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Letter to the Editor

Testing for Detailed Balance (Microscopic Reversibility) in Ion Channel Gating

The gating of ion channels is subject to the thermodynamic principle of detailed balance (microscopic reversibility). In the absence of an external energy source driving the gating, the number of transitions in each direction between any two states should (on average) be equal at thermodynamic equilibrium so that the single-channel record shows time reversibility (Colquhoun and Hawkes, 1982, 1995). For gating mechanisms in which states are connected in loops, detailed balance is preserved if the product of the rate constants in the clockwise direction around each loop is equal to the product of the rate constants in the counterclockwise direction around the same loop (Colquhoun and Hawkes, 1982, 1995). A violation of detailed balance would indicate the presence of an external energy source, as in the gating of Cl^- channels from Torpedo, where the external energy source is the Cl^- gradient (Richard and Miller, 1990).

In a recent paper published in the *Biophysical Journal*, Wagner and Timmer (2000) present a method to test for detailed balance in single-channel gating using the likelihood ratio test, and find that for certain models, the ability to detect violations of detailed balance depends on the identifiability of the transition rates. We write this letter to alert the readers of the *Biophysical Journal* to three additional methods that can also be used to test for detailed balance, including some that are model-independent.

In the first method, single-channel current records are analyzed in forward and backward directions (in time) to generate 2D dwell-time distributions of pairs of adjacent open and closed intervals. The 2D distributions contain information about both the frequency of occurrence and correlation between adjacent intervals of specified durations. If detailed balance is obeyed, then there should be no difference between the 2D distributions for the forward and backward analysis (Fredkin et al., 1985; Steinberg, 1987a, b). A χ^2 test for differences between the two distributions then gives a quantitative measure of whether detailed balance is violated (Song and Magleby, 1994). Wagner and Timmer (2000) refer to the application of this method by Song and Magleby (1994) as mainly a visual test, but the test is, in fact, quantitative. The visual plots are not necessary, but are generated to allow display and inspection of the data. A simplified form of the 2D method, in which the mean durations of open intervals in specific ranges are plotted against the mean durations of adjacent closed intervals in specific ranges (calculated separately for forward and backward analysis of the single-channel current records) can also be used to test quantitatively for detailed balance (Kerry et al., 1988; McManus and Magleby, 1989). More general cross-correlation and autocorrelation functions (Fredkin et al., 1985; Steinberg, 1986; Ball et al., 1988; Kerry et al., 1988; Colquhoun and Hawkes, 1987), in which the specific durations of the correlated intervals are not directly identified, can also be applied to forward and backwards analysis of the data to test for detailed balance. Although derived on the basis that channel gating is a discrete Markov process, these correlation methods are essentially model independent.

In the second method, 2D dwell-time distributions obtained from forward and backward analysis of the single-channel current records are fitted separately with sums of 2D exponential components (Rothberg et al., 1997). A component irreversibility factor (C_{IRR}) is then calculated for each 2D component using differences in the volumes of corresponding components in the forward and backward distributions (Rothberg and Magleby, 1998). The application of a *t*-statistic indicates whether there are significant violations of detailed balance. Calculating the C_{IRR} has the advantage over the other methods mentioned here, in that it directly determines the excess and deficit of interval pairs in each 2D component arising from any irreversibility in the gating. The C_{IRR} method uses an assumption of discrete state Markov gating together with the estimated numbers of states, but does not require an assumption of a specific kinetic scheme for the gating.

In the third method, the maximum likelihood fitting of specific gating mechanisms to single-channel data is used. The fitting is carried out in two ways: with the rate constants constrained so that detailed balance is preserved, and with the rate constants free so that detailed balance is not preserved. Application of the likelihood ratio test using the maximum likelihoods obtained for the constrained and unconstrained fitting then indicates whether the data are described significantly better when the detailed balance constraint is eliminated. Song and Magleby (1994) applied this test to the gating of BK channels and found that eliminating the constraint of detailed balance did not significantly improve the description of the data for the examined model. By imposing deviations from detailed balance, Song and Magleby (1994) then found, using the likelihood ratio test, that the upper limit on the percentage of irreversible opening-closing transitions for the examined model for the BK channel was $<$2 to 4%. As indicated by Wagner and Timmer (2000), the likelihood ratio test represents a powerful

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test for detailed balance. Song and Magleby (1994) found the likelihood ratio test to be more powerful in detecting violations of detailed balance than differences between 2D dwell-time distributions examined with the χ^2 test (detection of 2 to 4% irreversible gating with the likelihood ratio test vs. 6 to12% with the χ^2 test, for the examined model). The disadvantage of the likelihood ratio test, as applied by Song and Magleby (1994) and Wagner and Timmer (2000), is that it requires the assumption of a specific gating mechanism, and is therefore highly model-dependent.

Thus, there are a number of quantitative methods to examine whether the gating of an ion channel is consistent with thermodynamic equilibrium (including subconductance methods not detailed here). By applying these methods it is possible to test for detailed balance with and without the constraints of specific gating mechanisms.

REFERENCES

Ball, F. G., C. J. Kerry, R. L. Ramsey, M. S. P. Sansom, and P. N. R. Usherwood. 1988. The use of dwell time and cross-correlation functions to study single-ion channel gating kinetics. *Biophys. J.* 54:309–320.

Colquhoun, D., and A. G. Hawkes. 1982. On the stochastic properties of bursts of single ion channel openings and of clusters of bursts. *Philos. Trans. R. Soc. Lond. B.* 300:1–59.

Colquhoun, D., and A. G. Hawkes. 1995. The principles of the stochastic interpretation of ion-channel mechanisms. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 397–482.

Colquhoun, D., and A. G. Hawkes. 1987. A note on correlation in single ion channel records. *Proc. R. Soc. Lond. B.* 230:15–52.

Fredkin, D. R., M. Montal, and J. A. Rice. 1985. Identification of aggregated Markovian models: application to the nicotinic acetylcholine receptor. *In* Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack Kiefer. L.M. LeCam and R.A. Olshen, editors. Wadsworth Press, Belmont. 269–289.

Kerry, C. J., R. L. Ramsey, M. S. P. Sansom, and P. N. R. Usherwood. 1988. Glutamate receptor channel kinetics: the effect of glutamate concentration. *Biophys. J.* 53:39–52.

McManus, O. B., and K. L. Magleby. 1989. Kinetic time constants independent of previous single-channel activity suggest Markov gating for a large conductance Ca-activated K channel. *J. Gen. Physiol.* 94: 1037–1070.

Rothberg, B. S., R. A. Bello, and K. L. Magleby. 1997. Two-dimensional components and hidden dependencies provide insight into ion channel gating mechanisms. *Biophys. J.* 72:2524–2544.

Rothberg, B. S., and K. L. Magleby. 1998. Investigating single-channel gating mechanisms through analysis of two-dimensional dwell-time distributions. *Methods Enzymol.* 293:437–456.

Song, L., and K. L. Magleby. 1994. Testing for microscopic reversibility in the gating of maxi K^+ channels using two-dimensional dwell-time distributions. *Biophys. J.* 67:91–104.

Steinberg, I. Z. 1986. On the time reversal of noise signals. *Biophys. J.* 50:171–179.

Steinberg, I. Z. 1987a. Frequencies of paired open-closed durations of ion channels. Method of evaluation from single-channel recordings. *Biophys. J.* 52:47–55.

Steinberg, I. Z. 1987b. Relationship between statistical properties of single ionic channel recordings and the thermodynamic state of the channels. *J. Theo. Biol.* 124:71–87.

Richard, E. A., and C. Miller. 1990. Steady-state coupling of ion-channel conformations to a transmembrane ion gradient. *Science.* 247: 1208–1210.

Wagner, M., and J. Timmer. 2000. The effects of non-identifiability on testing for detailed balance in aggregated markov models for ion-channel gating. *Biophys. J.* 79:2918–2924.

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