KINETICS OF ACTIVATION OF ACETYLCHOLINE RECEPTORS IN A MOUSE MUSCLE CELL LINE UNDER A RANGE OF ACETYLCHOLINE CONCENTRATIONS

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ABSTRACT We studied, using the patch-clamp technique, the kinetics of single acetylcholine (ACh)-activated channels in a mouse muscle cell line. In the presence of high ACh concentrations we estimated the rate of channel isomerization into the open state (β) from the dwell time between openings. Also, we obtained estimates for β under low agonist concentrations by assuming a linear sequential model of channel activation and applying burst analysis. If the linear model is correct, then the two estimates of β should agree since β should be independent of ACh concentration. However, the estimates of β obtained under low ACh concentrations were slower than those obtained independently under high ACh concentrations. The discrepancy in the estimates of β suggests that the linear model is inadequate, but the discrepancy can be explained if open channels can close through two separate pathways. Two alternative kinetic models that can account for our data are discussed.

INTRODUCTION

Records of acetylcholine (ACh)-induced single-channel currents consist of separated groups of closely spaced openings. A group of openings is termed a "burst" and the brief closures within a burst are termed "gaps" (Nelson and Sachs, 1979; Colquhoun and Sakmann, 1981). This characteristic behavior of ACh-activated channels is observed at all concentrations of the agonist, even when the overall frequency of opening is low. An attractive interpretation of the gaps observed in frog muscle was suggested by Colquhoun and Sakmann (1981), based on a method developed by Colquhoun and Hawkes (1977). Their method of analysis can be applied to a linear sequential model of ACh-activated channels:

$$R \underset{k_{-1}}{\overset{2k_{+1}}{\rightleftharpoons}} RA \underset{2k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} RA_2 \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} RA_2^*$$

Scheme I

In this model two agonist molecules (A) are proposed to bind consecutively to a receptor (R) before an isomerization to the open state (RA_2^*) can occur. At low agonist concentrations, multiple openings can arise from a single ACh receptor when it is in a doubly liganded state (RA_2); the mean gap duration is given by $(\beta + 2k_{-2})^{-1}$ and the mean number of gaps per burst is given by $\beta/2k_{-2}$ (Colquhoun and Hawkes, 1977). Since the mean gap duration and the mean number of gaps per burst can be obtained from single-channel records it is possible, with this method, to calculate the channel opening rate, β, and the agonist dissociation rate, k_{-2}. Colquhoun and Sakmann (1981) first obtained an estimate of β and of k_{-2} for ACh-activated channels in frog muscle using this analysis. Similar burst analyses have since been used by others in various preparations (Dionne and Leibowitz, 1982; Sine and Steinbach, 1984*a*; Sakmann et al., 1983).

This analysis depends on the details of the kinetic model that is used to describe channel activation. If, for example, activation is not sequential or if channels can spend time in a blocked state, then the above analysis will not be valid (Colquhoun and Sakmann, 1981). We have tested the adequacy of the sequential model in a muscle cell line by comparing single-channel records obtained under a low concentration of ACh to records obtained under a high concentration of ACh. In particular, these experiments provided us with two independent estimates of the opening rate β. The independent estimates of the opening rate enabled us to test the adequacy of various models of channel activation.

METHODS

Cells

The cells used in these experiments were derived from the mouse muscle cell line C2 (Yaffe and Saxel, 1977). Myotubes suitable for experiments were prepared as follows (Silberstein et al., 1982): 2,000 trypsinized myoblasts were plated onto glass coverslips (12 mm diameter). The plated

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cells were allowed to proliferate in a medium composed of Dulbeccos' modified medium (DME), 0.5% chick embryo extract, and 20% fetal calf serum. When the cells reached 70% confluency (day 2), the medium was changed to one containing DME and 10% horse serum. On day 5 most of the cells fused into multinucleated myotubes. The cells were used for experiments between days 5 to 10.

Recording

Patch-clamp recordings of single ACh-activated channels were obtained in the "cell-attached" or the "inside-out" configurations (Hamill et al., 1981). Experiments were performed at room temperature in a mammalian Ringer's solution containing in millimolars: 147.0 NaCl, 5.0 KCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 6.0 glucose, and 10.0 HEPES, buffered to pH 7.3 with NaOH. ACh was added to the pipette solution, which was similar in composition to the bath solution. Membrane currents were measured with a patch-clamp amplifier (Mark IV; Physiology Department, Yale University, New Haven, CT). The signal was amplified (model 113; PARC, Burlingame, CA) and stored on an FM tape recorder (Racal Recorders, Irvine, CA, Store 4) at a recording speed of 15 in/s. The bandwidth of the entire system was ~5 kHz.

Data Analysis

The records were filtered off-line (2–3 kHz, −3 dB) with an eight-pole Bessel filter (model 902LPF; Frequency-Devices, Haverhill, MA) and digitized (50 or 100 μs per sample) at a 12 bit accuracy (model DT2782; Data-Translation Marlboro, MA) with a PDP 11/23 microcomputer. Transitions between open and closed states were detected by setting a threshold at half of the mean unitary current amplitude. Since the patches of membrane we recorded from contained more than one channel, there was an occasional (<5%) overlap of open channels. When an overlap occurred, the entire period of open time was excluded from the analyzed record. The closed time period that preceded the overlap and the closed time period that followed it were included in the record. The output of the detection procedure was an array of open times, amplitudes, and closed times. A typical record had 1,000 individual events. The condensed data files produced in this way were used to generate histograms of burst duration and closed time. The histograms had a flexible bin size such that adjacent bins were combined if the count per bin was less than five to ten events. Histograms were fitted with a multiexponential function using a nonlinear least squares method.

At a filter setting of 3 kHz many of the brief openings or closures cannot be detected. However, we can estimate the true number of events from the area under the exponent, which was fit to the appropriate component in the histogram. We defined a burst as a series of openings that are separated by closures lasting <0.5 ms. The corrected mean number of gaps per burst was obtained by finding the true number of closures belonging to the fast component in the closed time histogram and dividing it by the true number of bursts belonging to the slow component in the burst duration histogram.

The mean duration of the long lasting open state t_o was derived by the following expression: $(t_{bl} - G_b t_g)/(1 + G_b)$, where t_{bl} is the mean duration of a burst, G_b is the mean number of gaps per burst, and t_g is the mean gap duration.

We verified that the estimation of rates in the closed or open time histograms was not biased by the recording apparatus or the analysis. We produced a computer simulation of a two-state single-channel record by applying a series of exponentially distributed command voltages to the patch-clamp amplifier with a 10^9 Ω resistor across the head-stage. By adjusting the magnitude of the voltage step we obtained a signal-to-noise ratio similar to that present in an actual recording. The usual recording techniques and analysis were applied to these synthetic records. Under these conditions we obtained unbiased estimates of rates up to 15,000 s^{-1} (at a 3 kHz filter setting) provided that the low end cutoff for the fit of the histogram was set at 125 μs.

RESULTS

Channel Conductance

The data that we present here were obtained with either "cell-attached" or "inside-out" recordings (Hamill et al., 1981). The current records used for analysis were measured using patch-pipettes with added ACh. A positive voltage was applied to the pipette, thus hyperpolarizing the membrane patch. In over 150 patches examined the amplitude histograms of the ACh-induced unitary currents had a single peak, suggesting that only a single type of channel was present. The conductance of the observed channel had a typical value of 35 pS. The absolute membrane potential in the "cell-attached" configuration was not known, but we were able to estimate it from measurement of the current amplitude to be in the range of −100 to −140 mV.

Channel Kinetics under a Low Concentration of ACh

In the presence of 1.0 μM ACh or less, few channel openings overlapped and individual openings were usually separated by closures lasting longer than 100 ms. As shown in Fig. 1 *A* (*inset*), quite a few closures were very brief; their duration was in the range of 0.1–0.2 ms. The closures between openings, taken from a continuous record, were used to generate the closed-time histogram (Fig. 1 *A*), which was fitted with a multiexponential function. We found that three distinct exponents were necessary to obtain a good fit. The average rates were 8,800 ± 1,200, 700 ± 270, and 12.3 ± 8.1 s^{-1} (average ± SD). The percentage of the area represented by each of these exponents was 15 ± 8%, 6 ± 3.6%, and 79 ± 12% (average ± SD) respectively (six experiments). Because the mean gap duration was three orders of magnitude shorter than the slow component in the closed-time histogram, it is likely that the gaps separate openings of the same channel (Colquhoun and Sakmann, 1981). The separation of the components of the closed-time histogram allows us to define a burst as a series of openings that are separated by closures lasting <0.5 ms. If we assume that ACh activates channels by a linear mechanism (Scheme I), then at a low agonist concentration multiple openings can arise from a single receptor that is doubly liganded. The channel can isomerise between open and closed states only when there are two ACh molecules bound. Under these conditions the mean gap time is $(\beta + 2k_{-2})^{-1}$ and the mean number of gaps per burst is $\beta/2k_{-2}$ (Colquhoun and Hawkes, 1977), where β is the opening rate and k_{-2} is the dissociation rate from the doubly liganded shut state. We verified that the concentration of activating agonist was sufficiently low by comparing data obtained using 1.0 μM ACh with that obtained using 0.2 μM ACh. No significant differences in the proportion or the duration of the gaps within bursts were found.

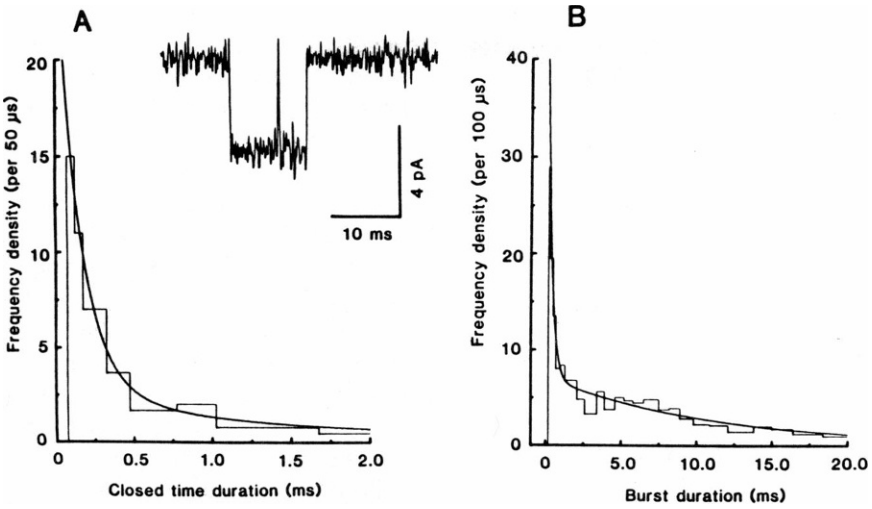


FIGURE 1 ACh-induced single channel currents recorded from multinucleated myotubes of the C_2 cell line in the presence of 1.0 μM ACh. (*A*) Closed-time histogram. The entire record of closed times lasting 0.125 μs to 2.0 s was simultaneously fitted with three exponents: 6,800 s^{-1} (7%), 1,017 s^{-1} (5%), and 8.0 s^{-1} (88%). (Inset) A burst with an apparent gap. (*B*) Burst-duration histogram. Same experiment as in *A*. Openings that were separated by a gap lasting <0.5 ms were treated as belonging to the same burst. Two exponents were fitted to the data with the following parameters: 3,580 s^{-1} (15%) and 92 s^{-1} (85%). The calculated membrane potential was −115 mV (assuming a reversal potential of 0 mV).

In principle, the total number of bursts can be found from the burst-duration histogram (Fig. 1 *B*). However, we found that the burst-duration histogram required two exponents of 7,200 ± 2,400 s^{-1} with 24 ± 14% of the total events and 139 ± 48 s^{-1} with 76 ± 14% of the events. The presence of two components in the burst-duration histogram, which has also been found by others (Colquhoun and Sakmann, 1981; Sine and Steinbach, 1984*a*), complicates the application of the theory of Colquhoun and Hawkes (1977) as stated above. It is necessary to determine the number of open states and which of these open states is associated with the gaps. Since the number of gaps per burst is small in our records, we could estimate the mean open time directly from open-time histograms. We found that the open-time histograms had two components similar to the burst-duration histograms. We assumed then that there are two open states. The gaps might be associated with either of these states or with both. If the gaps originate from a state that is directly connected to the brief open state then the mean of the open times, which are associated (preceding or following) with the gaps, should be short in duration. We found that the mean open time was 7.9 ± 2.8 ms before a gap and 7.4 ± 4.4 ms after a gap. The mean ratio of the open times before and after a gap to the slow open time was 0.85 ± 0.32 and 1.01 ± 0.41, respectively. The ratio of the open time before and after a gap to the fast open time was 58.5 and 54.1, respectively. These data indicate that the majority of the gaps are preceded with the long lasting open state and also end in opening into the long lasting open state. Consequently, we used the slow component of the burst-duration histogram to obtain the number of bursts in the calculation of the number of gaps per burst. With these assumptions, the corrected (see Methods) mean number of gaps per burst was 0.22 ± 0.1 and β and k_{-2} were estimated to be 1,587 ± 790 and 3,606 ± 250 s^{-1}, respectively. The mean slow (after correction) and fast closing rates were 170 ± 52 and 7191 ± 2444 s^{-1}, respectively.

Channel Kinetics under a High Concentration of ACh

A more direct method of estimating β is to examine channel behavior under a saturating concentration of agonist. Under these conditions, the dwell time between openings should approach $1/\beta$ because the binding steps should be faster than the isomerization, which will now be the rate limiting step. Although membrane patches contained several channels, we did not observe an increase in the frequency of overlapping channel openings with increasing ACh concentration. The increased probability of opening due to the high concentration of agonist was probably offset by receptor desensitization. We found that the records in the presence of high concentrations of ACh (10–100 μM) could be divided into periods of high burst activity of a single-channel and long lasting quiescent periods. This behavior was described by Sakmann et al. (1980) and corresponds to the process of desensitization, which is a slow but reversible process that prevents channels from being activated by agonist (Katz and Thesleff, 1957). The closed-time histograms in the presence of high concentrations of ACh could be fit with three to five exponents that ranged from 100 μs to 500 ms (Figs. 2 and 3). The increase in the number of components in the closed-time histogram probably reflects the additional shut states that are introduced by desensitization. Since desensi-

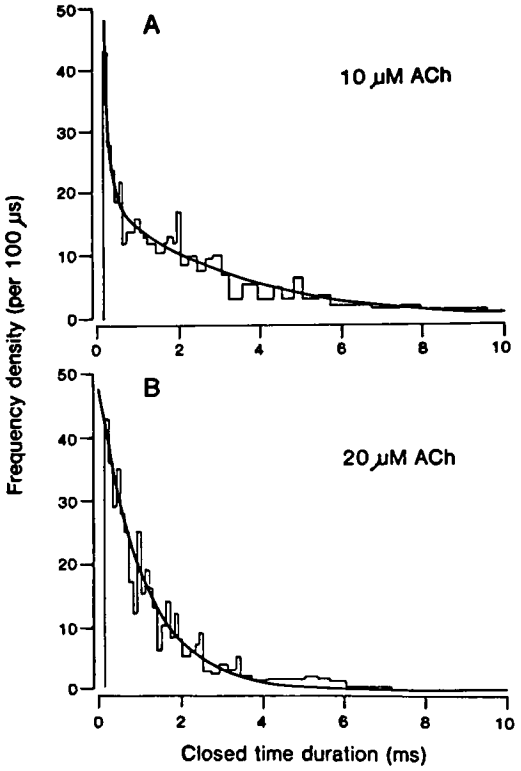


FIGURE 2 The histograms of the closed time intervals that were <10 ms in the presence of either 10 or 20 μM ACh. (*A*) 10 μM ACh. The histogram was fitted with two exponents, a fast component of 7,600 s^{-1} (32%) and a slow component of 320 s^{-1} (68%). (*B*) 20 μM ACh. The histogram was fitted with a single component of 910 s^{-1}.

tization is slow (Sine and Steinbach, 1984*a*; Fenwick et al., 1982) we can assign the fast components (<10 ms) to the activation process of individual channels. The first 10 ms of the closed time histogram could be fit with one to three exponents. At a concentration of 10 μm ACh, we found a major component (70%) of 400 s^{-1} and a minor component of 5,000 s^{-1}. At a concentration of 20 μM ACh, we found only one component of 1,000 s^{-1}. At 50 μM ACh, we found a major component (70%) of 6,000 s^{-1}, a second component (20%) of 400 s^{-1}, and a minor component of 20 s^{-1} (Figs. 2 and 3).

In an activation model of a single channel that has three shut states, the closed-time histogram should have three exponential components. As the concentration of the agonist increases, the three components will collapse into a single component with a time constant that converges to $1/\beta$ (Colquhoun and Hawkes, 1981). At an intermediate concentration of agonist, the rate and proportion of each component would depend upon all of the kinetic parameters including the rate of binding (k_{+1}, k_{+2}). However, individual components can be resolved only if they are sufficiently separated from all other components. The presence of a major component can mask the presence of a minor component of a similar rate. We found that at 10 μM ACh the rate of the major component was slow and we could resolve a minor fast component. At a concentration of 20 μM, the rate of the major component was such that no other components could be resolved. At a concentration of 50–100 μM, the rate of the major component was sufficiently fast to reveal a minor component with a slower rate. For concentrations of ACh between 10 and 100 μM there was a single major component in the closed-time histograms. The rate of this major component was 400 s^{-1} at 10 μM and it approached a saturating value of 6,700 s^{-1} at 100 μM (Fig. 3 *B*).

In the presence of an ACh concentration higher than 200 μM we observed a reduction in channel conductance. This effect has previously been described in other preparations as well, and has been ascribed to an agonist-dependent channel blockade (Sine and Steinbach, 1984*b*; Ogden and Colquhoun, 1983). If an agonist-dependent blockade had been present at a concentration of <100 μM then it would have been reflected in the closed-time

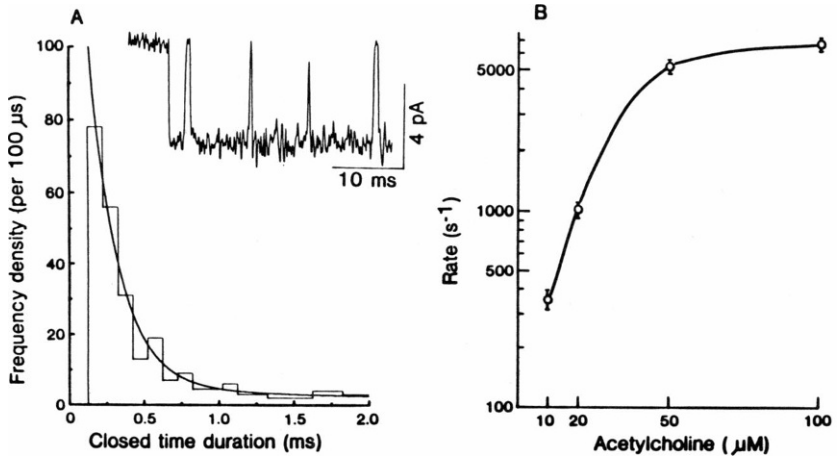


FIGURE 3 (*A*) The closed-time histogram in the presence of 50.0 μM ACh. Three exponents were used to generate a fit: 5,030 s^{-1} (67%), 310 s^{-1} (26%), and 18 s^{-1} (7%). Only the initial part of the histogram is shown. The calculated membrane potential was −132 mV. (*B*) The average major component (containing >50% of the area) of the closed-time histograms is plotted in relation to the concentration of ACh present. Bars represent SE; line is drawn by eye.

histogram. However, the major component that appeared in the presence of ACh concentrations of <100 μM is not likely to be related to blocking because we have not observed a reduction in the mean open time that would be predicted by a block mechanism. In addition, for a simple block mechanism, increasing the agonist concentration should not affect the mean duration of the blocked state, contrary to what was observed (Fig. 3 *B*). The most probable hypothesis is that the major component in the closed-time histograms is directly related to the activation process.

Given that at a concentration of 100 μM ACh the rate of the major component is close to its maximum level (Fig. 3 *B*) and assuming that the fast components in the closed-time histogram are not affected by desensitization (Sakmann et al. 1980; Ogden and Colquhoun, 1983; Sine and Steinbach, 1984*a*), we can estimate β, from the experiments at 100 μM ACh, to be at least 6,700 s^{-1} compared with 1,587 s^{-1} that was estimated at 1.0 μM ACh.

The inconsistent estimates of β provided by the linear model (Scheme I) suggest that it may not be adequate to describe the kinetics of ACh channel activation in the C2 muscle cell line. Moreover, the linear model contains only one open state compared with the two components observed in the burst-duration histogram (Fig. 1 *B*).

DISCUSSION

Colquhoun and Hawkes (1981) have shown that under particular assumptions about the mechanism of channel activation, burst analysis can yield an estimate of the channel opening rate and of the rate of agonist dissociation. However, we have found that for the C2 AChR, analysis of channel kinetics under a low concentration of agonist can be misleading.

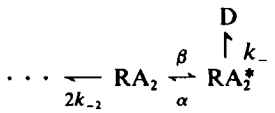
We exposed the membrane to a low concentration of agonist and analyzed the multi-exponential closed-time histograms. We obtained estimates of the mean number of gaps per burst and of the mean duration of the gaps within the burst. We then used the linear model analysis as described by Colquhoun and Hawkes (1981) and obtained an estimate for β of 1,587 s^{-1} and for k_{-2} of 3,606 s^{-1}.

The linear model of channel activation that was used by Colquhoun and Sakmann (1981) predicts that at a high concentration of agonist the closed-time histogram will be dominated by a single component with a rate equal to β (namely 1,587 s^{-1}). Indeed, in the range of 10.0–100.0 μM ACh a fast and dominant component appeared in the closed-time histograms. As we increased the concentration of the agonist the rate of that component increased and it became more prominent. It is not likely, as discussed in the Results section, that this major component is related to an agonist blocking mechanism. It appears then, that the major component is related directly to the individual activation of channels by the agonist. At a concentration of 100 μM ACh the rate of the major component approached a plateau of 6,700 s^{-1}. These observations lead us to conclude that the channel opening rate cannot be smaller than 6,700 s^{-1}, a value roughly fourfold larger than the 1,587 s^{-1} predicted by the linear model of Scheme I.

Alternative Kinetic Schemes for ACh-Activated Channels

The apparent discrepancy in the estimates of β can be explained if open channels are allowed to close in two separate pathways: to the fully occupied shut state (RA_2) and also to an additional closed state. If the rate of opening from the additional closed state is slow, then a transition to this state will terminate the burst. The effect of the additional pathway for channel closure is to reduce the number of gaps per burst under a low concentration of agonist. Therefore, the expression that predicts the opening rate β at a low concentration of agonist is no longer valid.

In Scheme II there are two pathways leading away from the open state (RA_2^*). Open channels can close to the doubly occupied shut state (RA_2) or by making a transition to the subset of states (D). We do not specify here the connections between (D) and the other kinetic states, other than the (RA_2^*) state. We will discuss two more concrete models below. If k_-, the rate of transition to (D) is comparable to the rate of closing to the (RA_2) state, then the number of gaps per burst will decrease.



Scheme II

We can estimate several of the kinetic parameters if we assume that the short gaps observed under a low concentration of agonist originate from the (RA_2) state. Under these conditions the mean gap duration should be $(\beta + 2k_{-2})^{-1}$ and we can use the estimate of β (6,700 s^{-1}) and the mean reciprocal duration the gaps (8,800 s^{-1}) to estimate k_{-2} to be 1,050 s^{-1}. At a low concentration of agonist the mean number of gaps per burst can be estimated by the following expression (see Appendix):

$$\frac{\beta}{2k_{-2}+\beta} \times \frac{\alpha}{k_- + \alpha} = \frac{n}{n+1},$$

where n is the mean number of gaps per burst. The mean rate of exit from the open state (170 s^{-1}) channel is equal to the sum of the rates leading out of that state ($\alpha + k_-$). Using the expression for the mean number of gaps per burst, we then obtained estimates for α (40.3 s^{-1}) and for k_- (129.7 s^{-1}).

The additional subset of states (D) can represent a closed state to the right of the open state. In this case it does not represent an additional path for opening channels but operates as "desensitised" state. This type of model will be discussed below. Another model that will be discussed is a

model in which the subset of states (D) represents an additional path for opening as well as for closing. That type of model will create a loop or a cycle in the kinetic model.

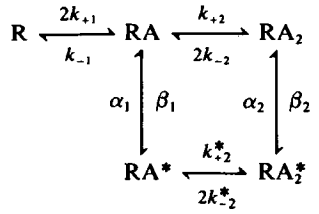
Sine and Steinbach (1984*a*) have suggested that the brief gaps under a low concentration of agonist reflect transitions into the additional closed state to the right of the open state (Scheme III), and that the intermediate component of the closed time histogram is related to the activation step.

$$R \underset{k_{-1}}{\overset{2k_{+1}}{\rightleftharpoons}} RA \underset{2k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} RA_2 \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} RA_2^* \underset{b}{\overset{f}{\rightleftharpoons}} RA_2'$$

Scheme III

Recently Sine and Steinbach (1986) estimated that at a low concentration of ACh, the opening rate is 321 s^{-1} (at 11°C) in the BC3H1 cell line. If we use the duration and proportion of intermediate gaps that we found, we calculate an opening rate of 52 s^{-1} and k_{-2} of 324 s^{-1} (at room temperature). The estimate that we obtained using this method is more than 100-fold smaller than the estimate we obtained under high concentration of agonist. In addition, this estimate is surprisingly small for a potent agonist such as ACh. We can, however, use Scheme III with a different interpretation. If we assume that the dissociation rate from the doubly occupied shut state is larger than the opening rate, then the duration of the expected gaps will be very brief and their proportion will be low. Because of the frequency limitation of the recording the component of the closed-time histogram, that corresponds to the openings from the (RA_2) state, will not be resolved. The reciprocal duration of brief gaps, 8,800 s^{-1}, is equal to b and the rate of opening from (RA_2) is 6,700 s^{-1}. From the mean number of brief gaps per burst and from the mean open time we can then estimate f to be 37.4 s^{-1} and α will be 132.6 s^{-1} so that the rate of exit from the open state will be 170 s^{-1}. Scheme 3 does not explain the fast component of the burst duration histogram. An additional open state and possibly additional closed states have to be added to this scheme before it can explain both the open-time and closed-time histograms (Sine and Steinbach, 1984, 1986).

In the cyclic model of Scheme 4, the subset of states (D) represents an additional path for opening as well as for closing.



Scheme IV

This model has been discussed in the literature from a theoretical point of view with little experimental support (Dionne et al. 1978; Colquhoun and Hawkes, 1981, 1983). In this model, as suggested by Colquhoun and Sakmann (1981), channels are allowed to open from both the singly and doubly liganded states. The conductance of the two open states is the same, but the lifetime of (RA*) is short compared with (RA_2^*), as required by the two components of the burst duration histogram (Fig. 1 *B*). The apparent discrepancy in the estimates of β can be explained if agonist molecules can unbind from the open state (RA_2^*); since (RA*) is short-lived this will lead to a transition to the closed state (RA) from which immediate opening at low concentration of agonist is not likely. The data that we obtained can be used to roughly estimate some of the kinetic parameters. From the burst duration histogram we can estimate α_1 to be 7,200 s^{-1}, assuming that the rate of binding to the singly occupied open channel is smaller than α_1, and that β_1 is likely to be much smaller than β_2. The last assumption is supported by the finding that there were fewer short-lived openings than long-lived openings. The estimates for β_2 and k_{-2} will be as in Scheme II, 6,700 and 1,050 s^{-1}, respectively. α_2 will be 40.3 s^{-1} and k_{-2}^* will be 64.8 s^{-1}.

Recently, Scheme IV was used by Colquhoun and Sakmann (1985), who estimated the rate of opening of the AChR in the frog neuromuscular junction at a low concentration of ACh to be 30,600 s^{-1} (at 10°C). This estimate is similar to an earlier estimate at a high concentration of ACh made by Ogden and Colquhoun (1983). The similarity of these estimates at different agonist concentrations is in contrast to our findings in C2. Also, the mean open time measured in the frog is much briefer than that measured in C2. If the dominant pathway of closing of the frog ACh receptor is to the doubly liganded closed state and is rapid, then the estimated value of β at both low and high concentration should be similar and the mean open time should be brief, as observed.

In Scheme IV (RA*) represents the brief open state. However, this is not supported by any direct evidence. If the brief openings derive from the singly-liganded receptor, their relative frequency should be dramatically reduced at a high concentration of agonist. Sine and Steinbach (1984*a*) found that this prediction is not met for the ACh receptor of the BC3H1 cell line. A modification to Scheme IV might be required to explain the brief open state.

Analysis of single channel records under a wide range of agonist concentrations provides greater insight into the mechanism of agonist action, and allows for more stringent tests of existing kinetic models. This approach may be complicated by the phenomena of desensitization and agonist blockade. However, in the C2 muscle cell line we found a major component in the closed time histogram that was directly related to the agonist concentration. At 100 μM ACh the rate of that component was 6,700 s^{-1}. We therefore estimated the rate of opening (β) to be close to this value. A kinetic model that allows channels to close in two separate pathways is consistent with the estimates of β at low and high concentrations of ACh.

APPENDIX

The Mean Number of Gaps Per Burst in a Cyclic Model

Since we are dealing with observed bursts only we will assume that a burst starts with the system in the long-lasting open state (see Results). A burst of openings is observed when there are transitions between the open state (RA_2^*) and the closed state (RA_2). Under the condition of a low concentration of agonist, a burst will terminate when a transition is made from (RA_2) to (RA) or when there is a transition from (RA_2^*) to (D). Let P_c be the probability to close to (RA_2), $\alpha/(\alpha + k_-)$, and P_o be the probability to open from (RA_2), $\beta/(\beta + 2k_{-2})$. It can be readily seen that the probability to have exactly i gaps in a burst $P(i)$ is

$$P(i) = (P_cP_o)^i [P_c(1 - P_o) + (1 - P_c)],$$

where $(P_cP_o)^i$ reflects i transitions between (RA_2^*) and (RA_2), $P_c(1 - P_o)$ reflects a burst termination by a dissociation from the shut state, and $(1 - P_c)$ reflects a burst termination by a dissociation from the open state. The mean number of gaps per burst is

$$n = \sum_0^\infty iP(i).$$

This is similar to a geometric distribution which converges to

$$n = P_cP_o/(1 - P_cP_o)$$

from which the final expression

$$\frac{\beta}{2k_{-2} + \beta} \times \frac{\alpha}{k_- + \alpha} = \frac{n}{n + 1}$$

can be readily derived.

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