brief communication

Diffusion model in ion channel gating

Extension to agonist-activated ion channels

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ABSTRACT Previously, we described a model which treats ion channel gating as a discrete diffusion problem. In the case of agonist-activated channels at high agonist concentration, the model predicts that the closed lifetime probability density function from single channel recording approximates a power law with an exponent of −3/2 (Millhauser, G. L., E. E. Salpeter, and R. E. Oswald. 1988*a*. *Proc. Natl. Acad. Sci. USA.* 85:1503–1507). This prediction is consistent with distributions derived from a number of ligand-gated channels at high agonist concentration (Millhauser, G. L., E. E. Salpeter, and R. E. Oswald. 1988*b*. *Biophys. J.* 54:1165–1168.) but does not describe the behavior of ion channels at low activator concentrations. We examine here an extension of this model to include an agonist binding step. This extended model is consistent with the closed time distributions generated from the BC_3H-1 nicotinic acetylcholine receptor for agonist concentrations varying over three orders of magnitude.

INTRODUCTION

With the cloning and sequencing of a number of ligand and voltage gated ion channels within the last few years, a number of plausible structures have been predicted for the membrane spanning regions. Clearly, the challenge is to relate the structure of ion channels to their functional behavior. The difficulties in studying the functional dynamics of ion channels with spectroscopic techniques have placed a great deal of emphasis on the interpretation of the microscopic kinetic behavior inferred from single channel recording. Lifetime histograms have traditionally been interpreted as sums of exponentials, whose time constants represent eigenvalues of a master equation for a kinetic mechanism with a small number of energetically distinct states. This approach assumes that the states are connected by Markov processes, which may or may not map directly to individual protein conformations, and has been used successfully to describe the dwell time distributions of a wide variety of ion channels (e.g., Colquhoun and Sakmann, 1985; Sine and Steinbach, 1986, 1987; McManus et al., 1989*b*; Papke et al., 1988; Korn and Horn, 1989). Molecular dynamics (Elber and Karplus, 1987) and spectroscopic studies (Ansari et al., 1985), however, suggest that a large number of minima may exist on the potential energy surface of a protein.

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Abbreviations used in this paper: AChR, Acetylcholine receptor; AIC, Akaike Information Criterion; DMEM, Dulbecco's modified Eagle's medium; LLR, log-likelihood ratio; PDF, probability density function; PIP, 1,1-dimethyl-4-acetylpiperazinium.

The complexity (particularly of closed time distributions) has led a number of groups to suggest that the dynamics associated with ion channel gating can be described by models other than discrete Markov models employing a small number of states (Läuger, 1988; Levitt, 1989; Liebovitch et al., 1987; Liebovitch and Sullivan, 1987; Millhauser et al., 1988*a*,*b*). Liebovitch et al. (1987) have described a model which postulates that the kinetic behavior of ion channels follows a fractal scaling scheme. In the limit of high fractal dimension ($D \rightarrow 2$), the model predicts a power law distribution for the probability density function (PDF). We have shown (Millhauser et al., 1988*a*,*b*) that an approximate power law distribution can arise from Markov processes, with a large number of states connected by equal rate constants (model 1):

$$O \overset{k_1}{\longleftarrow} C_1 \overset{k_2}{\longleftrightarrow} C_2 \overset{k_2}{\longleftrightarrow} C_3 \overset{k_2}{\longleftrightarrow} \cdots \overset{k_2}{\longleftrightarrow} C_N. \qquad (1)$$

This model is equivalent to a one-dimensional discrete diffusion problem among closed states (C), with a reflecting barrier at one end (C_N) and a partially absorbing barrier at the other (i.e., a finite probability of a transition to an open state, O). The forward and reverse rate constants between closed states are assumed to be equal (k_2). Under conditions where the opening rate is approximately equal to the transitions between closed states, the PDF follows an approximate power law decay ($f[t] = t^{-a}$) with an exponent (a) of 3/2 (Millhauser et al., 1988*a*). The effect of a finite number of states is a deviation from power law behavior at long times, in the form of an exponential tail (Millhauser et al., 1988*a*). A related model which uses the concept of defect diffusion

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has been proposed by Läuger (1988), which also, in the one-dimensional case, predicts a power law distribution with an exponent of 3/2 (Condat and Jäckle, 1989). Under specific conditions, at least four ion channel systems follow this decay law (Millhauser et al., 1988*b*). This approach has been extended to a physical model of channel gating based on reptation theory which predicts that channel gating will occur on the millisecond time scale with a power law dependency (Millhauser, 1990).

The original diffusion model does not include a ligand binding step and, therefore, cannot describe the behavior of ligand gated channels at nonsaturating concentrations of agonist (Millhauser et al., 1988*b*; see also Sansom et al., 1989). In the studies described in this communication, we show that a simple extension of the diffusion model is capable of describing the closed time behavior of the nicotinic acetylcholine receptor (AChR) from BC_3H-1 cells over a wide range of agonist concentrations.

AGONIST BINDING AND THE DIFFUSION MODEL

The diffusion model of ion channel gating can successfully predict the exponent of the approximate power law distribution of agonist-activated ion channels at high ligand concentration; however, the closed time distributions of many ion channels deviate markedly from power law behavior at nonsaturating agonist concentrations. As indicated previously (Millhauser et al., 1988*a*), the original diffusion model does not incorporate an agonist binding step and can only be valid for cases in which the agonist is saturating. At lower concentrations of agonist, the binding step becomes important to the kinetic behavior of the system, and the PDF would be expected to deviate from a simple power law. One possible extension of the diffusion model to include a ligand binding step is shown below (model 2):

$$\begin{array}{ccccccccc} & & L_1 & \overset{k_2}{\longleftrightarrow} & L_2 & \overset{k_2}{\longleftrightarrow} & L_3 & \cdots & L_N \\ & & k_5 \downarrow k_4 & & k_5 \downarrow k_4 & & k_5 \downarrow k_4 & & k_5 \downarrow k_4 \\ O & \overset{k_1}{\longleftarrow} & U_1 & \overset{k_3}{\longleftrightarrow} & U_2 & \overset{k_3}{\longleftrightarrow} & U_3 & \cdots & U_N \end{array} \qquad (2)$$

Two sets of closed states are postulated, with equal rate constants connecting the closed states within each set. Only the liganded closed states are connected to an open state, and the binding and unbinding of ligand represents the transition from one set of closed states to the other. In this scheme, O refers to the open channel state, L is the liganded form of the AChR, U is the unliganded form of the receptor, k_1 is the opening rate of the channel, k_2 is the backward and forward rate constants between the liganded states, k_3 is the forward and backward rates between the unliganded states, and k_4 and k_5 are the rates for the binding and unbinding of ligand (k_4 contains the ligand concentration and is a pseudofirst order constant). These five rate constants become the adjustable parameters (the number of L is equal to U and is set to a large value; i.e., 10 or greater). Thus, the model is an extension of the diffusion model in the sense that two one-dimensional diffusion pathways exist and the binding and unbinding of ligand switches the channel from one pathway to the other. Ligand binding places the protein in the correct diffusional configuration for channel opening. Although two agonist binding sites are known to exist on the nicotinic AChR, we have chosen a simple extension of the diffusion model as a starting point, using a minimal number of additional constraints. The model was also compared with fits to one, two, three, and four exponentials, which would correspond to arbitrary Markov models with one, two, three, and four closed states.

MATERIALS AND METHODS

BC_3H-1 cells (mouse brain tumor cell line; Schubert et al., 1974), obtained from American Type Culture Collection (Rockville, MD), were grown and maintained in Dulbecco's Modified Eagle's Medium with 10% fetal calf serum at 37°C in 10% CO_2 and passed weekly. After enzymatic dissociation, cells were plated on 35-mm dishes to be used for experiments. After 1 d, the cells are maintained in low serum medium (Olsen et al., 1983). Cells were used for recording 8–18 d after the serum change, with changes of medium every 4–5 d.

Single channels were recorded from nicotinic AChRs on BC_3H-1 cells using the cell attached recording configuration described by Hamill et al. (1981). A derivative of acetylcholine, 1,1-dimethyl-4-acetylpiperazinium (PIP; synthesized as described by Spivak et al., 1986) was used in all experiments. This agonist was used because its affinity is similar to acetylcholine but is not degraded by acetylcholinesterase and exhibits little or no channel blocking activity at the concentrations tested. A modified Ringer's solution consisting of 147 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, and 10 mM Hepes, pH 7.4, was used in both the bathing medium and inside the pipette. All data were collected at 15°C with the agonist in the pipette solution and a membrane potential of 90 mV hyperpolarized with respect to resting potential. The data were filtered at 5 kHz using an 8 pole Bessel filter (Frequency Devices) and transferred at 20 kHz to an IBM-AT computer. The data were then transferred to a VAXStation II computer for analysis. Semiautomated channel detection software with a threshold crossing algorithm and with user verification of all channels was used (software developed in the laboratory).

Predicted PDF's were calculated based on the kinetic model as described elsewhere (Millhauser and Oswald, 1988), and kinetic constants were adjusted to fit the data (dwell times were used without binning) using the Simplex algorithm (Caceci and Cacheris, 1984) with the maximum likelihood criterion used to determine convergence. Alternatively, one, two, three, and four exponential functions were fit to the data using the Simplex algorithm and the maximum likelihood criterion. Because model 2 and the exponential functions are not

nested hypotheses, we have used the Akaike Information Criterion (AIC) with bootstrap resampling (Horn and Korn, 1983) to compare these models. Forty resampled data sets at each concentration were generated on a VAXStation II computer using the VMS-supplied random number generator with reshuffling (Press et al., 1986). Maximum likelihood analysis of the resampled data was performed on a Sun 4/330 workstation (Sun Microsystems, Inc., Mountain View, CA).

RESULTS AND DISCUSSION

Closed time distributions at varying agonist concentrations

Closed dwell time distributions at four concentrations of PIP are shown in Fig. 1. Plotted on a log–log scale (McManus et al., 1987), the distribution clearly deviates from a power law at 1 and 10 μM PIP. Similar deviations from a power law distribution have been observed at low ligand concentration with ACh as the activating ligand (Sine and Steinbach, 1986; McManus et al., 1989*a*) and with other ligand gated receptors (Kerry et al., 1988; McManus et al., 1989*a*). On the other hand, the distributions begin to approximate a power law at higher concentrations, and the overall slope of the distribution is approximately −3/2 as predicted by the diffusion model (Millhauser et al., 1988*a*,*b*). As described below, these distributions can be fit adequately either by a sum of discrete exponentials or by model 2 (the expanded diffusion model).

Determination of the number of states

The expanded diffusion model (model 2) contains five rate constants and a variable number of closed states (N; using this notation, the number of closed states refers to the number of states in each manifold, giving $2N$ total closed states). For a given set of rate constants, the number of closed states dictates, in part, the time at which the PDF begins to decline exponentially. This is illustrated in Fig. 2 for a relatively low and a relatively high concentration of agonist (varied by changing k_4). As illustrated in Fig. 2 *A*, the distribution deviates markedly from a power law at the lower agonist concentration. As the number of states increases, the distribution begins to exhibit a regime with a power law decay. With the rate constants indicated in the legend, the power law regime becomes apparent at 32 states. At higher agonist concentration (Fig. 2 *B*), a power law is apparent with fewer states (observable in this example at four states), with the position of the exponential tail dependent upon the number of states (see Millhauser et al., 1988*b*). The time at which the exponential decay occurs is dependent upon not only the number of states but also the rate constants. For this reason the number of states cannot be determined by simple inspection of the data.

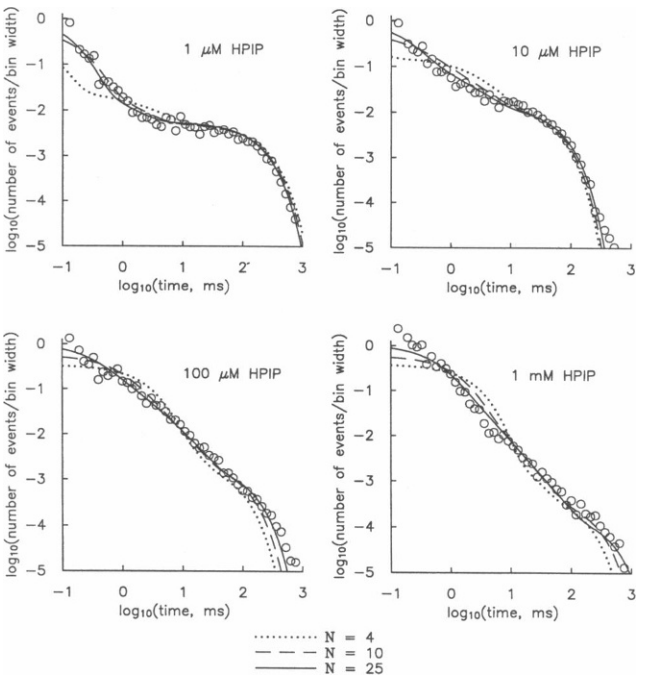

FIGURE 1 Closed time distributions for the activation of the BC_3H-1 nicotinic AChR at four agonist concentrations. The distributions are normalized based on the corrected number of events (Colquhoun and Sigworth, 1983) calculated from the open time distribution and contain the following number of events before correction: 2,800 for 1 μM, 2,700 for 10 μM, 3,788 for 100 μM, and 3,630 for 1 mM. The dotted, dashed, and solid lines through the data represent the *simultaneous* maximum likelihood fit to all four data sets for N's of 4, 10, and 25. The rate constants (ms^{-1}) for each value of N are given below. The value for k_4 (the pseudofirst order rate constant for agonist association) is given for 1 μM. The distribution for 10, 100, and 1,000 μM are generated by multiplying this constant by 10, 100, and 1,000, respectively.

N	k_1	k_2	k_3	k_4	k_5
4	0.382	0.00391	0.434	1.06	15.9
10	0.592	0.186	5.82	0.671	5.03
25	1.05	0.564	57.4	1.61	7.19

To study the number of states that provide adequate fits to the data, the four data sets shown in Fig. 1 were fit *simultaneously* to the expanded diffusion model (model 2), using the maximum likelihood criterion without binning, for N's of up to 75. As shown in Fig. 3, the maximum log likelihood increases to a plateau with an increasing number of states (note that the number of free parameters for each fit is the same regardless of the number of states). Simultaneous fits (using N's of 4, 10,

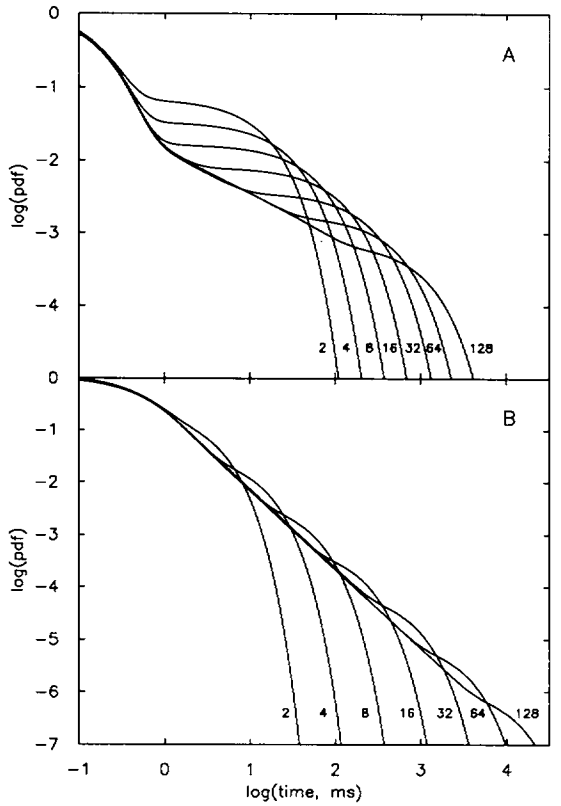

FIGURE 2 Simulated probability density functions at different values of N using the rate constants given in Fig. 1 for an N of 25. The value of N is indicated on the figure for each curve. The curves in A represent simulations at a "low concentration" of ligand, represented by k_4; whereas the curves in B are simulations at a "high concentration," corresponding to multiplying k_4 by 1,000.

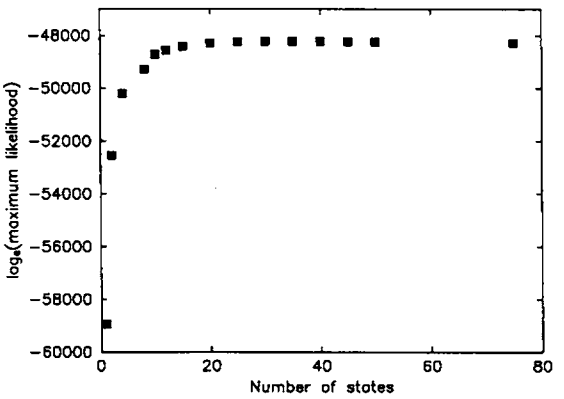

FIGURE 3 Maximum log likelihood of the simultaneous fit of the four data sets shown in Fig. 1 to model 2 (see text) at different values of N. The maximum log likelihood increases to a plateau with increasing numbers of states. The number of states tested was limited by computational demands. Increasing the number of states increases the length of the computation by $(2N)^3$ so that for models with 100 states or greater, the computation time becomes excessive.

and 25) to all four data sets are shown in Fig. 1. As suggested in Fig. 3, the overall fit to the data dramatically improves when 10 or more states are used. Marginal improvements are observed with increasing numbers of states. The actual values of the rate constants do not converge with increasing numbers of states but rather increase, particularly in the cases of k_1, k_2, and k_3, to account for the larger diffusional space. This indicates that the number of states cannot be uniquely determined from these data; however, the remarkably good correspondence between the predicted and observed results suggests that the model is adequate to describe the data.

Comparison with exponential fitting

Calculation of the PDF for the expanded diffusion model consists of generating a set of $2N$ exponential components which have time constants corresponding to the reciprocals of the eigenvalues of the kinetic matrix implied by model 2. The actual values of the time constants and their relative amplitudes are constrained by the five kinetic constants of the model. A discrete Markov model would likewise contain N eigenvalues (N in this case referring to the number of closed states); however, the number of constraints would be $2N - 1$. A considerable amount of controversy has been generated over the use of simplified kinetic schemes such as the original diffusion model and the fractal model of Liebovitch (Horn and Korn, 1989; Korn and Horn, 1989; Liebovitch, 1989; McManus et al., 1989*a*,*b*; Sansom et al., 1989). Although in a number of cases, statistical techniques have indicated that fractal and diffusion models do not describe the behavior of a number of ion channels (Korn and Horn, 1989; McManus et al., 1989*a*; Sansom et al., 1989), the comparisons were not always done at high ligand concentrations and were strictly limited to comparisons with a power law or models without the inclusion of a ligand binding step. For example, a locust muscle glutamate receptor at low glutamate concentrations is poorly described by the simple diffusion model (Sansom et al., 1989), but data collected at high ligand concentration (Kerry et al., 1988; unfortunately not included in the analysis done by Sansom et al., 1989) is much more consistent with the diffusion model (Millhauser et al., 1988*b*).

To compare the expanded diffusion model with exponential functions, the maximum likelihood for the fit to one, two, three, and four exponential relaxations was compared to model 2 with N of 25 for each *individual*

distribution. In each case, one and two exponentials were clearly inadequate to fit the data. On the other hand, three and four exponentials and model 2 were capable of reproducing the overall shape of the distribution (Fig. 4). The four exponential fit was statistically superior relative to the three exponential fit as judged by the log likelihood ratio (LLR; $p < 0.001$; Rao, 1973) at all but 1 μM HPIP. The fits to model 2 were then compared with the three and four exponential models using the Akaike Information Criterion (AIC = LLR − difference between the number of adjustable parameters). By placing the four exponential model in the numerator of the LLR (three exponentials in the case of 1 μM PIP), the AIC would indicate that the four exponential model is favored when the value is positive and model 2 would be favored if the value is negative. Unfortunately, however, the significance of this ranking is unknown. Horn (1987) has shown that bootstrap resampling of the data and recalculation of the AIC leads to a normal distribution of AIC values. From this, it is possible to assess the statistical significance of the AIC value *within the constraints of the assumptions of the AIC* (Leamer, 1983; Liebovitch and Toth, 1990).

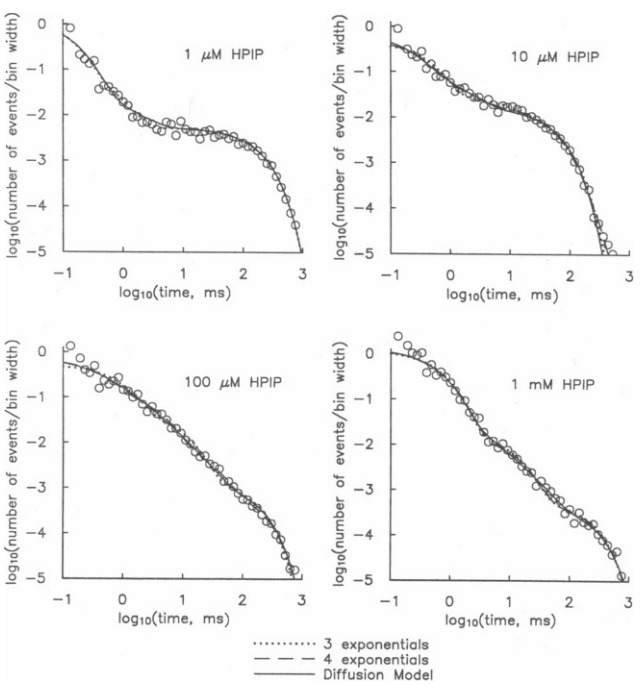

FIGURE 4 Comparison of the fit of the closed time distribution by the diffusion model with the fit to three and four exponential functions. The experimental data are the same as those presented in Fig. 1. In this case, however, the fit for both the exponentials and the diffusion model are to the *individual* distributions instead of all four distributions *simultaneously*.

Forty sets of resampled data were generated at each concentration and the AIC values were computed from the natural logarithms of the maximum likelihood values. A histogram was generated and fit to a Gaussian distribution using a least squares criterion. The results of the fit are shown in Table 1. The probabilities of obtaining AIC values of less than zero were 0.313, 0.0054, 0.342, and 0.604 for the data obtained at 1, 10, 100, and 1,000 μM, respectively. Although Fig. 4 does not show a major difference in the fit to model 2 and to four exponentials at 10 μM, the AIC criterion suggests that the four exponential model is statistically better at this concentration. This is not surprising because model 2 is a highly restrictive reaction scheme; whereas the fitting of three or four exponentials simply identifies potential exponential components, independent of a specific model. Because of the close correspondence between the data and both models, we conclude that both model 2 and discrete exponential components can describe the closed time distribution of the nicotinic AChR receptor from BC_3H-1 cells.

Conclusion

The diffusion model of ion channel gating (Millhauser et al., 1988*a*, *b*) and a similar model proposed by Läuger (1988) have been successful in predicting the behavior of a variety of ion channels under specific conditions. In the case of ligand-gated ion channels, the original models are valid only for cases in which the ligand binding step does not contribute significantly to the closed time distribution (i.e., saturating concentrations of ligand). We have shown here that a logical extension of the diffusion model to include a ligand binding step can produce behavior consistent with closed time distributions for the nicotinic AChR over a wide variation in agonist concentration.

Models which have been used previously to describe the single channel behavior of nicotinic AChRs (e.g., Colquhoun and Sakmann, 1985; Sine and Steinbach, 1986, 1987) are in most cases restricted to the manifold of states associated with receptor activation. A commonly used scheme (model 3) would predict that the closed time distribution approaches a single exponential component at high acetylcholine concentrations. This is, of course, not the case. The closed time distribution at high agonist concentration can be fit, however, with multiple exponential components (e.g., Sine and Steinbach, 1987) or by the diffusion model (Millhauser et al., 1988*a*). In a comprehensive study of the *Torpedo* nicotinic AChR, Sine et al. (1990) have used an extension of model 3 which includes open channel blockade and one or more exponential components to describe the closed time distribution. The additional exponential components

TABLE 1 **Comparison of the diffusion model with a sum of exponential components**

Conc. μM	Maximum likelihood					AIC	
	1 exponential	2 exponentials	3 exponentials	4 exponentials	Diffusion model	μ	σ
1	−16,388.2	−15,369.2	−15,351.9	−15,351.8	−15,352.9	1.48	3.04
10	−12,554.3	−11,961.3	−11,928.7	−11,917.4	−11,939.5	42.1	16.5
100	−16,897.1	−13,421.5	−13,115.6	−13,072.0	−13,074.7	4.97	12.2
1,000	−15,187.4	−8,645.6	−8,325.0	−8,318.2	−8,313.4	−3.22	12.1

Each closed time distribution in Fig. 4 was fit individually using the maximum likelihood criterion without binning to 1, 2, 3, and 4 exponentials and the diffusion model (model 2). The maximum log likelihood is shown above. For negative numbers, a smaller absolute value of the maximum log likelihood represents a better fit. These models have 1, 3, 5, 7, and 5 adjustable parameters, respectively. The mean (μ) and standard deviation (σ) for the AIC were calculated from a distribution generated from 40 resampled data sets. These values represent a comparison between the diffusion model and the 4 exponential model for 10, 100, and 1,000 μM and a comparison between the diffusion model and the 3 exponential model for 1 μM HPIP. A positive mean (μ) favors the exponential models.

are considered closures that arise from desensitization.

$$2A + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AR + A \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} A_2R \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} A_2R^*. \qquad (3)$$

A = agonist R = receptor

These results suggest that the diffusion model remains a viable alternative for the interpretation of closed time distributions of ion channels. Spectroscopic (Ansari et al., 1985) and molecular dynamic (Elber and Karplus, 1987) studies of proteins suggest that they are flexible structures comprised of many energy minima. The essential question is whether closed time distributions of ion channels are a reflection of a large number of similar energy minima or whether they are a reflection of a small number of different energy states. The diffusion model predicts that a one-dimensional diffusion process underlies the gating of the ion channel. Such a one-dimensional diffusion process could arise, for example, from a twisting motion such as that proposed for the gap junction (Unwin, 1986) or from an α-helix-screw process as has been suggested for the sodium channel (Catterall, 1988; Armstrong, 1981) and nicotinic AChR (Vogelaar and Chan, 1989). The use of polymer reptation theory has suggested that an α-helix → screw transition can occur on the time scales observed in the closed time distribution and that, in the absence of a ligand binding step, it follows a power law distribution described by the diffusion model (Millhauser, 1990). With regard to these models, one might question whether the assumption of uniform rate constants along the diffusion path (i.e., k_2 and k_3) is realistic. In a real protein, we might expect a variation of kinetic barrier heights as, for example, a helix twists in the channel interior. This aspect has been treated previously (Millhauser, 1990), and it was shown that a random distribution of barrier heights along the diffusion path may still result in a power law PDF with an exponent of 3/2. Thus, the extended diffusion model serves as a good approximation for a related model where k_2 and k_3 are randomly distributed quantities. The attractive feature of the model is the suggestion that ion channels may exhibit a common mechanism of gating that is controlled by a diffusive process. In its present form, the model accounts only for the closed time distribution under stationary conditions. It does not account for channel activation and desensitization after rapid agonist application (e.g., Dilger and Brett, 1990) or for nonstationary behavior under conditions of constant agonist application. Also, some uncertainty exists concerning the longest closed times due to the existence of variable numbers of receptors per patch. Obviously, the model will have to be expanded to account for the full complexity of channel gating (e.g., correlations between open and closed times, multiple open states, channel activation). We present here what we believe to be a useful working hypothesis for further investigation. Although we cannot rule out the use of discrete Markov models with small numbers of states, we feel that the diffusion model provides an alternative description of gating that is consistent with the structural features of ion channels.

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