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ACTION OF BRIEF PULSES OF GLUTAMATE ON AMPA/KAINATE RECEPTORS IN PATCHES FROM DIFFERENT NEURONES OF RAT HIPPOCAMPAL SLICES

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SUMMARY

1. Outside-out patches were isolated from granule cells of dentate gyrus and pyramidal cells of CA3 and CA1 regions of rat hippocampal slices. Patches were exposed briefly to L-glutamate using a piezo-driven double-barrelled application pipette.

2. Applications of glutamate (1 mM) of 1 ms duration activated patch currents which rose and decayed rapidly. The 20–80% rise time of these glutamate receptor (GluR)-mediated currents was usually 0·2–0·6 ms. At −50 mV the peak current varied from 10 to 500 pA in different patches.

3. The peak current–voltage relation for brief pulses of 1 mM glutamate was virtually linear in normal extracellular solution for patches from the three cell types (−100 to 60 mV).

4. The permeability of GluR channels activated at the peak to Ca^{2+}, relative to K^+, was less than 0·1 for all three cell types (under bi-ionic conditions with Ca^{2+} on the extracellular side and K^+ on the intracellular side of the membrane).

5. The offset decay time constant of the current following 1 ms pulses of 1 mM glutamate was brief, with mean values of $3{\cdot}0 \pm 0{\cdot}8$, $2{\cdot}5 \pm 0{\cdot}7$, and $2{\cdot}3 \pm 0{\cdot}7$ ms for dentate, CA3 and CA1 cell patches, respectively. Offset time constants were independent of membrane potential and independent of glutamate concentration (200 μM and 1 mM) for the three cell types.

6. Applications of 1 mM glutamate of 100 ms duration showed that glutamate responses desensitized rapidly. The time constants for desensitization were $9{\cdot}4 \pm 2{\cdot}7$, $11{\cdot}3 \pm 2{\cdot}8$, and $9{\cdot}3 \pm 2{\cdot}8$ ms for patches from dentate, CA3 and CA1 cells respectively. Desensitization time constants were only weakly dependent on glutamate concentration (200 μM and 1 mM) for the three cell types. Thus offset time constants are about four times faster than desensitization time constants for both glutamate concentrations.

7. Double pulse application of glutamate indicated that even a 1 ms pulse of 1 mM glutamate causes partial (about 60%) desensitization of GluR channels. The time

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course of recovery from desensitization was slower in dentate gyrus granule cell patches than in CA3 or CA1 pyramidal cell patches.

8. Desensitization was studied at equilibrium by exposing patches to low glutamate concentrations for at least 15 s before a 1 ms test pulse of 1 mM glutamate. The peak amplitude of the test pulse was halved by pre-equilibration with 9·6 and 4·2 μM glutamate in CA3 and CA1 cell patches; GluR channels in dentate gyrus granule cell patches were rather more sensitive, the concentration which halves the peak amplitude (IC_{50}) being 2·4 μM.

9. Currents activated by 1 ms pulses of 1 mM glutamate were blocked by the selective AMPA/kainate (KA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). The IC_{50} values were in the range 106 to 183 nM.

10. The currents activated by 1 ms pulses of 1 mM glutamate resembled excitatory postsynaptic currents (EPSCs) in many ways, suggesting that the transmitter is present only briefly in the synpatic cleft during excitatory synaptic transmission. Desensitization may be caused by a single EPSC, as well as by low ambient glutamate concentrations. Cell-specific differences in desensitization may influence the efficacy of synaptic transmission in the trisynaptic hippocampal circuit.

INTRODUCTION

Fast excitatory postsynaptic currents (EPSCs) in the central nervous system (CNS) are thought to be mediated by glutamate (see reviews by Dingledine, Boland, Chamberlin, Kawasaki, Kleckner, Traynelis & Verdoorn, 1988; Nicoll, Malenka & Kauer, 1990). Glutamate receptors (GluRs) can be divided into two categories, which may be termed AMPA/KA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate) and NMDA (*N*-methyl-D-aspartic acid) receptor channels. The EPSCs mediated by AMPA/KA receptors are much briefer (decay time constants 1–8 ms) than those mediated by NMDA receptors (decay time constants 10–200 ms). Thus fast transmission of excitation in CNS pathways is primarily mediated by the AMPA/KA type receptors.

The factors that determine the size and time course of EPSCs mediated by AMPA/KA receptors are not yet known; for example, neither the time course of the concentration of glutamate in the synaptic cleft, nor the density or the kinetic properties of the subsynaptic AMPA/KA receptors, have been determined experimentally. If glutamate disappeared very rapidly from the synaptic cleft then the decay of the AMPA/KA receptor-mediated EPSCs would, as at the neuromuscular junction, reflect primarily the distribution of channel lifetimes after removal of the agonist (Anderson & Stevens, 1973; Katz & Miledi, 1975). On the other hand, if the glutamate persisted in the synaptic cleft for some time, the fast decay of the AMPA/KA receptor-mediated EPSC could be determined by fast desensitization of these receptors (Dudel, Franke, Hatt, Ramsey & Usherwood, 1988; Trussell & Fischbach, 1989; Dudel, Franke & Hatt, 1990*a*).

The particular subtypes of AMPA/KA receptors that mediate EPSCs are not known either. Several genes encoding for subunits that form AMPA/KA receptor channels have been cloned and functional properties of the recombinant GluR channels assembled from these subunits have been studied (Hollmann, O'Shea-Greenfield, Rogers & Heinemann, 1989; Boulter, Hollmann, O'Shea-Greenfield,

Hartley, Deneris, Maron & Heinemann, 1990; Keinänen, Wisden, Sommer, Werner, Herb, Verdoorn, Sakmann & Seeburg, 1990; Nakanishi, Shneider & Axel, 1990; Egebjerg, Bettler, Hermans-Borgmeyer & Heinemann, 1991). However, the molecular composition of the native AMPA/KA receptors is not known, nor is it known if native receptors are homogeneous or form a mosaic of several subtypes. Furthermore, it is quite possible that native receptors differ from one area of the brain to another, and that they also differ between subsynaptic and extrasynaptic membrane regions of the same neurone.

In order to cast light on some of these questions, we have done experiments on granule cells of the dentate gyrus and pyramidal cells of the CA3 and CA1 subfields of rat hippocampus, the cells that constitute the trisynaptic excitatory pathway in the hippocampus (Andersen, Bliss & Skrede, 1971). The patch clamp technique in brain slices (Edwards, Konnerth, Sakmann & Takahashi, 1989) made it possible to characterize the functional properties of GluR channels in membrane patches from the somata of visually identified cell types. The aim was to apply a very brief pulse of glutamate to the receptors; the duration should ideally be not longer than the rising phase of fast EPSCs, i.e. not longer than 1 ms or so. In fact pulses of 1 ms duration proved feasible, so the responses that we measured should mimic the EPSC in a glutamatergic synapse if such central synapses do in fact resemble the neuromuscular junction in having a very brief time course of increase and decrease of transmitter concentration in the synaptic cleft. In so far as the time course of an EPSC can be mimicked, it will be possible to investigate, under controlled conditions, questions such as how much desensitization occurs during a single EPSC, and to study the effect of ambient glutamate on peak EPSC amplitude. Furthermore, when similar experiments are performed on recombinant AMPA/KA receptors that have been expressed in host cells, it should be possible to judge how closely the responses mediated by recombinant AMPA/KA receptors assembled from different subunit combinations resemble EPSCs at central synapses.

Some of the results have already been reported to the Physiological Society (Colquhoun, Jonas & Sakmann, 1992*a*, *b*).

METHODS

Brain slice preparation

Wistar rats 14 to 22 days old were decapitated and brains were removed quickly. Transverse hippocampal slices 300 μm thick were cut with a vibrating slicer and cell somata in the dentate gyrus and the CA3 and CA1 subfields were cleaned for patch clamping (Figs 1 and 2) essentially as described by Edwards *et al.* (1989; see also Hamill, Marty, Neher, Sakmann & Sigworth, 1981; Jonas & Sakmann, 1992*a*). Patch pipettes were pulled from borosilicate glass tubing (2·0 mm outer diameter, 0·5 mm wall thickness). When filled with internal solution, they had a resistance of 4–7 MΩ. Outside-out patches were obtained from neurones that had resting potentials more negative than −60 mV and which generated action potentials upon depolarization. Slices were used only within about 4 h after being placed in the maintenance chamber (Edwards *et al.* 1989), because patches obtained after this time were much less stable. All experiments were performed under a 40× water immersion objective (Nikon, Tokyo, Japan; 2 mm working distance), and at room temperature (20–24 °C).

Fast application pipette

The fast application of agonists was performed using a modification of the method originally developed by Franke, Hatt & Dudel (1987). Concentration jumps of agonist were performed by using an application pipette fabricated from theta glass tubing (Hilgenberg, Malsfeld, Germany;

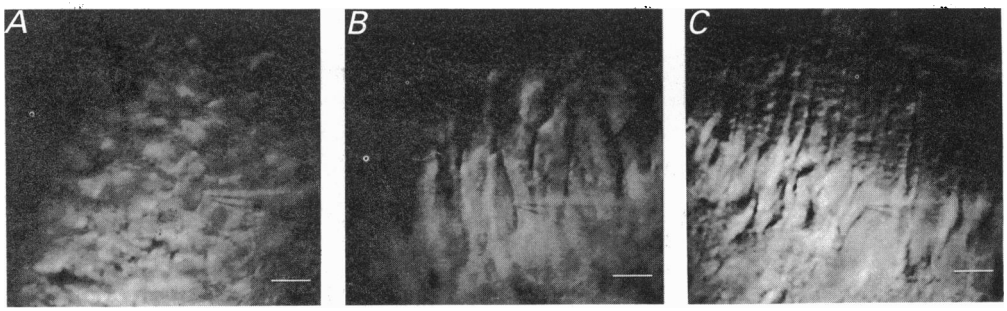

Fig. 1. Photomicrographs of dentate gyrus granule cell layer (*A*), CA3 subfield (*B*), and CA1 subfield (*C*) of a hippocampal slice after cleaning procedure, with a patch pipette attached to the cell soma. Micrographs were obtained with an infrared differential interference contrast camera system (Dodt & Zieglgänsberger, 1990) and were taken from TV screen. Scale bar 20 μm.

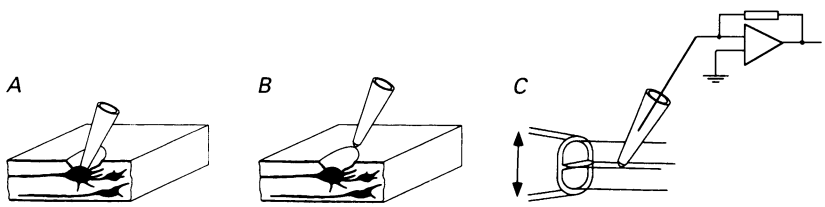

Fig. 2. Schematic drawing of fast application of glutamate to outside-out patches isolated from neurones in brain slices. *A*, whole-cell configuration obtained after cleaning procedure. *B*, isolation of outside-out patch. *C*, fast agonist application to excised outside-out patch with a piezo-driven theta glass application pipette.

1·5 mm outer diameter, 0·22 mm wall thickness, 0·16 mm septum; for some experiments: 1·6 mm outer diameter, 0·24 mm wall thickness, 0·093 mm septum was used) pulled out to a tip diameter of about 200 μm (see Figs 2 and 3). The application pipette was directly mounted on a piezo translator (P-245.20) operated by a piezo power switch (272.00, Physik Instrumente, Waldbronn, Germany). Control solution flowed down one barrel of the pipette, and the test solution (e.g. 1 mM glutamate) flowed down the other barrel. The solution reservoirs were slightly pressurized (e.g. 70 mbar, with N_2) to obtain an optimum flow rate (100 μm ms^{-1}). The tip of the patch pipette with the outside-out patch was positioned close to the interface between the two solutions, near the centre of the stream; when the piezo translator was activated, so as to move the application pipette by about 15 μm, the interface crossed the patch and produced a rapid concentration change.

In order to obtain fast, and symmetrical, concentration steps, the most important requirement appeared to be a sharp interface between the two solutions. To achieve this the flow rate should not be too high or too low (approximately 100 μm ms^{-1}), the application pipette tip should not be too small ($>$ 100 μm tip diameter), and the tip of the patch pipette should not be too close to the application pipette ($>$ 50 μm). Usually the tip of the patch pipette was positioned about 100 μm away from the tip of the application pipette. This arrangement (Fig. 3) had the additional advantage that the electrical artifact, which results from the charging and discharging of the piezo elements, had subsided before the response begun (for 1 ms pulses).

Data analysis

Currents were recorded with an EPC-7 patch clamp amplifier (List, Darmstadt, Germany), and filtered at 3 or 4 kHz bandwidth ($-$3 dB) with an 8-pole low-pass Bessel filter (Frequency Devices, Haverhill, MA, USA). Experiments were run online with a 486-based PC and a CED 1401-plus

interface (Cambridge Electronic Design, Cambridge, England). The piezo translator was controlled by one digital-to-analog converter (DAC), and the membrane potential was controlled by another DAC. The membrane current was sampled at 5–100 kHz via analog-to-digital converters (ADC), and was stored on the hard disk for later analysis. The ADCs and DACs were all interrupt-driven,

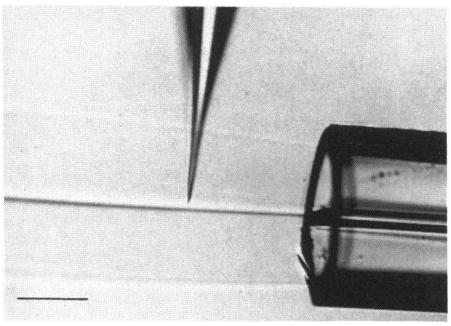

Fig. 3. Photomicrograph of application pipette and patch pipette as seen during the experiment. The two barrels of the application pipette were perfused with NRR and 10% NRR. Scale bar 100 μm. Because of the different refractive index of the NRR and the 10% NRR solution, the interface between them ('liquid filament') was clearly visible. Note the sharp interface between the two solutions.

so any combination of voltage steps, concentration steps and ADC sampling protocols could be done in each sweep. For testing the speed of the solution change on intact patches (Fig. 4), an event detector AI 2020A (Axon Instruments, Foster City, CA, USA), was used to trigger the CED 1401-plus interface with the opening of a large-conductance Ca^{2+}-activated potassium channel.

Peak and baseline currents were marked with cursors. The rise times of responses were determined as the difference between the times when the current (interpolated by the cubic spline method) reached 20 and 80% of the peak current. For display purposes the digitized currents were sometimes interpolated (cubic spline method). Current decays were fitted with one or more exponentials using an unweighted least-squares criterion, and Hill equations etc. were fitted by weighted least-squares.

Solutions and drugs

The extracellular physiological saline solution contained (mM): 125 NaCl, 25 $NaHCO_3$, 25 glucose, 2·5 KCl, 1·25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, and was bubbled with 95% O_2 and 5% CO_2. The HEPES-buffered solution (normal rat Ringer solution NRR) contained (mM): 135 NaCl, 5·4 KCl, 1·8 $CaCl_2$, 1 $MgCl_2$, 5 *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid) (HEPES), pH adjusted to 7·2 with NaOH. The 10% NRR used for testing the application pipettes was NRR diluted 1:10 with water. The K-NRR used for the experiments with the large-conductance Ca^{2+}-activated potassium channels (see Fig. 4*B* and *C*) contained (mM): 140 KCl, 1·8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH adjusted to 7·2 with KOH. The high-Ca^{2+} external solution contained (mM): 100 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH adjusted to 7·2 with $Ca(OH)_2$.

The internal solution contained (mM): 140 KCl, 10 ethylene glycol-bis(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), 2 $MgCl_2$, 2 Na_2ATP, 10 HEPES, pH adjusted to 7·3 with KOH. For the experiments with the large-conductance Ca^{2+}-activated potassium channels (Fig. 4*B* and *C*) the internal solution containing (mM): 150 KCl, 2 $MgCl_2$, 2 Na_2ATP, 10 HEPES, and 10 μM $CaCl_2$, pH adjusted to 7·3 with KOH, was used. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonopentanoic acid (D-APV) were from Tocris (Essex, England), all other substances from Sigma (St Louis, MO, USA). L-Glutamate was stored in a 10 mM stock solution in NRR (pH adjusted to 7·2 with NaOH), which was kept frozen for at most 2 months, and diluted before each experiment.

RESULTS

Tests of the agonist application method

The properties of individual application pipettes tended to vary, and they were tested before and after each day of experiments by perfusing the two barrels with

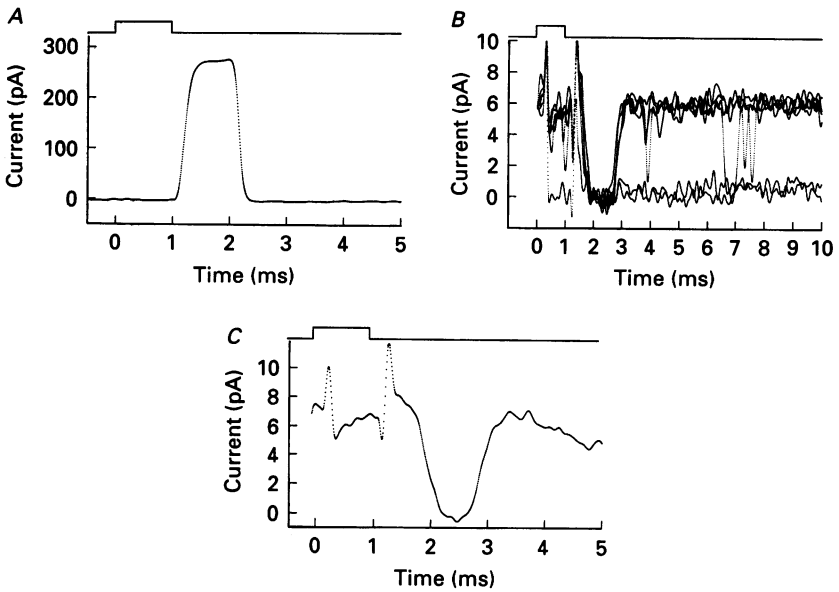

Fig. 4. Rapid solution changes and application of brief pulses of solutions with the theta glass application pipette. *A*, current recorded when a 1 ms solution change between NRR and 10% NRR was performed by movement of the application pipette with the piezo element. The 20–80% rise time in this recording was 150 μs for the onset and 100 μs for the offset. *B*, six superimposed traces of openings of a large-conductance Ca^{2+}-activated potassium channel. The openings of the channel, displayed as upward deflections, were used to trigger a 1 ms solution change between NRR solution and K-NRR solution. Pipette solution was internal solution without EGTA and with 10 μM free Ca^{2+} added. Note that in one of the traces the channel closed before the concentration jump. Holding potential 0 mV. *C*, average of twenty-four traces where the large-conductance potassium channel was open during the solution change. The 20–80% rise time was 250 μs for the onset and for the offset.

normal rat Ringer solution (NRR) and 10% NRR, respectively, and measuring the change in current with an open patch pipette during a solution change. The best application pipettes gave 20–80% rise times of the order of 100 μs, as shown in Fig. 4*A*; many were worse than this, and pipettes were used only if the onset and offset were reasonably symmetrical, and the rise time was better than about 200 μs. The speed of solution outflow, measured by moving the pipette tip to different distances from the application pipette opening, was adjusted to about 100 μm ms^{-1}.

An intact patch will inevitably equilibrate rather more slowly than a bare pipette, so more stringent tests of the performance of the system were performed by applying a pulse of a solution containing a high potassium ion concentration (K-NRR) to an outside-out patch while a large-conductance Ca^{2+}-activated potassium channel was open. The signal resulting from the changed driving force across the open channel

should give a good measure of the rate of concentration change in intact outside-out patches. The membrane potential was held at 0 mV, and the movement of the piezo-controlled application pipette was triggered 0·1 ms after the opening of a potassium

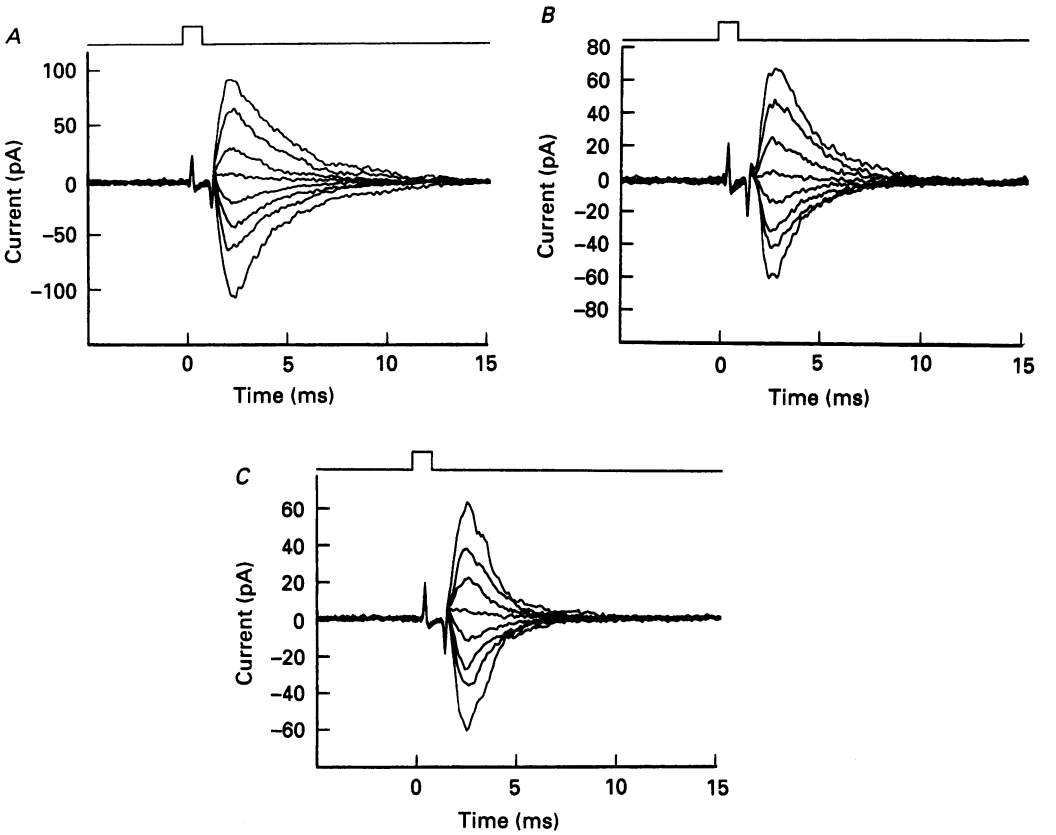

Fig. 5. Records of currents evoked by 1 ms applications of 1 mM glutamate at different membrane potentials in patches from dentate gyrus granule cell (*A*), CA3 pyramidal cell (*B*), and CA1 pyramidal cell (*C*). Single traces, holding potential 0 mV; membrane current was stepped to potentials between −80 and 60 mV (20 mV intervals) 50 ms before the trace was recorded.

channel was detected. Individual traces (superimposed) are shown in Fig. 4*B*; channels that stayed open long enough for the concentration jump to be completed were averaged, and an example is shown in Fig. 4*C*. The mean 20–80 % rise times for onset and offset determined in this way were 204 ± 32 and 233 ± 46 μs, respectively ($n = 9$). The fastest values, about 150 μs, were not much longer than the 20–80 % rise times for the opening and shutting of the potassium channels, which were 80–90 μs, values that indicate the response of the recording system to an essentially square input.

Response to brief (1 ms) pulses of a high concentration (1 mM) of glutamate

Figure 5 shows currents activated by 1 ms pulses of 1 mM glutamate, at various membrane potentials, in outside-out patches isolated from neurones of different hippocampal subfields. The response rises rapidly to a peak, with 20–80 % rise times

as little as 0·15 ms, though more typical values were 0·3–0·6 ms. The time from the first detectable response to the peak response (time-to-peak), though less well defined than the 20–80 % rise time, was about 1 ms for 1 ms pulses. Patches which gave 20–80 % rise times longer than 1 ms were excluded from the analysis. There was no

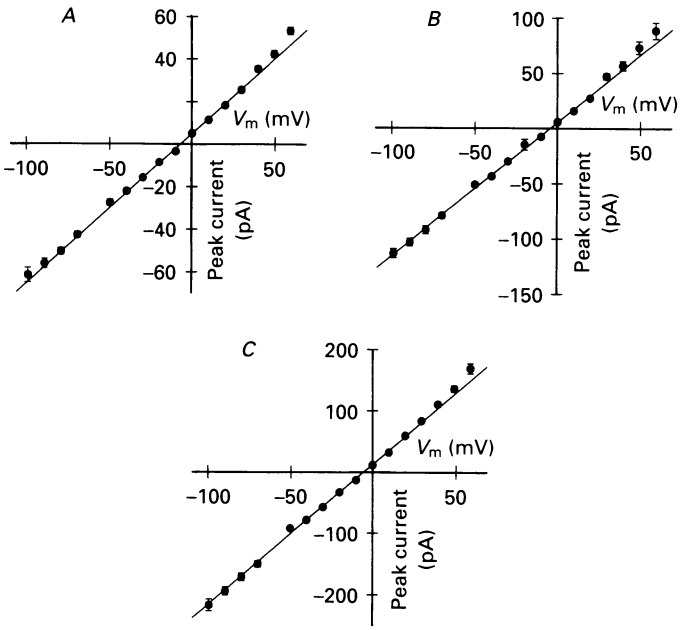

Fig. 6. Peak current–voltage relations in patches from dentate gyrus granule cells (*A*), CA3 pyramidal cells (*B*), and CA1 pyramidal cells (*C*). Currents were evoked by 1 ms pulses of 1 mM glutamate. Current values from different experiments were normalized to the value at −60 mV and averaged for each potential. The average values were then multiplied by the overall mean current at −60 mV. Data points were fitted with a straight line using a weighted least-squares fit. Reversal potentials are −7·1, −4·5, and −5·4 mV. Data pooled from four, five, and three experiments, respectively.

detectable dependence of the rise time on the membrane potential. The concentration of glutamate employed, 1 mM, is expected to produce a near-maximum peak current response (Jonas & Sakmann, 1992*a*). At −50 mV the peak current varied from a few picoamps to about 500 pA in different patches, before decaying with a time constant varying between 1·1 and 4·3 ms ($n = 26$, all cell types, see below).

Because of the fast kinetics, the currents activated by 1 ms pulses of 1 mM glutamate at −50 mV are likely to be entirely mediated by AMPA/KA type GluR channels. Moreover, the currents are blocked by low concentrations of the specific AMPA/KA receptor antagonist CNQX (see below). At more positive membrane potentials, however, some patches in addition to the rapidly decaying component showed a component with slow decay (within hundreds of milliseconds) and small amplitude (a few per cent of the peak current) which was presumably mediated by NMDA receptors. For the investigation of the voltage dependence of peak amplitude and decay time constant, patches with such a slow component were excluded from the analysis.

Peak current–voltage relations of currents activated by 1 ms pulses of 1 mM glutamate

The current–voltage relation (I–V) of the peak of the AMPA/KA component of the EPSC has been reported to be linear in dentate gyrus granule cells (Keller, Konnerth & Yaari, 1991), and CA1 pyramidal cells (Hestrin, Nicoll, Perkel & Sah, 1990), whereas the equilibrium current activated by AMPA or kainate in CA3 or CA1 cell patches is outwardly rectifying (Jonas & Sakmann, 1992*a*). We therefore investigated the I–Vs of the peak current activated by 1 ms pulses of 1 mM glutamate. The membrane potential (V_m) was switched to seventeen potentials (from −100 to +60 mV in 10 mV increments) 50 ms before applying the 1 ms concentration jump. There was usually a 5 s interval between jumps. Preliminary experiments showed that run-down of the responses, though not prominent, was sometimes enough to produce noticeable distortion of the I–V curve if potentials were tested in ascending order. Therefore the order in which the seventeen potentials were tested was randomized, and the computer program that controlled the experiment chose a new random permutation of seventeen for each run. Before averaging I–Vs from different patches, the currents were normalized to the mean current at −60 mV. The averaged normalized responses were then multiplied by the overall mean current at −60 mV, so that the currents shown in Fig. 6 have realistic values. The I–V relations obtained in patches from dentate, CA3, and CA1 cells are illustrated in Fig. 6. They were almost linear, as reported for synaptic currents, though there may be a slight outward rectification at large positive membrane potentials. The reversal potential was close to zero for patches from the three cell types, indicating a similar permeability of the channels for Na^+ and K^+.

Ca^{2+} permeability of GluR channels activated during peak currents

Previous experiments have indicated that the Ca^{2+} permeability of AMPA/KA type GluR channels activated by kainate in CA3 and CA1 neurones is low (Jonas & Sakmann, 1992*a*). In order to find out whether the channels mediating the fast response have a higher Ca^{2+} permeability, we measured the peak response with a high Ca^{2+} concentration on the extracellular face of the membrane patch.

Figure 7 illustrates membrane current traces at different membrane potentials (Fig. 7*A*) and the corresponding I–V relation (Fig. 7*B*) for the peak response to 1 ms pulses of 1 mM glutamate in a CA3 cell patch with Ca^{2+} as the predominant cation on the outer and K^+ on the inner face of the membrane. The I–V relation has an interpolated reversal potential of −49 mV corresponding to a relative permeability $P_{Ca}/P_{Na} < 0{\cdot}1$ (Lewis, 1979). Similar results were obtained for dentate gyrus granule cell patches and CA1 cell patches (Table 1). Three experiments were performed with 100 μM APV in both barrels of the application pipette, and no differences between the results of these experiments and those from experiments without APV were noted. The relative Ca^{2+} permeability for the glutamate-activated peak current was therefore low, as for the equilibrium current activated by kainate (Jonas & Sakmann, 1992*a*).

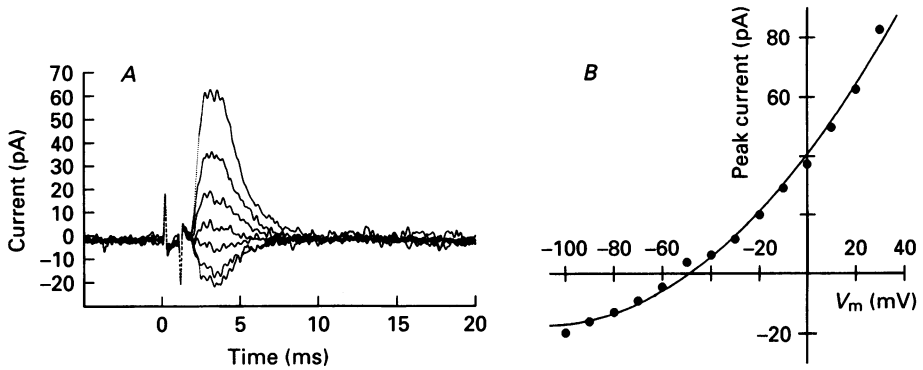

Fig. 7. Ca^{2+} permeability of AMPA/KA type GluR channels in CA3 cell patches during the peak current. *A*, current traces at different potentials, 1 ms pulse of 1 mM glutamate, Ca^{2+} external solution. Membrane potential varied between −100 and 20 mV in 20 mV steps for the traces shown. Single traces. *B*, peak current–voltage relation; data were fitted with a 2nd order polynomial using a least-squares fit. Reversal potential −49 mV. *A* and *B* from the same experiment.

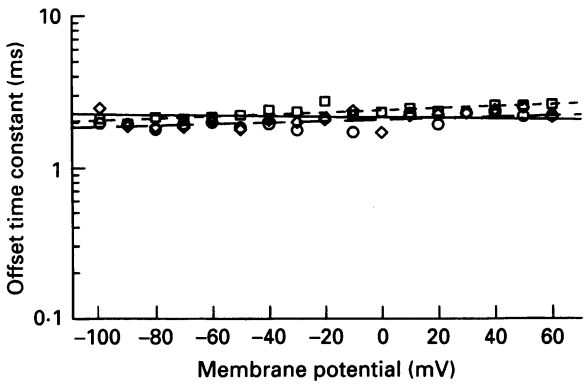

Fig. 8. Offset decay time constants of currents evoked by 1 ms pulses of 1 mM glutamate plotted against voltage. □: dentate gyrus granule cell patches; ○: CA3 pyramidal cell patches; ◇: CA1 pyramidal cell patches. Data pooled from two, four, and three experiments, respectively. The fitted lines have the form $\tau(V) = \tau(0)\exp(V/H)$, where H, the number of millivolts of membrane potential change for an e-fold increase in τ, is 634, 898 and −2250 mV for dentate, CA3 and CA1 cells respectively.

Decay of the current after removal of agonist

When 1 ms pulses of 1 mM glutamate were applied, the time from the first detectable response to the peak was about 1 ms; in other words there was no detectable decline in the response until the agonist was removed. The current decay illustrated in Figs 5 and 9*A* therefore reflects predominantly the random closure of GluR channels after removal of the agonist. The mean decay time constants, referred to hereafter as the *offset* decay time constants, for the currents activated by the 1 ms exposure at −50 mV were 3·0±0·8, 2·5±0·7, and 2·3±0·7 ms (mean±standard deviation) for patches from dentate gyrus granule cells, CA3 pyramidal cells, and

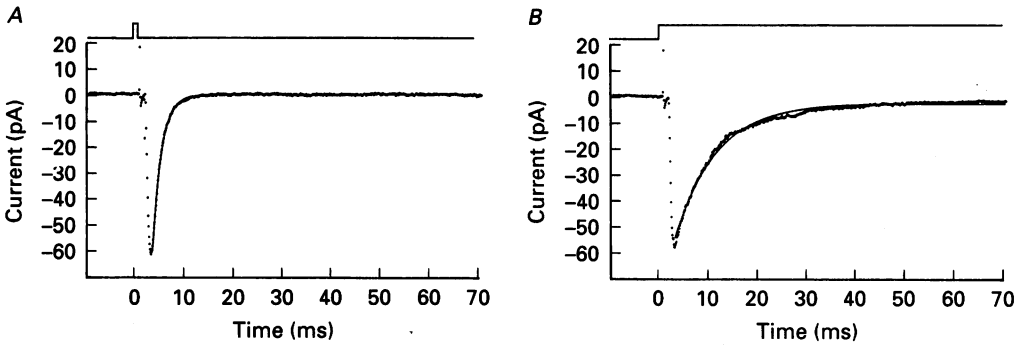

Fig. 9. Comparison of offset decay time constant and desensitization time constant in a CA3 cell patch. *A*, current evoked by a 1 ms application of 1 mM glutamate. The current offset was fitted with a single exponential function, $\tau = 1{\cdot}6$ ms. *B*, current evoked by a 100 ms pulse of 1 mM glutamate. The desensitization phase was fitted with a single exponential, $\tau = 8{\cdot}3$ ms. Five traces averaged, membrane potential −50 mV; data in *A* and *B* from the same experiment.

TABLE 1. Comparison of functional properties of AMPA/KA type GluR channels in patches from dentate gyrus granule cells, CA3, and CA1 pyramidal cells

	Dentate gyrus granule cells	CA3 pyramidal cells	CA1 pyramidal cells
Reversal potential in high Ca^{2+} external solution (mV)	−54·6, −53·7 (2)	−49·0, −50·9 (2)	−47·9, −44·8 (2)
Decay 1 ms 1 mM glutamate pulse (ms)	2·98 ± 0·80 (8)	2·49 ± 0·74 (8)	2·32 ± 0·70 (10)
Decay 100 ms 1 mM glutamate pulse (ms)	9·36 ± 2·70 (9)	11·26 ± 2·75 (5)	9·29 ± 2·82 (7)
Decay 1 ms 200 μM glutamate pulse (ms)	2·64 ± 0·42 (4)	1·82, 1·74 (2)	2·56 ± 0·72 (5)
Decay 100 ms 200 μM glutamate pulses (ms)	10·33 ± 0·71 (3)	n.d.	11·75 ± 2·43 (3)
GluRs desensitized after 1 ms pulse (%)	74 (41*, 33†)	60	53
τ for recovery from desensitization (ms)	33*, 450† (7)	48 (4)	58 (4)
Equilibrium desensitization			
IC_{50} (glutamate, μM)	2·4 ± 0·1	9·6 ± 0·5	4·2 ± 0·3
Hill coefficient	1·1 ± 0·03 (6)	1·2 ± 0·07 (4)	1·1 ± 0·05 (6)
CNQX block			
IC_{50} (CNQX, nM)	183 ± 8	117 ± 11	106 ± 9
Hill coefficient	1·5 ± 0·08 (3)	1·2 ± 0·08 (5)	1·5 ± 0·1 (4)

Number of patches indicated in parentheses. Membrane potential −50 mV. Approximations of the standard errors of the IC_{50} values were obtained from the Hessian matrix of the least-squares algorithm. For further details see text. *Associated with fast, †associated with slow component; n.d., not determined.

CA1 pyramidal cells, respectively. No obvious differences in time course between the patches isolated from different hippocampal cell types were observed (see Table 1). Figure 8 shows the offset time constant obtained with a 1 ms pulse of 1 mM glutamate, plotted against membrane potential for the three cell types. There was no appreciable voltage dependence of the decay time constants for any of the cell types.

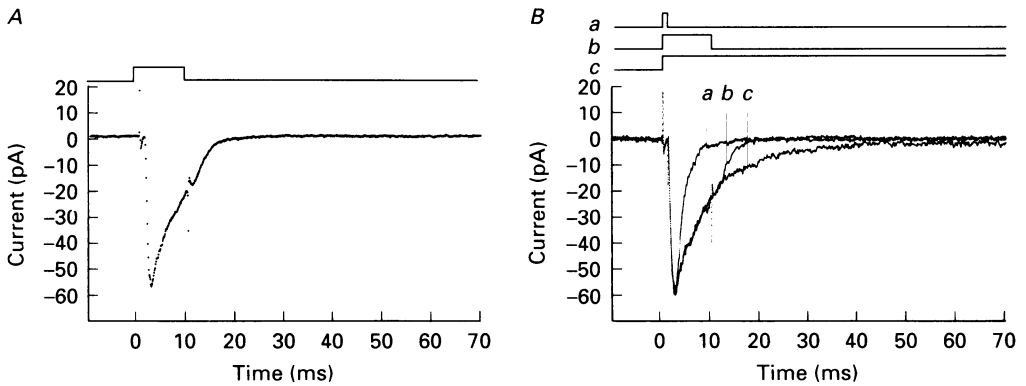

Fig. 10. Decay of current activated by 10 ms pulses of 1 mM glutamate. *A*, membrane current evoked by a 10 ms pulse of 1 mM glutamate; five responses averaged. *B*, single superimposed current traces evoked by 1 ms (*a*), 10 ms (*b*), and 100 ms (*c*) pulses of glutamate. Membrane potential −50 mV; data in *A* and *B* from the same experiment; same patch as Fig. 9. Note that the decay after the end of the 10 ms pulse is faster than the initial decay.

Comparison of channel closure after removal of the agonist with desensitization in the presence of the agonist

Long (100 ms) exposures. Currents activated by 100 ms pulses of 1 mM glutamate were compared with those activated by 1 ms pulses. Figure 9 shows membrane currents from a CA3 cell patch during exposure to 1 mM glutamate for 1 ms (Fig. 9*A*) and 100 ms (Fig. 9*B*) at a membrane potential of −50 mV. The rise time and the peak amplitude of glutamate-activated currents have almost the same value for the two exposure times (rise times of 0·43 and 0·42 ms in this particular experiment). But the desensitization time course, i.e. the decay in the presence of 1 mM glutamate, was considerably slower than the offset time course, i.e. the decay after removal of the agonist (8·3 ms as compared to 1·6 ms in this experiment).

For all three cell types, the offset decay time constant after 1 ms pulses was shorter, by a factor of about four, than that found for long agonist exposure (Table 1). The mean offset decay time constants for the 1 ms pulses were 3, 2·5 and 2·3 ms, whereas the mean desensitization time constants for the 100 ms pulses were 9·4, 11·3, and 9·3 ms, for dentate gyrus granule cells, CA3 pyramidal cells, and CA1 pyramidal cells, respectively. However, the size of the peak current was similar for brief and long pulses of 1 mM glutamate in all cell types.

It was quite often observed, especially for the 100 ms pulses, but sometimes also for the 1 ms pulses, that the decays were fitted slightly better by the sum of two exponential components than by a single exponential. The deviation from a single exponential usually appeared as a slow tail,

usually of low amplitude. However, there was little consistency in the fitted parameters for the slow component (possibly because, in the case of desensitization, the 100 ms pulses were insufficiently long). Therefore the few experiments where the slow component was prominent were excluded from the analysis. In cases where the slow component was small, but clearly needed, the time constant for the faster component was used for the averages given above and in Table 1.

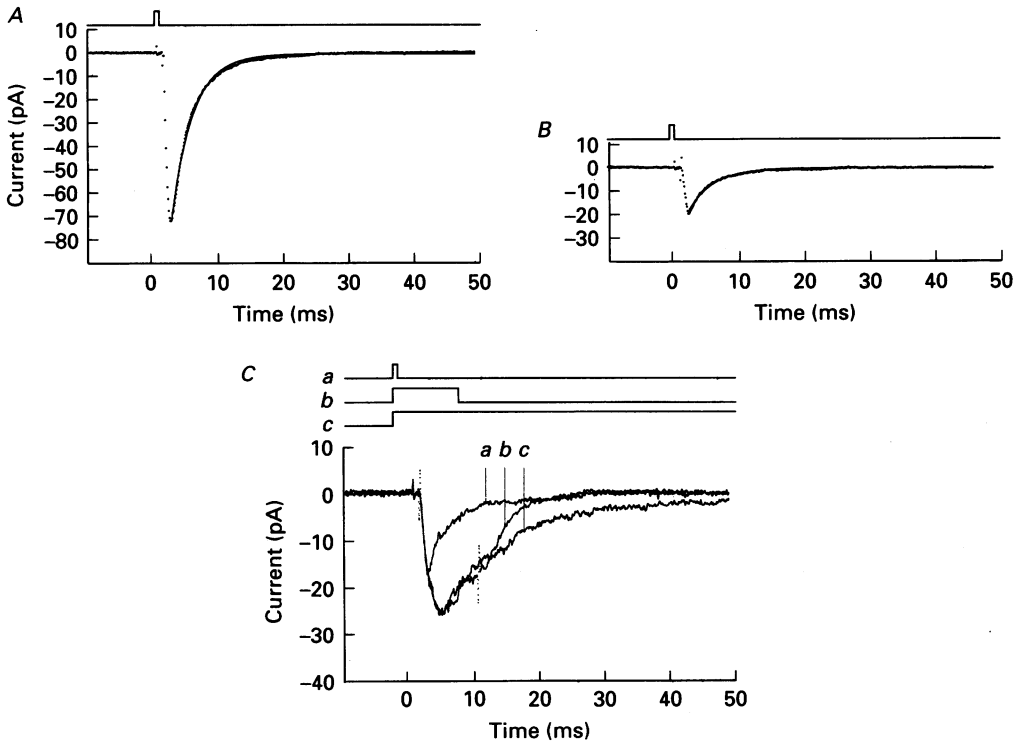

Fig. 11. Offset decay time constants in a CA1 cell patch for different glutamate concentrations. *A*, current evoked by a 1 ms pulse of 1 mM glutamate. The current offset was fitted with an exponential function, $\tau = 3{\cdot}3$ ms. *B*, current evoked by a 1 ms pulse of 200 μM glutamate, $\tau = 3{\cdot}9$ ms. Five responses averaged in *A* and *B*. *C*, current traces evoked by 1 ms (*a*), 10 ms (*b*), and 100 ms (*c*) applications of 200 μM glutamate; single traces. Membrane potential -50 mV; all data from the same experiment.

The equilibrium response to 1 mM glutamate, measured at the end of the 100 ms pulse, was very small, usually around 2% (range 0–5%) of the peak current. This could be an overestimate if there were slower components of desensitization. Such small currents could not be measured with great precision.

Intermediate length (10 ms) exposures. Figure 10*A* shows the response of a CA3 cell patch to a 10 ms pulse of 1 mM glutamate, and Fig. 10*B* shows superimposed responses to 1, 10 and 100 ms pulses. For 10 ms pulses, as expected, the response declined initially (for the first 10 ms) exactly as for 100 ms pulses; when the agonist was removed (at which point the response is about two-thirds desensitized) the decay clearly got faster, the time constant for the offset being much the same as that after 1 ms pulses. These superimposed current traces illustrate most clearly that offset after removal of glutamate was faster than desensitization in the presence of glutamate.

Dependence of responses on agonist concentration

It is of interest to know whether the offset decay time constant depends on the agonist concentration. If there were only one open state, so open and shut times are uncorrelated (e.g. Colquhoun & Hawkes, 1977, 1987), then it would be expected that

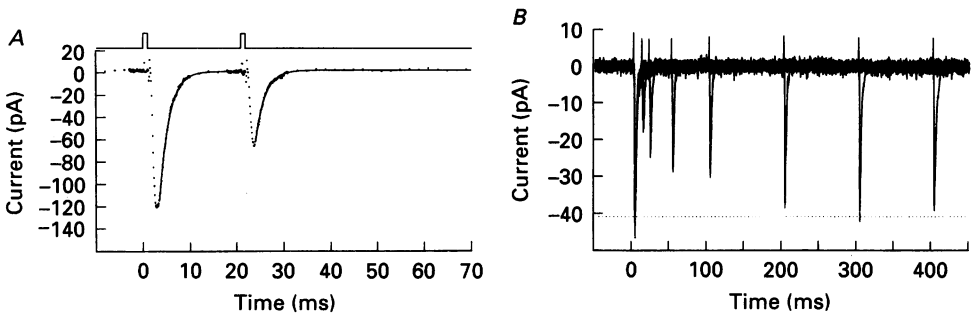

Fig. 12. Current traces from double-pulse experiments on dentate gyrus granule cell patches. *A*, current evoked by two successive 1 ms pulses of 1 mM glutamate separated by a 20 ms gap (single trace). Decay τ of the current evoked by the first and second pulse was 2·0 ms in both cases. Note that the response to the second pulse is smaller than the response to the first pulse. *B*, superimposed traces evoked by two pulses separated by different recovery intervals. Membrane potential −50 mV.

the offset time constant (zero concentration at $t > 0$) would not depend on the concentration of agonist to which the receptors had been exposed previously (at $t < 0$). However, there are, in many systems, correlations between open and shut times; for example they have been shown for nicotinic acetylcholine receptors (Colquhoun & Sakmann, 1985), and for NMDA receptors (Gibb & Colquhoun, 1992). If such correlations exist for AMPA/KA receptors too, then the offset time course might depend on how the system was distributed between the various open and shut states at $t = 0$, and this in turn would depend on the agonist concentration (and, if not equilibrated, on the length of exposure to agonist) before $t = 0$. It is known that AMPA/KA receptors have more than one open state (e.g. Cull-Candy & Usowicz, 1987), so there could, in principle, be a dependence of the offset time course on the agonist concentration.

Responses to 1 mM glutamate were compared with responses to 200 μM glutamate, using 1 ms pulses for both. In earlier experiments with long (300 ms) pulses of glutamate, it appeared that 200 μM produces less than 50% of the maximum response, whereas 1 mM was near-maximal (Jonas & Sakmann, 1992*a*). Results from a CA1 cell patch are shown in Fig. 11. The peak current elicited by 1 ms pulses of 1 mM glutamate (Fig. 11*A*) was much larger than with 200 μM glutamate. It is clearly seen that the decay time constants after the 200 μM glutamate pulse were comparable with those seen following application of 1 mM glutamate (decay time constants are 3·3 ms for 1 mM and 3·9 ms for 200 μM glutamate in this experiment). No significant difference was found between the offset decay time constants measured at the two concentrations in other experiments with the three cell types (Table 1).

Responses to pulses of 200 μM glutamate of different length are shown in Fig. 11*C*. In agreement with a previous investigation (Jonas & Sakmann, 1992*a*), the time constant for onset of desensitization obtained with 100 ms pulses appeared to be only weakly concentration dependent, the onset being slightly slower at the lower agonist

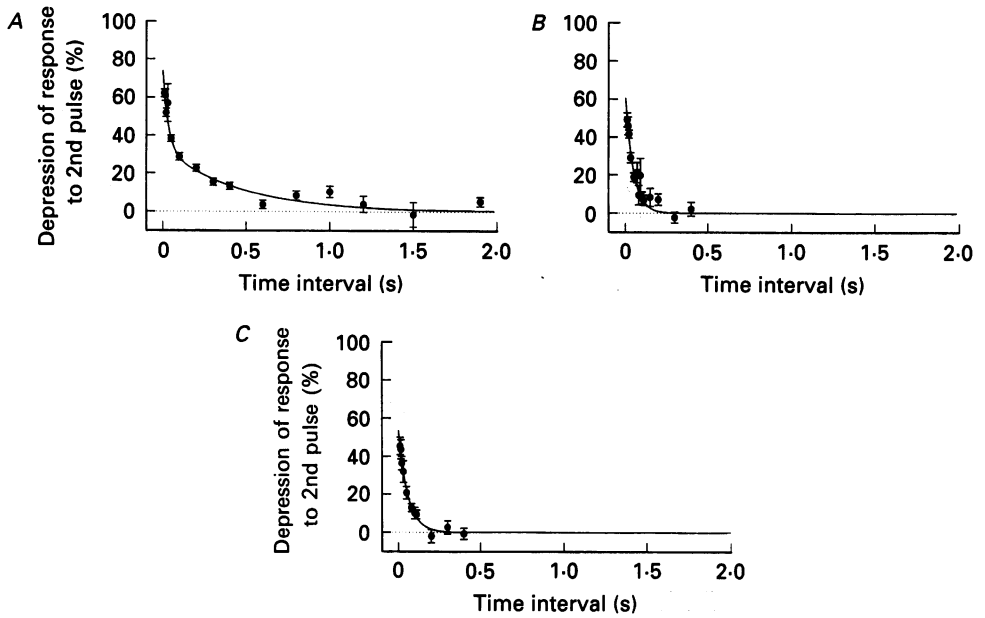

Fig. 13. Time course of recovery from desensitization in patches from dentate gyrus granule cells (*A*), CA3 pyramidal cells (*B*), and CA1 pyramidal cells (*C*). Reduction of peak current amplitude in response to the second as compared to the first pulse was plotted against the interval between the end of the first and the beginning of the second pulse. Note the slower recovery from desensitization in dentate gyrus granule cell patches. Data were fitted with the sum of two exponentials (*A*) or a single exponential function (*B* and *C*). Time constants and amplitudes of the two components were $\tau_1 = 33$ ms, $\tau_2 = 450$ ms and $A_1 = 41\%$, $A_2 = 33\%$ (*A*), and the time constant and amplitude of the single component was $\tau = 48$ ms and $A = 60\%$ (*B*), $\tau = 58$ ms and $A = 53\%$ (*C*). Membrane potential -50 mV: data pooled from seven, four, and four experiments, respectively.

concentration (Table 1). The desensitization rate with 200 μM glutamate was thus substantially slower than the offset rate, as it was with 1 mM glutamate (see Fig. 9). The response to the 10 ms pulse of 200 μM glutamate (Fig. 11*C*) initially superimposed on the response to the 100 ms pulse, and decayed more rapidly after the end of the pulse as for 1 mM glutamate (Fig. 10*B*).

Recovery from desensitization following brief exposure to glutamate

The experiments described above suggest that desensitization is not a major factor in determining the decay rate following brief exposures (1 ms) of patches to 1 mM glutamate. This does not, however, exclude the possibility that a fraction of channels may become desensitized during the 1 ms application. Double-pulse experiments were done to address this question. Figure 12*A* shows the response of a dentate gyrus granule cell patch to two 1 ms pulses of 1 mM glutamate, separated by an interval of

20 ms at a membrane potential of −50 mV. The response to the second pulse has a substantially reduced amplitude. Figure 12*B* shows, for another dentate gyrus granule cell patch, several superimposed current traces obtained with a double-pulse sequence, each with a different interval between the two pulses, the first pulse being

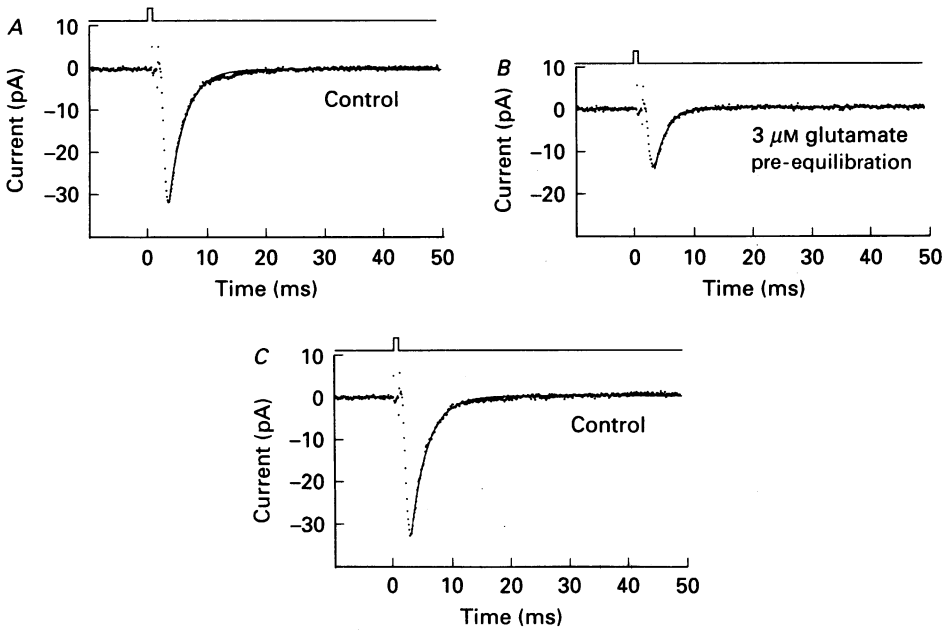

Fig. 14. Current traces of prepulse experiment on dentate gyrus granule cell patch. *A*, response to 1 ms pulse of 1 mM glutamate. *B*, response to the same pulse after pre-equilibration for 15 s with 3 μM glutamate in the control barrel. *C*, recovery of response after removing glutamate from the control barrel. Five responses averaged; membrane potential −50 mV. The decay time constants were 2·77, 2·13 and 2·87 ms, respectively.

at $t = 0$ in each case. The time course of the recovery extended over several hundred milliseconds.

In Fig. 13 the average reduction of the response to the second pulse was plotted against the recovery interval between the two pulses. The curves were fitted with one or two exponentials. Figure 13*A* shows that, in dentate gyrus granule cell patches, a considerable fraction of the channels were desensitized by the first pulse, as estimated by extrapolation of the fitted curve to $t = 0$. The recovery was initially quite fast, but then proceeded more slowly. Two exponential components were needed for an adequate fit; the time constants were 33 and 450 ms, with an amplitude at $t = 0$ of 74% (41% was associated with the fast, and 33% with the slow component).

The recovery from desensitization in CA3 and CA1 pyramidal cell patches is shown in Fig. 13*B* and *C*. The time course of recovery could be fitted satisfactorily by a single exponential for both. For CA3 cell patches the recovery time constant was 48 ms (the amplitude at $t = 0$ being 60%), and for CA1 cell patches the recovery times constant was 58 ms (the amplitude at $t = 0$ being 53%). Thus in CA3 and CA1 cell patches the recovery occurs at a comparable rate to that seen initially in dentate

gyrus granule cells, but the much slower component in the latter was not detectable in CA3 and CA1 cell patches.

Equilibrium desensitization of AMPA/KA glutamate receptor channels

It is possible that the ambient extracellular concentration of glutamate *in vivo* is sufficiently high to cause a functionally important degree of desensitization of AMPA/KA type GluR channels (Benveniste, Drejer, Schousboe & Diemer, 1984; Lerma, Herranz, Herreras, Abraira & Martin del Rio, 1986; Sah, Hestrin & Nicoll, 1989). The channels would, of course, be at equilibrium with the ambient glutamate. The question of how GluR channels might be affected by ambient glutamate was investigated by measuring the effect of pre-equilibration with low glutamate concentrations on the peak response to 1 ms pulses of 1 mM glutamate. At least 15 s was allowed for pre-equilibration with low glutamate concentrations. Preliminary experiments showed this to be more than long enough, as expected from the measured time constants for desensitization. We did not investigate the possibility that there might exist slower components (> 15 s) of desensitization; if such slow components do exist they must have small amplitudes, at least in outside-out patches, because the current remaining at the end of the 100 ms pulse was only a few per cent of the peak current (see above).

A control response to a 1 ms pulse of 1 mM glutamate, in a dentate gyrus granule cell patch, is shown in Fig. 14*A*; after 15 s pre-equilibration with 3 μM glutamate the peak current to the 'test pulse' was reduced substantially (Fig. 14*B*). After removal of glutamate from the control barrel the response recovered completely in this case (Fig. 14*C*). In other cases there was a tendency for the control responses to the 1 ms pulse to decline with time. In such cases the peak control responses were plotted against the time at which the pulse was applied, and the points were fitted, arbitrarily, with polynomials or exponentials; the peak control response expected at the time when a test pulse was applied was interpolated from the fitted curve, and this interpolated control was used to calculate the percentage inhibition after pre-equilibration with the low concentration of glutamate.

The fractional inhibition of peak currents activated by a 1 ms test pulse of 1 mM glutamate plotted against the glutamate concentration used for pre-equilibration is shown in Fig. 15 for the three cell types. The results show that GluR channels in patches from dentate gyrus granule cells were more susceptible to desensitization than for CA3 and CA1 cells, in agreement with the results of the double-pulse experiments. The concentrations of glutamate needed for a 50% reduction of the peak response to 1 ms pulses of 1 mM glutamate were 2·4, 9·6, and 4·2 μM for the dentate, CA3 and CA1 cell patches, respectively, and the Hill coefficients were 1·1, 1·2, and 1.1.

Inhibition by CNQX of peak responses to 1 ms pulses of 1 mM glutamate

The competitive inhibitor of AMPA/KA receptors, CNQX, blocks fast EPSCs in the CNS. Investigations of the action of CNQX have mostly been done under conditions where the agonist and antagonist have been allowed to come to equilibrium, so that equilibrium constants can be estimated (e.g. by the Schild method, see e.g. Verdoorn, Kleckner & Dingledine, 1989), and tests for com-

petitiveness performed. During excitatory synaptic transmission, however, the agonist is present for a short time only and there is unlikely to be time for the system to come to equilibrium, so a competitive antagonist that is normally regarded as freely reversible may behave in an irreversible manner when acting at a synapse, as has been shown at the neuromuscular junction (Pennefather & Quastel, 1981).

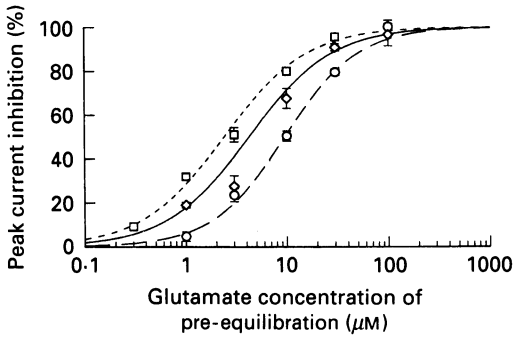

Fig. 15. Equilibrium desensitization. Responses to 1 ms pulses of 1 mM glutamate were measured before and after equilibration (for at least 15 s) with the glutamate concentration specified on the abscissa. Inhibition of the peak current is plotted against glutamate concentration. □: dentate gyrus granule cell patches; ○: CA3 pyramidal cell patches; ◇: CA1 pyramidal cell patches. Curves were drawn according to a weighted least-squares fit with the Hill equation. The IC_{50} values were 2·4, 9·6, and 4·2 μM, and the Hill coefficients were 1·1, 1·2, and 1·1. Membrane potential −50 mV; data pooled from six, four, and six experiments, respectively.

In order to investigate the action of CNQX on GluR channels on a time scale comparable to that of postsynaptic currents, we have measured inhibition of the peak responses to 1 ms pulses of 1 mM glutamate by various concentrations of CNQX. Initial experiments indicated that the extent of block was similar whether (a) CNQX was present only in the control solution, but not in the 1 mM glutamate solution that was applied for 1 ms to elicit the response, or (b) CNQX was present in both solutions (two experiments, data not shown). Moreover, it was found that when the patch was perfused with control solution, and then a 1 ms pulse was applied of a solution containing 1 mM glutamate and 2 μM CNQX, the response was not measurably reduced in comparison with the test responses evoked by 1 mM glutamate alone. It may therefore be concluded that CNQX re-equilibrates slowly compared with the 1 ms time scale of these experiments (and therefore slowly on the time scale of synaptic transmission). All subsequent experiments were done with CNQX present only in the control solution, not in the solution containing the glutamate.

Figure 16 shows the effect of CNQX on the current activated by a 1 ms pulse of 1 mM glutamate on a CA3 cell patch. The control responses before and after application of CNQX are shown in Fig. 16*A* and *C*, respectively, and the response in the presence of 1 μM CNQX is shown in Fig. 16*B*. Between 30 and 60 s was allowed for pre-equilibration with the antagonist. The peak current was almost completely inhibited by 1 μM CNQX. The peak current was plotted against time during wash-

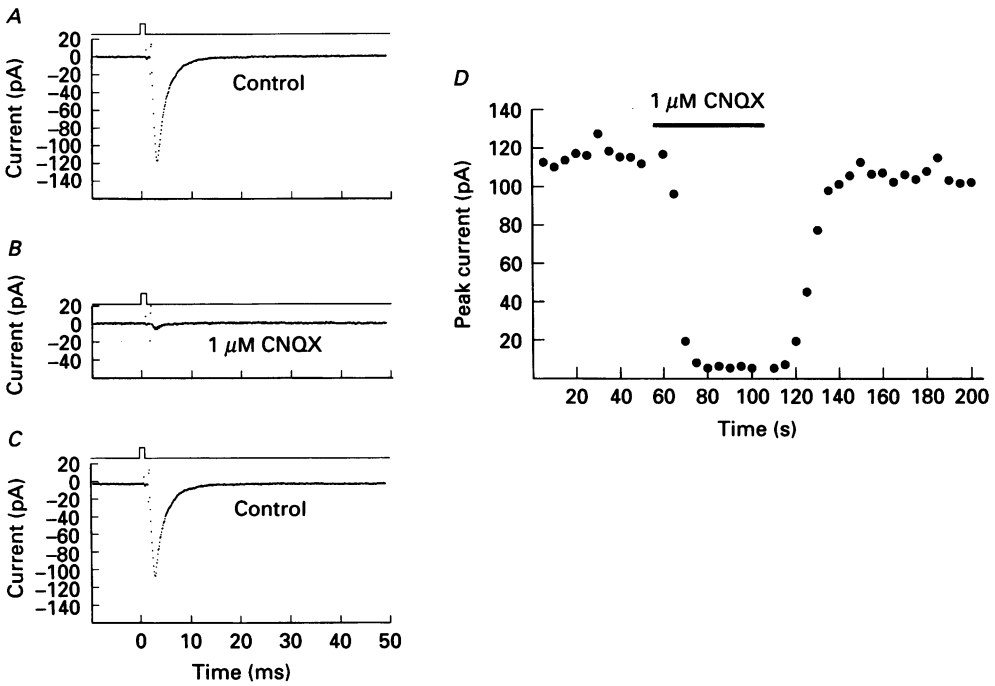

Fig. 16. Blocking effect of CNQX in a CA3 pyramidal cell patch. *A* and *C*, current with 1 ms pulse of 1 mM glutamate without CNQX. *B*, current evoked by a 1 ms pulse of 1 mM glutamate after equilibration with 1 μM CNQX for 30 s in the control barrel. Sequence of recordings *A*, *B*, *C*. *D*, peak current plotted against time during application of 1 μM CNQX. The horizontal bar indicates the time interval during which CNQX was applied. At the time indicated a switch to the reservoir with CNQX-containing solution was made. The delay between a switch and the solution change at the tip of the pipette is about 10 s. Five responses averaged; membrane potential −50 mV; all data from the same experiment and the same drug application.

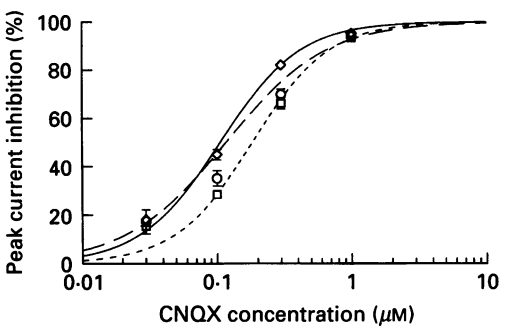

Fig. 17. CNQX dose–response curves. Inhibition of the peak current plotted against concentration of CNQX. □: dentate gyrus granule cells; ○: CA3 pyramidal cells; ◇: CA1 pyramidal cells. Curves show the weighted least-squares fit with the Hill equation. The IC_{50} values were 183, 117, and 106 nM, and the Hill coefficients were 1·5, 1·2, and 1·5. Membrane potential −50 mV; data pooled from three, five, and four experiments respectively.

in and wash-out of 1 μM CNQX in Fig. 16*D* and shows that the inhibitory effect of CNQX on the peak current developed rapidly and was almost entirely reversible upon washing in this experiment. A decrease of peak current with time in other experiments was allowed for by interpolation of the control values (see above).

The CNQX dose–response curves for patches from the three cell types at a membrane potential of -50 mV are shown in Fig. 17. The concentrations for 50% peak current inhibition (IC_{50}) were 183, 117, and 106 nM for dentate, CA3 and CA1 cells, respectively (see Table 1), and the Hill coefficients were between 1·2 and 1·5. The fact that nanomolar CNQX concentrations inhibited the currents activated by 1 ms pulses of 1 mM glutamate at negative membrane potentials indicated that these were almost entirely mediated by GluR channels of the AMPA/KA type.

DISCUSSION

The main aim of this study was to mimic EPSCs in CNS synapses by applying very brief pulses of glutamate to membrane patches, and to investigate activation, desensitization and antagonism of glutamate responses under controlled conditions. The results can cast some light on the time course of excitatory transmitter action at central synapses, and on the possible subunit structure of synaptic AMPA/KA receptors.

The response to brief glutamate pulses

Solution changes in intact patches. The performance of the application system on intact patches was tested by applying a brief pulse of high-potassium solution while a large-conductance Ca^{2+}-activated potassium channel was open (a method used, with long pulses, by Trussell & Fischbach, 1989). The responses to application and removal of the high-potassium solution were symmetrical, equilibration taking a few hundred microseconds, so roughly rectangular 1 ms applications were possible.

The rising phase. The 20–80% rise times for responses to 1 ms pulses of 1 mM glutamate were not greatly different from the rise times found for the 1 ms K^+-concentration jumps on open potassium channels (i.e. $< 0{\cdot}5$ ms). The rising phase of the responses evoked by 1 mM glutamate is therefore likely to be almost entirely determined by the application system, rather than by the rate at which glutamate interacts with the receptor. At the lower glutamate concentration, 200 μM, the rise time was little different for the 1 ms pulses, but this is largely because the activation process does not get near to equilibrium within 1 ms (Fig. 11*C*). The responses to longer (10 and 100 ms) pulses of 200 μM glutamate have longer rise times and longer times-to-peak than with 1 mM glutamate, so the slower rate of receptor interaction at the lower concentration is detectable, though probably still distorted by diffusion of the agonist.

The peak height. The peak current amplitudes for brief and long pulses are almost identical with 1 mM glutamate, whereas the peak current for brief pulses as compared to long pulses was much smaller with 200 μM glutamate. This indicates that the activation process does not reach equilibrium within less than 1 ms with 200 μM glutamate. The peak currents evoked by brief pulses of glutamate showed a substantial amount of variability, which made averaging necessary. Part of this

variability might be stochastic variability in the number of channels opened during each pulse.

The offset decay rate. The mean values of the offset time constant are remarkably similar for experiments in the three different hippocampal cell types, with mean values between 2·3 and 3·0 ms. The mean time constants are almost independent of membrane potential and the offset decay rates were independent of the concentration of glutamate (1 mM or 0·2 mM) during the pulse. Nevertheless some consistent variation of the value of the offset decay time constant was observed between different patches. Repeated 1 ms pulses of 1 mM glutamate on some individual patches consistently gave, for example, time constants of around 1 ms, whereas other patches consistently gave values of around 3 ms. The source of this variability is as yet unclear.

The response to long glutamate pulses: onset rate of desensitization

The time constant for the onset of desensitization is similar in the three hippocampal areas, with mean values between 9·3 and 11·3 ms. In the three hippocampal areas, the mean desensitization time constants are thus about four times longer than the mean offset time constants. Similarly, for glutamate-gated channels of crayfish muscle the decay after a brief pulse is faster as compared to a long pulse of glutamate (Dudel *et al.* 1990*a*). In contrast, the decays of currents activated by brief and long pulses of glutamate almost superimposed in cultured chick spinal neurones (Trussell & Fischbach, 1989). This suggests either a species difference or a different expression pattern of GluR channel subunits in spinal neurones, though this remains to be demonstrated in *in situ* hybridization experiments. The extent of desensitization at equilibrium was substantial; the response at the end of a 100 ms pulse of 1 mM glutamate was only a few per cent of the peak response at most.

The results reported here were from outside-out patches. It has been reported that desensitization is more pronounced in outside-out patches than in intact cells both for NMDA receptors (Sather, Dieudonné, MacDonald & Ascher, 1992), and for neuronal nicotinic acetylcholine receptors in chromaffin cells (Inoue & Kuriyama, 1991). It is not known whether this phenomenon occurs also with AMPA/KA receptors.

Comparison of responses to brief pulses with EPSCs

Rise and decay rates, and the persistence of transmitter. In many areas of the brain the dendritic location of synapses has made it difficult or impossible to achieve voltage clamp conditions good enough to measure reliably the time course of the EPSCs. Using patch clamp techniques in brain slices, well-clamped EPSCs were recorded, for example, in stellate cells of rat visual cortex (Stern, Edwards & Sakmann, 1992), and in rat cerebellar granule cells (Silver, Traynelis & Cull-Candy, 1992). In these cases EPSCs with rise times of 0·2 ms, and decay time constants of about 2·4 or 1·3 ms, respectively, have been found, and these values are very similar to those observed here with 1 ms pulses of 1 mM glutamate reported here under similar experimental conditions. This suggests that in visual cortex stellate cell and cerebellar granule cell synapses the decay rate of EPSCs may reflect primarily the

distribution of channel lifetimes after removal of the agonist. In other words, it seems likely that in these synapses transmitter is present in the synaptic cleft for a short time only (of the order of a millisecond or less), and that they may resemble the neuromuscular junction in this respect (Anderson & Stevens, 1973). For synapses of the trisynaptic hippocampal circuit, however, both slower rise times and slower decay time constants have been reported. The rise times reported are 0·5–1·9 ms for dentate gyrus granule cells (Keller *et al.* 1991), 0·3–1·5 ms for CA3 pyramidal cells (Jonas & Sakmann, 1992*b*), and 1·1–4·2 ms for CA1 cell synapses (Hestrin *et al.* 1990). The corresponding decay time constants vary considerably between different individual cells, being 3–9, 4–10, and 4–8 ms in dentate, CA3, and CA1 cells, respectively. The conditions are not favourable for voltage clamp of (especially) dentate and CA1 synapses, so these results are likely to be somewhat too slow. The mossy fibre synapses on CA3 pyramidal cells are, however, localized relatively close to the cell soma (electrotonic distance 0·006–0·06, Johnston & Brown, 1983), and they show the fastest rise of all synapses of the trisynaptic circuit, but they also have a relatively slow decay (Jonas & Sakmann, 1992*b*), intermediate between the offset and desensitization time constants determined in this paper. This could imply that in mossy fibre synapses on CA3 pyramidal cells the transmitter may remain in the synaptic cleft for a longer time. Alternatively, it is also possible that postsynaptic and extrasynaptic receptors differ in their functional properties.

Voltage dependence of peak current and offset decay. The finding that the offset time constant of currents activated by brief pulses of glutamate was essentially independent of membrane potential is consistent with reports that the decay rate of fast EPSCs, mediated by AMPA/KA receptors, is not voltage dependent (Hestrin *et al.* 1990; Keller *et al.* 1991). Moreover, the peak current amplitude evoked by glutamate pulses varies linearly with membrane potential, and the same is true for the peak of EPSCs in hippocampal synapses (Hestrin *et al.* 1990; Keller *et al.* 1991; Jonas & Sakmann, 1992*b*). On the other hand the equilibrium current activated by AMPA or kainate is outwardly rectifying in CA3 and CA1 pyramidal cell patches (Jonas & Sakmann, 1992*a*). The reason for this difference in rectification is not known. It could indicate that one of the steps to the desensitized state(s) of the AMPA/KA receptor channels are voltage dependent. Alternatively, different subtypes of AMPA/KA receptor channels could mediate the early and the late components of glutamate-activated currents.

Block by CNQX. The IC_{50} values for the inhibition of the peak response to 1 pulses of 1 mM glutamate were between 183 and 106 nM for the different cell types. If essentially no dissociation of CNQX occurs during a 1 ms glutamate pulse, as our results suggest, therefore if receptors are identical and independent, the IC_{50} should be close to the equilibrium constant for CNQX binding. At the other extreme, if agonist were applied for long enough for complete equilibration to occur between receptor, CNQX, and glutamate, the antagonist would appear less potent in reducing the equilibrium response. Estimates of the equilibrium constant (K) for CNQX binding found by Schild analysis are rather variable, but, in cases where the slope of the Schild plot was shown to be near 1, values of $K = 0{\cdot}49$ μM (for cerebellar granule cells; Wyllie, 1992) and $K = 0{\cdot}3$ μM (for receptors expressed in oocytes from whole rat brain RNA; Verdoorn *et al.* 1989) have been reported. These values are larger than,

but not grossly different from, our IC_{50} values. Two studies on hippocampal cells have suggested rather larger K values, but in neither case was validity of the estimate demonstrable: Blake, Yates, Brown & Collingridge (1989) found $K = 1{\cdot}4\ \mu M$ in the CA1 subfield of slices (but the slope of the Schild plot was less than 1 in these experiments), and Yamada, Dubinski & Rothman (1989) found $K = 2{\cdot}5\ \mu M$ in cultured hippocampal cells (but only one concentration was tested, so no validity test was possible). The IC_{50} of 350 nM for the inhibition of the AMPA/KA component of mossy fibre EPSCs in CA3 pyramidal cells (P. Jonas & B. Sakmann, in preparation) is also slightly higher than the IC_{50} values for the currents activated by brief glutamate pulses. However, the interpretation of the IC_{50} of a competitive antagonist on an intact synapse depends on many factors, such as receptor density, length of the transmitter action, etc. (Pennefather & Quastel, 1981) that are not known for glutamatergic synapses.

Calcium permeability. The experiments reported here show that the Ca^{2+} permeability of GluR channels mediating the fast peak current activated by 1 ms pulses of 1 mM glutamate is low ($< 0{\cdot}1$) but not negligible. In all three cell types the shift in reversal potential upon switching from high Na^+ to high Ca^{2+} extracellular solution is about -50 mV, very similar to the shift seen in equilibrium currents activated by kainate in CA3 and CA1 cell patches (Jonas & Sakmann, 1992*a*). The fact that there is a measurable Ca^{2+} permeability might indicate that Ca^{2+} entry into a cell through AMPA/KA type GluRs may occur during fast excitatory synaptic transmission.

Recovery from desensitization following brief pulses

Even a pulse of 1 mM glutamate as brief as 1 ms may produce substantial desensitization (about 60% when extrapolated back to the time at which the pulse was applied). This suggests that a fraction of the receptors in the patch are already desensitized at the peak of the response, the amplitude of which may therefore be attenuated by desensitization. This observation also leads to the conclusion that the offset rate could, to some extent, itself by influenced by desensitization; this could happen if, for example, channels that were desensitized at the peak had to return via the open state(s) before returning to the resting state. The recovery from desensitization reported here after 1 ms pulses is substantially slower than that reported for chick spinal neurones (Trussell & Fischbach (1989) found a time constant of about 10 ms) and crayfish muscle (Dudel *et al.* (1990*a*) found complete recovery within less than 10 ms). On the other hand, GluR channels with different functional properties seem to be present in locust muscle, and the time intervals necessary for complete recovery from desensitization varied between tens of milliseconds and seconds (Dudel, Franke, Hatt, Ramsey & Usherwood, 1990*b*).

Depression of excitatory synaptic transmission may occur at high firing frequencies of the presynaptic neurone, but it is controversial whether this is attributable to presynaptic or postsynaptic factors. In the hippocampus, the amplitude of the excitatory postsynaptic potential decreases with increasing frequency of stimulation in dentate gyrus granule cells synapses, but increases in CA3 and CA1 pyramidal cell synapses (Alger & Teyler, 1976). In the light of our results, it is possible that these cell-specific differences of excitatory synaptic transmission in the three hippocampal

subfields are caused by differences in recovery of postsynaptic AMPA/KA type GluR channels from desensitization. It seems likely that desensitization of postsynaptic AMPA/KA receptor channels at high stimulation frequencies may contribute to synaptic depression, at least in dentate gyrus granule cells.

Desensitization at equilibrium

Equilibration with glutamate concentrations below 1 μM can produce a reduction in the amplitude of the peak current evoked by the brief pulse of 1 mM of this agonist. This is in agreement with previous data for cultured hippocampal neurones obtained with much slower application on whole cells (Kiskin, Krishtal & Tsyndrenko, 1986). The ambient glutamate concentration in the cerebrospinal fluid is thought to be between 1 and 3 μM (Benveniste *et al.* 1984; Lerma *et al.* 1986). The same conclusion has been reached by consideration of the characteristics of the glutamate uptake mechanism (Nicholls & Attwell, 1990). Moreover, it has been demonstrated that in brain slices the ambient glutamate concentration is sufficiently high to activate NMDA receptors (Sah *et al.* 1989). Since our results suggest that 50% of receptors may be desensitized, at equilibrium, by 2–10 μM glutamate, it is possible that the ambient concentration of glutamate might be a relevant factor in setting the efficacy of synaptic transmission, especially in dentate gyrus granule cells, which were affected by lower concentrations than CA3 or CA1 cells. It could be possible, for example, that in ischaemic conditions where the extracellular potassium concentration is raised, the effect of this on the glutamate uptake carrier mechanism could cause the ambient glutamate concentration to rise (Benveniste *et al.* 1984; Nicholls & Attwell, 1990). It is tempting to speculate that either changes in the concentration of ambient glutamate or shifts of the dose–response curves for equilibrium desensitization could cause long-term changes in synaptic efficacy.

Implications of results for the subunit structure of AMPA/KA receptor channels

In situ hybridization experiments indicated that some subunit-specific AMPA/KA receptor transcripts are expressed ubiquitously, whereas others show a differential expression pattern in the subfields of rat hippocampus (Hollmann *et al.* 1989; Boulter *et al.* 1990; Keinänen *et al.* 1990; Nakanishi *et al.* 1990; Sommer, Keinänen, Verdoorn, Wisden, Burnashev, Herb, Köhler, Takagi, Sakmann & Seeburg, 1990; Bettler, Boulter, Hermans-Borgmeyer, O'Shea-Greenfield, Deneris, Moll, Borgmeyer, Hollmann & Heinemann, 1990; Egebjerg *et al.* 1991). Several of the properties of native GluR channels that we have measured are essentially the same in dentate gyrus granule cells, in CA3, and in CA1 pyramidal cells. The offset decay, the desensitization time constants, and the sensitivity to CNQX are similar in the three cell types. In particular, the linear peak I–V relation and the low Ca^{2+} permeability of the GluR channels mediating the fast peak current are consistent with our previous suggestion that the GluR-B (or GluR-2) subunit, expressed in all subfields of the trisynaptic pathway, dominates the conductance properties of the native AMPA/KA receptor channels (Hollmann, Hartley & Heinemann, 1991; Jonas & Sakmann, 1992*a*; Burnashev, Monyer, Seeburg & Sakmann, 1992).

Cell-specific differences were observed in the desensitization properties of native AMPA/KA type GluR channels. The recovery from desensitization by 1 ms pulses

is slower in dentate gyrus granule cells than in CA1 and CA3, and the equilibrium desensitization, for a given glutamate concentration, is more pronounced in dentate gyrus granule cells than for the other cell types. Although many properties of the recombinant GluR channels remain to be determined, the differences could be related either to the fact that the mRNA for the GluR-D (or GluR-4) subunit and the GluR-6 subunit are predominantly expressed in the dentate gyrus, and only to a smaller extent in the other subfields (Keinänen *et al.* 1990; Egebjerg *et al.* 1991). The differences could be also due to the reported preponderance of the alternatively spliced *flop* forms of the AMPA/KA receptors in the dentate gyrus and CA1 region (Sommer *et al.* 1990). It is possible that the neuronal cell types in the trisynaptic circuit of the hippocampus set synaptic efficacy in a cell-specific way by differential expression of GluR subunits and assembly of these subunits into native GluR channels with different desensitization properties.

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