathrm{h}} + \lambda x(t)\,\Delta K_c(t) + v\Delta K_c(t)$$
$$= x_0\,\bar{i}_{x,\,\mathrm{h}} \exp\left(-t/\tau_x\right) + \lambda x_0\,\Delta K_c(t)\exp\left(-t/\tau_x\right) + v\Delta K_c(t), \qquad (3)$$

where $x(t)$ is assumed to decay exponentially to zero from the initial value x_0 with time constant τ_x. The change of the concentration K_c with time is described by the continuity equation:

$$\frac{dK_c}{dt} = \frac{1}{VF}\left(i_x(E, K_c, t) + i_{K_1}(E, K_c) + ri_\mathrm{p}(K_c) - FP(K_c - K_\mathrm{b})\right)$$

or, referring to the steady state at E_h:

$$\frac{d\Delta K_c}{dt} = \frac{1}{VF}\left(i_x + \Delta i_{K_1} + r\Delta i_\mathrm{p} - FP\Delta K_c\right). \qquad (4)$$

During a voltage clamp to E_h, $\bar{i}_x$, Δi_{K_1}, and Δi_p are proportional to the change in concentration ΔK_c and (4) can finally be written as

$$\frac{d\Delta K_c(t)}{dt} = \frac{1}{VF}(x_0\,\bar{i}_{x,\,h}\exp(-t/\tau_x) + \lambda x_0\Delta K_c(t)\exp(-t/\tau_x) + \sigma\Delta K_c(t)). \qquad (5)$$

Given the boundary condition $\Delta K_c(t=0) = \Delta K_{c,\,0}$, eqn. (5) can be solved by making use of regular perturbation theory (see Appendix A). The current time course will then be found by substituting $\Delta K_c(t)$ in eqn. (3).

2. *Time course of the recorded current in the absence of i_x*

When a depolarization from E_h is small enough not to activate x, the concentration change ΔK_c on returning to E_h is simply described by

$$\frac{d\Delta K_c}{dt} = \frac{\sigma}{VF}\Delta K_c, \qquad (6)$$

as obtained from eqn. (5) with $x_0 = 0$. The solution of eqn. (6) is

$$\Delta K_c(t) = \Delta K_{c,\,0}\exp(-t/\tau_a) \qquad (7)$$

where
$$\tau_a = -VF/\sigma.$$

Substituting eqn. (7) in (3) with $x = 0$ we obtain

$$\Delta i(t) = \nu\Delta K_{c,\,0}\exp(-t/\tau_a). \qquad (8)$$

It should be noted that σ is proportional to the rate at which K is accumulated in the clefts by the flow of i_{K_1} minus the rate at which it is removed by diffusion to the bulk solution and by pumping (see the definition of σ in the list of symbols). The above treatment is essentially the same as that of Attwell *et al.* (1979) who showed the dependence of τ_a on the cleft volume and on the rates at which the cleft is accumulated and depleted. An exponential decay of ΔK_c is, of course, necessarily obtained when the processes by which accumulation occurs (i.e. i_{K_1}) and decays (i.e. diffusion and pumping) are proportional to the concentration change ΔK_c. For small changes in K_c concentration it is then expected that the current change will also be exponential (cf. Noble, 1976, eqn. (11)).

It is worth noting that when $\Delta K_c > 0$, i.e. when the depolarization preceding the return to E_h has induced accumulation of cleft K, the stability condition of eqn. (6) requires that $\sigma < 0$. According to the definition of σ (see list of symbols) this means that the total K flux entering the cleft decreases for increasing K_c.

Equation (8) may describe the time course of current change when the membrane potential lies below the threshold for activation of i_x. In frog atrium this threshold lies at about -40 mV. Current changes following voltage steps near -70 mV have been recorded by Noble (1976, Fig. 14). Fig. 1 shows these records plotted semilogarithmically against time. To a first approximation the current changes are exponential, though there is a tendency for a faster decay to occur very early in the records (see Appendix B). Note also that the time constant varies with the potential. This result is expected since i_{K_1} is strongly voltage-dependent and $\partial(i_{K_1} + i_p)/\partial K_c$ varies with potential (Brown *et al.* 1980). It is this variation of τ_a with potential that

enables the kinetics of current change in the absence of i_x to resemble those of a Hodgkin–Huxley mechanism (see Attwell *et al.* 1979). It is also clear from Fig. 1 that the voltage dependence of τ_a can be very large. τ_a changes from 9·5 sec to 1·5 sec on hyperpolarizing from -70 to -81 mV.
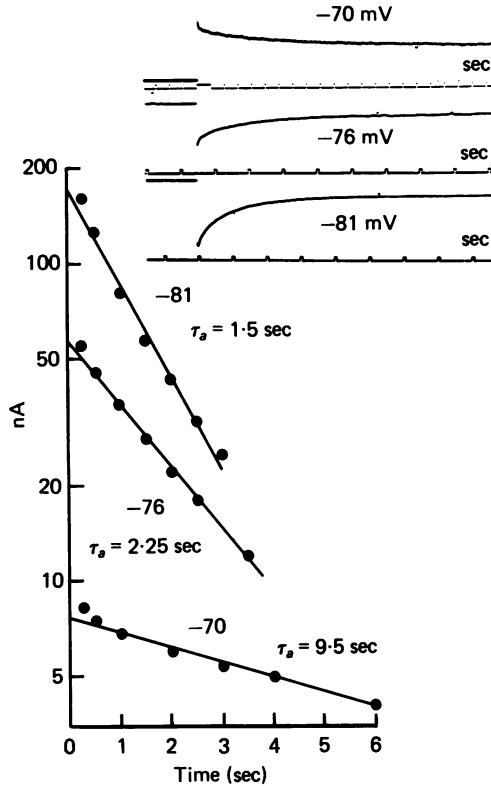

Fig. 1. Top (insert): Current changes following voltage steps in frog atrial muscle negative to the threshold (about -40 mV) for activating the current i_x. The holding potential was -74 mV. Depolarization to -70 mV produces a decaying outward current. Hyperpolarizations produce decaying inward current (from Noble, 1976, Fig. 14). Bottom: semilogarithmic plots of current change (measured as a deviation from the steady-current level at each potential). Each plot has been fitted by eye with a single exponential. Note the strong voltage dependence of the time constant, τ_a. Note, also, a very small deviation from exponentiality at beginning of each record. This deviation is extremely small compared to the i_x currents and has been ignored in the major part of this paper (but see Appendix B for further analysis and discussion).

In the experimental results we shall consider later in this paper, the potentials involved lie in the range (positive to -70 mV) over which τ_a is large. Values of τ_a in the region of 10 sec are then usually obtained (Brown *et al.* 1980, Figs. 5 and 6).

We may now consider how the time course of Δi deviates from eqn. (8) when i_x is present. We shall approach this problem in three stages. We shall first obtain the solution when i_x is a simple exponential, as is virtually the case over a wide range of current amplitudes in the numerical model. We shall then generalize this treatment to the case where i_x is represented by a sum of exponentials, either because more than

one conductance component is present or because changes in K_c perturb the time course of a single component. In each of these cases a particular time course of i_x must be *assumed* in advance of calculating Δi. Our third, and most general, approach will be to derive the complete time course of i_x as it is influenced by changes in K_c. It is convenient to develop the mathematics in these three stages since each may be used to introduce important results.

3. *Time course of Δi when i_x decays as a single exponential*

This case is relevant when a single Hodgkin–Huxley type component like i_x is present *and* when $\lambda = 0$, i.e. when we may neglect the influence of K_c changes on $\bar{i}_x$. Then from eqn. (5), setting $\lambda = 0$,

$$\frac{\mathrm{d}\Delta K_c}{\mathrm{d}t} = \frac{1}{VF}\left(x_0\,\bar{i}_{x,\,\mathrm{h}}\exp\left(-t/\tau_a\right)+\sigma\Delta K_c(t)\right). \tag{9}$$

This equation can be solved with the method used in the Appendix A for eqns. (A 4). The result is

$$\Delta K_c(t) = -\frac{\bar{i}_{x,\,\mathrm{h}}\,x_0\tau_x/VF}{1-\tau_x/\tau_a}\exp\left(-t/\tau_x\right)+\left(\Delta K_{c,\,0}+\frac{\bar{i}_{x,\,\mathrm{h}}\,x_0\tau_x/VF}{1-\tau_x/\tau_a}\right)\exp\left(-t/\tau_a\right). \tag{10}$$

The time course for the current Δi is given by substituting eqn. (10) in (3):

$$\begin{aligned}\Delta i(t) = \bar{i}_{x,\,\mathrm{h}}\,x_0\left(1-\frac{\nu\tau_x/VF}{1-\tau_x/\tau_a}\right)\exp\left(-t/\tau_x\right)\\ +\nu\Delta K_{c,\,0}\left(1+\frac{(\bar{i}_{x,\,\mathrm{h}}\,x_0\tau_x)/(VF\cdot\Delta K_{c,0})}{1-\tau_x/\tau_a}\right)\exp\left(-t/\tau_a\right),\quad\end{aligned} \tag{11}$$

from which it is clear that the total current will be given by the sum of two simple exponentials with time constants τ_x and τ_a. Semi-exponential splitting will therefore give these time constants exactly.

Notice, however, that the intercepts at $t = 0$ are not exactly the same as $\nu\Delta K_{c,\,0}$ (i.e. the true initial value of $\Delta i_{K_1} + \Delta i_p$) and $\bar{i}_{x,\,\mathrm{h}}\,x_0$. Thus, the intercept that would be obtained by semi-exponential splitting for the term in $\exp\left(-t/\tau_x\right)$ is

$$\bar{i}_{x,\,\mathrm{h}}\,x_0\left(1-\frac{\nu\tau_x/VF}{1-\tau_x/\tau_a}\right).$$

Since the only parameter changing with the depolarizing pulse preceding the return to E_h is x_0, the intercept will be proportional to it. This intercept may therefore be used to construct an activation curve for i_x. The method of semi-exponential curve-stripping will not itself distort this activation curve.

To obtain $\bar{i}_x$ from the results, however, we need more information. Since τ_x and τ_a are known exactly from the semi-exponential analysis, we need to know ν and V. This requires information of the kind obtained by Brown et al. (1980) and involves measuring $\partial(i_{K_1}+i_p)/\partial K_c$ and obtaining an estimate of the cleft space volume. In practice, it is unlikely that such information will be available in most experiments since it requires an extensive experimental protocol in addition to that required to measure activation curves.

However, it is worth noting that the error involved may be given an upper limit. The difference between the real value of $\Delta(i_{K_1}+i_p)$ at $t=0$, i.e. $\nu\Delta K_{c,0}$, and the intercept obtained by semi-exponential analysis is given by

$$A = \bar{i}_{x,h} x_0 \frac{\nu\tau_x/VF}{1-\tau_x/\tau_a}$$

which is the same as the error in estimating i_x by exponential splitting, but with opposite sign. Thus, as A is positive

$$\frac{A}{\bar{i}_{x,h} x_0} < \frac{\nu\Delta K_{c,0}+A}{\bar{i}_{x,h} x_0} < \frac{\nu\Delta K_{c,0}+A}{\bar{i}_{x,h} x_0 - A}$$

and therefore the percent error in estimating $\bar{i}_{x,h} x_0$ (i.e. $A/\bar{i}_{x,h} x_0$) is smaller than the ratio between the intercepts of $\exp(-t/\tau_a)$ and $\exp(-t/\tau_x)$ (i.e. $(\nu\Delta K_{c,0}+A)/(\bar{i}_{x,h} x_0 - A)$).

Thus, in the atrial analyses illustrated in Brown *et al.* (1976*a*, *b*), Figs. 6*A* and 7*A*, the intercept of $\exp(-t/\tau_a)$ is about 10% of the total tail current. Thus when the above treatment applies, the error in the estimate of i_x would be less than $(10/90)\times100 = 11\%$. In the frog sinus results described by Brown, Giles & Noble (1977) the intercept of $\exp(-t/\tau_a)$ forms about 30% of the total current which gives an upper limit of 43% for the error in estimating i_x.

4. *Time course of Δi where i_x is multi-exponential*

In this section we will suppose that i_x, during its decay, can be represented with the series

$$i_x(t) = \sum_0^\infty (q_n \exp(u_n t)). \tag{12}$$

This description will be appropriate either when more than one K conductance mechanism is present (in which case the u_n values may be true rate constants if $\lambda = 0$, i.e. if i_x is not significantly influenced by changes in the concentration K_c) *or* when a single or multicomponent i_x is perturbed by changes in K_c concentration. In the latter case, the relation of the u_n values to the true conductance time constants requires further analysis (see section 5).

Equation (4) then becomes

$$\frac{d\Delta K_c}{dt} = \frac{1}{VF}\left(\sum_0^\infty (q_n \exp(u_n t)) + \sigma\Delta K_c\right), \tag{13}$$

which can be integrated as done with eqn. (9):

$$\Delta K_c(t) = \left(\Delta K_{c,0} - \sum_0^\infty \left(\frac{q_n/VF}{u_n+1/\tau_a}\right)\right)\exp(-t/\tau_a) + \sum_0^\infty \left(\frac{q_n/VF}{u_n+1/\tau_a}\exp(u_n t)\right). \tag{14}$$

As in the simpler case considered in the previous section, the time course of ΔK_c acquires components with the time constants of i_x in addition to the time constant τ_a. The total current will be given by substituting (14) and (12) in (2):

$$\Delta i(t) = \nu\left(\Delta K_{c,0} - \sum_0^\infty \left(\frac{q_n/VF}{u_n+1/\tau_a}\right)\right)\exp(-t/\tau_a) + \sum_0^\infty \left(q_n\left(1+\frac{\nu/VF}{u_n+1/\tau_a}\right)\exp(u_n t)\right), \tag{15}$$

so that semi-exponential splitting will again give the exact time constants τ_a, $-1/u_0$, $-1/u_1$, $-1/u_2$, etc. The error in estimating the magnitude of each component of i_x will now vary with its time constant since $(1+(\nu/VF)/(u_n+1/\tau_a))$ varies with u_n. Clearly, the error will be least when u_n is very large, i.e. the time constant of the nth

component is very small, so that (for $\nu > 0$ and $u_n + 1/\tau_a < 0$) faster components will be underestimated less than slower components.

Note also that for each component, the fractional error $((\nu/VF)/(u_n + 1/\tau_a))$ is constant. If each component corresponds to a separate conductance mechanism and if $\lambda = 0$ then semi-exponential analysis will not itself distort the separate activation curves, even though their respective amplitudes are modified to differing degrees. Of course, if the multi-exponential time course of i_x is itself attributable to accumulation then the representation of i_x by separate conductance processes is not relevant. We shall discuss this question further in section 5.

5. *Time course of total current when i_x and $i_{K_1} + i_p$ are perturbed by accumulation*

In this section, for simplicity, we shall assume only one true conductance mechanism of the Hodgkin–Huxley type, i.e. i_x. However, the analysis may, in principle, be extended to the situation when more than one mechanism is present. Part of our reason for exploring the situation when one mechanism is involved is that we wish to determine whether its perturbation by accumulation may give rise to bi-exponential decay patterns of the kind usually found for the delayed K currents in atrium (Brown *et al.* 1976*a*, *b*;), frog sinus (Brown *et al.* 1977); mammalian SA node (DiFrancesco, Noma & Trautwein, 1979); Purkinje fibres (Noble & Tsien, 1969) and ventricular muscle (Katzung & Morgenstern, 1977; McDonald & Trautwein, 1978).

According to the assumptions made in section 1, to represent the effect of changes in K_c on i_x we use the equation

$$\bar{\imath}_x(t) = \bar{\imath}_{x,\mathrm{h}} + \lambda \Delta K_c(t), \qquad (16)$$

which assumes that, for small enough current changes, the partial derivative $d\bar{\imath}_x/dK_c$ is constant. Note that λ and ν can have very different values. The results described in the preceding paper (Brown *et al.* 1980) show that the value of λ is negative, whereas ν is positive over the range of potentials generally used for tail analysis.

The cleft K concentration will change according to eqn. (5). With the definitions given in the Appendix, eqn. (5) can be re-written as

$$\frac{d\Delta K_c}{dt} = a\Delta K_c + b\Delta K_c \exp(vt) + c \exp(vt), \qquad (17)$$

whose solution is eqn. (A 8) in Appendix A.

The total current is now, according to eqn. (A 13)

$$\Delta i(t) = \Omega \nu \exp(-t/\tau_a) + \bar{\imath}_{x,\mathrm{h}} x_0 \left(1 - \frac{\nu\tau_x/VF}{1 - \tau_x/\tau_a}\right) \exp(-t/\tau_x)$$

$$-\frac{\Omega}{\tau_x/VF} \sum_1^\infty \left(\frac{(-x_0\lambda\tau_x/VF)^n}{n!}(n - \nu\tau_x/VF) \exp(-t(n/\tau_x + 1/\tau_a))\right)$$

$$-\frac{\bar{\imath}_{x,\mathrm{h}}}{\lambda\tau_x/VF} \sum_1^\infty \left(\frac{(-x_0\lambda\tau_x/VF)^n}{(1 - \tau_x/\tau_a)(2 - \tau_x/\tau_a)\dots(n - \tau_x/\tau_a)}(n - \nu\tau_x/VF - \tau_x/\tau_a) \exp(-t(n/\tau_x))\right)$$

$$(18)$$

where Ω is the series defined by eqn. (A 9). Note that when $\lambda = 0 (b = 0)$ only the first two terms appear and the solution becomes the same as eqn. (11). When $\lambda \neq 0$ i.e.

i_x varies significantly with K_c, then additional time constants will appear. All these time constants $(1/((n+1)/\tau_x)$ and $1/(n/\tau_x + 1/\tau_a)$ $(n = 1, \ldots \infty))$ will be shorter than τ_x.

In principle therefore it is possible to reproduce components faster than x. Thus, if in the experimental results, $\tau_{x,\text{slow}}$ is the genuine conductance time constant, it is conceivable that $\tau_{x,\text{fast}}$ may represent the faster components produced by the perturbation of the time course of i_x by changes in K_c.
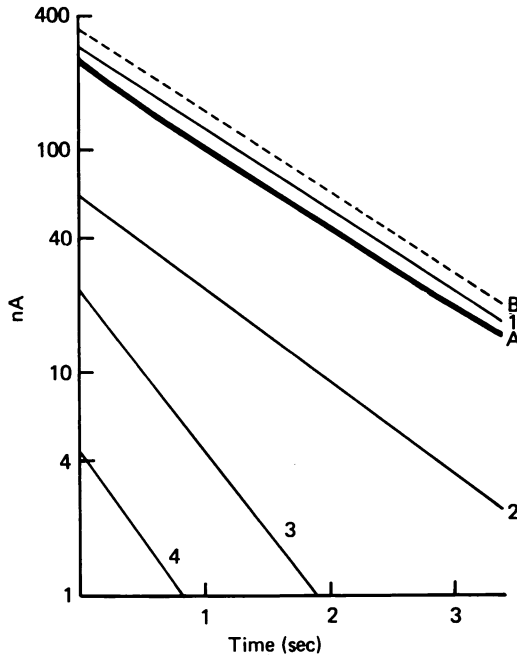
Fig. 2. Semilogarithmic plot of the components of current in eqn. (19). Component 1 is the component in $\exp(-t/\tau_x)$. Line B (interrupted) shows the amplitude that this component would have had if it were not reduced by K accumulation (eqn. (20)). Line 2 shows the component in $\exp(-t(1/\tau_x + 1/\tau_a))$. This component is negative, but is plotted as positive here for convenience. Lines 3 and 4 show the components in $\exp(-2t/\tau_x)$ and $\exp(-t(2/\tau_x + 1/\tau_a))$ respectively. Curve A (thick line) shows the sum of the four components, i.e. i_{split}. Note that except for a slight difference at early times, the total current decays with the same time course as the component in $\exp(-t/\tau_x)$.

We will now consider whether this idea may be correct by using eqn. (18) with known or plausible values of the coefficients to determine whether bi-exponential decays of i_x may be reproduced.

Using parameters from the numerical model of Brown *et al.* (1980), at $E_h = -40 \text{ mV}$ and $K_{c,h} = 4 \text{ mM}$ we have approximately $\nu = 30 \text{ nA/mM}$, (i.e. per 1 mM concentration change), $\lambda = -33 \text{ nA/mM}$ and $i_{x,h} = 868 \text{ nA}$. With $1/VF = 4 \cdot 10^{-3} \text{ mM/nA sec}$ and assuming $\Delta K_{c,0} = 4 \text{ mM}$, $x_0 = 0 \cdot 4$, $\tau_a = 8 \text{ sec}$ and $\tau_x = 1 \cdot 2 \text{ sec}$ we obtain from eqn. (A 12) a value of $\Omega = 5 \cdot 7 \text{ mM}$. (The series in (A 12) converges very rapidly so that fourth and higher order terms are less than $0 \cdot 1 \%$). Substituting in eqn. (18) gives a coefficient $\Omega\nu = 170 \text{ nA}$ for the component $\exp(-t/\tau_a)$. This is the component that

would be subtracted first using semi-exponential splitting. The faster components can be calculated to be

$$i_{\text{split}} = 288 \exp\left(-t/\tau_x\right) + 24 \exp\left(-2t/\tau_x\right) - 64 \exp\left(-t\left(\frac{1}{\tau_x} + \frac{1}{\tau_a}\right)\right)$$
$$- 4\exp\left(-t\left(\frac{2}{\tau_x} + \frac{1}{\tau_a}\right)\right). \quad (19)$$

Higher order terms have coefficients less than 1 and have therefore been neglected. Fig. 2 shows these components plotted together with their algebraic sum, i_{split} which is the current that would be obtained after subtracting the term in $\exp\left(-t/\tau_a\right)$. Also plotted is the exponential

$$\bar{\imath}_{x,\,\text{h}}\, x_0 \exp\left(-t/\tau_x\right) = 347 \exp\left(-t/\tau_x\right) \quad (20)$$

which represents the time course of i_x that would have been obtained in the absence of accumulation. The total current actually obtained can be seen to be smaller but to decay with a time constant that is very close to τ_x.

The reason for the accuracy with which the apparent time constant approximates to τ_x is that only the coefficients for the terms in $\exp\left(-t/\tau_x\right)$ and $\exp\left(-t(1/\tau_a + 1/\tau_x)\right)$ are large. Moreover, when $\tau_a \gg \tau_x$, $(1/\tau_a + 1/\tau_x)$ approximate to $1/\tau_x$. Faster components, such as $\exp\left(-2t/\tau_x\right)$ contribute less than 10 % of the total current and, in this case, would not be separable within the likely error of experimental results.

Thus, with the parameters similar to those used by Brown *et al.* (1980) (which were based on their experimental results) the faster components in eqn. (18) do not produce a strong enough deviation from an exponential time course to enable a single component of conductance to give the appearance of two components. This result is in agreement with the numerical calculations of Brown *et al.* (1980, Figs. 4 and 7).

In Fig. 3 we have extended our calculations to cover the complete range of activation of x. In each case the same parameters were used as in Fig. 2 except for the values of x_0 and $\Delta K_{c,\,0}$. x_0 was set to 0·2, 0·4, 0·6, 0·8 and 1·0. The values of $\Delta K_{c,\,0}$ were chosen by allowing this parameter to vary linearly with the degree of activation. This assumption was tested by using the numerical model of Brown *et al.* (1980) to compute the values of $\Delta K_{c,\,0}$ after 10 sec pulses to various potentials. The resulting variation of $\Delta K_{c,\,0}$ with x_0 is shown in Fig. 3A. The relation is close to linear up to $x_0 = 0\cdot 8$. For the solutions to eqn. (18) we allowed $\Delta K_{c,\,0}$ to be 10 x_0 mM, which is larger than the maximum value in Fig. 3A. Nevertheless, over the whole range, the decay tails (after splitting off the term in $\exp\left(-t/\tau_a\right)$) are nearly parallel, with time constants very close to τ_x. The 'fast component' is always very small. (Fig. 3B). Fig. 3C shows the 'activation curve' that would be obtained. A small degree of distortion (a negative shift of 6 mV in the half activation point) occurs as the initial value of i_x is reduced at the larger values of $\Delta K_{c,\,0}$. Attwell *et al.* (1979) have already drawn attention to effects of accumulation on the activation curve.

It should be noted, however, that a maximum value of $\Delta K_{c,\,0}$ of 10 mM is much larger than that in the analysis of Brown, Clark & Noble (1976b). The maximum coefficient of the term $\exp\left(-t/\tau_a\right)$ in their analysis is about 0·025 μA (see Figs. 6A and 7A), which corresponds to an increase in K concentration of only 1 mM.

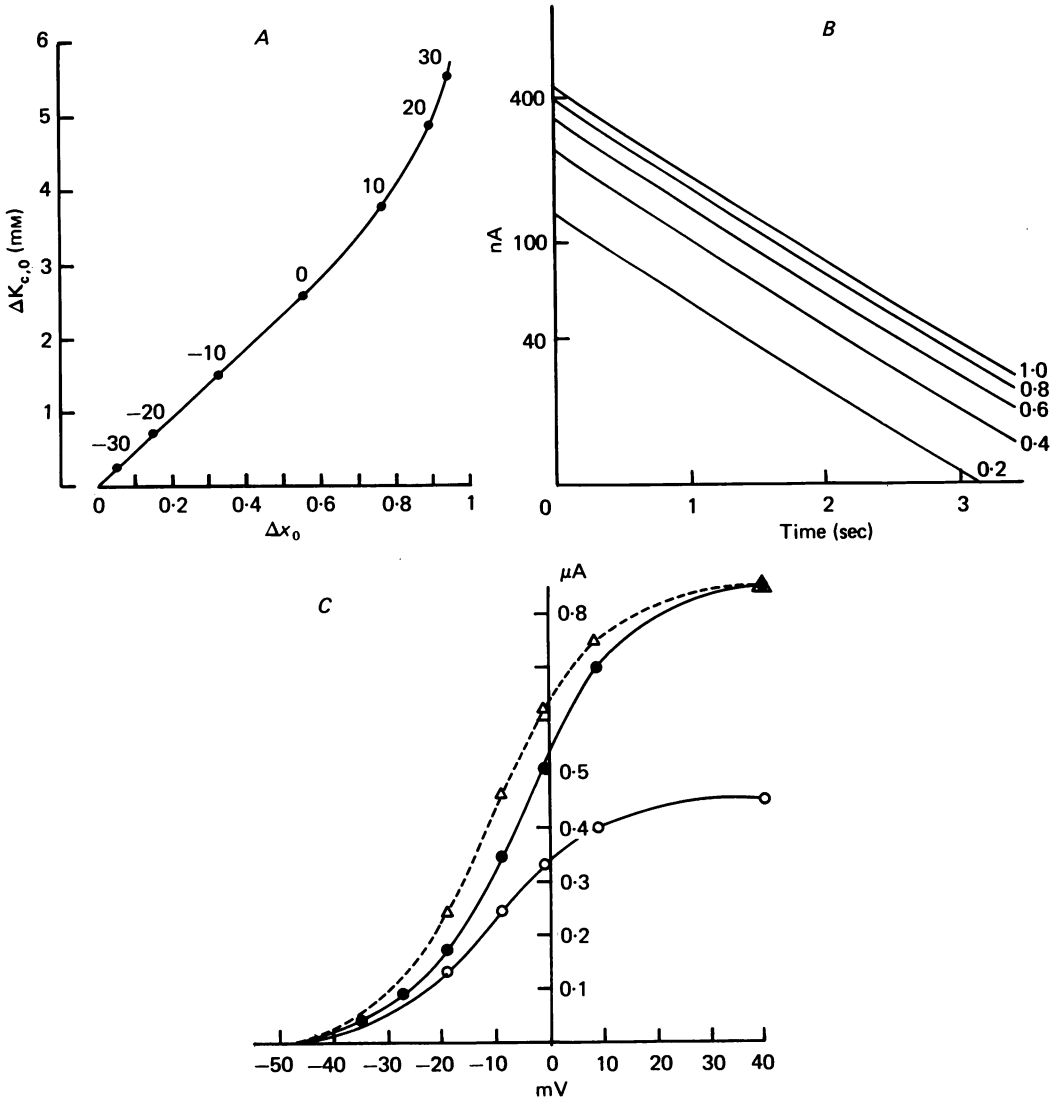It is however worth exploring the possibility that other parameters may be signi-

Fig. 3. *A*, relation between x activation and $\Delta K_{c,0}$ obtained using numerical model of Brown *et al.* (1980) as described in text. *B*, semilogarithmic plots of decay tails obtained using eqn. (18) after subtracting component in $\exp(-t/\tau_a)$. The lines are in fact slight curves but the deviation from a single exponential is very small. The time constants obtained using a single exponential are virtually identical with τ_x. *C*, activation curves. The filled symbols show the time activation curve that would be obtained without accumulation. The open circles show that obtained from the tails shown in *B*. The open triangles are the same results 'scaled up' to compare with the true curve. There is a -6 mV shift in the half activation point. This computation assumes 10 mM of K accumulation in the largest tail which, in a typical preparation would produce about 100–200 nA change in $(i_{K_1} + i_p)$ at -40 mV (cf. Brown *et al.* 1980, Fig. 2; Noble, 1976, Fig. 12). The intercept of $\exp(-t/\tau_a)$, which *over-estimates* $\Delta(i_{K_1} + i_p)$, obtained experimentally is in fact only about 10–20 nA (Brown *et al.*, 1980 (Fig. 8). The results shown here therefore show more distortion than would occur in the experimental analysis in this range (but see Fig. 5 for the situation when K accumulation is deliberately made very large).

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ficantly different from the numerical model and results of Brown *et al.* (1980). The obvious parameter to choose is $\partial \bar{\imath}_x / \partial K_c$, i.e. λ, since this determines the magnitude of the perturbation of the time course of i_x for a given amount of accumulation. We have therefore repeated the calculations of eqn. (18) for various values of λ larger than -33 nA/mM. Over a wide range of values we have been unable to reproduce the simple bi-exponential nature of the experimental results on i_x. Fig. 4 shows the
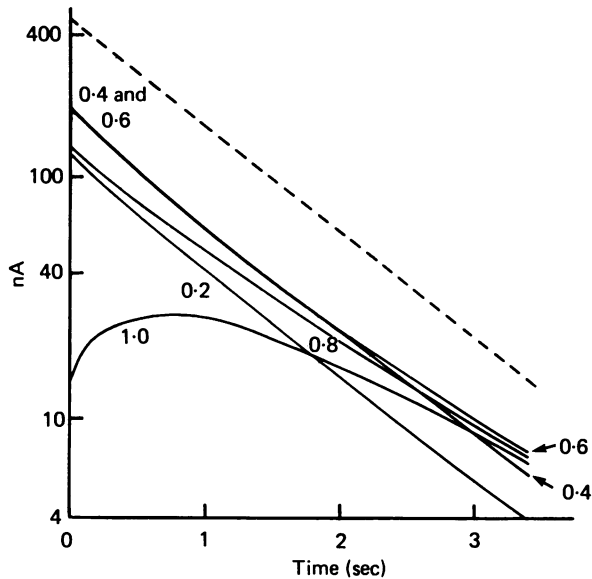
Fig. 4. Semilogarithmic plots of decay tails obtained after increasing the dependence of $\bar{\imath}_x$ on K_c by a factor of 5. This corresponds either to a sensitivity of $\bar{\imath}_x$ to K_c much greater than that usually found experimentally or to a situation in which K accumulation is deliberately made very large (e.g. by using very long pulses). In each case, the component in $\exp(-t/\tau_a)$ has been subtracted. For discussion see text.

computations done with a value of $\lambda = -150$ nA/mM, which is considerably larger than the experimental results suggest (see Brown *et al.* 1980, Fig. 1) since it would require only a 6 mM increase in K_c to reduce $\bar{\imath}_x$ to zero at the holding potential whereas increasing K_b from 2 to 8 mM in fact leaves i_x still large and positive at -40 mV (Brown *et al.* 1980, Fig. 1). For values of x_0 up to 0·4 we still find only a moderate deviation from the true time course of x. Between $x_0 = 0.4$ and 0·8 the deviation becomes more significant but is in the *wrong* direction to account for $i_{x,\,\text{fast}}$, i.e. the major effect is that the decay tail is slowed rather than accelerated. At $x_0 = 1$ the decay tail amplitude is greatly reduced and the tail ceases to be monotonic. These effects are however very similar to the results obtained experimentally when *large* amounts of accumulation are produced. Thus, Brown *et al.* (1976*b*, Fig. 3) show an example of a distorted 'activation curve' obtained following very long pulses (10 sec) which shows a decrease in tail amplitude following large depolarizations; and Noble (1976, Fig. 5) shows an example of a decay tail that ceases to be monotonic (panel 2) before it reverses direction (panel 3). The decay tail is either slowed or initially rises with time. This result is typical of those obtained when amounts of accumulation

occur that nearly reverse the sign of the decay tail (Brown, H. F. & Noble, S. J., un-published). Fig. 5 shows a comparison of the 'distorted' activation curves obtained experimentally by using very long pulses and that obtained from our equations.

Thus, although it is relatively easy to reproduce some of the effects of substantial amounts of accumulation on the time course of i_x, eqn. (18) does not readily allow the
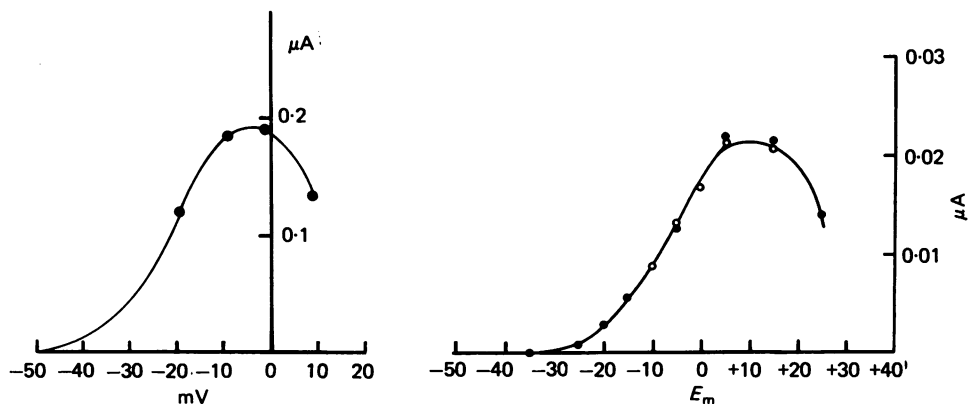

Fig. 5. Left: Strongly distorted 'activation' curve obtained by plotting initial ampli-tudes of those tails in Fig. 4 that are monotonic. Right: 'activation' curve obtained experimentally by Brown, Clark & Noble (1976b, Fig. 3) using very long (10 sec) depolarizations. Their kinetic analysis was in fact restricted to using much shorter pulses at large depolarizations where x activates more rapidly. The difference between results of the kind shown in Fig. 3 and those shown here is therefore attributable to the protocol used.

component $i_{x, \text{fast}}$ to be reproduced. A small fast component is evident in Fig. 3 but its magnitude is quite insufficient to account for the $i_{x, \text{fast}}$ observed experimentally. This component can in fact be as large as $i_{x, \text{slow}}$ (see Brown et al. 1976b, Figs. 6A and 7A).

DISCUSSION

Before discussing the particular conclusions we may draw from our results it may be helpful to the reader to give some physical insight into the equations we have obtained for the complete time course of current decay when both i_x and Δi_{K_1} are present and are both perturbed by K accumulation and its decay.

In the simplest case, when $i_{K_1} + i_p$ is involved without i_x (i.e. at potentials below the x current threshold) and when the K_c concentration changes involved are small enough to use linearized equations, the current will change with a single time constant, τ_a. To a first approximation, this is true for the experimental records below the x current threshold (see Fig. 1; also Appendix B). Similarly, if only one Hodgkin–Huxley mechanism is present and no significant accumulation occurs, the current will decay with the time constant of the gating mechanism, τ_x. This reproduces the experimental situation for very small voltage pulses or for the small current recorded by clamping at the beginning of a pace-maker potential (Brown et al. 1980, Fig. 5). When both processes occur, the current i_x will produce a perturbation in $i_{K_1} + i_p$ and will itself in turn be distorted by the change in concentration K_c occurring when

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the total K flow across the membrane changes. If, in a first approximation, we ignore the effects of accumulation on the time course of i_x, (as done in section 3 with the assumption $\lambda = 0$), then the perturbation of ΔK_c, and hence of $\lambda(i_{K_1} + i_p)$, will also display the time constant τ_x (eqn. 10). The perturbation associated with i_x decay generates the terms

$$\delta\Delta(i_{K_1} + i_p) = -x_0\,\bar{i}_{x,\mathrm{h}}\frac{\nu\tau_x/VF}{1-\tau_x/\tau_a}\exp\left(-t/\tau_x\right) + x_0\,\bar{i}_{x,\mathrm{h}}\frac{\nu\tau_x/VF}{1-\tau_x/\tau_a}\exp\left(-t/\tau_a\right) \tag{22}$$

which are obtained from eqn. (11) by subtracting the 'pure' components

$$x_0\,\bar{i}_{x,\mathrm{h}}\exp\left(-t/\tau_x\right) \text{ and } \nu\Delta K_{c,0}\exp\left(-t/\tau_a\right).$$

In eqn. (22) the symbol δ is used to indicate a first-order perturbation. The part of (22) decaying with time constant τ_x will be identified in a semi-exponential analysis as part of i_x and will introduce an error in the estimate of its amplitude, though not of its time constant. Since this term is negative it will reduce the estimate of i_x, and since it is proportional to x_0, it will not introduce any distortion of the shape of the activation curve, i.e. the error in each case will be simply proportional to the degree of activation.

   Thus, the time course of $\Delta(i_{K_1} + i_p)$ and of ΔK_c will have two components decaying with time constants τ_a and τ_x. If we now allow i_x to change (i.e. $\lambda \neq 0$) the first-order perturbation of i_x due to changes of K_c will be composed of two terms, obtained by multiplying eqn. (10) for $\Delta K_c(t)$ by $\lambda x(t)$. One of these terms is

$$\delta_1 i_x = \lambda x_0\left(\Delta K_{c,0} + \frac{x_0\,\bar{i}_{x,\mathrm{h}}\tau_x/VF}{1-\tau_x/\tau_a}\right)\exp\left(-t\left(\frac{1}{\tau_a}+\frac{1}{\tau_x}\right)\right). \tag{23}$$

The time constant $(1/\tau_a + 1/\tau_x)$ arises from the perturbation of a process decaying with time constant τ_x by a process with time constant τ_a. Note that when $\tau_a \gg \tau_x$ this new time constant is not very different from τ_x itself. Since λ is negative, this perturbation also reduces the estimated i_x but does not greatly change its apparent time constant.

   Eqn. (23) allows for the perturbation of i_x by changes in K_c concentration with time constant τ_a. In addition we have a further first-order perturbation

$$\delta_2 i_x = \lambda x_0\left(-\frac{x_0\,\bar{i}_{x,\mathrm{h}}\tau_x/VF}{1-\tau_x/\tau_a}\right)\exp\left(-t\left(\frac{1}{\tau_x/2}\right)\right) \tag{24}$$

The time constant $\tau_x/2$ is produced by the multiplication of a process decaying with time constant τ_x (i.e. by itself) by another process with the *same* time constant, i.e. the perturbation of the concentration K_c by i_x. This component is proportional to $(x_0)^2$ and will produce a term that decays exactly twice as fast as i_x when unperturbed by accumulation. Since this varies as $(x_0)^2$ it can be made very small by considering tails produced by very short or very small depolarizations, when x_0 is small. If λ is sufficiently large it will, however, produce a significant current as x_0 is increased and this may give rise to an apparent second component, perhaps the $i_{x,\mathrm{fast}}$ of the experimental results, at a more depolarized range of potentials.

   At first sight, this appears to be a very attractive explanation of $i_{x,\mathrm{fast}}$ since in

atrium (Brown *et al.* 1976*a*, *b*) and in sinus venosus (Brown *et al.* 1977) $i_{x,\,\text{fast}}$ does appear at a more depolarized range of potentials than $i_{x,\,\text{slow}}$. There are however two major difficulties with this explanation of $i_{x,\,\text{fast}}$:

(1) τ_{fast} is typically not half of τ_{slow} but rather lies between $0\cdot2$ and $0\cdot33$ of τ_{slow}. It might be thought that this difficulty could be met by considering even higher order perturbations. Thus, the second-order perturbation in the time course of i_x also contains the term

$$\frac{\bar{i}_{x,\,\text{h}}}{\lambda\tau_x/VF}\frac{(x_0\lambda\tau_x/VF)^3\,(3-\nu\tau_x/VF-\tau_x/\tau_a)}{(1-\tau_x/\tau_a)\,(2-\tau_x/\tau_a)\,(3-\tau_x/\tau_a)}\exp\left(-t\left(\frac{1}{\tau_x/3}\right)\right) \qquad (25)$$

which is in fact 3 times faster than x. Note however that this term is proportional to $(x_0)^3$ and will therefore be extremely small for low values of x_0. This gives rise to the difficulty that, on this explanation of $i_{x,\,\text{fast}}$, it should first appear with time constant $\tau_x/2$ and then acquire faster components ($\tau_x/3, \tau_x/4$ etc.) as x_0 increases. In fact, however, within the limits of experimental error. $\tau_{x,\,\text{fast}}$ is approximately invariant with the degree of activation (Brown *et al.* 1980, Fig. 6*B*).

(2) The more serious difficulty is that, as our results (see Figs. 2 and 4) show, the inclusion of second- and higher order perturbations does not in fact reproduce a component resembling $i_{x,\,\text{fast}}$. This failure arises from the fact that as the positive decay terms in $\exp(-t/(\tau_x/2))$ and $\exp(-t/(\tau_x/3))$ are added, *negative* decay terms in $\exp(-t(2/\tau_x+1/\tau_a))$, $\exp(-t(3/\tau_x+1/\tau_a))$ must also be added (see eqn. (18)). As is clear from Fig. 4, it is these negative terms that dominate and that give rise to the non-monotonic time course of i_x as the effects of accumulation are further increased.

So far as the origin of $i_{x,\,\text{fast}}$ is concerned we are left with three other possibilities:

(*a*) $i_{x,\,\text{fast}}$ may in fact represent a genuine second conductance mechanism (or, perhaps, the x conductance mechanism does not obey simple exponential kinetics of the Hodgkin–Huxley type). This was the hypothesis favoured by Brown *et al.* (1976*a*, *b*). The attraction of this hypothesis lies partly in the fact that it has so far proved very difficult to reproduce $i_{x,\,\text{fast}}$ with models that represent it as a perturbation of $i_{x,\,\text{slow}}$, and partly in the experimental observation of Brown *et al.* (1980) that substantially increasing the bulk extracellular K concentration, which might be expected to reduce the proportionate effect of a given amount of accumulation, does not tend to reduce or eliminate $i_{x,\,\text{fast}}$.

(*b*) The assumption that, for small current changes, the currents are linearly dependent on K_c may be incorrect. In Appendix B we show that allowing a quadratic dependence of $(i_{K_1}+i_p)$ on ΔK_c readily enables the small deviation of this component from an exponential time course to be reproduced. An extension of this approach to the equation for total current, however, requires experimental information of a more detailed nature than is at present available. Furthermore, the numerical analysis of the three-compartment model, in which the dependence of currents on the concentration K_c is largely non-linear, does *not* reproduce a significant fast component (Brown *et al.* 1980). We conclude therefore that if the non-linearity between currents and K_c plays any role in determining the appearance of $i_{x,\,\text{fast}}$ it cannot be the only one.

(*c*) The assumption that the spaces in which K^+ ion accumulation occurs are well mixed and homogeneous in K concentration may be incorrect. We have therefore extended the numerical model of Brown *et al.* (1980) to the system represented by

the equation for diffusion in a cylindrical trabeculum:

$$\frac{\partial K_c}{\partial t} = D_K \left(\frac{\partial^2 K_c}{\partial x^2} + \frac{1}{x} \frac{\partial K_c}{\partial x} \right) + \frac{i}{FV} - kK_c, \quad (kK_c = (1-r) \, i_p/FV) \qquad (26)$$

where D_K is the diffusion constant for K^+ ions in the extracellular space, i is the ionic current given by eqns. (9)–(13) of Brown *et al.* (1980), and k is the rate constant for
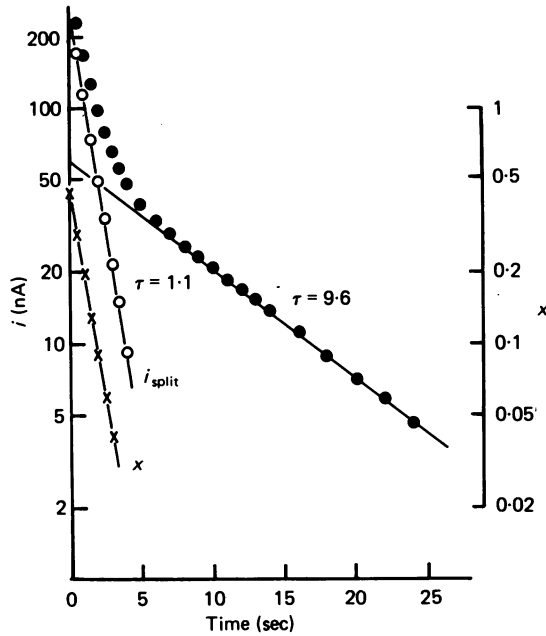

Fig. 6. Semilogarithmic plot of current decay tail produced following 5 sec depolarization to 0 mV and return to −40 mV. The current was computed using eqn. (26) with the net current, i, given by eqns. (9)–(13) in the numerical model described by Brown *et al.* (1980). The equations were solved using a matrix inversion procedure with the parameters given in the text. During the pulse the value of K_c increased by 5·5 mM (from 7·2 to 12·7 mM) at the centre of the preparation, while the surface K was kept constant at 2 mM. Despite this large degree of inhomogeneity, the resulting current decay tail is very similar to that obtained using the simpler three-compartment model (see Fig. 10 of Brown *et al.* 1980).

the Na–K exchange pump. This pump is assumed to be a linear function of K_c. Eqn. (26) was solved using the boundary conditions that $K_c = K_b$ at $x = r$ (the surface of the trabeculum) and $\partial K_c/\partial x = 0$ at $x = 0$ (the centre of the trabeculum). With a trabeculum radius of 100 μm we found we could reproduce the experimental current records, including the slow decay with time constant about 10 sec, with $D_K = 200 \, \mu$m² sec⁻¹ and $k = 0.0625$ sec⁻¹. The decay tail following a 5 sec depolarizing pulse to 0 mV followed by return to a holding potential of −40 mV (cf. Brown *et al.* 1980, Fig. 7) is in fact well fitted by two exponentials, the value of the fast time constant being very close to τ_x. Thus, assuming a non-uniform distribution of K^+ ions within the extracellular space does not reproduce the fast component (see Fig. 6).

Our conclusion therefore is that neither the three-compartment model in its simplest form, nor its extension using eqn. (26) to include non-uniformity in K_c concentration,

can reproduce $i_{x,\text{fast}}$. The possibility that this current is a genuine property of the i_x system, either as a separate component of membrane current or because i_x itself does not obey simple Hodgkin–Huxley kinetics, is therefore still an open one.

APPENDIX A

Solution of equation (5)

Setting

$$
\left.\begin{aligned}
a &= \sigma/VF, \\
b &= \lambda x_0/VF, \\
c &= x_0\,\bar{i}_{x,\text{h}}/VF, \\
v &= -1/\tau_x, \\
y &= \Delta\mathrm{K}_\mathrm{c},
\end{aligned}\right\} \tag{A 1}
$$

eqn. (5) of the text can be rewritten

$$
\mathrm{d}y(t)/\mathrm{d}t = ay(t) + by(t)\exp(vt) + c\exp(vt). \tag{A 2}
$$

A series solution can be found for eqn. (A 2) making use of regular perturbation theory (Lin & Segal, 1974). Regarding the coefficient of the cross-term, b, as the perturbation parameter, y can be expressed as a power series in b:

$$
y(t) = \sum_0^\infty [y_n(t) \cdot b^n]. \tag{A 3}
$$

The functions $y_n(t)$ $(n = 0, \infty)$ can be found by substituting the expression (A 3) in (A 2) and equating the coefficients of the same powers of b in the two members. This gives the set of equations:

$$
\left.\begin{aligned}
\frac{dy_0}{dt} - ay_0 &= c\exp(vt), \\
\frac{dy_n}{dt} - ay_n &= y_{n-1}\exp(vt) \quad (n = 1, \ldots \infty).
\end{aligned}\right\} \tag{A 4}
$$

The eqns. (A 4) are of the general type $dy/dt + P(t) = Q(t)$ whose solution is

$$
y(t) = \exp\left(-\int P\,\mathrm{d}t\right) \cdot \left(k + \int \exp\left(\int P\,\mathrm{d}t\right) Q\,\mathrm{d}t\right)
$$

k being a constant which is determined by the border conditions. The solution of (A 4) is therefore given by the recurrent set:

$$
y_0(t) = k_0\exp(at) + (c/(v-a))\exp(vt), \tag{A 5a}
$$

$$
y_n(t) = k_n\exp(at) + \exp(at)\int \exp((v-a)t)\,y_{n-1}(t)\,\mathrm{d}t \quad (n = 1, \ldots \infty), \tag{A 5b}
$$

where the k_n values $(n = 0, \ldots \infty)$ are constant. Substituting (A 5a) in (A 5b) we obtain

$$
y_1(t) = k_1\exp(at) + \frac{k_0}{v}\exp((v+a)t) + \frac{c}{(v-a)(2v-a)}\exp(2vt).
$$

This expression can be used in (A 5b) to obtain $y_2(t)$ and so on. With this iterative procedure, the general expression for $y_n(t)$ is written as:

$$y_n(t) = \sum_{m \leqslant n} \left(\frac{k_{n-m}}{m! v^m} \exp((mv+a)t) \right)$$

$$+ \frac{c \exp((n+1)vt)}{(v-a)(2v-a) \dots (nv-a)((n+1)v-a)} \quad (n = 0, \dots \infty)$$

According to (A 6) the time dependence of $y(t)$ is completely described by the series of exponentials

$$\exp((nv+a)t), \exp((n+1)vt) \quad (n = 0, \dots \infty)$$

Substituting the expressions (A 6) in (A 3) the coefficients of the above exponentials can be calculated. With the definition

$$\Omega = \sum_0^\infty [k_n b^n] \tag{A 7}$$

this procedure leads to the explicit solution of (A 2):

$$y(t) = \Omega \sum_0^\infty \left(\frac{1}{n!} \left(\frac{b}{v} \right)^n \exp((nv+a)t) \right)$$

$$+ c \sum_0^\infty \left(\frac{b^n}{(v-a)(2v-a) \dots ((n+1)v-a)} \exp((n+1)vt) \right). \tag{A 8}$$

The two series in (A 8) are convergent, as is easily proved by observing that the first one is an exponential series whose value is $\exp(at + (b/v) \exp(at))$, while for the second one we have

$$\lim_{n \to \infty} \left| \frac{\exp(vt)(b \exp(vt))^n}{(v-a) \dots ((n+1)v-a)} \right| \Big/ \left| \frac{\exp(vt)(b \exp(vt))^{n-1}}{(v-a) \dots (nv-a)} \right| = \lim_{n \to \infty} \left| \frac{b \exp(vt)}{(n+1)v-a} \right| = 0$$

and thus according to d'Alembert's ratio test, the series is absolutely convergent.

The value of Ω is obtained by setting the border condition $y(t=0) = \Delta K_{c,0}$ in (A 8):

$$\Delta K_{c,0} = \Omega \sum_0^\infty \left(\frac{1}{n!} \left(\frac{b}{v} \right)^n \right) + c \sum_0^\infty \left(\frac{b^n}{(v-a)(2v-a) \dots ((n+1)v-a)} \right).$$

Now

$$\sum_0^\infty \left(\frac{1}{n!} \left(\frac{b}{v} \right)^n \right) = \exp(b/v)$$

and therefore

$$\Omega = \exp(-b/v) \left(\Delta K_{c,0} - c \sum_0^\infty \left(\frac{b^n}{(v-a)(2v-a) \dots ((n+1)v-a)} \right) \right), \tag{A 9}$$

Finally, defining

$$\tau_a = -1/a = -VF/\sigma \tag{A 10}$$

the time dependence of ΔK_c will be described by substituting (A 1) in (A 8). This results in the expression

$$\Delta K_c(t) = \Omega \sum_0^\infty \left(\frac{(-x_0 \lambda \tau_x / VF)^n}{n!} \exp\left(-t \left(\frac{n}{\tau_x} + \frac{1}{\tau_a} \right) \right) \right)$$

$$+ \frac{i_{x,h}}{\lambda} \sum_1^\infty \left(\frac{(-x_0 \lambda \tau_x / VF)^n}{(1-\tau_x/\tau_a)(2-\tau_x/\tau_a) \dots (n-\tau_x/\tau_a)} \exp(-t(n/\tau_x)) \right) \tag{A 11}$$

where
$$\Omega = \exp\left(x_0 \lambda \tau_x / VF\right)\left(K_{c,0} - \frac{\bar{\imath}_{x,h}}{\lambda}\sum_1^\infty\left(\frac{(-x_0\lambda\tau_x/VF)^n}{(1-\tau_x/\tau_a)(2-\tau_x/\tau_a)\dots(n-\tau_x/\tau_a)}\right)\right), \text{(A 12)}$$

From eqn. (3) in the text and eqn. (A 11) the expression for $\Delta i(t)$ can also be found. The substitution gives

$$\Delta i(t) = -\frac{\Omega}{\tau_x/VF}\sum_0^\infty\left(\frac{(-x_0\lambda\tau_x/VF)^n}{n!}\left(n-\frac{\nu\tau_x}{VF}\right)\exp\left(-t\left(\frac{n}{\tau_x}+\frac{1}{\tau_a}\right)\right)\right)$$
$$+\frac{\bar{\imath}_{x,h}}{\lambda\tau_x/VF}\sum_1^\infty\left(\frac{(-x_0\lambda\tau_x/VF)^n}{(1-\tau_x/\tau_a)(2-\tau_x/\tau_a)\dots(n-\tau_x/\tau_a)}\left(n-\frac{\nu\tau_x}{VF}-\frac{\tau_x}{\tau_a}\right)\exp\left(-t(n/\tau_x)\right).$$
$$\text{(A 13)}$$

An alternative solution

Another solution of equation (A 2) can be given by using the method adopted to integrate (A 4). In this case

$$y(t) = c\exp\left(at+(b/v)\exp(vt)\right)\left(h/c+\int\exp(vt)\exp\left(-at-b/v\exp(vt)\right)\mathrm{d}t\right). \quad \text{(A 14)}$$

Using the border condition $y(0) = \bar{y}\,(=\Delta K_{c,0})$ to determine k:

$$y(t) = \exp\left(at+(b/v)\exp(vt)\right)\left(\bar{y}\exp(-b/v)+c\int_0^t\exp(vt)\exp\left(-at-b/v\exp(vt)\right)\mathrm{d}t\right).$$
$$\text{(A 15)}$$

A series expression of (A 15) can be obtained by observing that, in general, given any real numbers p, q, s:

$$\int\exp\left(pt+q\exp(st)\right)\mathrm{d}t = \int\sum_0^\infty\left(\frac{q^n\exp(ns+p)t}{n!}\right)\mathrm{d}t = \exp(pt)\sum_0^\infty\left(\frac{(q\exp(st))^n}{n!(ns+p)}\right).$$
$$\text{(A 16)}$$

The following identity also holds (for any real numbers a, b, c):

$$\sum_0^\infty\left(\frac{a^n}{n!((n+1)b+c)}\right) = \sum_0^\infty\left(\frac{a^n}{n!}\right)\sum_0^\infty\left(\frac{(-ab)^n}{(b+c)\dots((n+1)b+c)}\right). \quad \text{(A 17)}$$

The latter identity is demonstrated by noting that the nth term of the product on the right hand side is

$$\sum_0^n\left(\frac{a^j}{j!}\frac{(-ab)^{n-j}}{(b+c)\dots((n-j+1)b+c)}\right) = (-ab)^n\sum_0^n\left(\frac{1}{j!(-b)^j(b+c)\dots((j+1)b+c)}\right)$$
$$= \frac{(-ab)^n}{n!(-b)^n((n+1)b+c)} = \frac{a^n}{n!((n+1)b+c)},$$

where we have used the property

$$\sum_0^n\left(\frac{1}{j!(-b)^j(b+c)\dots((n-j+1)b+c)}\right) = \frac{1}{n!(-b)^n((n+1)b+c)}.$$

Using (A 16) and (A 17) we can therefore express the integral in (A 15) as

$$\int\exp\left((v-a)t-(b/v)\exp(vt)\right)\mathrm{d}t = \exp\left((v-a)t\right)\sum_0^\infty\left(\frac{(-(b/v)\exp(vt))^n}{n!((n+1)v-a)}\right)$$
$$= \exp\left((v-a)t\right)\sum_0^\infty\left(\frac{(-b/v\exp(vt))^n}{n!}\right)\sum_0^\infty\left(\frac{(b\exp(vt))^n}{(v-a)\dots((n+1)v-a)}\right)$$
$$= \exp\left(-at-b/v\exp(vt)\right)\sum_0^\infty\left(\frac{b^n\exp((n+1)vt)}{(v-a)\dots((n+1)v-a)}\right), \quad \text{(A 18)}$$

and therefore (A 15) can be rewritten

$$y(t) = \exp{(at + (b/v)\exp{(vt)})}\left\{\bar{y}\exp{(-b/v)} + c\exp{(-at - (b/v)\exp{(vt)})}.\right.$$

$$\cdot \sum_{0}^{\infty}n\left(\frac{b^n\exp{((n+1)vt)}}{(v-a)\dots((n+1)v-a)}\right) - c\exp{(-b/v)}\sum_{0}^{\infty}n\left(\frac{b^n}{(v-a)\dots((n+1)v-a)}\right)\right\}$$

$$= \Omega\sum_{0}^{\infty}\left(\frac{(b/v)^n}{n!}\exp{((nv+a)t)}\right) + c\sum_{0}^{\infty}n\left(\frac{b^n\exp{((n+1)vt)}}{(v-a)\dots((n+1)v-a)}\right), \qquad \text{(A 19)}$$

where we have used (A 9). The expression (A 19) is identical to (A 8). An analytical form for the solution (A 15) can also be given using the incomplete Γ function which is defined as

$$\Gamma(x,y) = \int_{0}^{y}P^{x-1}\exp{(-p)}\,\mathrm{d}p$$

(Abramowitz & Stegun, 1964, p. 260).

With the substitution $p = (b/v)\exp{(vt)}$ the integral in (A 15) can be written

$$\int_{0}^{t}\mathrm{e}^{vt}\exp{(-at - b/v\,\mathrm{e}^{vt})}\,\mathrm{d}t = \frac{1}{v}\left(\frac{v}{b}\right)^{\frac{1-a}{v}}\int_{b/v}^{(b/v)\mathrm{e}^{vt}}p^{-a/v}\,\mathrm{e}^{-p}\,\mathrm{d}p$$

from which the final expression for $y(t)$ is derived as

$$y(t) = \exp{(at + (b/v)\,\mathrm{e}^{vt})}(\bar{y}\,\mathrm{e}^{-b/v} + (c/v)(v/b)^{1-a/v}(\Gamma(1-a/v,(b/v)\mathrm{e}^{vt}) - \Gamma(1-a/v,b/v)))$$

$$\text{(A 17)}$$

APPENDIX B

Time course of the current with a quadratic dependence on K_c in the absence of a Hodgkin–Huxley component

In the case of a quadratic dependence of i_{K_1}, i_p on K_c, and for $i_x = 0$, eqns. (3) and (4) of the text become

$$\Delta i(t) = \nu\Delta K_c + \gamma\Delta K_c^2, \qquad \text{(B 1}a\text{)}$$

$$\mathrm{d}\Delta K_c(t)/\mathrm{d}t = \frac{1}{VF}(\sigma\Delta K_c + \eta\Delta K_c^2), \qquad \text{(B 1}b\text{)}$$

where

$$\gamma(E) = \frac{1}{2}\left(\frac{\partial^2}{\partial K_c^2}(i_{K_1} + i_p)\right)_E, \qquad \text{(B 2}a\text{)}$$

$$\eta(E) = \frac{1}{2}\left(\frac{\partial^2}{\partial K_c^2}(i_{K_1} + ri_p)\right)_E. \qquad \text{(B 2}b\text{)}$$

With the border conditions $\Delta K_c(0) = \Delta K_{c,0}$, $\Delta K_c(\infty) = 0$ the solution to eqn. (B 1b) is

$$\Delta K_c(t) = \frac{\Delta K_{c,0}\exp{(-t/\tau_a)}}{1 + \Delta K_{c,0}(\eta/\sigma)(1 - \exp{(-t/\tau_a)})}. \qquad \text{(B 3)}$$

Where $\tau_a = -VF/\sigma$. Substituting in (B 1a)

$$\Delta i(t) = \frac{\nu\Delta K_{c,0}\exp{(-t/\tau_a)}}{1 + \Delta K_{c,0}(\eta/\sigma)(1 - \exp{(-t/\tau_a)})} + \gamma\frac{\Delta K_{c,0}^2\exp{(-t/(\tau_a/2))}}{(1 + \Delta K_{c,0}(\eta/\sigma)(1 - \exp{(-t/\tau_a)}))^2} \quad \text{(B 4)}$$

The continuity of $\Delta K_c(t)$ in $0 < t < \infty$ and the condition $\Delta K_c(\infty) = 0$ require that

$$\sigma < 0,$$

$$\Delta K_{c,0} \eta < -\sigma. \tag{B 5}$$

The current given by (B 4) obviously deviates from the single exponentiality obtained when linearity between currents and K_c is assumed (e.g. eqn. (8)). The ability of expression (B 4) to reproduce the experimental records has been checked on the data
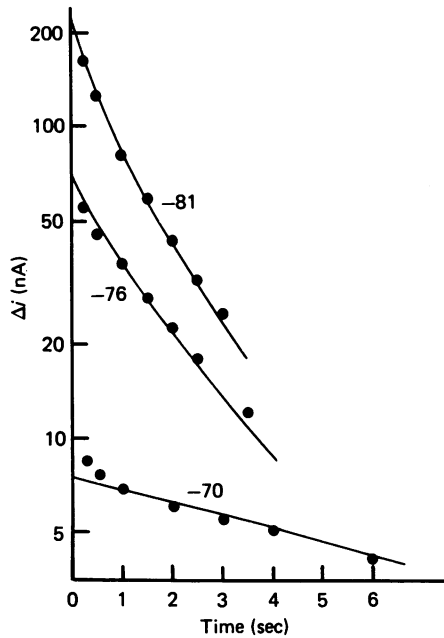

Fig. 7. The same experimental points as in Fig. 1 are now fitted with eqn. (B 4). Values of parameters used are $\eta = -80$ nA/mM and $\sigma = -50$ nA/mM for all three cases. Then, respectively, for -81, -76 and -70 mV: $\nu = -98\cdot14$, $-91\cdot4$ and $-77\cdot5$ nA/mM; $\Delta K_{c,0} = 1\cdot65$, $0\cdot65$ and $-0\cdot1$ mM; $\tau_a = 2\cdot2$, $2\cdot5$ and $8\cdot0$ sec. $1/VF = 0\cdot004$ mM/nA sec. Note that in the cases of the currents recorded during hyperpolarizations (-76 and -81 mV) eqn. (B 4) is able to reproduce the curvature observed experimentally, but fails to do so for the current recorded on depolarizing to -70 mV. See text for further discussion of this point.

shown in Fig. 1 of the text, and the results are shown in Fig. 7. As Fig. 7 is the result of a limited series of attempts, it has to be considered only an illustrative example. The data refer to currents recorded at different potentials, and therefore σ, η and γ could be varied in general. However, in order to limit the degrees of freedom the second derivatives η and γ have been kept constant (and negative).

Values for the parameters have been used which reproduce the appearance of a positive fast component for the curves at -81 and -76, while for -70 mV the second derivative at time zero is, if anything, negative. However the limitation on the reproducibility of a positive fast component on depolarizing pulses, as the case for -70 mV,

depends on the sign of the derivatives. The slope of the semilog plot is in fact calculated as

$$\frac{d\lg|\Delta i|}{dt} = \frac{1}{\Delta i}(\nu + 2\gamma\Delta K_c)\frac{d\Delta K_c}{dt} = \frac{1}{VF}\frac{\nu + 2\gamma\Delta K_c}{\nu + \gamma\Delta K_c}\cdot(\sigma + \eta\Delta K_c), \qquad (B\,6)$$

and its derivative as

$$\frac{d^2\lg|\Delta i|}{dt^2} = \frac{1}{(VF)^2}\Delta K_c(\sigma + \eta\Delta K_c)\left(\eta\frac{\nu + 2\gamma\Delta K_c}{\nu + \gamma\Delta K_c} + \gamma\nu\frac{\sigma + \eta\Delta K_c}{\nu + \gamma\Delta K_c^2}\right). \qquad (B\,7)$$

Considering the case of a depolarizing pulse, then $\Delta K_{c,0} < 0$ and $\Delta i_0 > 0$, which implies

$$\nu + \gamma\Delta K_{c,0} = \frac{\Delta i_0}{\Delta K_{c,0}} < 0 \qquad (B\,8)$$

and consequently $\nu < 0$ if $\gamma < 0$. According to (B 5) and (B 8) a negative slope at $t = 0$ is obtained only if

$$\nu + 2\gamma\Delta K_{c,0} < 0 \quad \text{(see eqn. (B 6)).} \qquad (B\,9)$$

On the other hand at $t = 0$ the second derivative will be positive if

$$\eta\frac{\nu + 2\gamma\Delta K_{c,0}}{\nu + \gamma\Delta K_{c,0}} + \gamma\nu\frac{\sigma + \eta\Delta K_{c,0}}{(\nu + \gamma\Delta K_{c,0})^2} > 0$$

which, assumed that η, γ are negative and according to (B 5), (B 8) implies

$$\nu + 2\gamma\Delta K_{c,0} > 0. \qquad (B\,10)$$

The contradiction between (B 9) and (B 10) can be removed using positive values for η, γ. In this case it is possible to obtain a curve with a positive fast component for -70 mV (not shown).

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