Increases in Effective Cleft Glutamate Concentration During Expression of LTP

Su Hyun Jung and Sukwoo Choi

Department of Neuroscience, Ewha Institute of Neuroscience (EIN), School of Medicine, Ewha Womans University, Seoul 110−783, Korea

Long-term potentiation (LTP) at hippocampal CA3-CA1 synapses is often associated with increases in quantal size, traditionally attributed to enhanced availability or efficacy of postsynaptic glutamate receptors. However, augmented quantal size might also reflect increases in neurotransmitter concentration within the synaptic cleft since AMPA-type glutamate receptors are not generally saturated during basal transmission. Here we report evidence that peak cleft glutamate concentration ($[glu]_{cleft}$) increases during LTP, as indicated by a lessening of the blocking effects of rapidly unbinding antagonists of AMPA. The efficacy of slowly equilibrating antagonists remained unchanged. The elevated $[glu]_{cleft}$ helps support the increased quantal amplitude of AMPA-type EPSCs (excitatory postsynaptic currents) during LTP.

Key Words: Synapse, Glutamate, LTP, EPSC, Quantal size

INTRODUCTION

LTP is thought to be a cellular substrate for learning and memory, and much attention has been paid to elucidate mechanisms underlying LTP induction and expression (Bliss & Collingridge, 1993). Especially, there has been a matter of debate concerning the site of LTP expression (presynaptic vs. postsynaptic). One major source for such debate has been controversial experimental results generated by the analysis of quantal synaptic responses (i.e. synaptic responses in response to the fusion of single vesicles) during LTP. Increases in quantal size, thought to be enhanced activity of postsynaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate receptors, have been reproducibly observed (Bekkers & Stevens, 1990; Malinow & Tsien, 1990; Kullmann & Nicoll, 1992). By contrast, increases in quantal frequency, an evidence for enhanced release probability from presynaptic terminals, have been also detected (Bekkers & Stevens, 1992; Kullmann & Nicoll, 1992; Malinow & Tsien, 1992). However, some studies (Malgaroli & Tsien 1992; Stevens & Wang, 1994) showed increases only in quantal frequency, but not in quantal size, thereby initiating the pre vs. post debate. A group of researchers who support the postsynaptic view then proposed postsynaptically 'silent synapse' hypothesis that silent synapses with only NMDA receptors, functionally silent at hyperpolarized membrane potential, gain AMPA receptors after LTP induction. Theoretically such a postsynaptic change could explain the increase in quantal frequency with LTP. There is now reasonably strong evidence to support several aspects of the postsy-

naptically silent synapse hypothesis (Shi et al, 1999), although alternative 'presynaptically silent synapse' hypotheses have been proposed (Kullmann & Asztely, 1998; Choi et al, 2000). Setting aside the issue of altered quantal frequency, no one has put doubts on a postsynaptic origin for altered quantal size. However, enhanced quantal size would be also produced by increases in presynaptic glutamate release if postsynaptic AMPA-type glutamate receptors were not saturated.

There are several reasons why changes in cleft transmitter concentration have been generally overlooked as a possible mechanism for LTP (but cf. Bliss & Collingridge, 1993; Perkel & Nicoll, 1993): First, the quantum has often been regarded as a fixed quantity of transmitter, even though the term was originally coined to express the idea of a unitary packet whose size or effectiveness might vary (Katz, 1969). Second, the prevailing view of excitatory transmission in the CNS has held that postsynaptic receptors are saturated even during basal transmission (Tang et al, 1994; Tong & Jahr, 1994; but cf. Frerking & Wilson, 1996; Silver et al, 1996). However, the recent study by Liu et al (1999) strongly suggested that postsynaptic AMPA-type glutamate receptors were not saturated. Third, there has been no practical method up until recently to monitor changes in peak transmitter concentration sensed by postsynaptic receptors within the cleft. One way to assess relative changes in $[glu]_{cleft}$ is to rely on quantitative measurements of the blocking effects of a low affinity, fast unbinding antagonist (Tong & Jahr, 1994; Diamond & Jahr, 1997; Liu et al, 1999). Principles of mass action predict that the higher the cleft transmitter concentration, the milder the inhibition by a fast unbinding antagonist; not so for a slow unbinding antagonist, since transmitter can only

Corresponding to: Sukwoo Choi, Department of Neuroscience, School of Medicine, Ewha Womans University, Ewha Dong-Daemoon Hospital, 70 Jongno 6-ga, Jongno-gu, Seoul 110-783, Korea. (Tel) 82-2-760-5504, (Fax) 82-2-760-5524, (E-mail) sukwoo@mm. ewha.ac.kr

ABBREVIATIONS: LTP, long-term potentiation; EPSC, excitatory postsynaptic current; CNS, central nervous system.

bind and activate those receptors unoccupied at the time of release, regardless of its cleft concentration (Clements et al, 1992; Tong & Jahr, 1994; Diamond & Jahr, 1997; Clements et al, 1998; Liu et al, 1999). This pharmacological approach was developed at synapses between cultured hippocampal neurons and verified by theoretical simulations and tests with iontophoretic application of glutamate to individual postsynaptic sites (Liu et al, 1999). In the present study, we tested a possibility that the augmented quantal size during LTP could be due to increases in $[glu]_{cleft}$ using the fast unbinding antagonist approach.

METHODS

Brain slices

Rats (5~12 d-old, mainly 8~12 d-old) were sacrificed by decapitation and the brains were cooled in ice-cold, modified artificial CSF (aCSF) containing (in mM): sucrose, 194; NaCl, 20; KCl, 3.5; $MgCl_2$, 1.3; NaH_2PO_4, 1.25; $NaHCO_3$, 26; glucose, 11 and gassed with 95% O_2/5% CO_2 at room temperature. Transverse hippocampal slices (300 mm) were cut in ice-cold modified aCSF using an automated vibroslicer. Slices were then transferred onto the recording chamber, submerged and superfused continuously at 3~4 ml/min with aCSF containing (in mM): NaCl, 120; KCl, 3.5; $MgCl_2$, 1.3; $CaCl_2$, 2~2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; glucose, 11; picrotoxin, 0.1, and gassed

with 95% O_2/5% CO_2 at room temperature. A cut was made between regions CA3 and CA1 to prevent epileptiform activity.

Electrophysiology

Whole-cell voltage-clamp recordings were made under differential interference contrast (DIC)-enhanced visual guidance from neurons 3~4 cell layers below the surface of slices at room temperature. Synaptic transmission was elicited at frequencies of 0.1~1 Hz (mainly 1 Hz) with monopolar stimulating electrodes (5 MΩ, A-M System, WA) positioned on stratum radiatum at a similar distance from stratum pyramidale. EPSCs were recorded with a patch electrode (2~4 MΩ tip resistance, no fire polishing or Sylgard coating) in the whole cell recording mode (Axopatch 1D). Pipette solution contained (in mM): Cesium gluconate, 100; EGTA, 0.6; HEPES, 10; CsCl, 10; NaCl, 5; TEAOH, 20; Mg-ATP, 4; Na-GTP, 0.3 (pH 7.3 with CsOH). The series resistance, which was not compensated, was typically within 10 MΩ and was monitored continuously using a current pulse generated with a 2 mV voltage command. Recordings showing >20% change in series resistance were discarded. Minimal stimulation, only slightly stronger than the highest stimulus that gave all failures, was implemented, although there was no certainty of stimulating a single input axon. We used recordings showing a uniform onset latency and a relatively constant potency of EPSCs. Furthermore, to acquire a reasonable number of EPSCs and
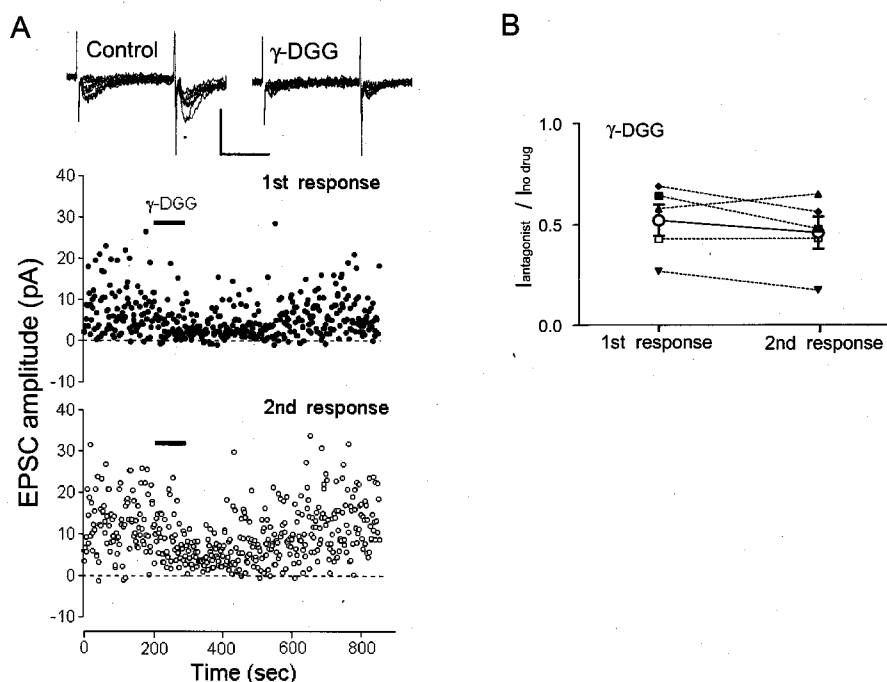

Fig. 1. The degree of inhibition by γ-DGG does not change significantly during paired-pulse facilitation of synaptic transmission (PPF). A, Representative experiment, comparing degree of inhibition by γ-DGG (500 μM) of first and second AMPA EPSCs in response to paired-pulse stimulation. Degree of inhibition is not significantly altered by PPF. Vertical bar, 25 pA; horizontal bar, 25 ms. B, Summary of 5 experiments of the kind shown in A. Proportion of AMPA EPSC amplitude remaining after γ-DGG inhibition in first or second response to paired stimulation. The degree of inhibition of AMPA EPSCs by g-DGG was not significantly altered with PPF (p> 0.2). PPF of AMPA EPSCs averaged $156 \pm 13\%$ of basal transmission.

allow reliable estimation of average EPSC amplitude values over a short time period (30~60 s), we generally avoided using recordings which contained failures in more than 70% of trials. Stimulation frequency was maintained constant throughout the entire experiment, without interruption in all experiments except that shown in Fig. 3f. LTP was induced by depolarizing a postsynaptic neuron to 0 mV while stimulation was continued at the same rate and intensity over a 60~120 sec period, unless otherwise noted. EPSCs were filtered at 1 kHz, digitized at 5 kHz and stored in a microcomputer.

Data analysis

AMPA receptor-mediated EPSC amplitudes recorded at −60 mV were determined by averaging the current over a 1~2 ms window at the peak responses and subtracting a baseline estimate from the same record. NMDA receptor-mediated EPSC amplitudes recorded at +40 mV were estimated by averaging the current over a 5~10 ms window 30~40 ms after the peak and subtracting a baseline estimate from the same record. A sharply-defined change in superfusing solutions was achieved by means of a three-way stop cock that switched between inflow of normal and drug-containing aCSF. Thus, the duration of antagonist application could be kept constant during repeated trials. To gauge the degree of antagonist inhibition, the

proportion of synaptic current remaining after application of antagonist was estimated as the ratio of the average EPSC amplitude in 20~60 trials just after the end of antagonist application and the average EPSC amplitude in an equal number of trials just before the start of antagonist application. All parameters for such determinations were kept constant for repeated antagonist applications in a given experiment. Control experiments with CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) (Fig. 2) confirmed the stability of measurements of antagonist inhibition with our solution exchange system. The concentrations of antagonists were chosen so as to produce ~50% inhibition of EPSCs. All averaged values are given as mean±SEM and were compared statistically by paired t-test (criterion for significance, $p < 0.05$).

RESULTS

To be most useful as an index of changes in $[glu]_{cleft}$ during synaptic plasticity at individual synapses, the inhibition by fast-off rate antagonists should not be confounded by changes either in vesicular release probability (P_r) or in the functional capability of glutamate receptors. The possible influence of altered P_r was examined by induction of paired-pulse facilitation (PPF), a well-accepted means of producing robust increases in P_r. When EPSCs
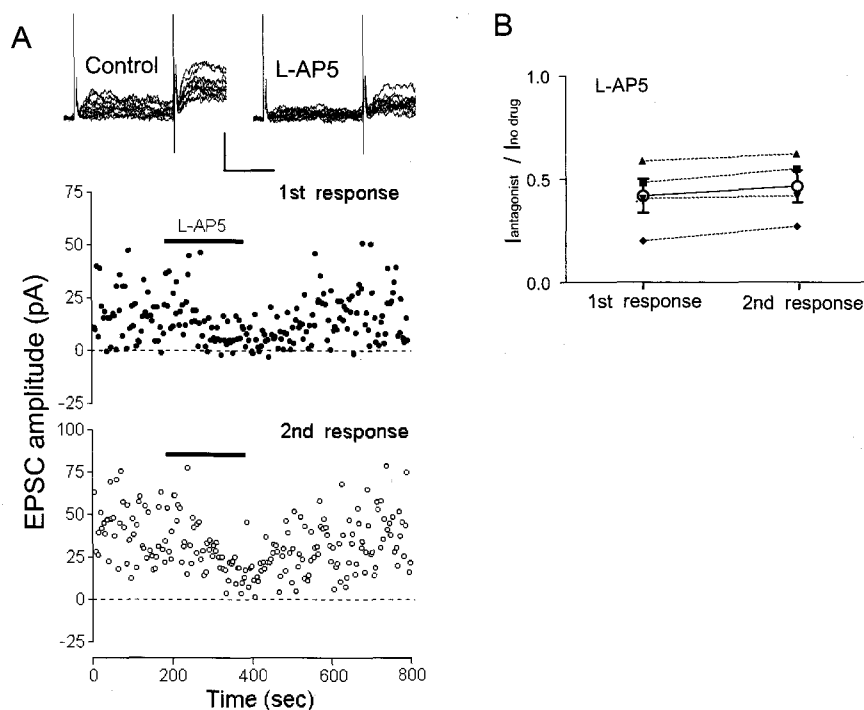

Fig. 2. The degree of inhibition by L-AP5 does not change significantly during paired-pulse facilitation of synaptic transmission (PPF). A, Representative experiment, comparing degree of inhibition by L-AP5 (250 μM) of first and second NMDA EPSCs in response to paired-pulse stimulation. Degree of inhibition is not significantly altered by PPF. NMDA EPSCs were recorded at +40 mV in the presence of 5 μM CNQX with 10 mM EGTA in the recording pipette. Vertical bar, 50 pA; horizontal bar, 25 ms. B, Summary of 4 experiments of the kind shown in A. Proportion of NMDA EPSC amplitude remaining after L-AP5 inhibition in first or second response to paired stimulation. The degree of inhibition by L-AP5 was not significantly altered with PPF ($p > 0.05$). PPF of NMDA EPSCs averaged 188±29% of basal transmission.

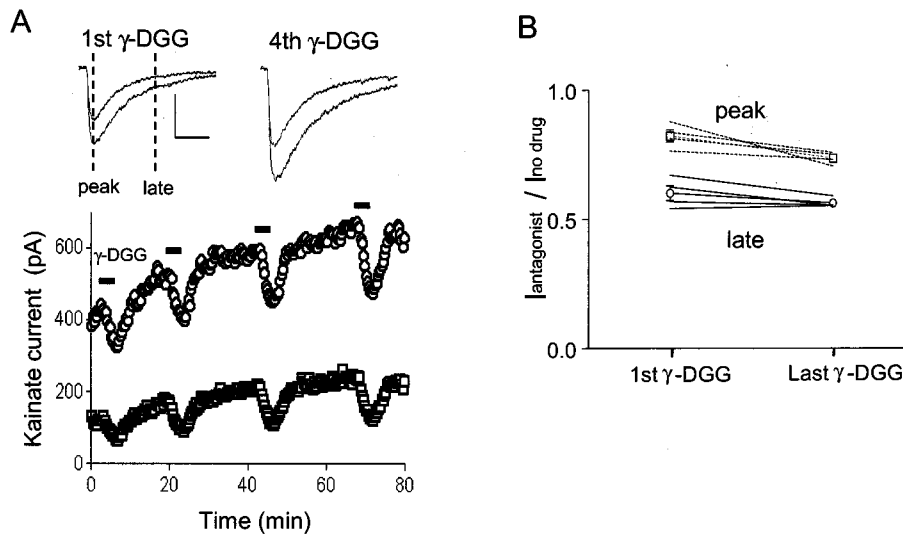Fig. 3. The degree of γ-DGG inhibition was not significantly altered during intracellular dialysis of constitutively active CaMKII. A, Representative experiment, comparing degree of inhibition by γ-DGG (500 μM) of kainate currents while the neuron was dialyzed with 200 nM constitutively active CaMKII (gift from Dr. T. Soderling). 10 mM EGTA was included in the recording pipette. AMPA-receptor mediated currents were induced by 7~10 ms pressure application of 100 μM kainate dissolved in aCSF onto cell soma. Vertical dotted lines in the current trace represent time points when the amplitude of kainate-induced current was measured. Decreased inhibition at the peak was also observed when γ-DGG was included in the puffer pipette. Note that the degree of γ-DGG inhibition does not change significantly during CaMKII-induced potentiation. Vertical bar, 250 pA; horizontal bar, 100 ms. B, Summary of 4 experiments of the kind shown in A. Proportion of kainate current amplitude remaining during exposure to γ-DGG in first or last application (p>0.05 & p>0.1 for peak and late amplitude, respectively). Potentiation of kainate currents with CaMKII dialysis averaged 153±11% of baseline (n=4).

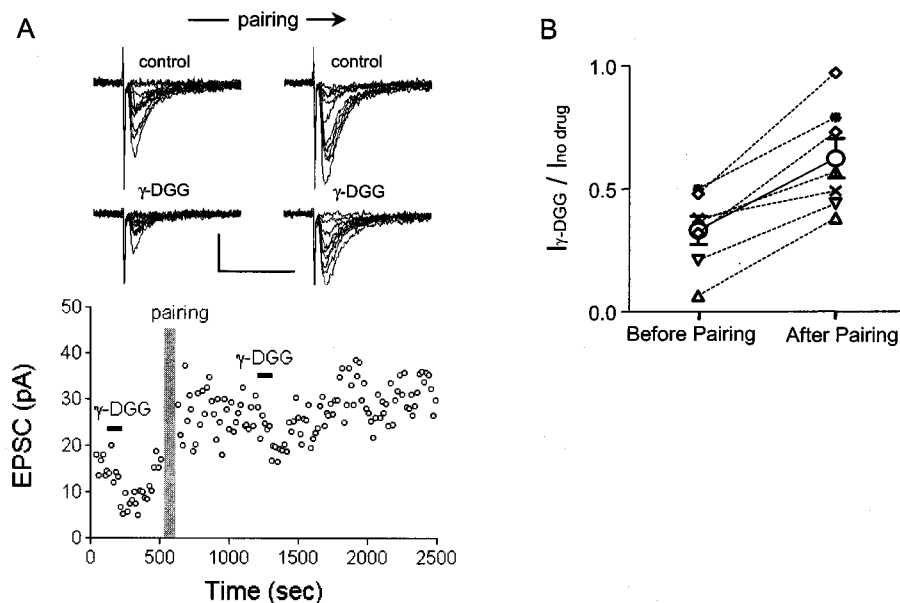Fig. 4. LTP is associated with lesser inhibition by γ-DGG. A, Representative experiment, showing that the degree of inhibition of AMPA EPSCs by γ-DGG (500 μM) decreases during LTP. Each symbol represents an average of 16 consecutive trials. Illustrative traces show groups of current records taken just before and just after exposure to antagonist drug in superfusate. Vertical bar, 25 pA; horizontal bar, 25 ms. B, Summary of 7 experiments of the kind illustrated in A. Degree of inhibition by γ-DGG ($I_{\gamma\text{-DGG}}/I_{no\ drug}$) before and after pairing. With LTP, proportion of EPSC amplitude remaining during exposure to γ-DGG was significantly increased (p<0.005). $I_{\gamma\text{-DGG}}/I_{no\ drug}$ were assessed 1138±221 s after pairing, respectively.
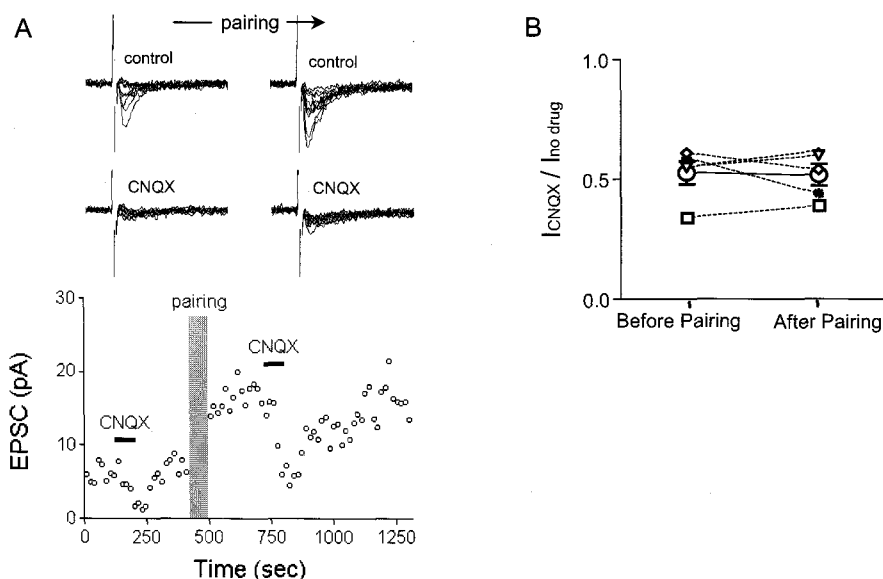
Fig. 5. Inhibition by CNQX does not change with LTP. A, No change after LTP in degree of inhibition by CNQX (0.7 μM). Each symbol represents an average of 16 consecutive trials. Illustrative traces show groups of current records taken just before and just after exposure to antagonist drug in superfusate. Vertical bar, 25 pA; horizontal bar, 25 ms. B, Summary of 5 experiments similar to that in A. Degree of inhibition by CNQX ($I_{CNQX}/I_{no\ drug}$) before and after pairing. With LTP, proportion of EPSC amplitude remaining during exposure to CNQX was unchanged (p > 0.5). $I_{CNQX}/I_{no\ drug}$ were assessed 1060 ± 251 s after pairing.

were evoked by pairs of stimuli separated by 50 ms, the second EPSC amplitude averaged $156 \pm 14\%$ of the first (Fig. 1). Nonetheless, the fractional EPSC amplitude remaining during exposure to γ-D-glutamylglycine (γ-DGG) was not significantly different for the first and second EPSCs (0.521 ± 0.077 vs. 0.458 ± 0.080 respectively, n=5, p > 0.2). The same kind of experiment was performed for NMDA EPSCs using the rapidly dissociating NMDA receptor antagonist L-(+)-2-amino-5-phosphonopentanoic acid (L-AP5) (Fig. 2). While a large PPF ($188 \pm 28\%$) was observed, the proportion of EPSC remaining during exposure to L-AP5 was not different for the first and second EPSCs (0.420 ± 0.082 vs. 0.465 ± 0.077, n=4, p > 0.05).

To find out whether the assessment of relative changes in [glu]$_{cleft}$ would be affected by increasing the functional capability of AMPA receptors, we induced such changes by directly phosphorylating AMPA receptors with CaMKII (calcium/calmodulin-activated protein kinase II) (Lledo et al, 1995; Barria et al, 1997; Shirke et al, 1997). Intracellular dialysis with a constitutively active form of CaMKII caused a slowly developing potentiation of kainate-induced currents (Fig. 3A), consistent with previous results (Lledo et al, 1995; Barria et al, 1997; Shirke et al, 1997). Possible changes in the degree of inhibition by fast off-rate antagonists were examined with repeated applications of g-DGG. The degree of inhibition was monitored at the peak of agonist-induced current and 100~200 ms after the peak. As expected from the fast unbinding nature of γ-DGG, the degree of inhibition was less at the peak, when the agonist concentration was highest. Nevertheless, the degree of γ-DGG inhibition at either time point remained unaffected by the CaMKII-induced recruitment of functional AMPA receptors (Fig. 3B).

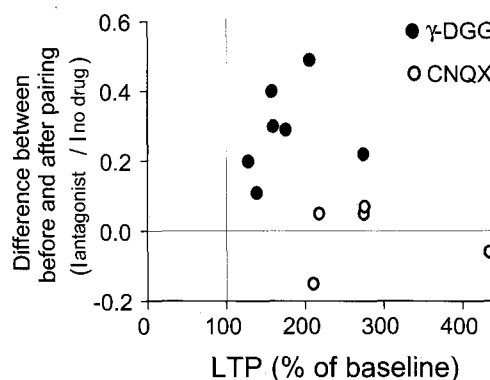Having established that changes in antagonist responsiveness, indicative of variations in transmitter concentra-

Fig. 6. Difference between inhibition by antagonists before and after pairing, plotted against the magnitude of the potentiation, for the experiments shown in Figs. 5 and 6.

tion sensed by postsynaptic receptors within the cleft, were not confounded by altered P$_r$ or by enhanced availability or functionality of glutamate receptors, we proceeded to look for possible changes in [glu]$_{cleft}$ in association with LTP. Potentiation at CA3-CA1 synapses was induced conventionally, by pairing synaptic stimulation and postsynaptic depolarization (Fig. 4A). LTP was found in 7/7 neurons, the enhanced synaptic current averaging $174 \pm 19\%$ of control. The pairing also caused a considerable reduction in the degree of inhibition of AMPA EPSCs by γ-DGG (Fig. 4A). In pooled results (Fig. 4B), the proportion of current amplitude remaining during exposure to γ-DGG averaged 0.332 ± 0.058 before pairing and 0.624 ± 0.081 after pairing-induced LTP, a highly significant change (p < 0.005).

In contrast to γ-DGG, CNQX-induced inhibition did not

change significantly with LTP (Fig. 5). The potentiated transmission in the experiments with CNQX averaged 282 ±4% of control, no less than that seen in the experiments with γ-DGG (Fig. 6). Nonetheless, the proportion of current amplitude remaining during exposure to CNQX before pairing (0.528±0.048) and following LTP (0.518±0.045) was not detectably different (n=5, p>0.5). The sharp contrast between the effects of rapidly and slowly unbinding AMPA-receptor antagonists strongly supports the idea that [glu]$_{cleft}$ increases in association with potentiated transmission.

DISCUSSION

Our experiments have uncovered a novel physiological event that occurs with LTP in hippocampal slices: an increase in excitatory neurotransmitter concentration at individual active synapses. The elevation of [glu]$_{cleft}$ was detected as a substantial lessening of the blocking effects of rapidly unbinding antagonists acting on AMPA-type glutamate receptors, and could be readily monitored without interference from possible increases in release probability or in the number of functional AMPA receptors (Figs. 1 and 2), which may also accompany LTP. Since AMPA receptors appear to be incompletely saturated (Liu et al, 1999), the elevated [glu]$_{cleft}$ is likely to participate in increases in quantal size that are often (Bliss & Collingridge, 1993; Larkman & Jack, 1995; Nicoll & Malenka, 1995; Stricker et al, 1996) but not always found associated with LTP (Malgaroli & Tsien, 1992; Stevens & Wang, 1994; Bolshakov & Siegelbaum, 1995). The increase in the peak [glu]$_{cleft}$ would also fit well with previous findings about LTP expression, such as increases in glutamate outflow with no change in PPF (Bliss & Collingridge, 1993).

One potential problem using γ-DGG would be that changes in γ-DGG inhibition could be due to altered properties of AMPA receptors possibly induced during LTP induction. In fact, previous studies suggested that AMPA receptors were phosphorylated by CaMKII during LTP (Lledo et al, 1995; Barria et al, 1997; Shirke et al, 1997). As shown in Fig. 3, γ-DGG inhibition of kainite-induced currents did not change with phosphorylation of AMPA receptors by CaMKII, suggesting that CaMKII-induced changes in AMPA receptors do not involve alteration in γ-DGG binding to AMPA receptors. However, it should be noted that the time scale of kainite application was a matter of seconds in contrast to a millisecond time scale of synaptic glutamate release, which might add unknown complexity to γ-DGG inhibition of AMPA receptors.

Potential mechanisms for the increase in [glu]$_{cleft}$ during LTP may be subdivided according to whether or not they involve changes in glutamate release from presynaptic terminals. Glutamate release could be enhanced by an increase in vesicular concentration, e.g. through increased activity of the vesicular glutamate transporter or by an increase in the openness or permeability of exocytotic fusion pores (Rahamimoff & Fernandez, 1997). The effectiveness of a fixed amount of released neurotransmitter might be improved by reducing the diffusional escape of glutamate, via changes in cleft geometry (Wahl et al, 1996; Kruk et al, 1997), possibly through actions of cell adhesion molecules (Luhl et al, 1994), or by decreased glutamate binding to glutamate transporters, abundantly distributed in pre- and postsynaptic structures and neighboring glial cells,

that normally restrict [glu]$_{cleft}$ (Diamond & Jahr, 1997). Changes in the effective glutamate concentration at postsynaptic receptors might even arise from improvement of their alignment with presynaptic release sites (Xie et al, 1997). It will be interesting to see whether increased [glu]$_{cleft}$ felt by AMPA receptors is also reflected in the time integral of glutamate concentration at more distant sensors such as glial glutamate transporters (Bergles & Jahr, 1997), as implied by some but not all of the mechanisms discussed above. Determining the precise cellular mechanism of the increased [glu]$_{cleft}$ will be helpful in assessing its quantitative contribution in conjunction with other putative LTP mechanisms such as increased P_r at presynaptic terminals (Steven & Wang, 1994; Bolshakov & Siegelbaum, 1995; Malgaroli & Tsien, 1998; Kullmann & Asztely, 1998) and upregulation of AMPA receptors on postsynaptic spines (Isaac et al, 1995; Lia et al, 1995; Durand et al, 1996).

ACKNOWLEDGMENTS

REFERENCES

Barria A, Muller D, Derkach V, Griffith LC, Soderling TR. Regulatory phosphrylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science 276: 2042−2045, 1997

Bekkers JM, Stevens CF. Presynaptic mechanism for long-term potentiation in the hippocampus. Nature 346: 724−729, 1990

Bergles DE, Jahr CE. Synaptic activation of glutamate transporters in hippocampal astrocytes. Neuron 19: 1297−1308, 1997

Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361: 31−39, 1993

Bolshakov VY, Siegelbaum SA. Regulation of hippocampal transmitter release during development and long-term potentiation. Science 269: 1730−1734, 1995

Clements JD, Feltz A, Sahara S, Westbrook GL. Activation kinetics of AMPA receptor channels reveal the number of functional agonist binding sites. J Neurosci 18: 119−127, 1998

Clements JD, Lester RA, Tong G, Jahr CE, Westbrook GL. The time course of glutamate in the synaptic cleft. Science 258: 1498−1501, 1992

Diamond JS, Jahr CE. Transporters buffer synaptically released glutamate on a submillisecond time scale. J Neurosci 17: 4672−4687, 1997

Durand GM, Kovalchuk Y, Konnerth A. Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381: 71−75, 1996

Frerking M, Wilson M. Saturation of postsynaptic receptors at central synapses? Curr Opin Neurobiol 6: 395−403, 1996

Isaac JT, Nicoll RA, Malenka RC. Evidence for silent synapses: implications for the expression of LTP. Neuron 15: 427−434, 1995

Katz B. The Release of Neural Transmitter Substances (Liverpool: Liverpool University Press) 1969

Kruk PJ, Korn H, Faber DS. The effects of geometrical parameters on synaptic transmission: a Monte Carlo simulation study. Biophys J 73: 2874−2890, 1997

Kullmann DM, Asztely F. Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. Trends Neurosci 21: 8−14, 1998

Kullmann DM, Nicoll RA. Long-term potentiation is associated with increases in quantal content and quantal amplitude. Nature 357:

240 – 244, 1992

Larkman AU, Jack JJB. Synaptic plasticity: hippocampal LTP. Curr Opin *Neurobiol* 5: 324 – 334, 1995

Liao D, Hessler NA, Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* 375: 400 – 404, 1995

Liu G, Choi S, Tsien RW. Variability of neurotransmitter concentration and nonsaturation of postsynaptic AMPA receptors at synapses in hippocampal cultures and slices. *Neuron* 22: 395 – 409, 1999

Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA. Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proc Natl Acad Sci USA* 92: 11175 – 11179, 1995

Luhl A, Laurent JP, Figurov A, Muller D, Schachner M. Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. *Nature* 372: 777 – 779, 1994

Malgaroli A, Tsien RW. Glutamate-induced long-term potentiation of the frequency of miniature synaptic currents in cultured hippocampal neurons. *Nature* 357: 134 – 139, 1992

Malinow R, Tsien RW. Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* 346: 177 – 180, 1990

Nicoll RA, Malenka RC. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* 377: 115 – 118, 1995

Perkel DJ, Nicoll RA. Evidence for all-or-none regulation of neurotransmitter release: implications for long-term potentiation. *J Physiol* Lond 471: 481 – 500, 1993

Rahamimoff R, Fernandez JM. Pre- and postfusion regulation of transmitter release. *Neuron* 18: 17 – 27, 1997

Shirke AM, Malinow R. Mechanisms of potentiation by calcium-calmodulin kinase II of postsynaptic sensitivity in rat hippocampal CA1 neurons. *J Neurophysiol* 78: 2682 – 2692, 1997

Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284: 1811 – 1816, 1999

Silver RA, Cull-Candy SG, Takahashi T. Non-NMDA glutamate receptor occupancy and open probability at a rat cerebellar synapse with single and multiple release sites. *J Physiol Lond* 494: 231 – 250, 1996

Stevens CF, Wang Y. Changes in reliability of synaptic function as a mechanism for plasticity. *Nature* 371: 704 – 707, 1994

Stricker AC, Field AC, Redman SJ. Changes in quantal parameters of EPSCs in rat CA1 neurons in vitro after the induction of long-term potentiation. *J Physiol* Lond 490: 443 – 454, 1996

Tang CM, Margulis M, Shi QY, Fielding A. Saturation of postynaptic receptors after quantal release of transmitter. *Neuron* 13: 1385 – 1393, 1994

Tong G, Jahr CE. Multivesicular release from excitatory synapses of cultured hippocampal neurons. *Neuron* 12: 51 – 59, 1994

Wahl LM, Pouzat C, Stratford KJ. Monte Carlo simulation of fast excitatory synaptic transmission at a hippocampal synapse. *J Neurophysiol* 75: 597 – 608, 1996

Xie X, Liaw J-S, Baudry M, Berger TW. Novel expression mechanism for synaptic potentiation: Alignment of presynaptic release site and postsynaptic receptor. *Proc Natl Acad Sci USA* 94: 6983 – 6988, 1997