Eur Biophys J (1989) 17: 1–4

European Biophysics Journal

Monotonic and non-monotonic single channel open time distributions with two sequential open states

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Received July 22, 1988/Accepted in revised form January 10, 1989

Abstract. Some conditions under which kinetic schemes including two sequential open states of identical conductance will display a non-monotonic (i.e. with a deficit of short open times and a maximum at $t > 0$) distribution of single channel open times are described theoretically. Neither a closed cyclic scheme nor exclusively irreversible transitions between states are required for non-monotonic distributions. A required condition for the schemes considered here is that all openings are to a state from which closing is not possible. It is the presence of a precursor process to channel closing that produces the non-monotonic distribution. Following each channel opening some time is required for a transition into the second open state from which all closings proceed. Simple schemes of this sort cannot provide the basis of any experimental reports of non-monotonic distributions.

Key words: Theoretical study, single channels, probability density function, monotonic and non-monotonic distributions

Introduction

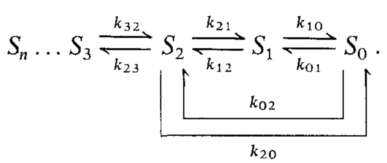
Non-monotonic distributions of single channel open times with a deficit of short open times and a maximum at a time greater than zero were reported experimentally by Nagy (1987), and a possible theoretical basis for such effects was provided by Colquhoun and Hawkes (1983). Colquhoun and Hawkes accounted for non-monotonic open time distributions with a closed cyclic scheme, including two sequential open states of the same conductance, in which all transitions between states were irreversible and proceeded in the same direction around the cycle. Such schemes require coupling to an energy source. I find that neither a closed cyclic scheme, exclusively irreversible transitions nor coupling to an energy source are required for non-monotonic distributions.

The schemes analyzed here are not proposed as the actual basis for any experimental results. In fact, Kerry et al. (1986) have pointed out that instrumental limits on resolution of brief openings can artifactually generate such distributions, and caution must be exercised in experimental demonstrations of this effect. The point of this communication is to more narrowly define conditions required for non-monotonic distributions and so clarify a possible physical basis. It is the presence of a precursor process to channel closing that generates a non-monotonic distribution of open times. A preliminary report of some of these results has been made (Goldman 1988).

Results and discussion

Conditions for monotonic distributions

We first examine Scheme 1:



Here S_0 and S_1 are open states of the same conductance, and $S_2, S_3 \ldots S_n$ are all closed. For the distribution of open times all the closed states may be lumped, and Scheme 1 becomes identical to that considered by Colquhoun and Hawkes (1983).

The open time distribution for Scheme 1 may be described by the probability density function, $N(t)$, given by

$$N(t) = N_1 k_{12} P_1(t) + N_0 k_{02} P_0(t), \quad (1)$$

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where $P_1(t)$ and $P_0(t)$ are the probabilities of occupancy of S_1 and S_0, and N_1 and N_0 are the numbers of independent openings to each state. Expressions for the probability of occupancy of any state in a fully generalized, i.e. six transition rate constant, three state scheme including expressions for the relaxation time constants, steady state occupancies, and initial conditions effects all in terms of the elementary transition rate constants have been given by Goldman (1976). Another problem for fully generalized three state schemes already treated (Goldman and Hahin 1979) is how to extract the six rate constants when observations are possible of the occupancy of only one state. The probabilities of occupancy are given by

$$P_i(t) = P_{i\infty} - \left[\frac{\dot{P}_{i0} + b(P_{i0} - P_{i\infty})}{a-b}\right]\exp(-at) + \left[\frac{\dot{P}_{i0} + a(P_{i0} - P_{i\infty})}{a-b}\right]\exp(-bt), \tag{2}$$

where P_{i0} and $P_{i\infty}$ are initial and steady state occupancy probabilities of any state S_i and $\dot{P}_{i0}$ is the time derivative of its occupancy at $t=0$. Expressions for a and b evaluated for Scheme 1 are given by (8) and (9) below.

For Scheme 1, $P_{1\infty} = P_{0\infty} = 0$ as all openings terminate in a closing to S_2. Channels open either to S_1, or to S_0. For openings to S_1 the initial probability of occupancy of this state, P_{10}, is unity with $P_{00}=0$. Similarly for the N_0 openings to S_0, $P_{00}=1$ and $P_{10}=0$. Initial conditions are then simply given by

$$\dot{P}_{10} = -(k_{12}+k_{10})P_{10} + k_{01}P_{00} = -(k_{12}+k_{10}) \tag{3}$$

$$\dot{P}_{00} = -(k_{02}+k_{01})P_{00} + k_{10}P_{10} = -(k_{02}+k_{01}). \tag{4}$$

$$N_1 = N_T\frac{k_{21}}{k_{21}+k_{20}} \tag{5}$$

$$N_0 = N_T\frac{k_{20}}{k_{21}+k_{20}}, \tag{6}$$

where N_T is the total number of openings. Equation (1) becomes

$$N(t) = \frac{N_T}{k_{21}+k_{20}}\left\{-\left[\frac{k_{21}k_{12}(b-(k_{12}+k_{10})) + k_{20}k_{02}(b-(k_{02}+k_{01}))}{a-b}\right]\exp(-at) + \left[\frac{k_{21}k_{12}(a-(k_{12}+k_{10})) + k_{20}k_{02}(a-(k_{02}+k_{01}))}{a-b}\right]\exp(-bt)\right\}, \tag{7}$$

with

$$a = \frac{k_{12}+k_{10}+k_{02}+k_{01}}{2} + \left[\left(\frac{k_{12}+k_{10}-k_{02}-k_{01}}{2}\right)^2 + k_{10}k_{01}\right]^{1/2} \tag{8}$$

$$b = \frac{k_{12}+k_{10}+k_{02}+k_{01}}{2} - \left[\left(\frac{k_{12}+k_{10}-k_{02}-k_{01}}{2}\right)^2 + k_{10}k_{01}\right]^{1/2}. \tag{9}$$

The conditions for a monotonic open time distribution (i.e. for the coefficients on the two exponential terms in (7) to both be positive) are that

$$a > (k_{12}+k_{10}) > b$$

and

$$a > (k_{02}+k_{01}) > b.$$

It can be readily shown that these inequalities are always satisfied whatever the values of the rate constants, given these initial conditions. All values of the rate constants are given by

$$n(k_{12}+k_{10}) = (k_{02}+k_{01}), \tag{10}$$

where n is any positive number. Equation (8) becomes

$$a = \left(\frac{1+n}{2}\right)(k_{12}+k_{10}) + \left[\left(\frac{1-n}{2}\right)^2(k_{12}+k_{10})^2 + k_{10}k_{01}\right]^{1/2}, \tag{11}$$

and similarly for (9). There are three cases to evaluate: $n<1$, $n=1$, and $n>1$. For $n<1$ terms under the radical always have a value $> \frac{1-n}{2}(k_{12}+k_{10})$. For $n=1$, a and b may be evaluated directly, and for $n>1$ terms under the radical always have a value $> \frac{n-1}{2}(k_{12}+k_{10})$ owing to the square on these terms. Hence, for $n<1$

$$a > \frac{1+n}{2}(k_{12}+k_{10}) + \frac{1-n}{2}(k_{12}+k_{10}) > (k_{12}+k_{10})$$

$$b < \frac{1+n}{2}(k_{12}+k_{10}) - \frac{1-n}{2}(k_{12}+k_{10}) < (k_{12}+k_{10}).$$

For $n=1$

$$a = (k_{12}+k_{10}) + k_{10}k_{01}{}^{1/2} > (k_{12}+k_{10})$$

$$b = (k_{12}+k_{10}) - k_{10}k_{01}{}^{1/2} < (k_{12}+k_{10}).$$

For $n>1$

$$a > \frac{n+1}{2}(k_{12}+k_{10}) + \frac{n-1}{2}(k_{12}+k_{10}) > (k_{12}+k_{10})$$

$$b < \frac{n+1}{2}(k_{12}+k_{10}) - \frac{n-1}{2}(k_{12}+k_{10}) < (k_{12}+k_{10}),$$

and the first inequality is satisfied for all values of the rate constants. Similarly,

$$m(k_{02}+k_{01})=(k_{12}+k_{10}), \tag{12}$$

where m is any positive number. Proceeding as above it can be shown that the second inequality is also always satisfied. Equation (7), then, is always the sum of exponentials whatever the values of the rate constants, and the open time distribution for Scheme 1 is always monotonic (given the initial conditions (3) and (4)) in agreement with the conclusions of Colquhoun and Hawkes (1983).

Two special cases of interest arise if an irreversible transition is included by setting either k_{01} or $k_{10}=0$. For $k_{01}=0$, Eqs. (8) and (9) become $a=k_{12}+k_{10}$ and $b=k_{02}$, and (7) becomes

$$N(t)=\frac{N_T}{k_{21}+k_{20}} \cdot \left[k_{21}k_{12}\exp(-(k_{12}+k_{10})t)+k_{20}k_{02}\exp(-k_{02}t)\right]. \tag{13}$$

For $k_{10}=0$, Eq. (7) becomes

$$N(t)=\frac{N_T}{k_{21}+k_{20}} \cdot \left[k_{21}k_{12}\exp(-k_{12}t)+k_{20}k_{02}\exp(-(k_{01}+k_{02})t)\right]. \tag{14}$$

By inspection Eqs. (13) and (14) are sums of exponentials, and open time distributions are monotonic as is required from the properties of the more generalized Eq. (7).

Conditions for non-monotonic distributions

Non-monotonic open time distributions are displayed by Scheme 2.

$$S_x \longrightarrow S_1 \underset{k_{01}}{\overset{k_{10}}{\rightleftharpoons}} S_0 \overset{k_{02}}{\longrightarrow} S_2$$

S_0 and S_1 are again open states of the same conductance, and S_2 is either a resting or an inactivated state. S_x represents any number of lumped closed states from which transitions into S_1 are possible, and could (but is not required to) include S_2. As written, Scheme 2 is not a closed cyclic scheme. Its essential feature (see also Colquhoun and Hawkes 1983) is that all openings are to S_1 and all closings are from S_0.

The probability density function for open times of Scheme 2 is given by

$$N(t)=N_T\,k_{02}\,P_0(t) \tag{15}$$

where $N_T=N_0=N_1$. The central difference between Schemes 1 and 2 is in the initial conditions here given by

$$\dot{P}_{00}=-(k_{01}+k_{02})P_{00}+k_{10}P_{10}=k_{10}, \tag{16}$$

as all openings are to S_1. $P_{0\infty}=0$ as all openings terminate in a closing to S_2, and Eq. (15) becomes

$$N(t)=N_T\,k_{02}\left[-\frac{k_{10}}{a-b}\exp(-at)+\frac{k_{10}}{a-b}\exp(-bt)\right], \tag{17}$$

with

$$a=\frac{k_{10}+k_{01}+k_{02}}{2} +\left[\left(\frac{k_{10}-k_{01}-k_{02}}{2}\right)^2+k_{10}k_{01}\right]^{1/2}, \tag{18}$$

$$b=\frac{k_{10}+k_{01}+k_{02}}{2} -\left[\left(\frac{k_{10}-k_{01}-k_{02}}{2}\right)^2+k_{10}k_{01}\right]^{1/2}. \tag{19}$$

Equation (17) is the difference of exponentials for all values of the rate constants and so Scheme 2 always predicts a non-monotonic distribution of open times. It is neither a closed cyclic scheme nor exclusively irreversible transitions that generates the non-monotonic distribution, but the presence of a precursor process to closing. There is a deficit of short open times and a maximum in the distribution at $t>0$ because there is no occupancy of S_0 at $t=0$, and some time is needed after each channel opening for the probability of occupancy of S_0 to increase.

In Scheme 2 all transitions are made irreversible by setting $k_{01}=0$. In this case $a=k_{10}$, $b=k_{02}$, and (15) becomes

$$N(t)=N_T\,k_{02} \cdot \left[-\frac{k_{10}}{k_{10}-k_{02}}\exp(-k_{10}t)+\frac{k_{10}}{k_{10}-k_{02}}\exp(-k_{02}t)\right]. \tag{20}$$

The rate constants of the two exponentials in the distribution are now just the reciprocals of the mean open times of S_1 and S_0 in agreement with Colquhoun and Hawkes (1983). These results agree with the conclusions of Colquhoun and Hawkes in that they also require opening to a state from which closing is not possible for non-monotonic distributions. They differ in that some of the conditions in the example treated by them have been relaxed.

Results intermediate between the pure monotonic and pure non-monotonic cases of Schemes 1 and 2 are obtained by relaxing the requirements of strictly irreversible transitions between states S_x and S_1 or S_0 and S_2 or by including a transition, from S_x directly into S_0. In these cases distributions are either monotonic or non-monotonic depending on the relative values of the rate constants. To illustrate this point consider Scheme 2 modified to include a non-zero rate constant between S_2 and S_0 given by k_{20}. All closings are still from S_0 and the probability density function is again given by Eq. (15). As before $P_{0\infty}=0$, but initial conditions are now given by

$$\dot{P}_{00} = -(k_{10}+k_{02})\,P_{00}+k_{10}P_{10}\,. \tag{21}$$

(15) becomes

$$\begin{aligned} N(t) &= N_T k_{02} \\ &\cdot\left[-\frac{-(k_{01}+k_{02})P_{00}+k_{10}P_{10}+bP_{00}}{a-b}\exp(-at)\right. \\ &\left.+\frac{-(k_{01}+k_{02})P_{00}+k_{10}P_{10}+aP_{00}}{a-b}\exp(-bt)\right]. \end{aligned} \tag{22}$$

When P_{00} is sufficiently small, i.e. when k_{20} is small relative to k_{x1}, both the quantities $k_{10}P_{10}+bP_{00}$ and $k_{10}P_{10}+aP_{00}$ are greater than $(k_{01}+k_{02})P_{00}$ yielding a non-monotonic distribution. At $t=0$

$$N(t)_0 = N_T k_{02} P_{00}\,. \tag{23}$$

In the limit of negligible P_{00} this becomes zero as for the pure non-monotonic case of Scheme 2. For

$$k_{10}P_{10} + bP_{00} < (k_{01}+k_{02})P_{00} < k_{10}P_{10} + aP_{00}\,,$$

the distribution is monotonic. For the opposite limiting case of $k_{20} \gg k_{x1}$, P_{10} is negligible, and the distribution is monotonic as can be shown by proceeding as described for Scheme 1.

Kijima and Kijima (1987) have examined the problem of the signs on the coefficients to the exponential terms in a probability density function for generalized schemes with any number of open and closed states. They showed that under the assumption of detailed balance, i.e. that steady state is a true equilibrium state, all coefficients are positive. The present results demonstrate, for the simple three state schemes considered here, that equilibrium is not required for all positive coefficients. Detailed balance was not assumed to obtain this result for Eq. (7), and Eqs. (13) and (14) explicitly violate detailed balance. Moreover, Scheme 2 which does not display all positive coefficients for the open time probability density function is an equilibrium scheme as the steady state is a true equilibrium state. S_2 could be an absorbing inactivated state, and coupling to a source of energy is not required for non-monotonic distributions if the scheme is not cyclic.

Conclusions

Scheme 2 cannot be the basis of the results of Nagy (1987). Nagy reported non-monotonic open time distributions for openings that occurred during the first 5 ms of a step in potential, but not for those that occurred later in that same step. For Scheme 2, openings at any period during a step in potential are still to S_1, and some time after channel opening will always be needed for the transition to S_0. Hence, open time distributions constructed from any time interval during the potential step will be non-monotonic.

Acknowledgements. I thank Dr. K. Nagy and Professor B. Neumcke for their comments on the manuscript. This work was supported by National Institutes of Health Grant NS 07734, and by a Fulbright Senior Professor award from the Fulbright Commission, Bonn, Federal Republic of Germany.

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