On the extraction of kinetic rate constants from experimental data

Dear Sir:

Recently Balser, J. R., D. M. Roden, and P. B. Bennett (1990) proposed a method for experimentally determining the values of the transition rate constants in a multistate kinetic scheme when direct observations are possible of the occupancy of only one of the states. They applied this method to the scheme

$$C_1 \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} C_2 \underset{k_{23}}{\overset{k_{32}}{\rightleftharpoons}} O_3,$$

(where C_1 and C_2 are closed and O_3 is the conducting state) used to describe potassium currents in guinea pig ventricular myocytes. They correctly noted that the time course of the membrane current during a single potential step is insufficient to uniquely define the values of the four transition rate constants. The k_{ij}'s are instantaneous functions of membrane potential.

Balser et al. (1990) claimed that this scheme provides only three independent values for a single step in potential, and that four are needed. They approached the problem by noting that the potential dependency of the rate constants should be given by either

$$k_{ij} = \exp [A_{ij} + B_{ij}V + C_{ij}V^2] \quad (1)$$

or, over a sufficiently limited voltage range

$$k_{ij} = \exp [A_{ij} + B_{ij}V]. \quad (2)$$

Using either Eq. 1 or 2 the four rate constants at any one potential imply 12 or 8 unknown terms. They expressed the rate constants in the form of e.g., Eq. 1, and concluded that a unique set of rate constants could be obtained by solving simultaneously at four different potentials. No analytical solutions were presented. Iterative methods, called a global fitting procedure, were utilized that were demanding of computer time. The Balser et al. (1990) analysis is in error in that in general more information is provided from the current time course at each potential than they claim, more information is required to solve the problem than they have recognized, and their global fitting method cannot in principle provide a unique set of values for the k_{ij} terms. I describe here exact, analytical methods for determining the values of the k_{ij}'s exclusively in terms of directly experimentally measurable quantities. Iterative methods are not required. The approach described can be extended to schemes with more than three states in certain special cases.

In the Balser et al. (1990) approach the total data set is the time course of the membrane current at the four selected potentials, always with the same initial conditions. In general five independent values can be obtained from the current time course at any potential, whereas six are needed (four k_{ij}'s and two initial conditions). Each additional voltage examined from the same initial conditions provides four more experimental values, and introduces four more unknown terms. Hence, of themselves, these data can be equally well described by an infinite number of sets of k_{ij}'s. Eqs. 1 and 2 introduce no additional measurable quantities which are functions of the unknown terms, but only the purported (albeit reasonable) form of the relation between unknown terms. This approach, then, can never uniquely define the rate constants. While a finite number of roots may exist, their number is expected to be too large to permit a practicable solution even with numerical methods. This was already suggested by their finding that the extracted values of the rate constants depended on the initial guesses.

Let P_1, P_2, and P_3 be the probabilities of occupancy of C_1, C_2, and the observable conducting state, O_3, respectively. The scheme is described by

$$dP_1/dt = -k_{21}P_1 + k_{12}P_2 \quad (3)$$

$$dP_2/dt = -(k_{12} + k_{32})P_2 + k_{21}P_1 + k_{23}P_3 \quad (4)$$

$$dP_3/dt = -k_{23}P_3 + k_{32}P_2, \quad (5)$$

with

$$P_1 + P_2 + P_3 = 1. \quad (6)$$

P_1 can be eliminated. Eqs. 4 and 5 become two coupled first order differential equations in P_3 and P_2. These can be combined into a single second order differential equation in P_3 with the solution

$$P_3(t) = P_3(\infty) - \frac{\dot{P}_3(0) + b[P_3(0) - P_3(\infty)]}{a - b} \exp(-at) + \frac{\dot{P}_3(0) + a[P_3(0) - P_3(\infty)]}{a - b} \exp(-bt), \quad (7)$$

where

$$a = (k_{21} + k_{12} + k_{32} + k_{23})/2 + \{[(k_{21} + k_{12} + k_{32} - k_{23})/2]^2 + k_{32}(k_{23} - k_{21})\}^{1/2} \quad (8)$$

$$b = (k_{21} + k_{12} + k_{32} + k_{23})/2 - \{[(k_{21} + k_{12} + k_{32} - k_{23})/2]^2 + k_{32}(k_{23} - k_{21})\}^{1/2}. \quad (9)$$

The steady-state probability of occupancy, $P_3(\infty)$, is given by

$$P_3(\infty) = k_{21}k_{32}/(k_{21}k_{32} + k_{21}k_{23} + k_{12}k_{23}). \quad (10)$$

The initial velocity of the occupancy probability is given by

$$\dot{P}_3(0) = -k_{23}P_3(0) + k_{32}P_2(0), \quad (11)$$

and $P_3(0)$ and $P_2(0)$ are the initial probabilities of occupancy of C_2 and O_3. In general five values are obtained from the current time course at any potential (a, b, $P_3(\infty)$, $\dot{P}_3(0)$, and $P_3(0)$). These are experimentally determined from the five terms needed to fit equations of the form of Eq. 7 to experimental data (two relaxation time constants, the steady-state value, and

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two coefficients on the exponential terms). Neither $\dot{P}_3(0)$ or $P_3(0)$ need be directly measured. They can be computed from the measured a, b, and $P_3(\infty)$ values and the coefficients on the two exponential terms using the definitions in Eq. 7. To extract the values of the k_{ij}'s Eqs. 8–11 must be solved simultaneously. As $P_3(0)$ is always experimentally determinable, there are four equations in five unknowns (k_{21}, k_{12}, k_{32}, k_{23}, and $P_2(0)$). As noted by Balser et al. (1990) $P_3(V, t)$ is not itself observed but can be computed from the measured conductance according to

$$P_3(V, t) = g(V, t)/g_{max}(\infty), \tag{12}$$

where $g_{max}(\infty)$ is the saturated value of the conductance. Alternatively, the tail current method of Balser et al. (1990) may be used. For a scheme displaying inactivation, the measured g_{max} is not sufficient to define P_3.

If $P_2(0)$ is independently known (and not zero) then the rate constants can be extracted for a single step in potential. Eqs. 8–11 yield

$$k_{23} = [(a + b)P_2(0) - \dot{P}_3(0)]/2[P(0) + P_3(0)] \pm ([\dot{P}_3(0) - (a + b)P_2(0)]^2 - 4[P_2(0) + P_3(0)][abP_2(0)[1 - P_3(\infty)]])^{1/2}/2[P_2(0) + P_3(0)]. \tag{13}$$

k_{32} is obtained from Eq. 11. The remaining rate constants are readily obtained by noting that

$$a + b = k_{21} + k_{12} + k_{32} + k_{23} \tag{14}$$

$$ab = k_{21}k_{32} + k_{21}k_{23} + k_{12}k_{23}, \tag{15}$$

and hence

$$abP_3(\infty) = k_{21}k_{32}. \tag{16}$$

The solution has two roots, and the correct set must be selected on physical criteria. For example, all rate constants must be real and positive, or Eq. 1 or 2 must be satisfied.

For the case where $P_2(0)$ is not known (but not zero), the problem can be solved in the following way. For any step to potential V from some fixed reference initial conditions we obtain a, b, $P_3(\infty)$, and $\dot{P}_3^*(0)$, where the star indicates a step from reference conditions. For some other step to V_c from reference we obtain a_c, b_c, $P_{c3}(\infty)$, and $\dot{P}_{c3}^*(0)$. This is eight equations in nine unknowns. Now again step from reference to V_c, but after some time interval, T, at V_c step again to the original V. At V when $t = 0$

$$\dot{P}_3(0) = -k_{23}P_3(0) + k_{32}P_2(0), \tag{11}$$

and for V_c at $t = T$

$$\dot{P}_{c3}(T) = -k_{c23}P_{c3}(T) + k_{c32}P_{c2}(T). \tag{17}$$

$\dot{P}_3(0)$ and $\dot{P}_{c3}(T)$ are different as the rate constants are instantaneous functions of potential. $\dot{P}_i$ in general is discontinuous across a step in potential. However, the variables P_2 and P_3 are continuous. Hence

$$P_3(0) = P_{c3}(T)$$

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P_2 can be eliminated between Eqs. 11 and 17. For simplicity, but without any loss of generality, T can be selected as steady state yielding

$$\dot{P}_3(0)/P_{c3}(\infty) = k_{c23}(k_{32}/k_{c32}) - k_{23}. \tag{18}$$

$\dot{P}_3(0)$ is measured from the time course of the current at V following the step to V_c of duration T, and expresses the well known effect of changing initial conditions on the values of the coefficients on the exponential terms. $\dot{P}_3(0)$ is a new experimental value not predictable from the current time courses at V and V_c separately as it includes the effect of the time course of the unknown P_2 variable during V_c. Eq. 18 introduces no new unknowns, yielding nine equations for the nine unknown terms. To obtain explicit values for the rate constants at V and V_c in terms of experimentally measurable quantities, solve simultaneously Eqs. 8–11 for V (from reference), an analogous set of four for V_c (from reference) and Eq. 18. We obtain

$$k_{c23} = -Y/2X \pm [(Y^2 - 4XZ)^{1/2}/2X], \tag{19}$$

where

$$X = \alpha[\alpha(a_c + b_c) - (a + b) - \beta] \tag{20}$$

$$Y = ab[1 - P_3(\infty)] + \beta[(a + b) - \alpha(a_c + b_c) + \beta] - a_cb_c\alpha^2[1 - P_{c3}(\infty)] \tag{21}$$

$$Z = \alpha\beta a_cb_c[1 - P_{c3}(\infty)], \tag{22}$$

and

$$\alpha = [\dot{P}_3^*(0)P_{c3}(\infty) - \dot{P}_3(0)P_3^*(0)]/\dot{P}_{c3}^*(0)P_{c3}(\infty) \tag{23}$$

$$\beta = \dot{P}_3(0)/P_{c3}(\infty). \tag{24}$$

k_{c23} is expressed only in terms of experimentally measurable quantities. k_{c32} is given by

$$k_{c32} = [k_{c23}[(a_c + b_c) - k_{c23}] - a_cb_c[1 - P_{c3}(\infty)]]/k_{c23}, \tag{25}$$

and

$$k_{23} = \alpha k_{c23} - \beta \tag{26}$$

$$k_{32} = \alpha k_{c32}. \tag{27}$$

The remaining rate constants can be obtained from Eqs. 16 and 14 for V, and from analogous expressions for V_c. Again, there are two roots.

Once a solution has been obtained for the first pair of potentials, V and V_c, $P_2^*(0)$ can be computed from k_{32} and k_{23} or from k_{c32} and k_{c23}. Values can then be obtained for any other potential using Eq. 13.

A modified procedure is required when $P_2^*(0) = P_3^*(0) = 0$. In this case Eq. 11 provides no information about the rate constants, and the time course of the membrane current is completely defined by just three terms (a, b, and $P_3(\infty)$). Steps from reference to V and V_c now provide only six equations, while eight are needed (the four rate constants at each potential, $P_2^*(0)$ is now known and equal to zero). Two additional experimental values can be obtained by proceeding

as above but now observing the effects of two different durations of V_c on the currents during V. One duration could be steady state as before, and the other need only be far enough from steady state for $P_{c2}(T)$ to be clearly different from $P_{c2}(\infty)$. We solve Eqs. 8–10, an analogous set for V_c, Eq. 18 and for the current during V following V_c of duration $T <$ steady state

$$\dot{P}_3^T(0) = [k_{c23}(k_{32}/k_{c32}) - k_{23}]P_3^T(0) + (k_{32}/k_{c32})\dot{P}_{c3}(T). \quad (28)$$

$\dot{P}_{c3}(T)$ can be computed from

$$\dot{P}_{c3}(T) = -[a_c b_c P_{c3}(\infty)/(a_c - b_c)] \exp(-a_c T) + [a_c b_c P_{c3}(\infty)/(a_c - b_c)] \exp(-b_c T). \quad (29)$$

Values for the rate constants can be obtained from Eqs. 19–27 as before except that α now has the value α^T given by

$$\alpha^T = [\dot{P}_3^T(0)P_{c3}(\infty) - \dot{P}_3(0)P_3^T(0)]/\dot{P}_{c3}(T)P_{c3}(\infty). \quad (30)$$

Alternatively, a different holding potential can be selected such that $\dot{P}_3^*(0) \neq 0$ when $P_3^*(0) = 0$.

Alternatively, the tail current time course at OFF can be used. If the potential preceding and following the test step is not the same then there are eight equations in nine unknowns. However, if the holding potential preceding and following the test step is the same then there are just eight unknowns (four transition rate constants each for the test and holding potentials) for the eight equations. Proceeding as above a solution is obtained by making use of the continuity of $P_2(t)$ and $P_3(t)$ at both ON and OFF. This approach cannot be used when the steady-state values of P_2 and P_3 at the holding potential are both zero or even if only that for P_3 is zero. In the first case there are only six equations for the seven unknowns (k_{21} at the holding potential is now zero). In the second, the coefficient on the exp $(-at)$ term vanishes, and the relaxation rate constant, a, is not experimentally determinable. The conditioning pulse, test pulse method of Eqs. 19–24 is valid for all values of $P_3^*(0)$.

This problem has also been solved for a generalized three-state scheme in which k_{31} and k_{13} are not assumed to be zero (Goldman and Hahin, 1979), but only for the case that $P_2^*(0) = P_3^*(0) = 0$. For the generalized scheme $\dot{P}_3^*(0)$ is not zero under these initial conditions. If the steady state is a true equilibrium (detailed balance) there are only five independent rate constants at each potential, and the two durations of V_c method of Eq. 30 provides a solution.

This method can be applied to schemes with any number of closed states if they extend in a straight chain with only a single closed state directly coupling to the open and with no nonzero rate constants connecting nonadjacent closed states. In this case for N states there are 2 $(N - 1)$ rate constants plus $N - 1$ initial conditions for V and again 2 $(N - 1)$ rate constants for V_c from the same reference initial conditions. The current time course at V provides $[2(N - 1) + 1]$ experimental values, and that at V_c an additional 2 $(N - 1)$. $N - 2$ durations of V_c are then needed while up to 2 $(N - 1)$ are available from this current time course. Hence a finite number of roots always exists when the number of initial conditions rather than the number of test potentials is increased. Numerical methods can be used for schemes with many states where analytical solutions are too cumbersome.

For fully generalized schemes there are N $(N - 1)$ rate constants at both V and V_c and again $N - 1$ initial conditions. This approach, then, does not permit a solution for three or more states in the fully generalized case when observations of the occupancy of only a single state are possible. For various special cases the outcome depends on the detailed properties of the proposed scheme, i.e., the number of nonzero rate constants assumed, the number of independent rate constants needed assuming detailed balance, the number of states with zero occupancy at reference initial conditions, the number of states whose occupancy can be directly observed, and whether single channel recording can provide additional values.

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