Kinetics of Homomeric GluR6 Glutamate Receptor Channels

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ABSTRACT We studied the kinetics of the unedited version of rat GluR6 glutamate (glu) receptor channels, GluR6Q, in outside-out patches using a system for submillisecond solution exchange. Half-maximum activation of the channels was reached with ~0.5 mM glu. The maximum slope of the double-logarithmic plot of the peak current versus glu was ~1.3, indicating that at least two binding steps are necessary to open the channels. Currents in response to a pulse of 10 mM glu had a short rise time (10–90% of peak current) of ~220 μs at ~20°C. The rise time increased with falling glu concentration, reaching ~6.0 ms with 10 μM glu. In the continued presence of glu, the channels desensitized, and this desensitization can be described with a single time constant of ~7.0 ms for a pulse of 10 mM glu. The steady-state current in response to a long pulse of 10 mM glu was below 1/280th of the peak current. The time constant of desensitization was found to be independent of concentration between 30.0 and 0.3 mM glu, but to be increased for lower concentrations. After a short pulse of 1 ms duration and 10 or 0.3 mM glu, currents decayed with a time constant of ~2.5 ms. Recovery from desensitization after a pulse took ~5 s, and the half-time of recovery was ~2.2 s. Continuous application of low concentrations of glutamate reduced the peak currents in response to a pulse of 10 mM glu markedly. Fifty percent response reduction was observed in the continuous presence of ~0.3 μM glu. Our results for homomeric GluR6 agree with a cyclical reaction scheme developed for completely desensitizing, glu-activated channels on crayfish muscles.

INTRODUCTION

Glutamate-gated ion channels mediate most of the excitatory synaptic transmission in the vertebrate central nervous system. All currently known ionotropic glutamate receptor subunits belong to the same gene family (Hollmann and Heinemann, 1994). Some are known to form functional channels on their own, whereas others need additional heterogeneous subunits (Seeburg, 1993). The GluR6 glutamate receptor subunit is expressed in many parts of the nervous system, and it is able to form functional homomeric channels. High levels of expression are found in the olfactory lobe, piriform cortex, dentate gyrus, hippocampus, and the granular cell layer of the cerebellum. In the hippocampus, a gradient was observed from rostral to caudal areas, with increased density in the CA3 region as compared to the CA1 region (Egebjerg et al., 1991). The desensitizing response to kainate, as well as the lack of activation of the channels by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate, classified them as kainate-type receptor channels. Measurements of the activation, desensitization, and recovery time course of homomeric GluR6 glutamate receptor channels are crucial for understanding their physiological role. In addition, this knowledge might help us to better understand the role of structural differences in determining the kinetics of glutamate receptor channels.

We applied glutamate (glu) to outside-out patches isolated from HEK 293 cells transiently transfected with the GluR6 subunit. To overcome the problem caused by the rapid desensitization of the channels, we used a system for very rapid solution changes. We applied a wide range of glu concentrations in succession to single patches. We gained insight into the kinetics of the channels and developed a reaction scheme that agrees with our experimental data.

MATERIALS AND METHODS

Expression of receptors

Human embryonic kidney cells (HEK 293) were transiently transfected with cDNA encoding the GluR6(Q) subunit. The cells were maintained in modified minimal essential medium with 2 mM L-glutamin, 100 IU/ml penicillin/streptomycin, and 10% fetal calf serum in a humidified incubator (5% CO_2) at 37°C. Approximately 24 h after the cells were plated at low density on polylysin-coated coverslips, a mixture of 1.13 μg/ml cDNA and 5 μl/ml Lipofektamine per ml Opti-MEM medium (both from Gibco BRL Life Technologies GmbH, Eggenstein, Germany) was added. The cells were incubated for 16–18 h, washed with phosphate-buffered saline, and incubated for another 24–48 h in medium.

Electrophysiology

Cells were viewed with an upright microscope (Zeiss, Oberkochen, Germany) and superfused with an extracellular solution at ~20°C, containing 162 mM NaCl, 5.3 mM KCl, 0.67 mM $NaHPO_4$, 0.22 mM KH_2PO_4, 15 mM HEPES, and 5.6 mM glucose, with the pH adjusted to 7.4 with 5 N NaOH. Patch pipettes were pulled from thick-walled borosilicate glass tubes with filament and an external diameter of 2.0 mm and an internal diameter of 1.16 mm (Clark, Pangbourne, England) with a DMZ-Universal Puller (Zeitz Instruments, Augsburg, Germany). The pipettes had resistances between 5 and 10 MΩ when filled with the intracellular solution containing 140 mM KCl, 2 mM $MgCl_2$, 11 mM EGTA, 10 mM HEPES, with the pH adjusted to 7.4 with 5 N NaOH. Outside-out patches were

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obtained as described previously (Hamill et al., 1981) and voltage clamped to −60 mV.

Fast application of agonists

The fast application of agonists was performed using the liquid filament switch developed by Franke et al. (1987). A P 810.30 piezo element, with a glass tube connected to it (Physik Instrumente, Waldbronn, Germany) and held in a brass cylinder, was used. The performance of the system was tested by switching the ionic concentration at the tip of open patch pipettes and measuring the change in liquid junction current. The 10–90% rise and decay time of this current was below 100 μs.

Data collection and analysis

Currents were recorded with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) filtered at 10-kHz bandwidth (−3 dB) with an 8-pole low-pass Bessel filter, passed through a modified pulse code modulator, and recorded with a videorecorder on tape. Data records were replayed from tape filtered at 5-kHz bandwidth and sampled at 30 kHz onto the hard disk of a PC using a Digidata 1200 Interface and the pCLAMP program (Axon Instruments). Further analysis was done with CLAMPEX, a part of the pCLAMP program.

RESULTS

To test for the presence of active glutamate-gated channels in a patch, we applied pulses of 50 or 100 ms duration of 10 mM glu at intervals of 5 s at a holding potential of −60 mV. In about every third patch we observed currents of glu-gated channels. The size of the currents varied between patches from a few pA to more than 1 nA. Application of pulses of AMPA did not elicit channel openings in the patches, whereas a pulse of kainate (both 1 mM), as expected, elicited fast rising currents that desensitized in the continued presence of the agonist (Egebjerg et al., 1991; Burnashev, 1993). We focused on the analysis of ensemble currents created by averaging several single responses to glu pulses at a given concentration. During applications of 10 mM glu, the currents rose fast to a peak and decayed within ~40 ms to less than 1% of the peak value. An ensemble average current of a patch is shown in Fig. 1 *A*. The current response to 10 mM glu reached a peak value of −980 pA in this patch. The trace is shown superimposed with averaged responses of the same patch to pulses of 1, 0.3, and 0.03 mM glu, which reached peak current values of −680, −370, and −28 pA, respectively.

We applied glu in concentrations ranging from 10 μM to 100 mM to patches and measured the peak current $\hat{\imath}$. The plot in Fig. 1 *B* shows the results of five such experiments. The $\hat{\imath}$ values were normalized with respect to the $\hat{\imath}$ values measured with 10 mM glu. The data are shown in a double-logarithmic plot. The dose-response curves measured for relative $\hat{\imath}$ were very similar in all patches. Below 30 μM glu concentration they rose with maximum double-logarithmic slopes (n_H) of ~1.3. The half-maximum activating concentration, EC_{50}, or the apparent K_m was ~0.5 mM and above 1 mM $\hat{\imath}$ approached a saturation level. The maximum double-logarithmic slope of the dose-response curve at agonist concentrations of $\ll K_m$ is of special interest, because its value is a lower limit to the number of agonist molecules that have to bind to the receptor to elicit channel opening

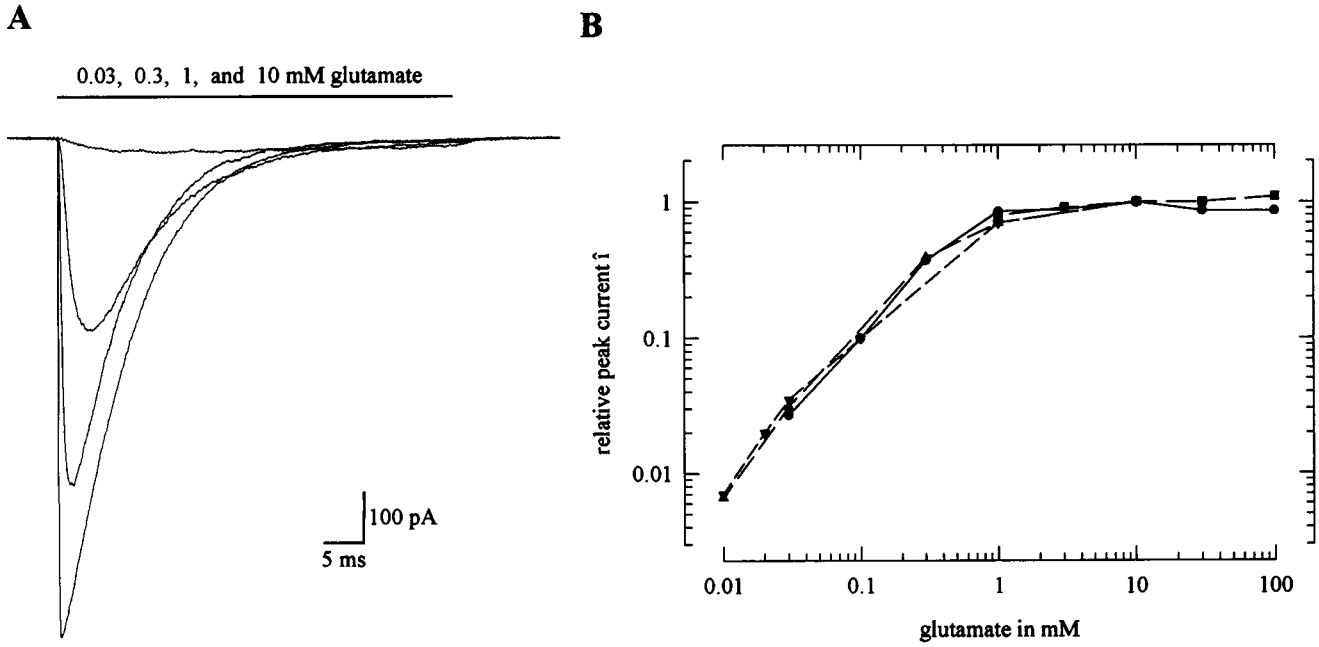
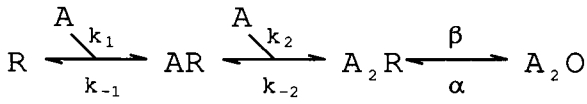


FIGURE 1 Activation of GluR6 glutamate receptor channels on outside-out patches: dependence of peak current on glutamate concentration. Glutamate was applied in 50-ms pulses every 5 s while the patches were held at −60 mV. (*A*) Superimposed averages of three to seven single responses of one patch at 0.03, 0.3, 1, and 10 mM glutamate concentration. The long horizontal bar above the traces indicates the time course of the application. (*B*) Double logarithmic plot of peak current, $\hat{\imath}$, versus glutamate concentration. $\hat{\imath}$ is normalized to the value of $\hat{\imath}$ at 10 mM glutamate. The results of five patches are shown.



SCHEME 1

(Katz and Thesleff, 1957; Dudel, 1977; Dreyer et al., 1978; Colquhoun and Ogden, 1988). For the present channels, the minimum number of glu binding sites seems to be two.

Consider a reaction as represented in Scheme 1, where R represents a closed channel, A represents the agonist molecules, and AR, A_2R, and A_2O represent the closed and the open channel with one or two agonist molecules bound (del Castillo and Katz, 1957). The affinity of the receptor channels for the agonist is determined by the binding rate constants (k_1 and k_2), in units of M^{-1} s^{-1}, and the dissociation rate constants (k_{-1} and k_{-2}), in units of s^{-1}. The channel opening and closing rate constants, in units of s^{-1}, are termed β and α.

More information about these rate constants can be obtained by investigating the concentration dependence of the response rise time after a step increase in agonist concentration, if agonist application is not rate limiting. The initial sections of the average currents in Fig. 1 *A* are shown in Fig. 2 *A*, scaled to the same size and expanded in time scale. To illustrate the speed of the liquid filament switch, the response of the open pipette to a change in the liquid junction potential as determined at the end of the experiment is also shown. The current rise time t_r of the open pipette from 0.1 to 0.9 $\hat{i}$ was 90 μs, and the t_r of the patch with a pulse of 10 mM glu was 170 μs. We tested the performance of the application system routinely in the course of the study, and t_r values below 100 μs with open pipettes were reliably obtained. For patches with high holding current (>10 pA), the change in the liquid junction current could also be measured with the patch in place. The t_r of this liquid junction current was only slightly longer (110 ± 10 μs, $n = 4$) than the value obtained for the open pipette at the end of the experiment. Occasionally we obtained patches with a prolonged t_r in response to pulses of glu (>1 ms for 10 mM). The liquid junction current also had a long t_r with the patch in place, and these patches were discarded, assuming that a non-optimal membrane configuration rather than intrinsic channel properties were responsible for the longer current rise.

The t_r of the patch shown in Fig. 2 *A* was 1.0, 1.8, and 5.3 ms with glu pulses of 1, 0.3, and 0.03 mM concentration, respectively. Fig. 2 *B* shows dose-response curves for t_r obtained in five patches in a double-logarithmic plot. The decrease in rise time with increasing concentrations provides estimates on the order of magnitude of the binding rate constants k_1 and k_2 in Scheme 1. The binding rates (binding rate constants multiplied by glu concentration) increase toward higher concentrations. The current rise time is expected to decrease until the binding rates cease to be rate limiting. For high concentrations a plateau of t_r is expected to be determined (for $\beta \gg \alpha$) by β, the channel opening rate constant. t_r approached a plateau level above 3

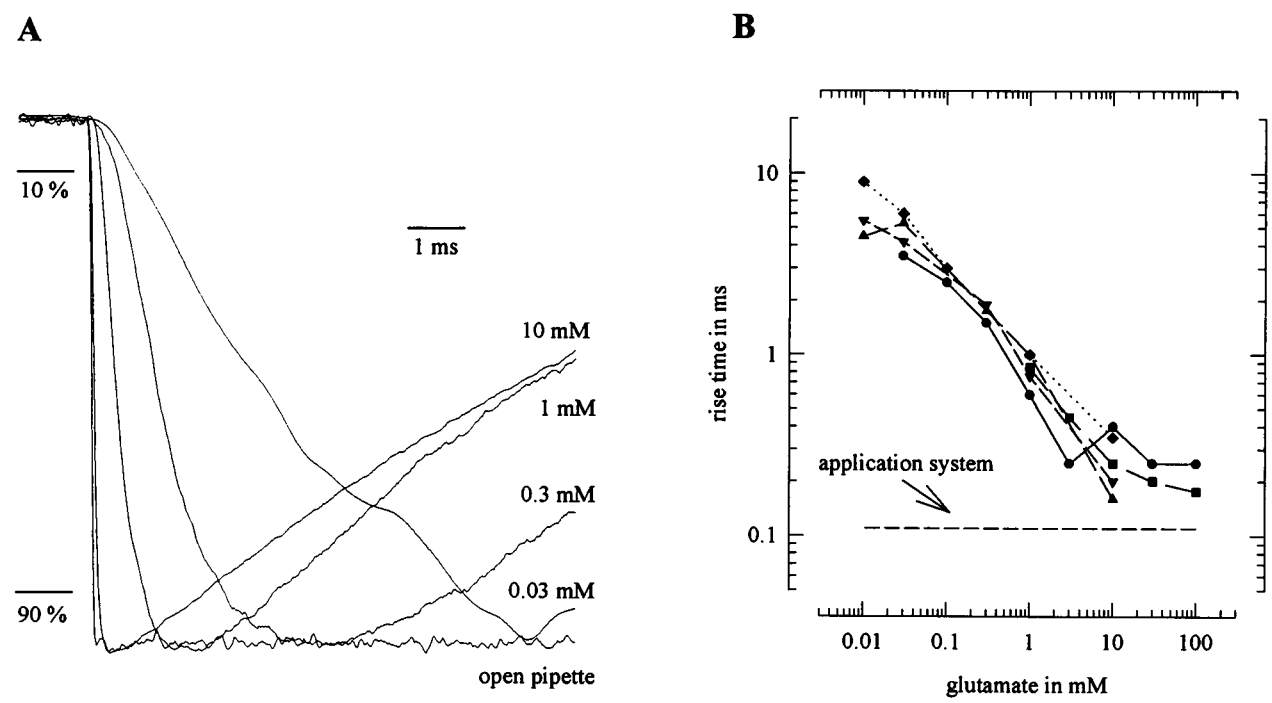
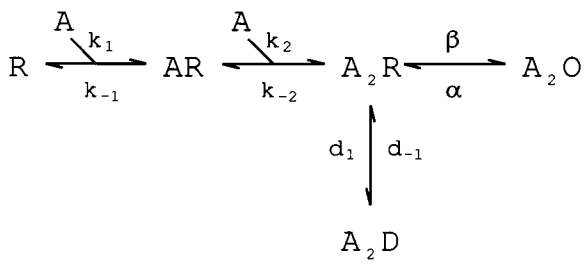


FIGURE 2 Dependence of current rise time on glutamate concentration. (*A*) The rising part of the recordings shown in Fig. 1 *A* are shown normalized to $\hat{i}$. The current traces obtained for 0.03 mM were filtered digitally at 0.5 kHz before averaging to reduce noise for this display. The response of the open pipette to a change in liquid junction potential was recorded at the end of the experiment. The short horizontal bars on the left side of the figure mark the 10% and 90% values of the currents $\hat{i}$ used to determine the rise time t_r. (*B*) Double logarithmic plot of the rise time t_r versus the glutamate concentration. The results of five patches are shown. The dashed line illustrates the mean rise time in response to changes of liquid junction potential with intact patches.

mM glu (Fig. 2 *A*). The mean rise time values in response to the highest glu concentrations used were ~220 μs. An estimate of 10,000 s^{-1} for β is obtained from $t_r = 2.2/\beta$, in which 2.2 is the ln (90/10), the conversion factor from the exponential time constant to the 10–90% rise time. The binding rates must exceed this value at saturating concentrations, which makes it possible to estimate a lower limit for the binding rate constants. Our measure for the speed of the application system determined with intact patches has been marked as a dashed line in Fig. 2 *B*. Because the response rise time of the patches does not reach the speed of the application system, we assume that the plateau for the rise time of the glu gated currents is determined by the channel opening rate constant. At low concentrations t_r should reach a plateau level or should pass through a maximum (Franke et al., 1991; von Beckerath et al., 1995). At limiting low concentrations, t_r is primarily determined by k_{-1}, k_{-2}, α, and β for a mechanism like Scheme 1 (Buchman and Parnas, 1992). In the majority of the experiments, the maximum t_r does not seem to have been reached with 0.01 mM glu. Provided that α and β can be determined, the low concentration rise time data make it possible to estimate the order of magnitude of k_{-1} and k_{-2}.

As one can see in Figs. 1 *A* and 3 *A*, the current in response to a 50-ms pulse of 10 mM glu decayed in the presence of the agonist, with no further openings after the end of the pulse. This desensitization could be described reasonably well with a single time constant τ_d of ~8 ms. Rapid desensitization of ligand-gated ion channels is assumed to be a direct consequence of binding the agonist. Two principal mechanisms have been proposed. In one, desensitization is a further conformational change after channel opening; desensitization occurs from the open state. In the other, a closed channel can change conformation to a desensitized state or can bind agonist and is converted to a desensitized state. Classical modifications of Scheme 1 to account for desensitization involve the addition of a state A_2D, that is connected to either A_2R, as in Scheme 2, or A_2O. Desensitization from A_2R is indistinguishable from that from A_2O in case of large β.

To reproduce the desensitization of the channels in response to a pulse of 10 mM glu, the rate constant of desensitization (d_1) and the rate constant of recovery from desensitization (d_{-1}) have to be adjusted. Desensitization in response to a pulse of 10 mM glu was almost complete, as



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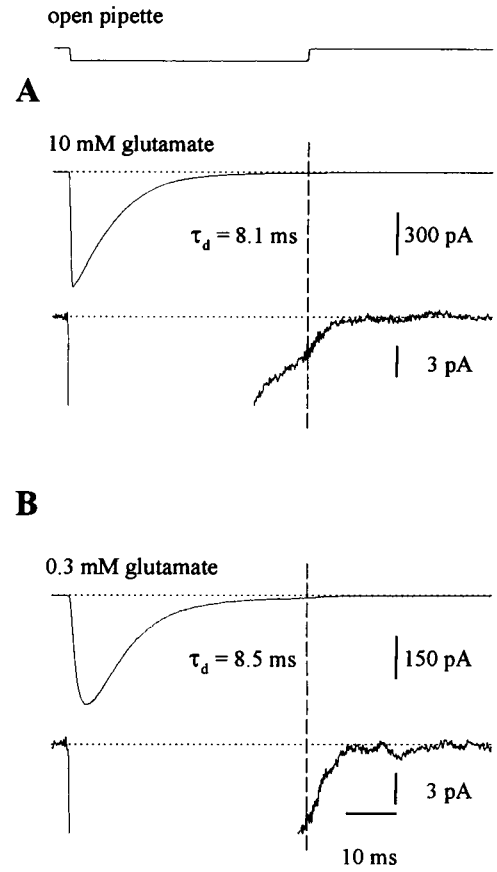


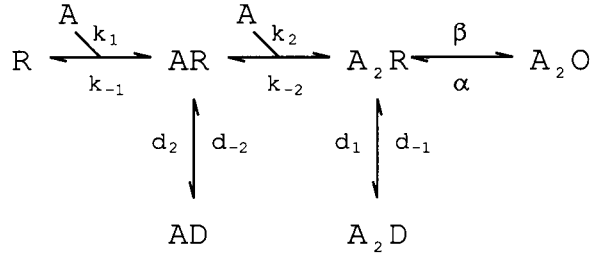
FIGURE 3 Desensitization of GluR6 glutamate receptor channels. (*A*) Average response of one patch to 20 pulses (50 ms) of 10 mM glutamate. The current reached a peak value of −840 pA. The time constant of desensitization was 8.1 ms. The lower trace shows the baseline (dotted line) of this average at increased amplification. The dashed vertical line marks the current amplitude of ~3 pA at end of the pulse. (*B*) Average response of the same patch to 23 pulses of 0.3 mM glutamate. The current reached a peak value of −395 pA. The time constant of desensitization was 8.5 ms. The lower trace shows the baseline at increased amplification, and the dashed vertical line marks the current amplitude of ~8 pA at the end of the pulse. Glutamate was applied every 5 s while the patches were held at −60 mV. The current traces were filtered at 3 kHz.

illustrated in Fig. 3 *A*. The upper trace shows the average of 20 single responses of a patch to 50-ms pulses of 10 mM glu. The average reached a peak current value of −840 pA and desensitized with a time constant τ_d of 8.1 ms. With a time constant τ_d of 8.1 ms, the exponential decay goes to 1/479th the peak value in 50 ms. The lower trace in Fig. 3 *A* shows the baseline of the same average at much higher amplification. It can be seen that at the end of the pulse (marked by a dashed vertical line), the current had decayed to a value of about 3 pA. The current thus decayed to about 1/280th of the peak value, and this allows us to estimate a limit for the ratio of d_1/d_{-1}. Note the decay with the average duration of bursts after the end of the application. The absence of new openings after the end of the application supports the suggestion that recovery from A_2D to A_2R must be slow compared to the rate constant k_{-2}. In Fig. 3 *B*

an average of 23 single responses of the same patch to pulses of 0.3 mM glu is shown. The current reached a peak value of −395 pA, and the time constant of desensitization was 8.5 ms. This is in conflict with Scheme 2. For a mechanism with desensitization occurring only from A_2R, like Scheme 2, a more pronounced decrease in desensitization is expected with such a reduction in the peak open probability.

We investigated the concentration dependence of desensitization in more detail. The data are shown in Fig. 4 *B* in a double-logarithmic plot of τ_d versus glu concentration. There was some variability in the τ_d from patch to patch; however, the time constant of desensitization for a patch and a pulse of glu at a given concentration did not change during the course of the experiment. Above 0.3 mM glu concentration, τ_d did not show concentration dependence, but rose steeply below this concentration. To reproduce this, a second pathway of desensitization has to be added (Scheme 3). The lower trace in Fig. 3 *B* shows the baseline of the averaged current in response to pulses of 0.3 mM glu at increased amplification. The dashed vertical line marks the residual current (~8 pA) at the end of the application. The residual current is 1/49th the peak value, whereas the exponential decay goes to 1/359th the peak value. From this an order of magnitude for the ratio of d_2/d_{-2} can be estimated.

We varied the duration of the pulses to compare channel closure after removal of glu with desensitization. The decay



SCHEME 3

after a short pulse of agonist is determined by α, β, k_{-2}, and d_1 if present in Schemes 1 to 3, and in Scheme 4, which will be presented below. Fig. 4 *A* shows currents from one experiment during exposure to 10 mM glu for 1, 3, and 50 ms. The rise time and the peak amplitude of the currents have almost the same values. The decay time constant in the presence of glu, the desensitization, was longer than the current decay time constant after removal of glu. The values were 5.1 and 2.8 ms for a 50-ms and a 1-ms pulse, respectively, in this experiment. As expected, the response to a 3-ms pulse declined initially like the response to a 50-ms pulse (due to desensitization), and after the removal of the agonist the decay became faster and the time constant was almost the same as that for a 1-ms pulse. With a pulse of 0.3 mM glu and 1 ms duration, the current, as expected, did

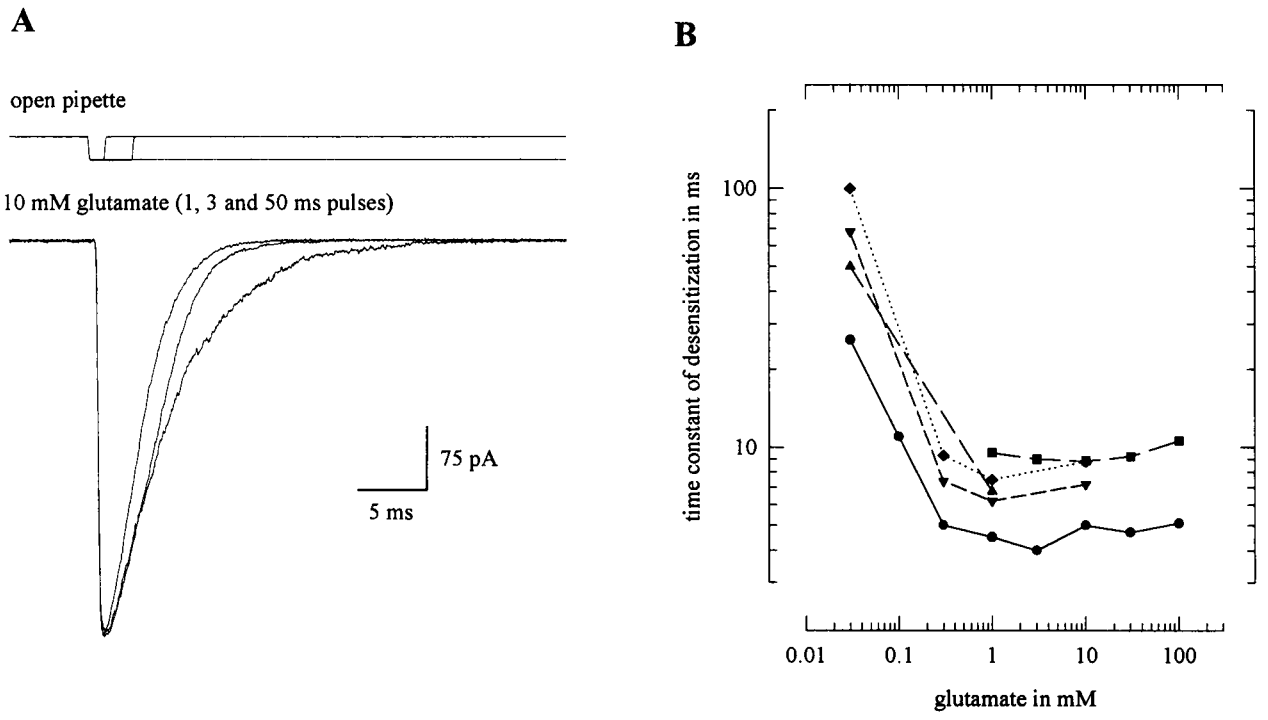
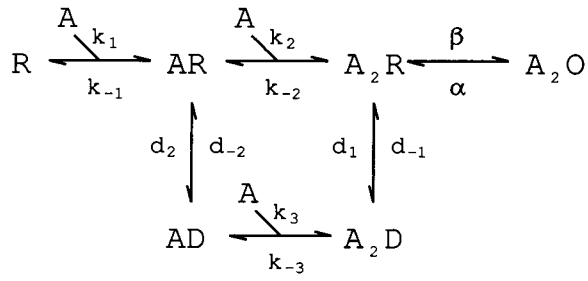


FIGURE 4 Effects of pulse duration and concentration on current decay. (*A*) Pulses of 10 mM glutamate concentration and 1, 3, and 50 ms duration were applied to an outside-out patch. The superimposed current traces are the average of five single responses. The small traces at the top (marked "open pipette") are liquid junction current changes. They were recorded at the end of the experiment after the patch was destroyed, to illustrate the time course of the application. (*B*) Plot of the time constant of desensitization τ_d versus glutamate concentration on a logarithmic scale. Averages of 3–10 single responses to 50- or 100-ms pulses of 0.03 to 100 mM glutamate concentration were evaluated. The results of five patches are shown.



SCHEME 4

reach a lower peak value than for a 50-ms pulse of the same concentration (not illustrated). The current decay after a 1-ms pulse of 0.3 mM glu (2.5 ± 0.2 ms, $n = 4$) was, however, very similar to the decay after a 1-ms pulse of 10 mM glu (2.7 ± 0.5 ms, $n = 6$). Provided β, k_{-2}, and d_1 are known, one could estimate from these measurements the channel closing rate constant α. The independence of the decay time constant after a short pulse from glu concentration is expected if it represents the burst duration.

Desensitization in response to long pulses of 10 mM glu was almost complete. The average rate of channel opening reached a steady state that amounted to no more than 1/280th of the maximum rate in Fig. 3 *A*. A time constant of desensitization of ~8 ms in response to a pulse of 10 mM glu, assuming a mechanism of desensitization like that in Scheme 3, would predict an off time constant from desensitization (A_2D to A_2R) ≥ 2.2 s. For low concentrations, an additional faster component of recovery from desensitization, recovery from AD determined by d_{-2}, is predicted.

To investigate the recovery of the channels from desensitization, we applied pairs of pulses of 50 ms duration and 0.3 or 10 mM glu. The delay between the first (control pulse) and the second (test pulse) was varied from 0.1 to 10 s. Fig. 5 *A* shows an overlay of such averaged pulse pairs in response to 0.3 mM glu. To regain full amplitude, the paired pulses needed to be separated by 5 s and the time constant of recovery from desensitization (τ_{rec}) was ~1.6 s. Repeating the protocol with a pulse of 10 mM glu gave a similar result (τ_{rec} ~ 2 s), as seen in Fig. 5 *B*. The relative amplitude of the test versus the control pulse is plotted against the interval between the pulses for the two concentrations. The data were fitted with one exponential with the time constants given above. There appears to be a slight dependence of recovery on glu concentration, but recovery from desensitization is faster than predicted by Scheme 3. A modification of the reaction scheme is necessary to allow a low enough d_{-1} and fast enough recovery from desensitization. Scheme 4 is a cyclical reaction scheme that allows recovery from desensitization from A_2D with the rate constant k_{-3} via AD. It contains an additional agonist binding step from AD to A_2D with the binding rate constant k_3.

Some receptor channels are desensitized by the continuous presence of low agonist concentrations. This can be

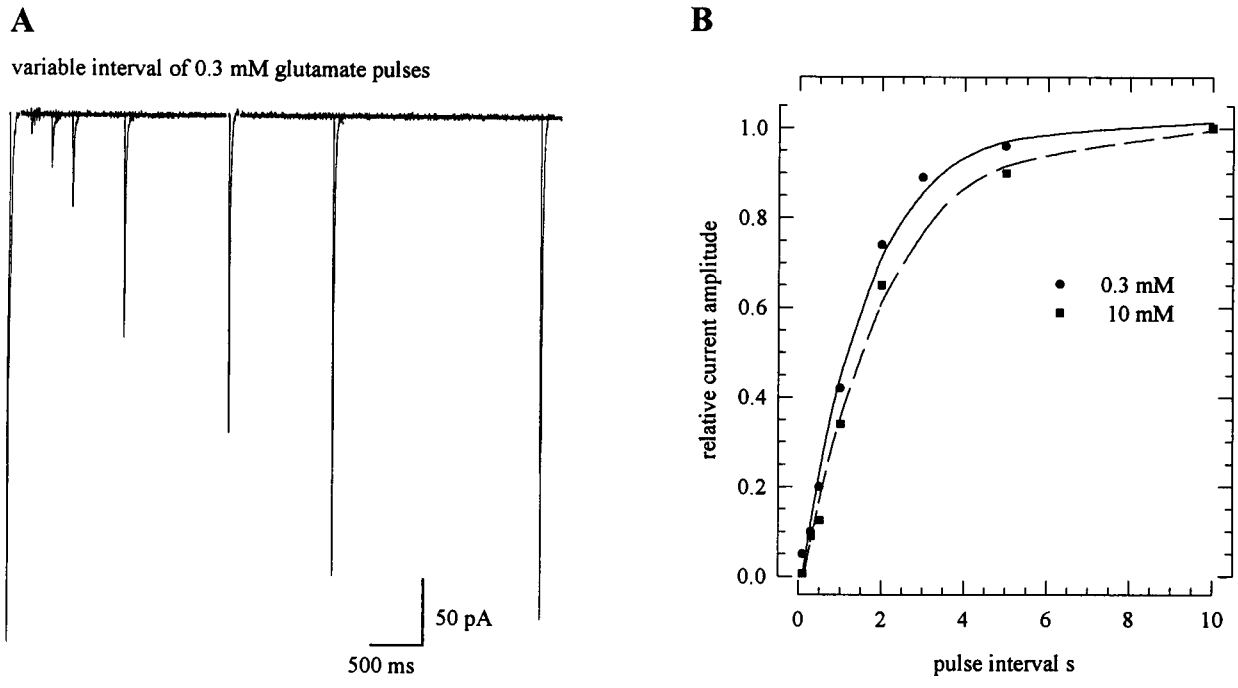


FIGURE 5 Time course of recovery from desensitization. (*A*) Fifty-millisecond 0.3 mM glutamate pulses that desensitized the channels completely were followed by a second pulse with variable interval. Three to five single responses were averaged for each interval and are shown superimposed. The responses were recorded with an interval of 10 s to allow recovery from desensitization. The control pulse was followed by a test pulse after 0.1-, 0.3-, 0.5-, 1-, 2-, 3-, and 5-s intervals. The amplitude of the response to the second pulse divided by the amplitude of the response to the first pulse indicates the extent of desensitization at the respective interval. (*B*) Plot of the relative current amplitude in response to pulses of 0.3 and 10 mM glutamate concentration versus pulse interval. The data were fitted with one exponential with time constants of 1.6 and 2 s (*solid* and *dashed lines*).

explained by assuming high affinity states of the channels. The appearance of high affinity can also be obtained solely from low-affinity binding, with a subsequent accumulation of channels in desensitized states yielding an apparent high affinity. To test for the effect of low glu concentrations on the channels, we added glu concentrations between 0.03 and 10 μM to the background solution, passing the patch between pulses, and recorded the response to 50-ms pulses of 10 mM glu every 5 s. In Fig. 6 *A* the result of a representative experiment is shown with the average current traces superimposed. The two largest currents (marked "control") were recorded at the beginning and the end of the predesensitization protocol, with no glu bathing the patch. The other averages were recorded while the glu concentration, indicated on the left side of the current traces, was present in the background solution. The presence of 0.1, 0.3, 1, 3, and 10 μM glu reduced the response to 0.77, 0.54, 0.19, 0.08, and 0.02 of the control values, respectively. The results of four experiments are shown in Fig. 6 *B* in a double-logarithmic plot. The concentration needed for a 50% reduction of the peak response (IC_{50}) was about 0.3 μM, and the slope of the dose-response curve was about -1.0.

With low glu concentrations access to desensitized states in Scheme 4 has to occur via AR, the binding rate constant k_1 being rate limiting. A low-frequency transit from R to AR might suffice to allow the channels to desensitize and accumulate in desensitized states, provided either d_{-2} or k_{-3} is low enough. One should also consider that k_1 could be substantially higher than k_2. A difference in affinity of the two binding sites, however, will affect the dose-response curve for peak current $\hat{\imath}$ and current rise time t_r. Based on our experiments, we propose Scheme 4 as the mechanism for the activation and desensitization of the channels.

DISCUSSION

We investigated the kinetics of homomeric GluR6 receptor channels by analyzing the relaxation of the channels after concentration steps. This approach is an alternative to the analysis of single-channel recordings and provides complementary kinetic data (Colquhoun and Hawkes, 1977; Dudel et al., 1990; Franke et al., 1991). It is particularly useful in the study of ion channels for which it is difficult to obtain single-channel recordings of sufficient quality because of a low single-channel conductance and/or multiple subconductance states or rapid desensitization of the channels in the continued presence of an agonist.

To gain insight into the mechanism of receptor function, a direct approach is to study the response as a function of agonist concentration. This strategy allows quantitative predictions by applying the principles of reaction kinetics to different reaction schemes. Our analysis led us to propose a

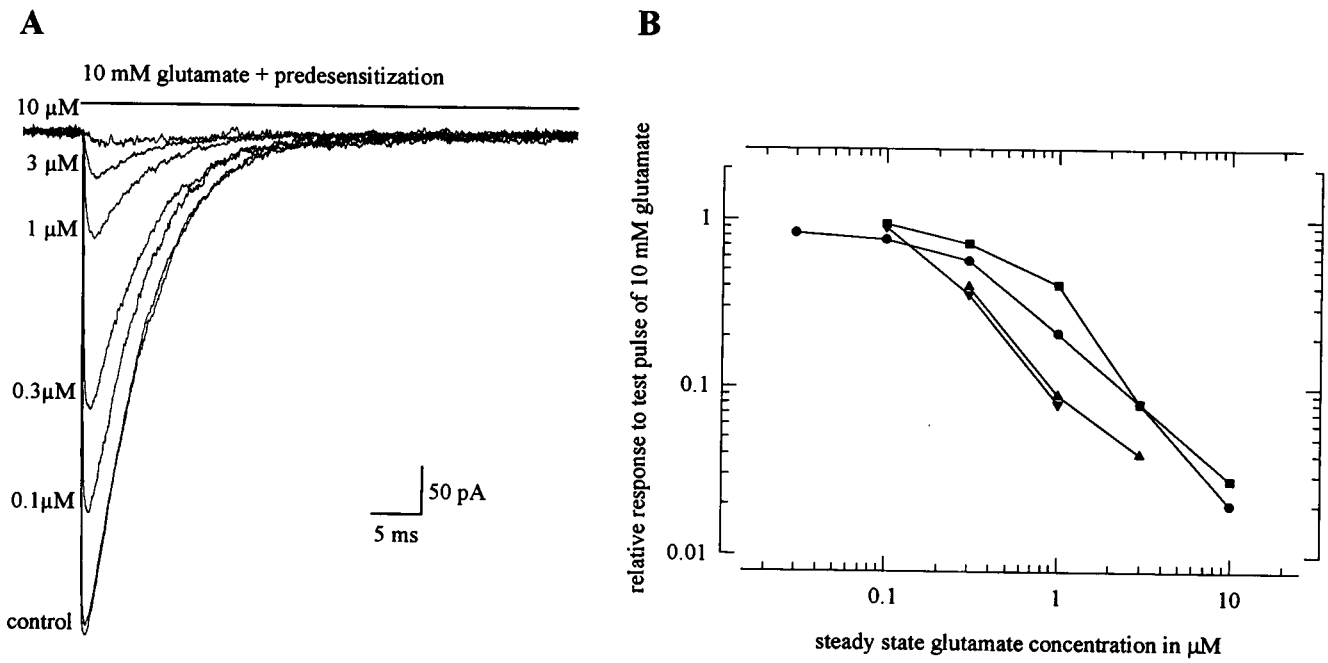


FIGURE 6 Dose response of predesensitizing, low glutamate concentrations applied in the steady state. The extent of desensitization was tested by applying 10 mM glutamate pulses, the elicited response indicating the fraction of nondesensitized channels. (*A*) Averages of four to seven single responses of one patch to pulses of glutamate during predesensitization to a variable extent by low glutamate concentrations are shown superimposed. The long horizontal bar above the traces indicates the time course of the pulse. The two largest responses (marked "control") were recorded at the beginning and the end of the experiment with no predesensitization of the channels. The other responses were recorded in the presence of 0.1 to 10 μM glutamate, as indicated on the left side of the traces before and between the pulses. (*B*) Double-logarithmic plot of the relative peak current versus steady-state glutamate concentration. The results of four patches are shown.

cyclical reaction scheme with five closed and one open or conducting state (Scheme 4). Two of the closed channel states are so-called desensitized states. It is possible to test the schemes using a computer and the appropriate software (Franke et al., 1993; Colquhoun and Hawkes, 1995). These simulations are likely to advance our understanding of the kinetics of the channels further and will be dealt with in detail elsewhere.

The peak currents, $\hat{\imath}$, elicited by rapidly applied agonist pulses of different concentrations, can yield dose-response curves avoiding the influence of desensitization. The maximum slope of the double-logarithmic dose-response curve n_H is often used to estimate a lower limit for the number of binding steps n necessary to open a channel (Werman, 1969; Colquhoun, 1975; Dudel, 1977; Colquhoun and Ogden, 1988). The value of 1.3 obtained in the present study indicates an $n = 2$. This is also the minimum number of binding steps that has been proposed to be necessary to provide sufficient energy for channel opening (Jackson, 1989). We obtained a slope greater 1 only for very low glu concentrations. The experimental support for the estimate of the number of binding steps is not strong. Two binding steps are not of fundamental importance for the proposed mechanism, because the desensitization and resensitization kinetics of the channels can be reproduced with a cyclical reaction scheme containing one binding step.

Surprisingly, the kinetics of the channels investigated in the present study are very similar to the kinetics of a glutamate-gated channel of crayfish muscle (Dudel et al., 1993). The scheme for the completely desensitizing channels of crayfish is also cyclical. It contains five binding steps of agonist before channel opening to account for the very steep dose response of the open probability. It allows desensitization to occur from A_5O, AR, and A. Desensitization from AR is most effective at intermediate glu concentrations (0.5 mM) and declines for higher glu concentrations due to the reduced lifetime of the AR state. Desensitization from AR causes a minimum of τ_d around 0.5 mM in this case. Scheme 4 is a scaled-down version of the circular scheme for the crayfish channels. The necessary modification is to reduce the number of glutamate binding steps to agree with the n_H of homomeric GluR6 channels. This agreement in reaction mechanism might be a sign of a common principle, although the crayfish channel is quisqualate or AMPA and not kainate type, underlying the kinetics of some glutamate-gated ion channels.

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