Gating Kinetics of the Quisqualate-Sensitive Glutamate Receptor of Locust Muscle Studied Using Agonist Concentration Jumps and Computer Simulations

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ABSTRACT Outside-out patches excised from extrajunctional membrane of locust muscle were subjected to "concentration jumps" of L-glutamate, using the liquid filament switch technique, to study channel opening and closing rates, desensitization onset, and recovery from desensitization of a quisqualate-sensitive glutamate receptor (qGluR). Based on data obtained from these experimental studies, computer modeling techniques have been used in an attempt to simulate the behavior of qGluR during a concentration jump of L-glutamate. A linear model with three closed states (one unliganded, one monoliganded, and one biliganded), one open state (binding two molecules of L-glutamate), and two desensitization states (the one monoliganded, the other biliganded) leading from the unliganded closed state simulated all of the experimentally observed behavior. The results are discussed in the context of previous equilibrium studies in which desensitization was inhibited with concanavalin A and for which a ten-state model was required to simulate the behavior of qGluR.

INTRODUCTION

The first single-channel studies of extrajunctional, quisqualate-sensitive glutamate receptors (qGluRs) (Usherwood, 1981) of locust muscle were undertaken using a megaohm seal patch clamp technique (Patlak et al., 1979; Gration et al., 1981). Gating of the qGluR channel (125–150 pS) was studied at equilibrium with L-glutamate, with desensitization inhibited by concanavalin A (con A) (Mathers and Usherwood, 1976; Patlak et al., 1979). Under these conditions, open and closed dwell time probability density functions for glutamate concentrations extending over a 1000-fold range indicated that the qGluR channel has at least four open states and four closed states. In addition, correlations between successive channel dwell times suggested the presence of multiple channel isomerization pathways connecting these states (Kerry et al., 1988). The results of further equilibrium studies using a gigaohm seal recording technique suggested a ten-state Markovian model for qGluR with five open states, five closed states, and multiple isomerization pathways connecting these states (Bates et al., 1990). Parameters for this model were derived using the maximum likelihood procedure of Ball and Sansom (1989).

Openings of the qGluR channel are rarely observed under equilibrium conditions in the absence of con A because of desensitization (Gration et al., 1980). Dudel et al. (1990a) recently used the liquid filament switch technique of Franke et al. (1987) to apply "concentrations jumps" of L-glutamate to patches of locust muscle that were not treated with con A. They found that desensitization of this receptor can arise from a closed channel state without a previous channel opening. Similar behavior has been subsequently suggested for a vertebrate nicotinic acetylcholine receptor (nAChR) (Ochoa et al., 1989).

In the study reported herein, a broad spectrum of L-glutamate concentrations and pulse application regimes was used to further characterize desensitization onset and recovery from desensitization and to provide insight into the opening and closing rates of the qGluR channel. In parallel with the concentration jump experiments, computer simulation studies have been undertaken in an attempt to identify a mechanism for gating of the qGluR channel during a step change in concentration of L-glutamate. As a starting point, computer modeling techniques were used to simulate the behavior of a desensitizing version of the complex model proposed by Bates et al. (1990) when it is subjected to concentration jumps of L-glutamate. The behaviors of some simpler Markov models, incorporating a range of desensitization mechanisms, were then simulated. It is clear from these studies that there are important differences between the data obtained in equilibrium studies and those obtained in concentration jump experiments.

MATERIALS AND METHODS

Experimental

Outside-out patches of membrane were excised from adult locust (*Schistocerca gregaria*) extensor tibiae muscle fibers, and the qGluRs that they contained were studied using the liquid filament switch technique described in Franke et al. (1987) and Dudel et al. (1990a). This technique produced changes in solution at the surface of a patch that were complete within 0.2 ms.

Single channel data were recorded using a List EPC7 patch clamp amplifier (List-Electronic, Darmstadt, Germany) and stored on videotape with a Sony 701 ES PCM. The recorded data were low-pass filtered at 3 kHz and then digitized at a sampling frequency of 25 kHz. Trains of identical pulses of L-glutamate were normally applied to a patch, and the responses to these were averaged. The averaged current provided an estimate of qGluR activity during a concentration jump of L-glutamate. The duration of channel activity in response to a single pulse of L-glutamate was defined as the time from

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the first channel opening during the pulse to the last channel closure. Recovery from desensitization was investigated with two techniques. First, trains of 30–40 L-glutamate pulses of different frequencies (0.1–2 Hz) were used. The amplitude of the averaged current recorded during a train was plotted against pulse frequency. Second, a sequence of seven L-glutamate pulses was applied repeatedly to a patch. Each sequence consisted of a conditioning pulse followed by six test pulses, with the interval between the conditioning pulse and a test pulse increasing progressively, but nonlinearly, from 0.1 s to 10 s. The patch was rested for 15 s between sequences. The ratio of averaged test current amplitude to averaged conditioning current amplitude was plotted against the interval between conditioning and test pulse.

Simulations

Sansom et al. (1989) demonstrated that the gating kinetics of the locust muscle qGluR were best modeled as Markov processes with relatively small numbers of open and closed states (Horn, 1984; Colquhoun and Hawkes, 1981). Consequently, the algorithm described by Clay and DeFelice (1983) for simulating single-channel dwell times was used to simulate applications of pulses of L-glutamate to qGluR. The simulation procedure was similar to that described by Ball et al. (1989) in their studies of post-perturbation behavior of the K^+ channel of a neuroblastoma cell line (NG108-15). The transition rate from channel state i to state j was termed q_{ij}, thus allowing two transition rate matrices ($Q1$ and $Q2$) to be defined, with elements q_{ij} and diagonal elements $q_{ii} = -\sum_{i \neq j} q_{ij}$. Matrix $Q1$ was constructed using the L-glutamate concentration of the liquid filament (i.e., the expected glutamate concentration at the membrane patch in the concentration jump experiments). Matrix $Q2$ was produced using the agonist concentration of the bath saline, which was zero in this study. A termination state was selected prior to the start of a simulation. When the model entered this state the simulation was halted and the program was reset, ready for the next simulated application of L-glutamate. The termination state is thus a completely absorbing ("desensitized") state and was used as a first approximation of the desensitization process. An initial state probability vector was obtained by using the post-pulse transition rates in $Q2$ to calculate the equilibrium probabilities of the channel states in the absence of L-glutamate. This vector determined the initial state of the qGluR channel at the start of the simulation. The initial state having already been selected, $Q1$ was used to simulate the channel's behavior for the duration of the L-glutamate pulse; the time spent by the channel in its open and closed conformations was noted as a dwell time vector. At the end of the pulse of L-glutamate, the transition rates were reset to the values in matrix $Q2$, and the simulation was continued until the channel entered the termination state (Fig. 1 *A*). Simulations were also undertaken without a termination state. These enabled the behavior of the qGluR channel to be examined continuously during a series of pulses of L-glutamate.

Dwell time vectors for successive, simulated L-glutamate pulses, sampled at a frequency of 25 kHz, were combined to produce time-sampled averaged currents.

Computational

Computer simulations and analysis were performed on a Masscomp MC5500 (Concurrent Computer Corporation, Westford, MA) equipped with a floating point processor. Data analysis and modeling programs were written in Fortran 77. The NAG library was used as a source of numerical subroutines.

The fitting of multiple exponentials to the time course of each averaged current was optimized using nonlinear least-squares Gauss-Newton and simplex (NAG routines EO4FDF and E04CCF) algorithms.

RESULTS

Experimental data

qGluR channel activation and onset of desensitization

Data from 65 outside-out patches were analyzed. Data obtained from patches containing the short duration S channels described by Dudel et al. (1988) were not analyzed; this communication refers exclusively to the long duration L channels described by these authors.

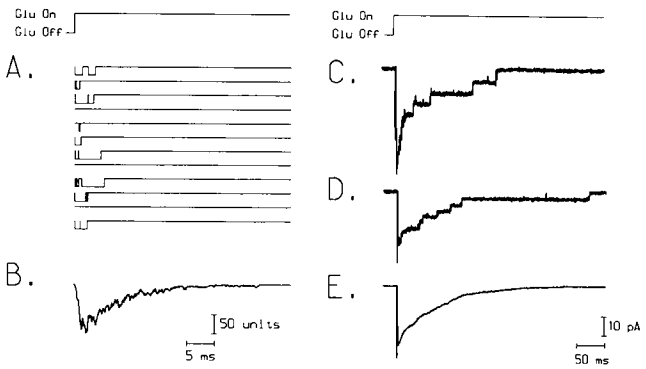

FIGURE 1 (*A*) typical single channel currents simulated by model II. Each trace was elicited by a pulse of 10^{-3} M L-glutamate. Channel openings are downward and have a unit conductance. (*B*) a typical time-sampled averaged current produced by combining 100 simulated single channel currents as described in (*A*). (*C,D*) individual responses of an outside-out patch of locust muscle membrane containing qGluR. The holding potential was −40 mV. Pulses (1 s) of 10^{-3} M L-glutamate were applied using a liquid filament switch. Responses to 35 such pulses produced the averaged current illustrated in (*E*). The averaged current, which decayed to zero (within 350 ms) before the end of the pulse (not shown), had a rise time (10–90% of maximum current) of less than 1 ms.

When outside-out patches excised from locust muscle were exposed to a brief pulse of L-glutamate, openings of qGluR channels of 115–150 pS were typically elicited within 0.5–2 ms of the beginning of the pulse (Fig. 1, *C* and *D*) (see also Dudel et al., 1988). No subconductance levels were observed. Maximal activation of the qGluR in a patch occurred within 10 ms, and this was followed by a decline in the patch current as these receptors desensitized (Fig. 1, *C–E*). Averaged currents obtained for a single patch for three concentrations (10^{-3}, 3.3×10^{-3}, and 10^{-2} M) of L-glutamate are illustrated in Fig. 2 *A*. The channel opening rate estimated from the rising phase time constant (τ_r) was 5.8×10^5 M^{-1} s^{-1} ($\pm$ 3.9×10^5 M^{-1} s^{-1} (SD), $n = 3$). Data for the this and three other patches gave a mean opening rate of 4.5×10^5 M^{-1} s^{-1} (Table 1).

For patches exposed to pulses of 10^{-3} M L-glutamate, the decays of the averaged currents were biphasic; that is, for each there was an initial fast decay followed by a later slow decay. In all cases, the decays of the averaged currents were best fitted by two exponentials. τ_f and τ_s (i.e., the time constants for the fast and slow decays, respectively) showed slight variations with time, but there was no evidence for a trend toward more rapid decays. Also, there was no indication of a time-dependent decline in the averaged current amplitude (Usherwood, 1989; Sather et al., 1992).

The amplitude of the averaged current increased when the concentration of L-glutamate was raised; for example, the amplitude of the averaged current for a train (frequency, 0.33 Hz) of pulses of 3.3×10^{-3} M L-glutamate was 5.86 $\pm$ 2.41 pA (SD; $n = 58$), whereas for 10^{-2} M L-glutamate it was 7.66 $\pm$ 7.62 pA (SD; $n = 58$) (Fig. 2 *A*). Qualitatively similar data have been obtained for an excitatory glutamate receptor of

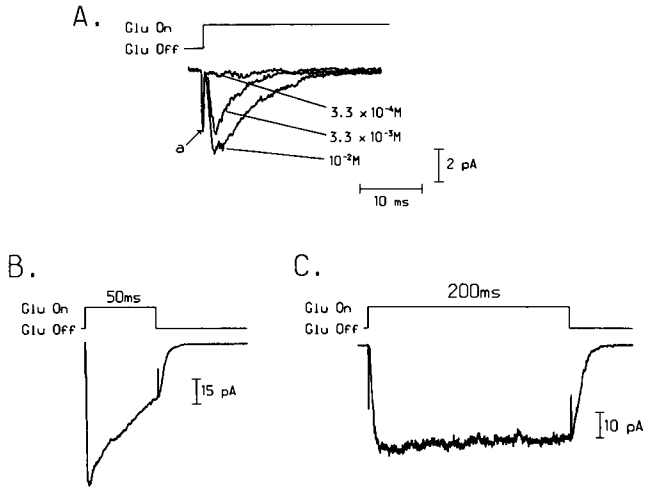

FIGURE 2 (*A*) averaged currents resulting from 50 pulses (100 ms duration) of 3.3×10^{-4} M, 3.3×10^{-3} M, and 10^{-2} M L-glutamate, respectively. The currents are superimposed to demonstrate the increase in both the amplitude of the averaged current and the duration of channel activity with increasing agonist concentration. A small pulse artefact (*a*) is present at the start of the current trace. (*B*) a train of 35 pulses (each of 50 ms duration) of 10^{-3} M L-glutamate was applied to a patch containing at least 20 qGluR. The averaged current decayed rapidly following termination of the agonist pulse. (*C*) averaged current produced by 40 pulses (each of 200 ms duration) of 10^{-3} M L-glutamate following pretreatment of a patch, containing about 9 qGluR, with 1 μM con A. Only slight desensitization of qGluR was seen during the presentation of the pulse of L-glutamate.

crayfish muscle (Dudel et al., 1990b) and a vertebrate nicotinic acetylcholine receptor (Franke et al., 1991b). There was always an increase in the duration of qGluR channel activity when the concentration of L-glutamate was raised. For example, in the patch illustrated in Fig. 2 *A* the responses resulting from pulses of 3.3×10^{-3} M L-glutamate had a mean duration of 4.6 ± 3.6 ms (SD; $n = 58$), and the averaged current decayed biexponentially with time constants of 0.4 ms (τ_f) and 3.3 ms (τ_s), whereas the responses resulting from pulses of 10^{-2} M L-glutamate had a mean duration of 8.8 ± 6.1 ms (SD; $n = 85$), and the averaged current decayed with time constants of 0.4 ms (τ_f) and 5.9 ms (τ_s).

Recovery from desensitization

Recovery of qGluR from desensitization was investigated by applying 10^{-3} M glutamate as trains of 30–40 pulses and by using trains with different pulse frequencies. Between trains there was a rest period of 1 min. The amplitude of the averaged current obtained in response to a train was 3.56 pA (± 3.83 (SD), $n = 40$) for a 2-Hz train and 10.76 pA (± 4.54 (SD), $n = 40$) for a 0.1-Hz train. When the pulse frequency in a train was reduced below 0.1 Hz, the averaged current amplitude did not increase. Therefore, it seems reasonable to assume that at 0.1 Hz the qGluR recovered completely from desensitization between pulses of L-glutamate. Recovery from desensitization was also studied by exposing patches to a repeated sequence of seven pulses of 10^{-3} M L-glutamate (Fig. 3 *A*). Each sequence consisted of a conditioning pulse of L-glutamate followed by six test pulses, with the interval between the conditioning pulse and a test pulse increasing progressively, but nonlinearly, from 0.1 s to 10 s. Each sequence was repeated approximately 15 times for a given patch. A patch was rested for 15 s between sequence presentations. The currents elicited by the conditioning pulse were averaged, as were those for each test pulse in the sequence. In Fig. 3 *B* the ratio of the averaged test current to the averaged conditioning current is plotted against the interval between the conditioning pulse and the test pulse. This plot is best fitted with two exponentials, with time constants

TABLE 1 Relationship between the time constant (τ_f) for the rising phase of the averaged current and concentration of L-glutamate for four outside-out patches of extrajunctional membrane of locust skeletal muscle

Patch number	τ_f (ms) for three concentrations of L-glutamate			Channel opening rate (M^{-1} s^{-1})
	10^{-2} M	3.3×10^{-3} M	10^{-3} M	
1	0.53	0.52	1.03	5.8×10^5
2	0.44	1.76	1.87	3.1×10^5
3	0.57	—	1.33	4.6×10^5
4	0.39	—	1.46	4.7×10^5

Mean channel opening rate = 4.5×10^5 M^{-1} s^{-1} (± 1.1×10^5 M^{-1} s^{-1} (SD), $n = 4$).

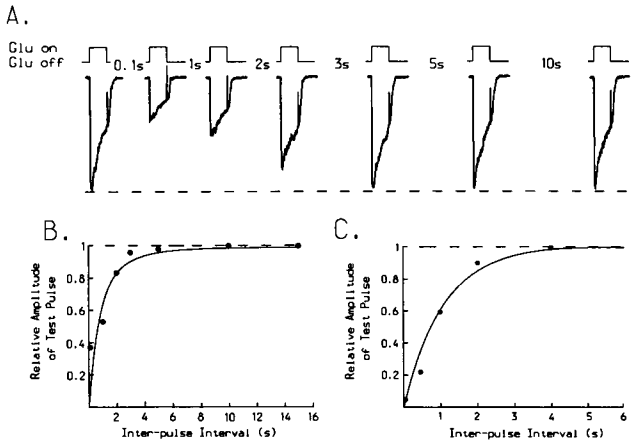

FIGURE 3 (*A*) An outside-out patch containing at least 12 qGluR was exposed to sequences of seven pulses (each pulse of 100 ms duration) of 10^{-3} M L-glutamate. The first pulse in a sequence was a conditioning pulse, and this was followed by six test pulses with intervals between the conditioning pulse and test pulses increasing progressively, but nonlinearly, from 0.1 s to 10 s. Repetition of the sequence (8–15 times) with a 15-s interval between sequences produced seven averaged currents, that is, for the conditioning pulse and for each of the six test pulses. (*B*) ratio of averaged test current amplitude to averaged conditioning current amplitude for the averaged currents shown in (*A*) plotted against the interval between conditioning and test pulses. The plot is biphasic and best fitted by two exponentials with time constants of 0.71 s and 2.21 s. (*C*) An outside-out patch was exposed to pairs of pulses (each comprising a conditioning pulse followed by test pulse) of 10^{-2} M L-glutamate. When the interval between the conditioning and test pulses was increased, the ratio of averaged test current amplitude to averaged conditioning current amplitude approached unity. When this ratio is plotted against the interval between conditioning and test pulses it is seen that recovery of qGluR from desensitization is biphasic. The recovery curve is fitted with two exponentials with time constants of 1.1 s and 1.4 s (modified after Dudel et al., 1990a).

of 0.71 and 2.21 s. The results of this study confirm the observations of Dudel et al. (1990a) that recovery from desensitization of qGluR L-channels is biphasic (Fig. 3 *C*).

Channel closure rate

In some patches a residual current was present at the termination of a pulse of L-glutamate. This current decayed rapidly as the qGluR channels closed. Its time course was best fitted with a single exponential with a time constant (τ_d) of 3–7 ms. Fig. 2 *B* shows the averaged current produced in response to a train of 50-ms pulses of 10^{-3} M L-glutamate (frequency, 0.1 Hz). The residual current had a τ_d of 5.5 ms.

Effects of concanavalin A

Pretreatment of membrane patches with 1 μM con A for approximately 1 min prior to the application of pulses of 10^{-3} M L-glutamate largely eliminated the desensitization-dependent decay of the averaged current (Fig. 2 *C*).

Simulations

A range of Markovian models was used in an attempt to simulate the behavior of the qGluR channel during the concentration jump experiments. These models fall into two basic classes. The first class contains the model described by Bates et al. (1990) that simulates the kinetics of the channel of the nondesensitizing qGluR when the receptor is at equilibrium with L-glutamate (Fig. 4, model I). This model has four identical L-glutamate binding sites. When the channel is in its open conformation the microscopic affinity of these binding sites is increased (Table 2). There are two L-glutamate binding rates, k^c_{on} and k^o_{on}, and three equilibrium parameters, the L-glutamate binding constant for the closed channel (K_B), the ratio of the L-glutamate binding constant for the open channel to that for the closed channel (α), and the closed-to-open equilibrium constant for the unliganded receptor channel complex (L). In addition, there are four channel opening rates h_i (i = 1 to 4). For the purposes of this study, the open unliganded state featured in the ten-state model proposed by Bates et al. (1990) is omitted; this state has a low equilibrium probability and does not contribute significantly to the behavior of the model. The majority of the gating models in this study belong to the second class. These are based on a kinetic scheme employed in two recent studies of vertebrate nAChR (Ogden et al., 1987; Papke et al., 1988). Model II (Fig. 4) assumes that there are two equivalent L-glutamate binding sites and that the qGluR channel opens only when two agonist molecules are bound. The model is characterized by only two equilibrium constants: K_B, the equilibrium L-glutamate binding constant, and K_O, the equilibrium constant for channel opening. The model contains two kinetic constants: k_{on}, the L-glutamate binding rate, and h, the opening rate of the channel (Table 2).

The rate constants of models II, III, and IV were adjusted by a process of trial and error until their simulated behavior matched that obtained experimentally from qGluR. The values of the rate constants required to achieve this convergence are well within the limits of physical plausibility (Table 2). It is accepted that this is not a rigorous method for fitting models to experimental data.

As a first approximation to desensitization, the termination state for the simulation was the closed unliganded state of the gating mechanism. During the application of each pulse of L-glutamate the simulation of channel activity was stopped immediately when the model returned to this state. When this approach was adopted for model I, the rate of activation of the receptor was very low and channel activity frequently continued long after the end of a L-glutamate pulse (Fig. 5 *A*). This behavior was a product of the relatively small rate constants in the model of Bates et al. (1990) (Table 2). When the rate constants in model I were increased, so as to simulate more closely the rapid qGluR activation displayed by the L-glutamate pulse data, it then ceased to simulate the full range of equilibrium behavior exhibited by qGluR in previous experimental studies (Kerry et al., 1987, 1988; Bates et al., 1990).

Model II simulated remarkably closely the behavior of qGluR seen in the experimental studies. The rate of rise of the qGluR current and the time course of the averaged current during a pulse of L-glutamate were strikingly similar to those observed experimentally (Fig. 5 *B*). However, since the simulation included a termination state, the decay of the current cannot be related to any intrinsic property of the qGluR gating mechanism, for example, desensitization. Desensitization of qGluR can be formally represented by adding one or more closed states (termed "desensitized states") to the kinetic mechanism, which have relatively long mean lifetimes and which replace the termination state.

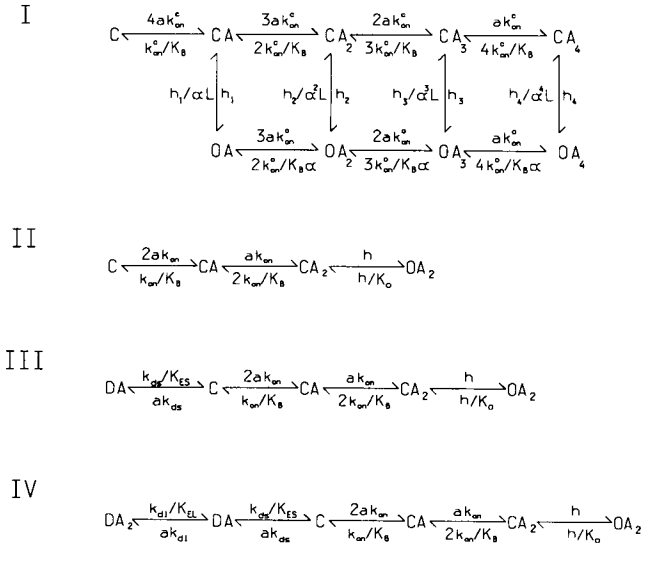

FIGURE 4 Gating models of the qGluR channel. *C* represents the closed channel conformation, *D* the desensitized closed channel conformation, *O* the open channel conformation, and *A* the agonist (L-glutamate) molecule. Rate constants are detailed in Table 2.

TABLE 2 Parameters for gating models

Model I			
$K_B = 270\ M^{-1}$	$L = 1.5 \times 10^{-4}$	$\alpha = 44$	
$k_{on}^c = 3.7\ M^{-1}\ ms^{-1}$	$k_{on}^o = 126\ M^{-1}\ ms^{-1}$		
$h_1 = 3.2 \times 10^{-2}\ ms^{-1}$	$h_2 = 0.69\ ms^{-1}$	$h_3 = 3.9\ ms^{-1}$	$h_4 = 6.4 \times 10^{-3}\ ms^{-1}$
Model II			
$K_B = 1.3 \times 10^4\ M^{-1}$	$K_O = 3$		
$k_{on} = 1 \times 10^5\ M^{-1}\ ms^{-1}$	$h = 0.9\ ms^{-1}$		
Model III			
$K_B = 1 \times 10^4\ M^{-1}$	$K_O = 0.27$	$k_{ds} = 1 \times 10^4\ M^{-1}\ ms^{-1}$	
$k_{on} = 1 \times 10^4\ M^{-1}\ ms^{-1}$	$h = 0.9\ ms^{-1}$	$K_{ES} = 5 \times 10^7\ M^{-1}$	
Model IV			
$K_B = 7692\ M^{-1}$	$K_O = 1.8$	$k_{ds} = 1 \times 10^4\ M^{-1}\ ms^{-1}$	$K_{ES} = 4.7 \times 10^7\ M^{-1}$
$k_{on} = 1 \times 10^4\ M^{-1}\ ms^{-1}$	$h = 0.9\ ms^{-1}$	$k_{dl} = 2.14\ M^{-1}\ ms^{-1}$	$K_{EL} = 21{,}435\ M^{-1}$

These were obtained by process of trial and error as described in the text.

In model III, entry into the desensitized state (DA) required the binding of a molecule of L-glutamate (Fig. 4, model III). This ensured that the model was in its closed unliganded state at the start of each L-glutamate pulse. The simulated responses of model III to a range of L-glutamate concentrations were similar to those observed in the concentration jump experiments (Fig. 5 *C*).

Two further mechanisms were considered. The first represents only a slight modification of model III in that only a single molecule of L-glutamate is needed to gate an opening of the qGluR channel. With this mechanism, changes in L-glutamate concentration had no effect on the rate of onset of desensitization. The second possibility allows desensitization from a biliganded closed state CA_2. This resulted in an increase in the rate of onset of desensitization when the concentration of L-glutamate was increased. Both of these results are clearly at odds with the experimental data.

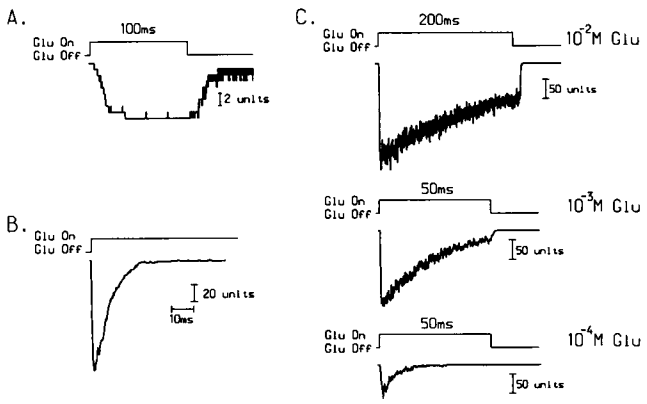

FIGURE 5 Time-sampled averaged currents produced by simulating the effects of a series of brief pulses of 10^{-3} M L-glutamate. The simulation was stopped when the model returned to the closed unliganded (*C*) state (the termination state). (*A*) averaged current simulated by model I in response to 100 pulses of L-glutamate. The averaged current has a slow rise time, and channel gating continues after removal of the agonist. (*B*) averaged current simulated by model II using 400 pulses of L-glutamate. The time course of this current closely follows that observed experimentally (Fig. 1 *E*); it has a rise time (10–90%) of 2 ms, and complete desensitization occurs within 25 ms of the start of the L-glutamate pulse. (*C*) simulated averaged currents produced by model III for a range of L-glutamate concentrations. Each averaged current results from 500 pulses (each of 100 ms duration) of L-glutamate. When the concentration of L-glutamate was increased, the amplitude of the averaged current and the duration of channel activity increased.

The requirement for a biphasic recovery of qGluR from desensitization necessitated the addition of a second desensitized state to the channel gating mechanism (Fig. 4, model IV). Entry into this state (DA_2) involves the binding of a second L-glutamate molecule to the monoliganded, desensitized qGluR. Model IV is characterized by four equilibrium constants: K_B, K_O, and the L-glutamate binding constants for the short- and long-lived desensitized states (K_{ES} and K_{EL}, respectively), and by four kinetic constants: k_{on}, the opening rate of the channel h, and k_{ds} and k_{dl}, the L-glutamate binding rates for the short- and long-lived desensitized states (Table 2).

In simulations using model IV, the decay phase of the L-glutamate-evoked current contained two exponential components with time constants of 8.3 and 33.8 ms (Fig. 6 *A*). Model IV showed the same response as model III to changes in L-glutamate concentration, that is, an increase in concentration of L-glutamate caused an increase in the length of time over which channel activity was observed and a corresponding decrease in the rate of onset of desensitization.

The relationship between the frequency of pulses in a train and the amplitude of the averaged current produced by the train is shown in Fig. 6 *B*. The amplitude of the averaged current was maximal for frequencies of 0.05 Hz or less. At 1 Hz the amplitude of the averaged current was 50% of that obtained at 0.05 Hz. The relationship can be fitted with two exponentials with time constants of 0.9 and 7.5 s.

When subjected to trains of seven pulses of L-glutamate, that is, a conditioning pulse followed by six test pulses with the interval between the conditioning pulse and the test pulse increasing progressively, but nonlinearly, from 0.1 s to 15 s, model IV exhibited behavior similar to that displayed experimentally by qGluR (cf. Figs. 3 *B* and 6 *C*). The amplitude of the averaged current for a test pulse was less than that for the conditioning pulse until the interpulse interval reached 15 s. The relationship the ratio of the averaged test pulse amplitude to the averaged conditioning pulse amplitude and the

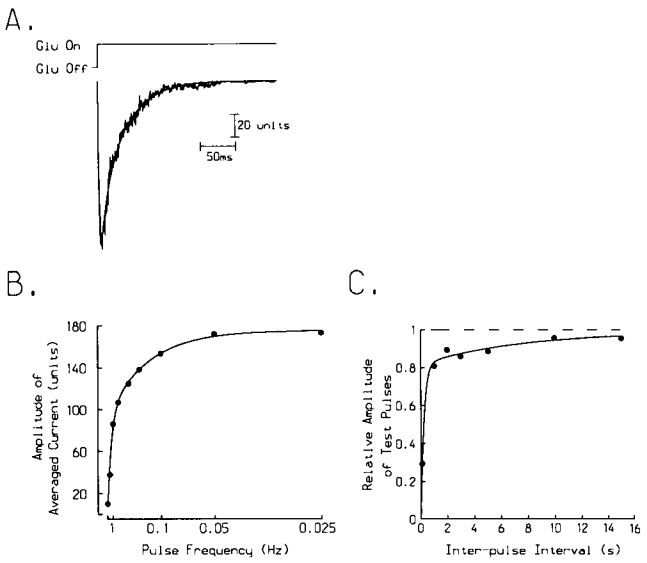

FIGURE 6 (*A*) averaged current simulated by model IV with a two-exponential fit (*smooth line*) superimposed. The averaged current is derived from 500 pulses (each of 300 ms duration) of 10^{-3} M L-glutamate. (*B*) relationship between the frequency of a train of 500 consecutive pulses (each of 200 ms duration) of 10^{-3} M L-glutamate and the amplitude of the averaged current. (*C*) simulation by model IV. The relationship between the ratio of averaged test current amplitude to averaged conditioning current amplitude and the interval between a conditioning pulse and six test pulses in a repeated sequence. Each pulse of L-glutamate was 50 ms in duration.

interval between conditioning pulse and test pulse was best fitted by two exponentials with time constants of 0.2 and 7.5 s (Fig. 6 *C*).

When the duration of the pulse of 10^{-3} M L-glutamate was reduced to 20 ms in model IV, approximately 60% of the channels were still open at the end of the pulse. These channels closed with a time constant of 2.7 ms (Fig. 7 *A*). When the two desensitized states (DA and DA_2) were removed (the rate constants for the rest of the model remained unaltered), the averaged current did not decay during the agonist pulse. The removal of DA and DA_2 had no effect on the channel closing rate (Fig. 7 *B*).

DISCUSSION

The liquid filament switch technique (Franke et al., 1987) has revealed a number of previously unobserved features of the gating mechanism of desensitizing qGluR of extrajunctional membrane of locust skeletal muscle, most notably: an unexpectedly high opening rate for the qGluR channel, the biphasic decay of the averaged current in the presence of L-glutamate, the rapid decay of the residual current following removal of the L-glutamate, and the apparently biphasic recovery of qGluR from desensitization.

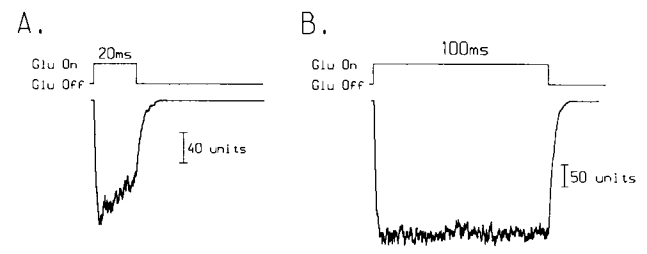

FIGURE 7 (*A*) Following a brief (20 ms) pulse of 10^{-3} M *l*-glutamate approximately 60% of the simulated qGluR channels in a patch were still open. These channels closed at a rate that was faster than the rate of desensitization. (*B*) When the two desensitized states of model IV were deleted the averaged current during a pulse of 10^{-3} M L-glutamate predictably did not decay. The closing rate of the qGluR channel was unaffected.

The qGluR channel has a channel opening or activation rate that is higher than that predicted by the equilibrium studies of Bates et al. (1990) on nondesensitizing qGluR. However, it is lower than that reported for some other ligand-gated channels (Franke et al., 1991a,b; Dudel et al., 1990b; Colquhoun et al., 1992). Model I simulates the slow, state-switching behavior observed under equilibrium conditions that is governed by the slow association and dissociation of L-glutamate. This limits the rate of activation of the qGluR channel to such an extent that the rise time (10–90%) of the averaged current produced by a simulated pulse application of L-glutamate is at least an order of magnitude greater than that obtained in the experimental studies. The activation rate for model I can be increased by altering its rate constants. However, when this is done the correlations between successive dwell times observed in the experimental equilibrium studies of Kerry et al. (1987, 1988) and Bates et al. (1990) are no longer apparent.

Previous experimental studies of qGluR and those reported herein using pulses of L-glutamate show that the decay of the averaged current resulting from desensitization of qGluR is biphasic. It is not clear whether this is the result of a single complex kinetic mechanism or it is due to the presence in patches of locust muscle of two populations of qGluR with markedly different desensitization onset kinetics. It is of interest to note that averaged currents recorded during the application of agonist concentration jumps to membrane patches containing vertebrate *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors also decay biphasically because of desensitization (Colquhoun et al., 1992; Lester and Jahr, 1992). In the case of qGluR, attempts to investigate experimentally the decay of the averaged current were confounded by the influence of L-glutamate concentration. The rate of decay of the averaged qGluR current decreased when the concentration of L-glutamate was raised. This was not entirely unexpected, however, in view of the results of our previous equilibrium studies of this receptor (e.g., Gration et al., 1981; Bates et al., 1990). This effect of agonist concentration on gating kinetics may not be unique to qGluR. Equilibrium studies of an excitatory glutamate receptor of crayfish muscle have shown an increase in channel "burst" duration when the concentration of L-glutamate was raised (Dudel and Franke, 1987), and concentration jump studies of this receptor have also revealed a decrease in the rate of decay of the averaged current following elevation of the L-glutamate concentration (Dudel et al., 1990b).

Following the demonstration by Dudel et al. (1990a) of an apparently biphasic recovery from desensitization for locust qGluR, a qualitatively similar phenomenon was reported for vertebrate hippocampal NMDA and non-NMDA receptors

(Colquhoun et al., 1992; Lester and Jahr, 1992). What accounts for the marked variability in the time course of recovery of qGluR from desensitization? Perhaps the qGluR of extrajunctional membrane of locust muscle are genotypically homogeneous but phenotypically constrained by environmental factors such as population density (Clark et al., 1979). The two types of qGluR (L and S) described by Dudel et al. (1988) may represent extreme examples of such phenotypes, although there are other equally plausible explanations to account for the sometime presence of S-channels (Usherwood, 1989).

Model IV predicts that the recovery of qGluR from desensitization is dependent on the agonist affinity (K_B). Previous macrosystem studies of qGluR have shown that an increase in the agonist affinity (e.g., when applying L-quisqualate rather than L-glutamate) results in a decreased rate of recovery from desensitization (Anis et al., 1981). This has also been shown to be the case for a rat neuronal NMDA receptor (Lester and Jahr, 1992). The effect of agonist affinity on the recovery of qGluR from desensitization clearly has important implications for the modeling of this receptor, and studies are currently being undertaken to characterize this phenomenon at the single-channel level.

Model II simulated the averaged current of patches containing qGluR with a time course close to that recorded in the L-glutamate pulse experiments, but it failed fully to simulate the experimentally observed desensitization properties of this receptor. However, model IV, which evolved from model II, simulated all of the experimentally observed behavior of qGluR. It is worth noting that a simple linear model of type II with the addition of two desensitized states accessible via a closed monoliganded state would be expected to behave in a manner very similar to that of model IV. A methodology that allows a rigorous testing of the fit of kinetic mechanisms such as model IV to the experimental data is currently being developed with the aim of differentiating between such models.

Why are greatly different models required to simulate the agonist pulse data of desensitizing locust muscle qGluR on the one hand and the equilibrium data of nondesensitizing locust muscle qGluR on the other? The open time probability density functions obtained in the equilibrium studies of Bates et al. (1990) predict the presence of a minimum of four open states. It is possible that when con A blocks the desensitization of qGluR, L-glutamate may still bind to the two postulated desensitization sites. If the filling of these sites gates the qGluR channel, this would increase the number of open states of qGluR. We are currently comparing the behavior of nondesensitizing qGluR under equilibrium and nonequilibrium conditions in an attempt to test this hypothesis. Unfortunately, technical constraints have so far prevented us from studying the effects of L-glutamate concentration on τ_d. This is a pity, because the model of Bates et al. (1990) predicts a complex relationship between τ_d and L-glutamate concentration, whereas in model IV τ_d is independent of agonist concentration.

A number of authors have proposed channel gating models of a nature similar that of model III. For example, the cyclic model for the desensitization of embryonic mouse muscle nAChR proposed by Franke et al. (1992) has one desensitized state entered from the closed unliganded state and a second desensitized state connected to the channel open state. A more complicated cyclic model has been proposed for crayfish muscle excitatory GluR by Dudel et al. (1992) in which desensitization states are entered from unliganded, monoliganded, and biliganded closed states. Clements and Westbrook (1991) have proposed a mechanism to explain the activation kinetics of a vertebrate NMDA receptor that features desensitization from a closed but liganded state and requires the binding of two agonist molecules for channel opening. Although our simulation studies suggest that these models may be incompatible with the experimentally observed behavior of desensitizing qGluR, it is important to note their underlying similarity to model IV.

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