Desensitization and Resensitization Kinetics of Glutamate Receptor Channels from *Drosophila* Larval Muscle

M. Heckmann and J. Dudel
Physiologisches Institut der Technischen Universität, 80802 München, Germany

ABSTRACT Outside-out patches from wild-type *Drosophila* larval muscle were exposed to L-glutamate (glu) using a piezo-driven application system. Glu receptor channels opened and desensitized in response to rapid applications of 10 mM glu. Desensitization was fitted with an exponential function with a mean time constant of desensitization (τ_d) of 15 ms in response to 10 mM glu. The τ_d was concentration dependent and decreased to 6 ms (on average) with 0.7 mM glu and increased again to 12 ms (on average) in response to 0.5 mM glu. Desensitization in response to longer applications of glu was almost complete, but surprisingly, even a 1-ms pulse of 3 mM glu produced about 30% desensitization. In the presence of low glu concentrations, the response to a pulse was reduced and was about halved by preequilibration with 30 μM glu. Recovery from desensitization was not concentration dependent and was fitted with an exponential function with a mean time constant of 150 ms. During recovery the channels rarely opened. Kinetic schemes were fitted to these results, and a circular reaction scheme was found to fit the data best. An important feature of the scheme is desensitization from a lower ligated closed state. This allows substantial desensitization of synaptic receptor channels in response to quantal release of transmitter, in part without opening of the channels. Desensitization reduces the probability of the channels opening in response to a subsequent release for a period of time determined by the rate of recovery from desensitization and might serve as a form of molecular short-term memory.

INTRODUCTION

Glutamate (glu) serves as an excitatory neurotransmitter in vertebrate and invertebrate nervous systems and activates a number of different receptors. Fast postsynaptic currents after glu release at a synapse are mediated by glu receptor channels (Usherwood, 1994; Edmonds et al., 1995). Glu receptor channels can be subdivided into two main subtypes, the AMPA/KA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate) and NMDA (*N*-methyl-D-aspartic acid) receptor channels (Seeburg, 1993; Wisden and Seeburg, 1993; Hollmann and Heinemann, 1994). Postsynaptic currents mediated by AMPA/KA receptor channels rise and decay faster than those mediated by NMDA receptor channels (Jonas and Spruston, 1994), and rapid desensitization within milliseconds in response to glu appears to be typical for AMPA/KA receptor channels (Franke et al., 1987; Dudel et al., 1988, 1990b; Colquhoun et al., 1992). The time course of desensitization and resensitization of AMPA/KA receptor channels might therefore influence the transfer of information at a synapse (Dudel et al., 1992; Trussell et al., 1993).

In the present study we investigated the desensitization and resensitization kinetics of glu receptor channels from *Drosophila* larval muscle. These channels are the functional equivalent of vertebrate muscle nicotinic acetylcholine receptors and are considered to be distantly related to vertebrate AMPA/KA receptor channels (Schuster et al., 1991). To gain insight into the kinetics of these channels, we applied glu rapidly in a number of protocols to outside-out patches of muscle membrane. We explored several reaction schemes in computer simulations and found a scheme that agrees with our experimental data. Based on our results, desensitization is likely to be of functional relevance at glutamatergic synapses with similar receptor channels.

MATERIALS AND METHODS

Preparation

Wild-type *Drosophila melanogaster* strain Oregon R was reared on standard *Drosophila* medium in bottles as described (Heckmann and Dudel, 1995). Third-instar larvae were dissected, pinned on a thin layer of sylgard (Jan and Jan, 1976), and moved to the stage of an upright microscope fitted with a 20× 0.4 NA objective (LD Achroplan, 20 mm working distance; Zeiss, Oberkochen, Germany). The preparation was bathed in Schneiders *Drosophila* medium (Life Technologies GmbH, Eggenstein, Germany) containing ~30 μg/ml collagenase type 1A (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) for approximately 15 min before patching and then superfused with an extracellular solution containing 135 mM NaCl, 5 mM KCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$, and 5 mM HEPES, with the pH adjusted to 7.4 with 5 N NaOH.

Electrophysiology

The measurements were made at room temperature (18–22°C). Patch pipettes were pulled from thick-walled borosilicate glass tubes with filament (outer diameter 2.0 mm, internal diameter 1.16 mm; Clark, Pangbourne, England), with a DMZ-Universal Puller (Zeitz Instruments, Augsburg, Germany). The pipettes had resistances of ~5 MΩ when filled with intracellular solution containing 150 mM K-propionate, 5 mM Na-propionate, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, and 10 mM Tris-maleate buffer, with the pH adjusted to 7.4 with 5 N NaOH. Outside-out

Received for publication 31 October 1996 and in final form 7 February 1997.

Address reprint requests to Dr. Manfred Heckmann, Physiologisches Institut der Technischen Universität München, Biedersteiner Strasse 29, D-80802 München, Germany. Tel.: 0049-89-4140-3144; Fax: 0049-89-4140-3377; E-mail: heckmann@physiol.med.tu-muenchen.de.

0006-3495/97/05/2160/10 $2.00

patches were obtained as described previously (Hamill et al., 1981). They were taken from muscles 6 and 7 of the abdominal segments of the larvae (Atwood et al., 1993) and were voltage clamped to −60 mV.

Fast application of agonists

Glu was applied with a liquid filament switch (Franke et al., 1987). A P 810.30 piezo element (Physik Instrumente, Waldbronn, Germany) held in a brass cylinder was used with a glass tube (outer diameter 0.5 mm, wall thickness 0.175 mm; Hilgenberg, Malsfeld, Germany) connected to it. The tube was perfused with extracellular solution, to which glu was added at the desired concentration. The performance of the application system was tested by perfusing the glass tube with diluted extracellular solution and switching the ionic concentration at the tip of patch pipettes. The 10–90% rise and decay times of this change in liquid junction current with open pipettes were below 100 μs.

Data collection and analysis

We used an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) and filtered the data at 5 kHz bandwidth (−3 dB) with an 8-pole low-pass Bessel filter. The data were recorded with ISO_2 software (MFK Computer, Niederhausen, Germany) and a PC 486 (sampling rate of 50 kHz, 12-bit resolution) and stored on the hard disk of a computer. Averaging and fitting of exponential curves was done off line with ISO_2.

Computer simulations

Simulations of reaction schemes and sets of rate constants were performed with a program using a variable-coefficient ordinary differential equation solver, with fixed-leading coefficient implementation (Brown et al., 1989) for solving the sets of differential equations on a workstation.

RESULTS AND DISCUSSION

Several paths to desensitization

Rapid application of glu to outside-out patches from *Drosophila* larval muscle elicits openings of glu receptor channels. Responses to 100-ms applications of 10 mM glu to a patch held at −60 mV are shown in Fig. 1. Two single responses and the average current response to 100 consecutive applications at an interval of 1 Hz are shown. In this patch up to two channels opened simultaneously. The channel openings started with a very short delay after the beginning of the applications. For some milliseconds the channels flickered rapidly between open and closed conformations and then remained closed in the continued presence of glu. The average current reached a peak value of −8 pA. The 10–90% current rise time was 200 μs, and the current decay was fitted well by a single exponential function with a time constant of desensitization (τ_d) of 10 ms.

We investigated the dependence of channel open probability and current rise time on glu concentration (Scheme 1). R represents a closed channel, A represents a glu molecule and AR, A_2R, A_3R, A_4R, A_5R, and A_5O represent a closed

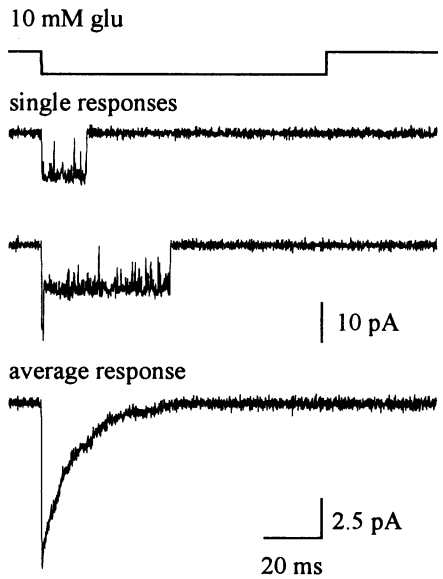
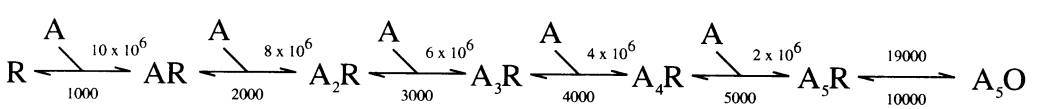


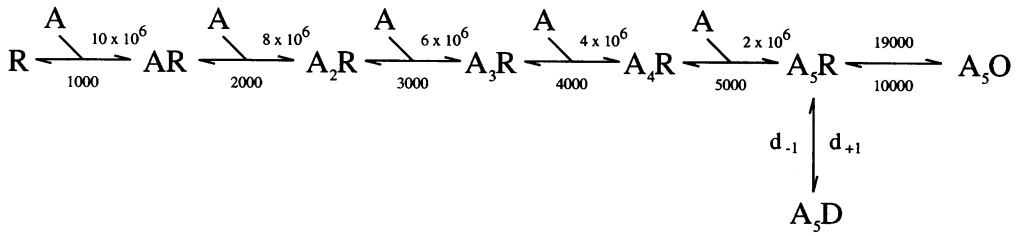
FIGURE 1 Desensitization of glutamate receptor channels on an outside-out patch. Two single responses to 100-ms pulses of 10 mM glutamate and the average current response to 100 applications are shown. The decay of the average current was fitted by a single exponential function with a time constant of 10 ms. The patch was held at −60 mV, the pulse frequency was 1 Hz, and the data were filtered to a final cutoff frequency of 5 kHz. The time course of the application is indicated at the top.

and an open channel with one, two, three, four, and five glu molecules bound. The five binding steps were necessary to reproduce the current increase proportional to the power $n = 4.4$ of the glu concentration between 0.3 and 0.5 mM glu. The units of the rate constants are s^{-1}, and the binding rate constants are $M^{-1}\ s^{-1}$ (Heckmann et al., 1996b). We increased the channel opening rate constant, β, from 15,000 s^{-1} to 19,000 s^{-1}, because the latter value reproduces our data slightly better.

In response to 10 mM glu in 23 patches we observed values for the τ_d ranging from 7 to 29 ms, with a mean value of 14.6 ± 5.4 ms. The τ_d was thus quite variable between patches but was well fitted with one exponential, indicating that individual patches contained channels with similar kinetics. Variability of the τ_d from patch to patch has been reported for channels from a number of preparations (Dudel et al., 1990a,c; Franke et al., 1993; von Beckerath et al., 1995; Heckmann et al., 1996a) and might be explained by differences in subunit combinations (Seeburg, 1993; Lomeli et al., 1994) or reversible modifications like phosphorylation or dephosphorylation of the channels (Huganir and Greengard, 1990). A reversible mechanism seems more likely in our case, because we observed that the τ_d increased or decreased occasionally during experiments (data not shown).



SCHEME 1



SCHEME 2

To allow for desensitization of the channels, Scheme 1 must be modified. We assumed desensitization to be a conformational change, like channel opening or closing. Desensitization might, for example, occur from the open state or the fully ligated closed state (Scheme 2). The two mechanisms are almost equivalent, because the rate constant of desensitization d_{+1} will have to be orders of magnitudes lower than β and α, the channel opening and closing rate constants, to reproduce our data. In either case the channel must approach the open state to desensitize. Decreasing the probability that the channels will open should decrease their probability of desensitizing. One expects, therefore, according to Scheme 2 (or a scheme with desensitization starting from A_5O), an increase in the τ_d of the average current in response to lower glu concentrations (Franke et al., 1993).

Single responses from another patch to 100-ms pulses of 1 mM glu are shown in Fig. 2 *A*. Only one channel opened in this patch. Channel openings started with a slightly longer delay after the beginning of this glu application than in response to 10 mM glu. In addition, compared with the response to 10 mM, the last channel opening after the beginning of the application came sooner, and there appeared to be longer closed periods between groups of openings. Longer closings, resulting from periods in A_4R, before returning to A_5O, are expected with the rate constants we gave for Scheme 1. The average current of 169 single responses of this patch to 1 mM glu, shown in Fig. 2 *B*, decayed much faster (τ_d = 2.8 ms) than the response to 10 mM glu (τ_d = 13.3 ms). Fig. 2 *C* shows single responses of the same patch to 1-ms pulses of 1 mM glu. It is evident that channel openings ceased even earlier than in response to the long pulses, and the average current response to 192 short pulses, shown in Fig. 2 *D*, decayed with a time constant of 0.9 ms (0.6 ms is predicted by Scheme 1 for the current decay after removal of glu).

We measured average τ_d values of 5.8 ± 1.2 ms, n = 10, and 6.0 ± 2.4 ms, n = 8, in response to pulses of 1 and 0.7

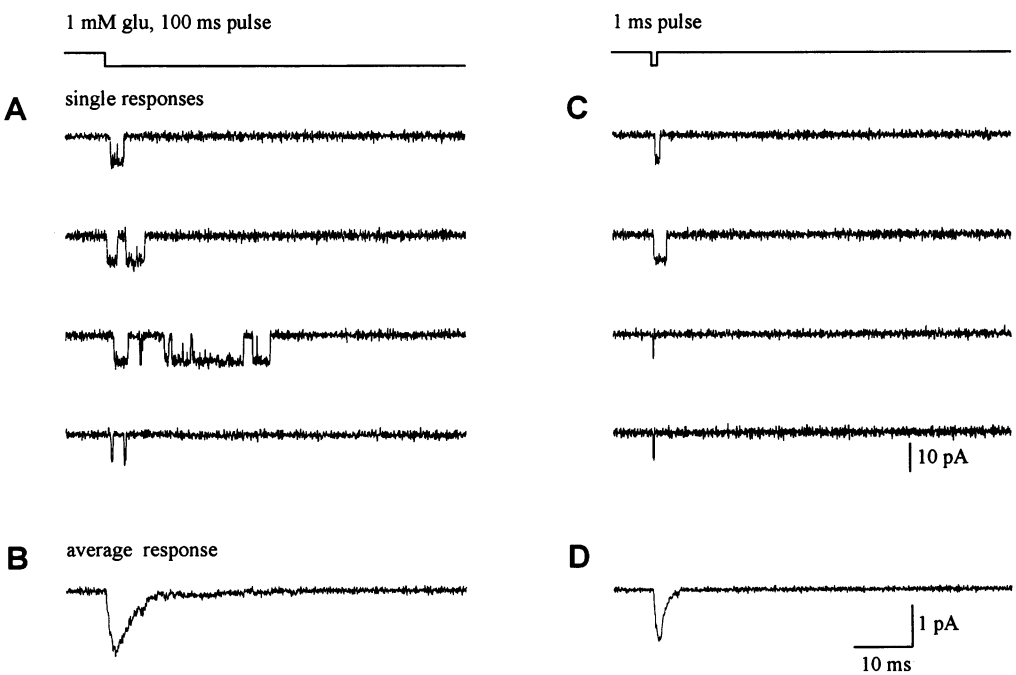


FIGURE 2 Effect of pulse duration on current decay. (*A*) Single responses of a patch containing one active glutamate receptor channel to 100-ms pulses of 1 mM glutamate. (*B*) The decay of the average response to 169 pulses was fitted by a single exponential function with a time constant of 2.8 ms. (*C*) Single responses of the same patch to 1-ms pulses of 1 mM glutamate. (*D*) The average response to 192 pulses decayed with a time constant of 0.9 ms. The holding potential, pulse frequency, and filter cutoff were as in Fig. 1.

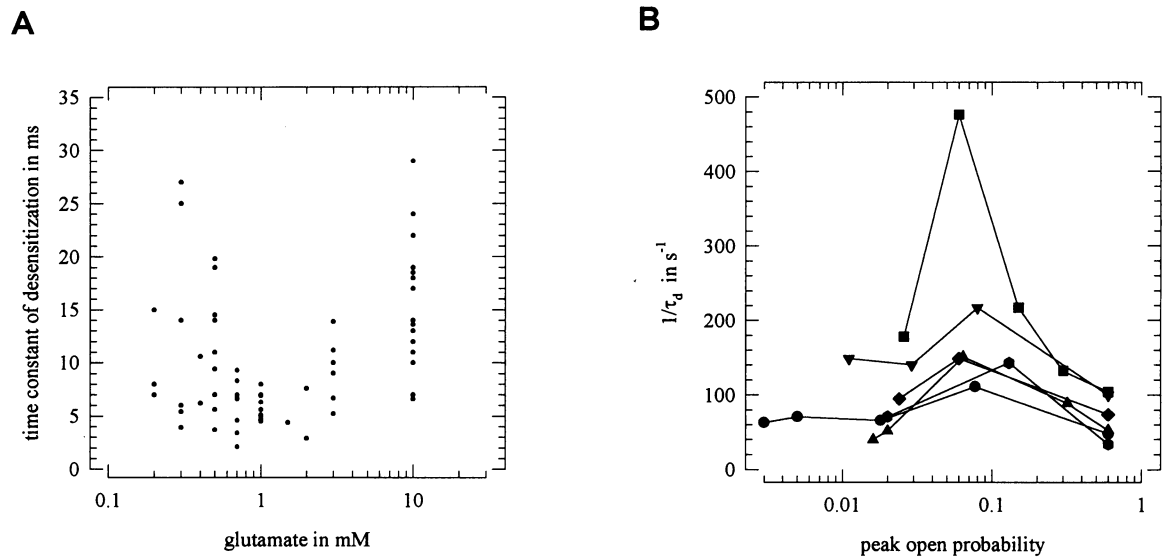


FIGURE 3 The time constant of desensitization is concentration dependent. (*A*) Plot of the time constant of desensitization (τ_d) versus glutamate concentration on a logarithmic scale. Average currents from 50–300 single responses to 100-ms pulses of 0.2–10 mM glutamate concentration were evaluated. The results are from 30 patches. (*B*) Plot of the rate of desensitization, $1/\tau_d$, versus the peak open probability of the channel (p_o). The p_o with 10 mM Glu was taken to be 0.6; each symbol represents a different patch ($n = 6$).

mM glu, respectively. In response to 0.5 mM glu concentrations, the τ_d increased again to an average value of 11.6 ± 5.7 ms, $n = 9$. We compared the τ_d values by using a rank-sum test allowing multiple comparisons (Dunn, 1964; Rosner, 1990) and obtained a $p < 0.01$ for the responses to 10 mM not to be different from those to 1 mM glu, and a $p < 0.06$ for the responses to 1 mM not to be different from those to 0.5 mM glu. The τ_d values measured

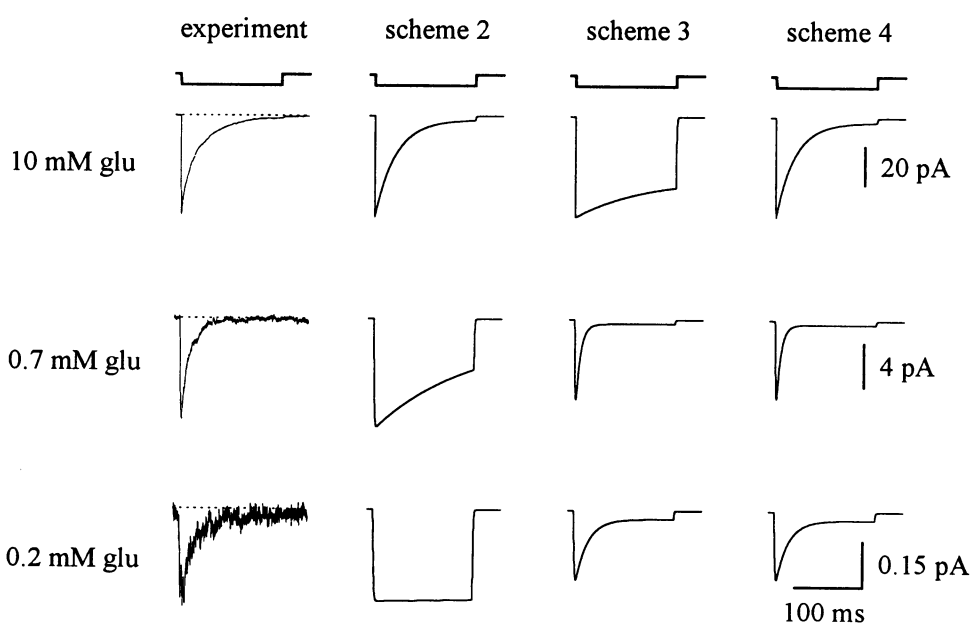


FIGURE 4 Modeling the concentration dependence of desensitization. Average current responses of a patch to 60, 95, and 391 applications of 10, 0.7, and 0.2 mM glutamate, respectively, are shown in the left column, normalized with respect to the peak current. The time constants of desensitization were 24, 10, and 14 ms with 10, 0.7, and 0.2 mM glutamate, respectively. The pulse duration was 150 ms. Dotted lines mark the baseline current level; the other experimental conditions were as in Fig. 1. The results of computer simulations of this experiment with Schemes 2, 3, and 4 are shown in the other columns. The simulated current traces are scaled with respect to the experimental data obtained in response to 10 mM glutamate. The concentration dependence of the average peak current is fitted reasonably by all three reaction schemes, but only Scheme 4 reproduces the time course of desensitization over the whole concentration range. We used the following rate constants of desensitization and resensitization for the simulations: $d_{+1} = 100\ s^{-1}$, $d_{+2} = 500\ s^{-1}$, $d_{-1} = 1.2\ s^{-1}$, and $d_{-2} = 6\ s^{-1}$.

with 30 patches in response to glu pulses of concentrations ranging from 0.2 to 10 mM versus glu concentration on a logarithmic scale are plotted in Fig. 3 *A*. The substantial scatter for the values of the τ_d mentioned above is evident at all concentrations. A minimum of the τ_d for glu concentrations at about 1 mM is nevertheless obvious.

In Fig. 3 *B* the rate of channel desensitization $1/\tau_d$ is plotted against the peak open probability (p_o) for six patches, represented by different symbols. The p_o in response to 10 mM glu was taken to be 0.6 (Heckmann et al., 1996b). Apparently the rate of desensitization reaches a maximum for a p_o around 0.1 for these channels. It can also be seen that patches with higher rates of desensitization compared with patches with lower rates of desensitization at high p_o also desensitize faster at lower p_o.

To find a mechanism of desensitization that reproduces this concentration dependence, we simulated several reaction schemes with the help of a computer and appropriate software. We confined the simulation to modifications of Scheme 1 without changing the rate constants of this scheme. Results of some of these simulations are shown in Fig. 4, together with data from another patch. In response to 10 mM glu, up to 12 channels opened simultaneously in this patch. This relatively high number of channels allowed us to measure the τ_d down to a very low p_o. Average current responses to 10, 0.7, and 0.2 mM glu are shown normalized with respect to the peak current in the left column of Fig. 4. The τ_d was 24, 10, and 14 ms in response to 10, 0.7, and 0.2 mM glu, respectively, and desensitization was thus compared to the experiments shown in Figs. 1 and 2, relatively slow in this patch.

The simulations were scaled with respect to the response to 10 mM glu, taking into account the single-channel current amplitude and the number of channels assumed to be active in this patch. All three schemes reproduced the concentration dependence of the peak current response to 0.7 and 0.2 mM glu closely. Scheme 2, with $d_{+1} = 100\ s^{-1}$ and $d_{-1} = 1.2\ s^{-1}$, reproduces the time course of desensitization in response to 10 mM glu. However, the time course of desensitization in response to the low glu concentrations was not accurately reproduced. This could be improved by increasing d_{+1}, but then desensitization with 10 mM glu was too fast.

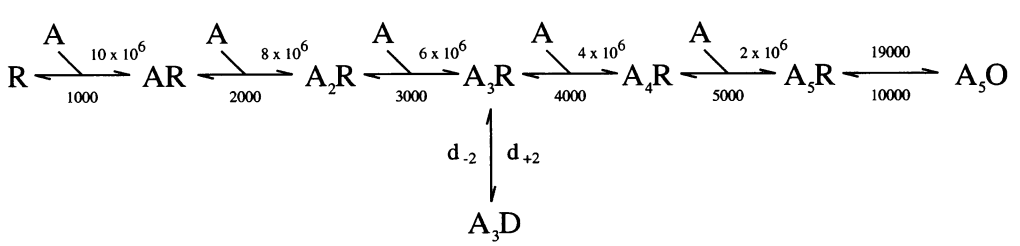
Scheme 3 is a modification of Scheme 2. It allows desensitization from a lower ligated state, A_3R. With the rate constants $d_{+2} = 500\ s^{-1}$ and $d_{-2} = 6\ s^{-1}$, it reproduces the time course of desensitization in response to pulses of low glu concentration. However, like Scheme 2, Scheme 3 cannot reproduce the data over the whole concentration range. Scheme 4 is a combination of Scheme 2 and Scheme 3, and allows desensitization from A_3R and A_5R with the rate constants given above. It accurately reproduces the desensitization kinetics in response to all three glu concentrations.

Mechanisms with desensitization from a lower ligated channel state and the fully ligated open or closed state have also been proposed for completely desensitizing channels from crayfish muscle (Dudel et al., 1993) and vertebrate AMPA/KA receptor channels from a number of preparations (Jonas et al., 1993; Raman and Trussel, 1995; Heckmann et al., 1996a). Another mechanism with desensitization starting from the unligated closed state and involving two glu bindings has been proposed for glu receptor channels from locust muscle (Standley et al., 1993). Because it does not allow both a rapid desensitization and a high peak open probability, this mechanism cannot reproduce our data.

Recovery from desensitization

According to Scheme 4, recovery from A_5D proceeds with the rate constant d_{-1} via A_5R. Once in A_5R, the probability of opening is about 0.74 (1–5,000/19,000) and is determined predominantly by the ratio of the rate of glu dissociation from A_5R (5,000 s^{-1}) and the channel opening rate constant (19,000 s^{-1}). In response to 200-ms applications of 10 mM glu, up to 20 channels opened in the patch from which current traces are shown in Fig. 5. In 5 of 100 pulses, channel openings started after the end of the applications. Two current traces with reopenings are shown, and the openings are shown again at increased amplification and on an increased time scale in the insets. The insets show brief bursts of single-channel openings as normally seen during recordings with low glu concentrations. Taking into account the number of channels (at least 20 in this patch), the 100 pulses, and the probability that 0.74 will open in A_5R, according to Scheme 4, 1480 reopenings are expected in this experiment. Because recovery via A_5R is much rarer than predicted, this result is in conflict with Scheme 4.

According to Scheme 4 with rate constants as determined above, the time course of recovery from desensitization is slower after a pulse of high glu concentration than after a



SCHEME 3

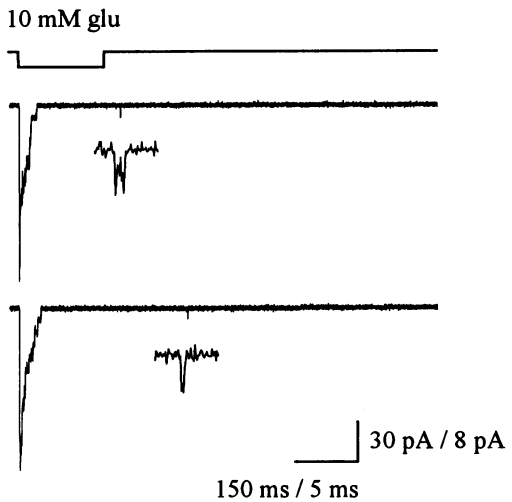


FIGURE 5 Channel openings during recovery from desensitization are rare. In response to 200-ms applications of 10 mM glutamate, up to 20 channels opened simultaneously in this patch. After 5 out of 100 pulses, we observed brief openings of channels after the end of the application. Two traces with channel openings during recovery from desensitization are shown. These "reopenings" are shown again at higher amplification and on an increased time scale in the insets. The filter cutoff frequency in this experiment was 2 kHz; the other experimental conditions were as in Fig. 1.

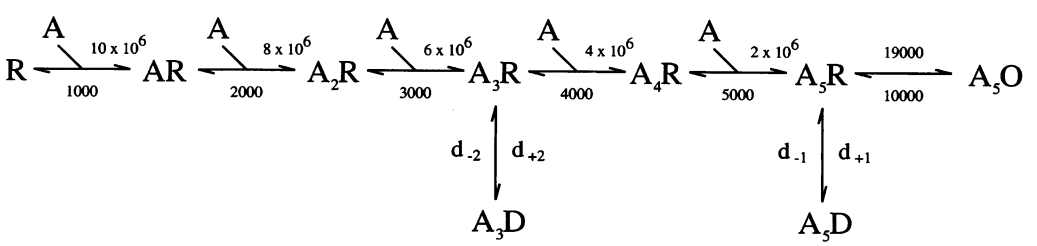
pulse of low glu concentration. The reason is that the channels should desensitize predominantly to A_5D and A_3D during the high and low concentration pulses, respectively. The rate of recovery from A_5D has to be lower than that from A_3D to reproduce the desensitzation in response to a long pulse. For a reasonable fit of current traces like the ones shown in Figs. 1, 2, and 4, values of about 1 s^{-1} and 6 s^{-1} are necessary for d_{-1} and d_{-2}.

We investigated the time course of recovery from desensitization in response to 100-ms double pulses of 10 and 0.7 mM glu. Average current responses from one patch in response to pulses of 10 mM glu are shown in Fig. 6 *A*. With only 30 ms between the first and second pulses, the response to the second pulse was markedly reduced because of desensitization of the channels. After 1 s the response to the second pulse reached the amplitude of the first response. Results from four such experiments with pulses of 10 and 0.7 mM glu are shown as mean values ± standard deviation in Fig. 6, *B* and *C*. Recovery was fitted with a single exponential function with a time constant of about 150 ms in response to pulses of the two concentrations. The data are shown together with results from simulations of these experiments with Scheme 4 and Scheme 5, which will be introduced below. Recovery from desensitization after the 0.7 mM glu pulses is well reproduced by Scheme 4, but this mechanism does not reproduce the recovery after the 10 mM glu pulses.

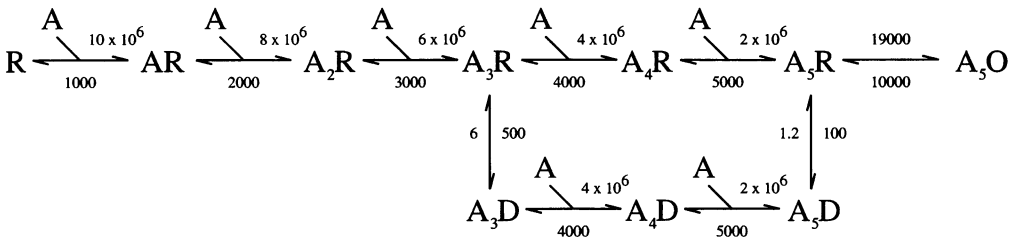
A circular reaction scheme

To overcome the two deficits of Scheme 4, the excessive number of reopenings, and the too slow recovery from A_5D, an alternative route for recovery from A_5D must be provided. A simple solution is to introduce an additional desensitized state A_4D (Scheme 5) that connects the states A_3D and A_5D in a circular reaction scheme (Katz and Thesleff, 1957; Cachelin and Colquhoun, 1989; Franke et al., 1993; Heckmann et al., 1996a). All we know about the rate constants for the additional binding and dissociation steps is that they should reduce the number of reopenings and speed up the time course of recovery from A_5D. For a circular interconversion the principle of microscopic reversibility requires, in addition, that under equilibrium conditions, the transition frequencies (the product of the rate constants) be the same in both directions (Läuger, 1995). Microscopic reversibility can be achieved with the rate constants determined for Scheme 4 by simply assuming that the binding and dissociation rate constants of the desensitized states are the same as those of the respective *R* states. With these rate constants Scheme 5 reproduced the time course of recovery from desensitization (Fig. 6, *A* and *B*) and allowed recovery from desensitization with fewer reopenings.

Some glu receptor channels are desensitized by transmitter concentrations much lower than those needed to open the channels (Dudel et al., 1990b; Colquhoun et al., 1992; Heckmann et al., 1996a). To test for this effect we added glu in low concentrations to the solution superfusing the patches between applications and recorded the responses to 10 mM glu pulses. The average response to 10 mM glu was reduced to half-amplitude by about 30 μM glu (data not shown). In the left column of Fig. 7 are shown the average current responses from one experiment recorded without and during superfusion of the patch with 0.1 mM glu, which reduced the response to about one-quarter the control value. A simulation of this experiment with Scheme 5 reproduced this result and is shown in the next column. Scheme 3



SCHEME 4



SCHEME 5

produced a similar extent of desensitization in this protocol, as shown in the right column; even so, it did not allow additional glu binding to the desensitized state. The slowly rising component of the average current is due to recovery from A_3D during the response to the 10 mM test pulse.

Quantal synaptic currents and receptor channel desensitization

Even after transmitter pulses much shorter than the time constant of desensitization, desensitization of some glu receptor channels can be substantial (Colquhoun et al., 1992; Hestrin, 1992; Raman and Trussel, 1995). To examine this we measured the desensitization after 1-ms pulses of glu. The average current response to two 1-ms pulses of 3 mM glu with an interval of 10 ms is shown in Fig. 8. The response to the second pulse reached about 70% of the amplitude of the average current response to the first pulse. A simulation of this experiment with Scheme 5, shown below the experimental data, reproduced the experiment fairly accurately.

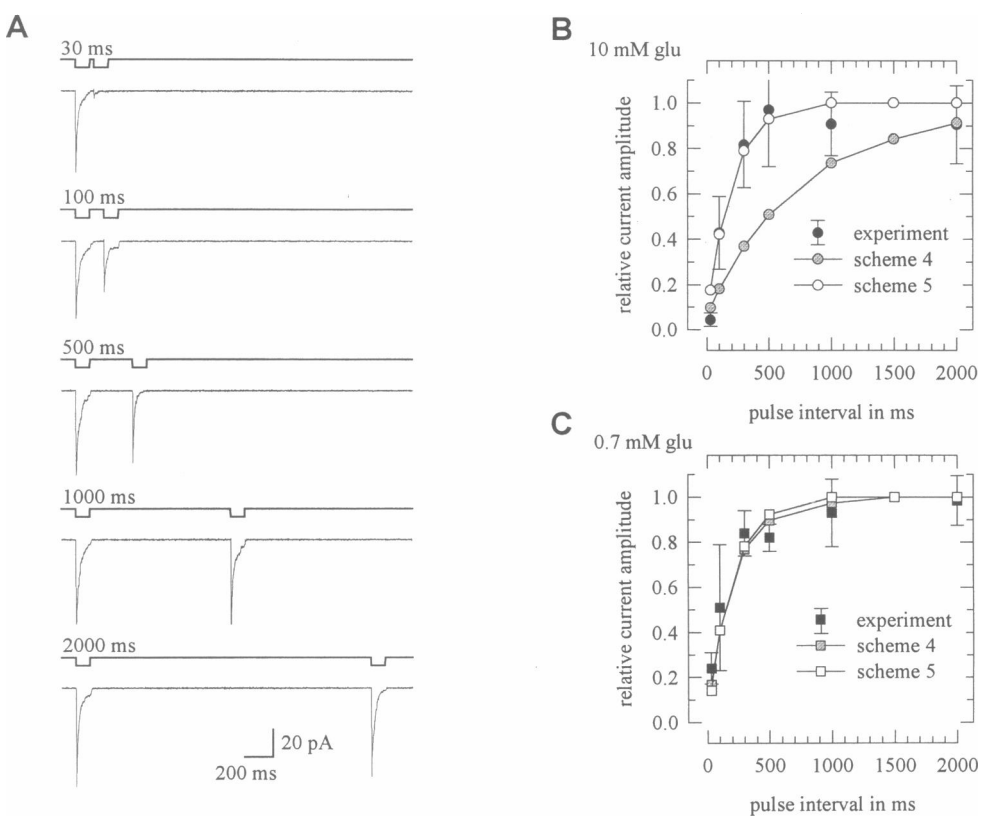


FIGURE 6 Recovery from desensitization is not concentration dependent. (*A*) A control pulse (100-ms duration and 10 mM glutamate concentration) that desensitized the channels was followed by a test pulse with a variable interval. For each interval 10 single responses were averaged. The interval between the control pulses was 5 s. The amplitude of the response to the test pulse divided by the amplitude of the response to the control pulse indicates the extent of desensitization at the respective interval. (*B*) Plot of the time interval between control and test pulse versus the relative current amplitude. The responses from four patches to 10 mM glutamate are shown as mean values ± standard deviation. The results of computer simulations of this experiment with Schemes 4 and 5 are shown together with the experimental data. The rate constants given in the legend of Fig. 4 and in Scheme 5 were used for the simulations. Scheme 5 reproduces the time course of recovery from desensitization, whereas Scheme 4 does not fit the experimental data. (*C*) Responses of the same patches to pulses of 0.7 mM glutamate are shown as mean values ± standard deviation together with the results from computer simulations of Schemes 4 and 5. Both schemes reproduce the experimental data at this concentration.

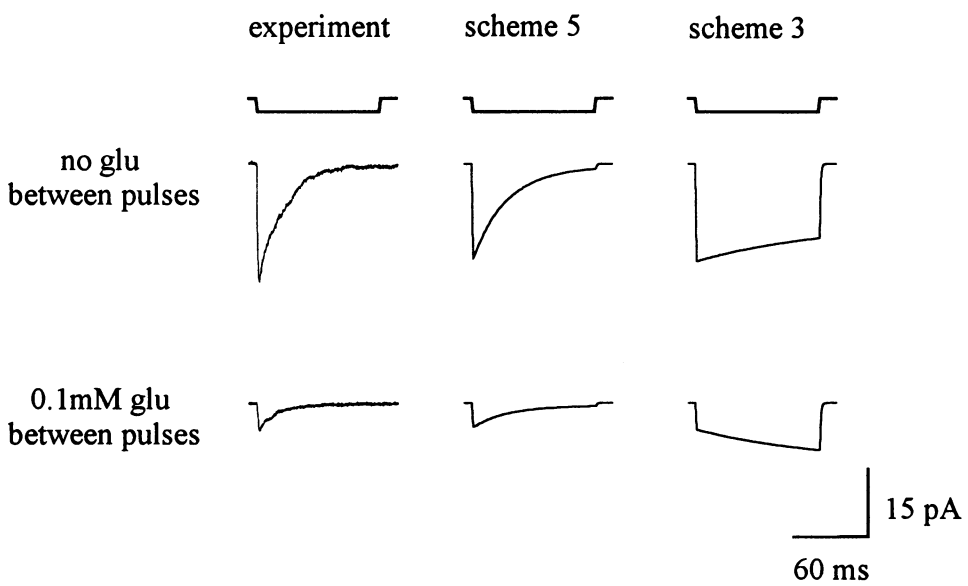


FIGURE 7 Low glutamate concentrations desensitize the channels. Average current responses of a patch to applications of 10 mM glutamate are shown in the left column. The lower trace was recorded while 100 μM glutamate superfused the patch between the pulses, and the upper trace was recorded without such a predesensitization. The other experimental conditions were as in Fig. 1. The results of computer simulations of this experiment with Schemes 5 and 3 are shown in the other columns. The simulated current traces are scaled with respect to the experimental data obtained without predesensitization.

Why do the channels desensitize so much in response to the brief pulse? A closer look at Scheme 5 helps to understand this. The time course of the probabilities of five of the nine states of Scheme 5 in response to a 1-ms pulse of 3 mM glu are plotted in Fig. 9 *A*. Before the pulse, the channel is in the unligated closed state (R). The probability of being in this state decreases rapidly after the beginning of the pulse, and the probabilities of the triple ligated closed state (A_3R) and then the open state (A_5O) increase. The probabilities of the desensitized states A_3D, A_4D, and A_5D remain low during the pulse. After the pulse, as the probability of the state A_3R increases again, the probability of the triple ligated desensitized state (A_3D) rises. Most of the desensitization therefore occurs after the pulse during dissociation of glu and results from desensitization from a lower ligated state combined with sufficiently slow dissociation of glu from the channels. In response to a 1-ms pulse of a lower concentration (for example, 0.5 mM glu, as illustrated in Fig. 9 *B*), the open state reaches a maximum probability of only 2%; however, the probability of A_3D reaches 10%.

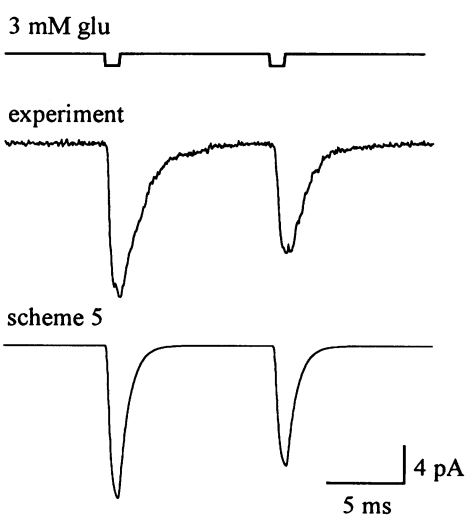


FIGURE 8 Desensitization by brief pulses of glutamate. Two 1-ms pulses of 3 mM Glu were applied to a patch at an interval of 10 ms. The current trace is the average of 100 single responses; the other experimental conditions were as in Fig. 1. The result of a computer simulation of this experiment with Scheme 5 is shown scaled with respect to the peak current value of the response to the first pulse in the experimental average.

What is the advantage of this kinetic mechanism? The release of glu might be viewed as serving two roles. The first is to create a fast-rising excitatory postsynaptic current of sufficient size and duration to transmit excitation. The concentration of glu in the synaptic cleft probably reaches millimolar concentrations within less than one-tenth of a millisecond within about 100 nm of the point of release (Clements, 1996). According to our kinetic mechanism, receptor channels within this area will open with about 50% probability. Farther from the center of release, lower peak glu concentrations are reached in the synaptic cleft. Because five glu must bind to open the channels, whereas three suffice to desensitize them, the probability of the channels desensitizing falls more slowly than the probability of opening with increasing distance from the center of glu release. Therefore it might actually be that at a synapse more channels are desensitized than opened by glu, depending on the structural relation between the presynaptic release site and postsynaptic receptor channel localization.

A testable hypothesis

We attempted to find a reaction scheme that allows us to reproduce the data with a minimum number of states and rate constants. Rate constants might have to be changed and states and pathways added as additional information becomes available. Scheme 5 is intended to serve as a working hypothesis, a summary of previous studies, and a guide for further investigations. The five glu binding sites probably

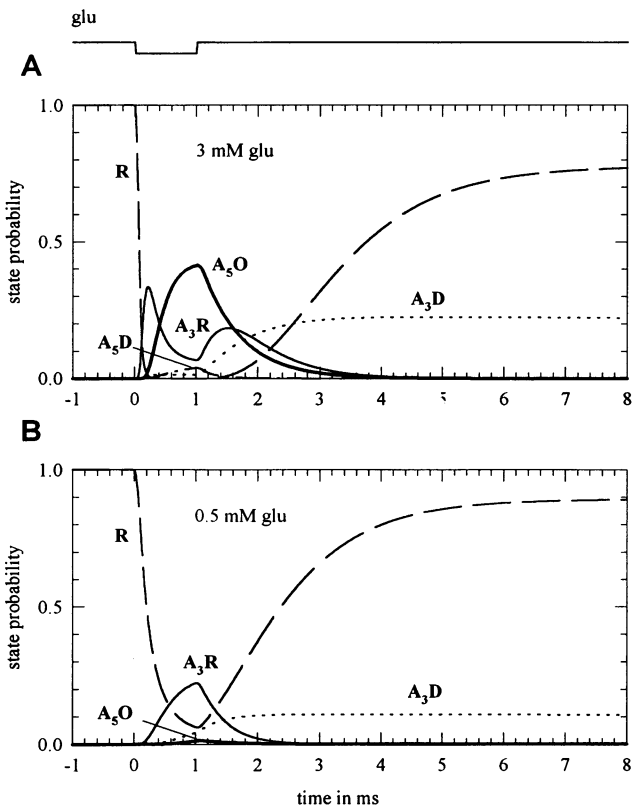


FIGURE 9 Desensitization of the channels by brief pulses. The results of computer simulations of the time course of the probabilities of the states R, A_3R, A_5O, A_5D, and A_3D in response to a 1-ms pulse of 3 mM glutamate (*A*) and of the states R, A_3R, A_5O, and A_3D in response to 0.5 mM glutamate (*B*) with Scheme 5 are shown. Note the extent of desensitization after the pulses (A_3D).

reside in different subunits of the channel (Sutcliffe et al., 1996). The sububits each have the same rate constants of binding and unbinding of glu, but only with three and five glu bound is the channel subject to appreciable desensitization. This may indicate that the channel is a heteropentamer with three nondesensitizing and two desensitizing subunits and might be supported by investigations of the molecular structure of the channel. However, even a homopentamer could change its ability to desensitize with the number of bound glu molecules.

We thank Dr. J. Eilers and Dr. M. Larkum for comments on the manuscript.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 391).

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