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Reduction of Excitatory Postsynaptic Responses by Persistently Active Metabotropic Glutamate Receptors in the Hippocampus

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Losonczy, Attila, Peter Somogyi, and Zoltan Nusser. Reduction of excitatory postsynaptic responses by persistently active metabotropic glutamate receptors in the hippocampus. *J Neurophysiol* 89: 1910–1919, 2003; 10.1152/jn.00842.2002. The release of glutamate from axon terminals is under the control of a variety of presynaptic receptors, including several metabotropic glutamate receptors (mGluRs). Synaptically released glutamate can activate mGluRs within the same synapse where it was released and also at a distance following its diffusion from the synaptic cleft. It is unknown, however, whether the release of glutamate is under the control of persistently active mGluRs. We tested the contribution of mGluR activation to the excitatory postsynaptic responses recorded from several types of GABAergic interneuron in strata oriens/alveus of the mouse hippocampus. The application of 1 μM (αS)-α-amino-α-[(1S,2S)-2-carboxycyclopropyl]xanthine-9-propanoic acid (LY341495), a broad-spectrum mGluR (subtypes 2/3/7/8) antagonist at this concentration, increased evoked-excitatory postsynaptic current (eEPSC) amplitudes by 60% (n = 33). On identified cell types, LY341495 had either no effect (7 of 14 basket and 7 of 13 oriens-lacunosum moleculare, O-LM cells) or resulted in a 32 ± 30% (mean ± SD) increase in EPSC amplitudes recorded from basket cells and a seven-times greater (216 ± 102%) enhancement of EPSCs in O-LM cells. The enhancement of the first EPSC of a high-frequency train indicates persistent mGluR activation. During antagonist application, the relative increase in EPSC amplitude evoked by the second and subsequent pulses in the train was not larger than that of the first EPSC, showing no further receptor activation by the released transmitter. The effect of mGluR subtype selective agonists [3 μM L(+)-2-amino-4-phosphonobutyric acid (L-AP4): mGluR4/8; 600 μM L-AP4: mGluR4/7/8; 1 μM (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)-glycine (DCG-IU): mGluR2/3] and an antagonist (0.2 μM LY341495: mGluR2/3/8) suggests that persistently active mGluR2/3/8 control the excitability of hippocampal network.

INTRODUCTION

One of the most striking features of synaptic neurotransmission in the CNS is that it operates within a very broad dynamic range. The dynamic behavior of a synapse can be modified on different time scales by several activity-dependent processes. One possible way of activity-dependent regulation is through presynaptic neurotransmitter receptors (Frank and Fuortes 1957; Vizi and Kiss 1998). It is well established that the release of glutamate can be modulated by several heteroreceptors (e.g., $GABA_B$, muscarinic, adenosine receptors) and receptors activated by glutamate [e.g., kainate, *N*-methyl-D-aspartate (NMDA), and metabotropic glutamate receptors]. In case of heteroreceptor activation, the transmitter has to reach the receptors on glutamatergic terminals from a distance, and therefore the receptor activation will probably reflect the overall activity of the population and not only a single releasing neuron. The classical view of autoreceptor activation is that presynaptic receptors are activated by the transmitter released at the same synapse where the receptors are located, providing a sensitive feedback of the activity of a single releasing neuron. However, recent experiments provided evidence that glutamate can diffuse to neighboring synapses where it can activate metabotropic glutamate receptors (mGluRs) (Conchilla and Alford 1998; Dube and Marshall 2000; Mitchell and Silver 2000; Scanziani et al. 1997; Semyanov and Kullmann 2000). Thus high-frequency activity of the presynaptic neurons will result in a more pronounced build-up of glutamate around the active synapses, resulting in population activity-dependent regulation of transmitter release. One way of monitoring the overall network activity is through sufficiently sensitive presynaptic receptors, which are persistently activated by the ambient level of transmitter in the extracellular space. Indeed, it has been shown in vitro that persistently active presynaptic mGluRs regulate glutamate release in the hypothalamus (Oliet et al. 2001; Schrader and Tasker 1997). In the present study, we tested whether persistently active mGluRs play a role in the regulation of transmitter release from hippocampal glutamatergic terminals in a uniform or a differential manner.

Synaptic connections exhibit strikingly different short-term plasticity (Zucker 1989). Some synapses show facilitation or depression, but some others display a combination of facilitation and depression. It has been demonstrated that in the hippocampus and neocortex, short-term plasticity of synapses along the same axon depends on the identity of the postsynaptic target cell (Markram et al. 1998; Reyes et al. 1998; Scanziani et al. 1998; Thomson 2000). For example, in the CA1 area of the hippocampus excitatory postsynaptic potentials in basket and bistratified interneurons display paired-pulse depression, whereas those onto oriens-lacunosum moleculare (O-LM) interneurons exhibit marked short-term facilitation (Ali and Thomson 1998; Ali et al. 1998). Similarly, a presyn-

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aptic metabotropic glutamate receptor subtype (mGluR7a) also shows a postsynaptic target cell-dependent expression (Shigemoto et al. 1996); glutamatergic axon terminals making synapses onto hippocampal O-LM interneurons contain a high density of mGluR7a, whereas terminals making excitatory synapses on pyramidal cells and other types of interneuron, including basket cells, have a much lower density of mGluR7a. It is, therefore possible that the cell-type-specific differential expression of the amount of mGluR7a contributes to the distinct short-term plasticity observed at these connections. In this study, we also tested whether activation of presynaptic mGluRs plays a role in determining the short-term synaptic plasticity at glutamatergic synapses contacting distinct interneurons.

METHODS

Acute slice preparation and whole cell recording of evoked EPSCs

Twelve to 24-day-old [16.2 ± 2.6 (mean ± SD), $n = 46$] C57Black6 mice were anesthetized first with halothane and then with ketamine (50 mg/animal) in accordance with the guidelines of the Institute of Experimental Medicine Protection of Research Subjects. After decapitation, the brains were removed and were placed into ice-cold artificial cerebrospinal fluid (ACSF). Horizontal slices were cut at 300–350 μm thickness with a Vibratome (Leica VT1000S; Leica Microsystems, Vienna) and were stored in continuously oxygenated ACSF (pH = 7.4), containing (in mM) 85 NaCl, 75 sucrose, 2.5 KCl, 25 glucose, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 4 $MgCl_2$, and 0.5 $CaCl_2$. After 30 min, this medium was replaced by an ACSF containing (in mM) 126 NaCl, 2.5 KCl, 25 glucose, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 2 $MgCl_2$, and 2 $CaCl_2$. After another hour of incubation at 30°C, the slices were transferred to a recording chamber where they were perfused with ACSF containing the $GABA_A$ receptor antagonist SR95531 (20 μM). Recordings were performed at 26°C from the somata of visually identified (Olympus BX50WI microscope with infrared differential interference contrast optics with a ×40 water immersion objective) interneurons located mostly in the stratum oriens/alveus. In horizontal slices, the boundaries between the targeted CA1 area and the CA3 area or the subiculum are not always clear, and after recovering the cells, the somata of several of them were found outside the CA1 area. Recordings were made with a K-gluconate-based intracellular solution, containing (in mM) 130 K-gluconate, 5 KCl, 2 $MgCl_2$, 0.05 EGTA, 10 HEPES, 2 Mg-ATP, 0.4 Mg-GTP, 10 creatinine-phosphate, and 0.013 biocytin. The intracellular solution was titrated to a pH of 7.25 and osmolarity of 305–315 mosmol. Excitatory synaptic currents were evoked by extracellular stimulation (stimulus isolator made by Supertech, Pécs, Hungary, 0.1 ms pulse width), and the stimuli were delivered through a theta glass pipette filled with ACSF and placed 30–100 μm away from the somata. The synaptic currents were recorded with an Axopatch 200B or a MultiClamp 700A amplifier (Axon Instruments, Foster City, CA). All recordings were low-pass filtered at 2 kHz and digitized on-line at 10 kHz using a PCI-MIO 16E-4 data acquisition board (National Instruments, Austin, TX) using either a WinWCP3.0.6 software (courtesy of Dr. J. Dempster, University of Strathclyde, Glasgow, UK) or EVAN1.3 software (Nusser et al., 2001). Patch electrodes were pulled (Narishige PP-830, Tokyo or Zeitz Universal Puller, Zeitz-Instrumente Vertriebs GmbH, Munich, Germany) from thick-walled borosilicate glass (1.5 mm OD, 0.86 mm ID, Sutter Instruments, Novato, CA). The DC resistance of the electrodes was 2–8 MΩ when filled with pipette solution. Series resistance (R_s) and whole cell capacitance were estimated by compensating for the fast current transients evoked at the onset and offset of 10 ms, 5 mV voltage-command steps and were checked every 2 min during the recording. If the compensated series resistance increased by >40%, the recording was discontinued. The series resistance remaining after 70–90% compensation (with 7–8 μs lag values) was 3.1 ± 1 MΩ. In the second series of (αS)-α-amino-α-[(1S,2S)-2-carboxycyclopropyl]-xanthine-9-propanoic acid (LY341495) experiments, the Rs compensation was adjusted between 70 and 90% in a way that the compensated Rs was constant throughout the recordings.

In the first series of antagonist experiments ($n = 15$ cells included), the effect of 1 μM LY341495 on the short-term plasticity of excitatory inputs to interneurons was tested using a train of 10 stimuli at 33 Hz, followed by a single pulse at recovery time intervals from 150 to 1800 ms. This protocol was repeated every 20 s. In these experiments, the stimulation was turned off during the ~10 min drug wash-in period to minimize the number of stimuli delivered to the fibers. In most of these experiments, postsynaptic responses could not be kept stable for the wash-out period probably due to the large number of stimuli delivered during the experiments. For the second series of antagonist experiments using 0.2 μM ($n = 4$ cells) and 1 μM ($n = 19$) LY341495 and for the mGluR agonist experiments, we applied three stimuli at 33 Hz in every 15 s, but the stimulation was not turned off during the drug wash-in and -out periods.

The drugs LY341495, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)-glycine (DCG-IV), and L(+)-2-amino-4-phosphonobutyric acid (L-AP4) were purchased from Tocris (Bristol, UK). All other chemicals and drugs were obtained from Sigma. Data are given throughout the manuscript as means ± SD with the exception of data in Fig. 4, where means ± SE are plotted and the numbers of observations are indicated in the legend. The effect of the drugs was tested on the distribution of individual evoked-excitatory postsynaptic current (eEPSC) amplitudes (~20 events in 5 min. of control and in drug) using the Mann-Whitney U test and significance was determined at $P < 0.01$. For the LY341495 experiments where the drug was washed out, the EPSC amplitudes in control, drug, and wash-out were compared with Kruskal-Wallis test followed by Mann-Whitney U test using Bonferroni correction ($P < 0.01$).

Anatomical identification of interneurons

The recorded interneurons were identified by intracellular labeling with biocytin (0.5%) and immunocytochemistry using antibodies to somatostatin, pro-CCK, parvalbumin, calretinin, calbindin, mGluR1α, and mGluR7a as described by Losonczy et al. (2002). Following electrophysiological recording, slices were fixed in a fixative containing 4% paraformaldehyde, 0.05% glutaraldehyde, and 15% (vol/vol) saturated picric acid in 0.1 M phosphate buffer (pH 7.4). Fixed slices were then embedded in gelatin and re-sectioned at 60 μm thickness. Biocytin was visualized with 7-amino-4-methylcoumarin-3-acetic acid-conjugated streptavidin (1:1000, Vector Laboratories, Burlingame, CA), and primary antibodies were visualized by Alexa Fluor 488- (diluted 1:1000, Molecular Probes, Leiden, The Netherlands), Cy3- or Cy5-conjugated secondary antibodies (diluted 1:400, Jackson ImmunoResearch, West Grove, PA) in an indirect immunofluorescence procedure. All reagents were diluted in Tris-buffered saline (TBS) containing 0.1% Triton X-100. Cells were studied using a Leica dichromatic mirror system, as described previously (Losonczy et al. 2002), recorded on a CCD camera, analyzed, and displayed using the Openlab software (Improvision, Coventry, UK). Brightness and contrast were adjusted for the whole frame and no part of a frame was enhanced or modified in any way. The immunonegativity of a cell for a given marker could be due to damage caused by the recording, an undetectably low level of the molecule or the genuine absence of the molecule. Therefore only the positive detection of immunoreactivity is informative after extensive whole cell recording. After immunocytochemical evaluation, the sections were de-mounted, and the recorded cells were further labeled by avidin-biotinylated HRP complex (Vector Laboratories) followed by peroxidase reactions using diaminobenzidine (0.05%). The sections were then dehydrated and

permanently mounted on slides. The axonal and dendritic patterns of each neuron were analyzed at high magnifications. Some recovered neurons were subsequently reconstructed using a drawing tube. The identification of some of the recorded cells is described in details in Losonczy et al. (2002). In five cells, the axonal arbor was not sufficiently complete for classification, but the neurochemical content allowed them to be categorized as O-LM cells (*A084* and *A100*: somatostatin positive, mGluR1α strongly positive; *A094, A243,* and *A595*: somatostatin positive, mGluR1α positive, innervated by strongly mGluR7a positive terminals). In addition, the truncated axonal arborizations and the lack of immunocytochemical results did not allow us to unequivocally classify four cells, therefore we present them as "nonclassified" in the tables [*A128*: parvalbumin positive, can be axo-axonic or basket cell; *A122*: CCK positive, can be basket or bistratified cell; *A097* and *A632*: somatostatin positive, mGluR1α negative, can be O-LM or oriens bistratified (O-Bi) cell]. The anatomical identification of 19 cells (*A584-A632*) is not presented in Losonczy et al. (2002). These cells were classified according to the same criteria used by Losonczy et al. (2002). Of the 19 cells, 18 had sufficient axon for classification. The soma of one O-LM cell was not recovered, but its axon unequivocally identified the cell. Sixteen of the cells were tested for somatostatin immunoreactivity, and of these 6 O-LM cells, 1 O-Bi cell and an unidentified cell were immunopositive. Eleven of the 19 cells were tested for parvalbumin immunoreactivity, and of these, 4 basket cells and an unidentified cell were immunopositive. Fourteen cells were tested for mGluR1α immunoreactivity, and of these, 6 O-LM cells were strongly immunopositive and an additional O-Bi cell was weakly immunopositive.

Some of the cells classified as basket cells may have been axo-axonic cells (Maccaferri et al. 2000) that innervate the axon initial segment of pyramidal cells in the rat. Because both basket and axo-axonic cells have their boutons mainly in the pyramidal cell layer, the only adequate method to differentiate the two cell types is using electron microscopic analysis of the synaptic terminals. The present sample of cells was not prepared for electron microscopic analysis; therefore this test could not be performed. Nevertheless, it is very unlikely that the potential presence of axo-axonic cells in the basket cell population would affect the conclusion on the effect of drugs. On careful checking of the axonal structure of each cell, in the absence of knowledge about the effect of the drug, cells with indistinguishable axonal patterns, which showed no resemblance to axo-axonic cells, showed significantly different responses to the drugs.

RESULTS

To investigate the role of mGluR activation at excitatory synaptic inputs to GABAergic interneurons in s. oriens/alveus of mouse hippocampus, we performed whole cell voltage-clamp recordings from the somata of visually identified neurons and evoked postsynaptic currents with weak extracellular stimulation in the presence of the $GABA_A$ receptor antagonist SR95531 (20 μM). In our first series of experiments, we applied 10 stimuli at 33 Hz to characterize the short-term plasticity of the eEPSCs, to determine the effect of mGluR activation on the short-term synaptic dynamics, and to investigate whether the released transmitter during the train of stimuli results in significant mGluR activation. Using a K-gluconate-based intracellular solution and holding the cells at −60 mV, eEPSCs are inward with 10–90% rise time of 1.25 ± 0.64 ms (n = 44) and fast exponential decays (50% decay time = 2.24 ± 0.82 ms). The rapid kinetics of the eEPSCs indicate that mainly AMPA receptors mediate these synaptic currents. In eight cells (4 O-LM, 2 basket, and 2 O-Bi), we applied >20 μM 2,5-dioxo-6-nitro-1,2,3,4-tetrahydrobenzol-[f]quinoxaline-7-sulphonamide (NBQX) at the end of the recordings and found that this non-NMDA receptor antagonist abolished the postsynaptic responses (Fig. 2*A*). During the train of stimuli, the amplitude of eEPSCs showed depression or facilitation or a combined facilitating-depressing pattern.

The cells were filled with biocytin and were subsequently characterized by their somatostatin, cholecystokinin, parvalbumin, calretinin, and mGluR1α content, and tested whether they were decorated by strongly mGluR7a immunoreactive axon terminals as well as according to the dendritic and axonal arborizations. We have distinguished the following three anatomical classes of interneurons according to their axonal and dendritic arborizations (Losonczy et al. 2002): basket cells (n = 27) mainly innervate s. pyramidale (Ramon y Cajal 1893); O-LM cells (n = 23) have dendrites restricted to s. oriens/alveus and mainly innervate s. lacunosum moleculare (McBain et al. 1994); and O-Bi (n = 14) cells have dendrites similar to O-LM cells but innervate s. radiatum and s. oriens (Maccaferri et al. 2000) as well as project outside the CA1 area. The detailed analysis of the short-term synaptic dynamics of eEPSCs and the anatomical identification of some of the recorded cells are described in Losonczy et al. (2002).

Persistently active mGluRs reduce evoked EPSCs

To test whether the activation of presynaptic mGluRs regulates glutamate release from axon terminals making synapses on GABAergic interneurons, we applied the broad-spectrum mGluR antagonist LY341495 (1 μM), which is known to inhibit mGluR2/3/7/8 at this concentration (Kingston et al. 1998; Leazza et al. 1999; Turner and Salt 1999; Wittmann et al. 2001). The application of 1 μM LY341495 resulted in a significant increase (from 198 ± 259 to 253 ± 279 pA, paired t-test, $P < 0.001$) in the amplitude of the first response of the train in 33 successfully identified interneurons. When the drug effect was investigated within anatomically identified interneuron populations, significant increase was detected for basket (from 337 ± 345 to 393 ± 368 pA, n = 14, paired t-test, $P <$

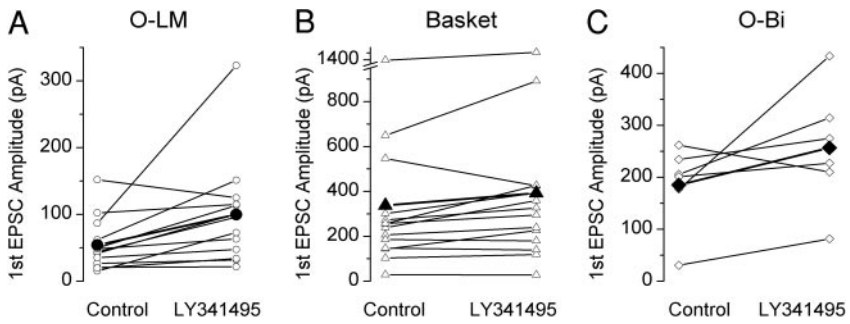

FIG. 1. Summary plots of the effect of (αS)-α-amino-α-[(1S,2S)-2-carboxycyclopropyl]xanthine-9-propanoic acid (LY341495, 1 μM) on the amplitude of the 1st evoked-excitatory postsynaptic currents (eEPSCs) in identified cell types. Note the variability in the degree of enhancement by LY341495 between and within each cell class. ○, △, and ◇, individual cells; ●, ▲, and ◆, means. *A*: oriens-lacunosum moleculare (O-LM) cells; *B*: basket cells; *C*: oriens bistratified (O-Bi) cells.

0.05, Fig. 1*B*) and O-LM cells (from 54 ± 39 to 100 ± 78 pA, $n = 13$, paired *t*-test, $P < 0.05$, Fig. 1*A*) but not for O-Bi cells (paired *t*-test, $P = 0.15$, Fig. 1*C*). Because there was a very large heterogeneity (CV = 0.55) in the degree of change in the amplitude (Fig. 1 and Table 1), we evaluated the effect of LY341495 in each individual cell. In 6 of 13 O-LM, 7 of 14 basket, and 4 of 6 O-Bi cells, the amplitudes of the eEPSCs were significantly (~20 responses in control and drug periods, Mann-Whitney *U* test, $P < 0.01$) altered in at least one stimulus time point (Fig. 2 and 3; Table 1). In 16 of these 17 responding cells, the amplitude of the first eEPSC was already significantly altered following the application of the mGluR antagonist (Figs. 2 and 3), showing persistent mGluR activation. The application of LY341495 increased the EPSC amplitudes in all but one cell (Table 1). To test whether glutamate released during the stimulus train causes further activation of presynaptic mGluRs, we plotted the relative amplitude increase ($Peak_{LY341495}/Peak_{Control}$) against the stimulus number at every stimulus during the 33-Hz train for all anatomically identified basket and O-LM cells (Fig. 4*A*) and for those cells that showed a significant drug effect (Fig. 4B). As shown in Fig. 4, the $Peak_{LY341495}/Peak_{Control}$ ratios did not increase as a function of stimulus number in either cell type. The degree of

TABLE 1. *Effects of 1 μM LY341495 on the amplitude of eEPSCs*

Cell ID	Cell Type	Peak 1_{Drug}/ Peak 1_{Contr}	Peak 2_{Drug}/ Peak 2_{Contr}	Peak 3_{Drug}/ Peak 3_{Contr}
A064	O-LM	**2.42**	**1.74**	**1.63**
A069	O-LM	**3.60**	**2.44**	**2.84**
A078	O-LM	**2.19**	**2.17**	**2.22**
A077	O-LM	1.29	1.01	1.19
A084	O-LM	0.98	0.97	1.13
A584	O-LM	1.03	1.08	1.57
A585	O-LM	0.82	1.02	1.20
A593	O-LM	1.18	1.38	0.94
A594	O-LM	1.72	1.07	1.45
A595	O-LM	**4.72**	**4.24**	**3.33**
A596	O-LM	**2.28**	**2.35**	**2.57**
A597	O-LM	1.35	1.41	1.04
A620	O-LM	**3.73**	**3.66**	**3.28**
A073	Basket	**1.37**	**1.20**	1.19
A074	Basket	1.16	**1.46**	**1.46**
A075	Basket	**1.51**	**1.36**	1.27
A076	Basket	**0.78**	1.34	1.33
A071	Basket	0.94	1.21	1.21
A072	Basket	1.14	1.13	1.19
A080	Basket	1.05	0.95	0.78
A083	Basket	1.31	1.16	1.05
A607	Basket	1.04	1.09	1.09
A610	Basket	**1.57**	**1.48**	**1.51**
A614	Basket	0.97	0.88	0.90
A625	Basket	**1.66**	**1.81**	**1.58**
A626	Basket	**1.20**	1.17	**1.13**
A627	Basket	1.16	1.29	1.34
A081	O-Bi	1.13	1.12	0.93
A079	O-Bi	**2.44**	**2.44**	**2.41**
A589	O-Bi	**1.53**	**1.52**	**1.53**
A600	O-Bi	**2.68**	**1.88**	**1.41**
A605	O-Bi	0.80	1.03	0.89
A623	O-Bi	**1.17**	**1.34**	**1.33**
A632	non-classif.	**2.74**	**2.80**	**2.49**

Significant changes ($P < 0.01$ with the Mann-Whitney *U* test) in the amplitude of excitatory postsynaptic currents (EPSCs) ($Peak_{Drug}/Peak_{Contr}$ ratio) following drug application are indicated by bold numbers. The soma of most cells was in the CA1 area; some somata were in the CA3 area (*A593; A605; A614; A625*), the axons of the last three innervating the CA1 area as well; a few cells were at the border of the CA1 area with the subiculum (*A078, A077, A073*), or in the subiculum (*A071*). LY341495, (αS)-α-amino-α-[(1S,2S)-2-carboxycyclopropyl]xanthine-9-propanic acid; eEPSC, evoked EPSC.

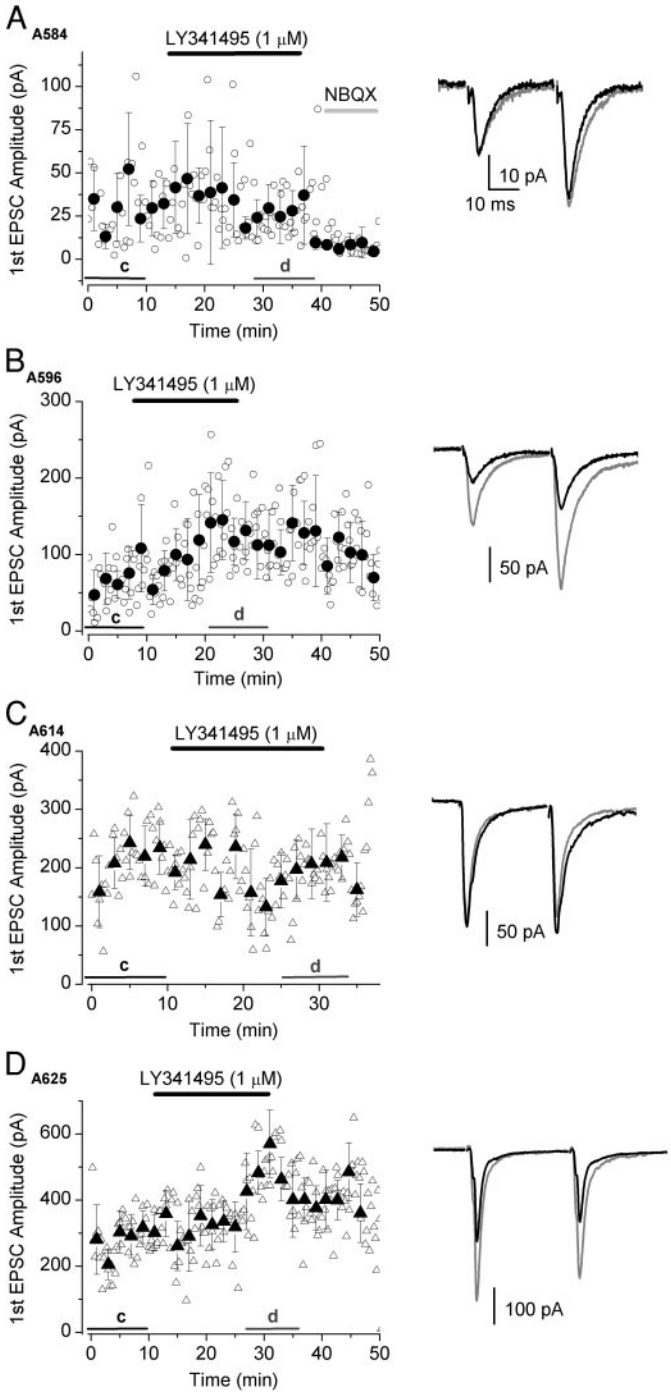

FIG. 2. Effect of 1 μM LY341495 on the amplitude of eEPSCs in identified O-LM (*A* and *B*) and basket (*C* and *D*) cells. *Left*: the amplitudes of the 1st eEPSCs during the recordings (small open symbols) and superimposed are the mean ± SD (filled symbols) calculated every 2 min. Bars c and d indicate the control and drug periods, respectively. *Right*: the averaged synaptic responses for the 1st 2 stimuli (control: black traces; LY341495: gray traces). The application of the metabotropic glutamate receptors (mGluR) antagonist either increased (*B* and *D*) or resulted in no significant change (*A* and *C*) of the amplitude of the 1st eEPSCs. The increase in the amplitude in *B* was reversible on the wash-out of the drug (Kruskal-Wallis, followed by Mann-Whitney *U* test, $P < 0.01$). The eEPSCs were blocked by the AMPA/kainate receptor antagonist NBQX (*A*).

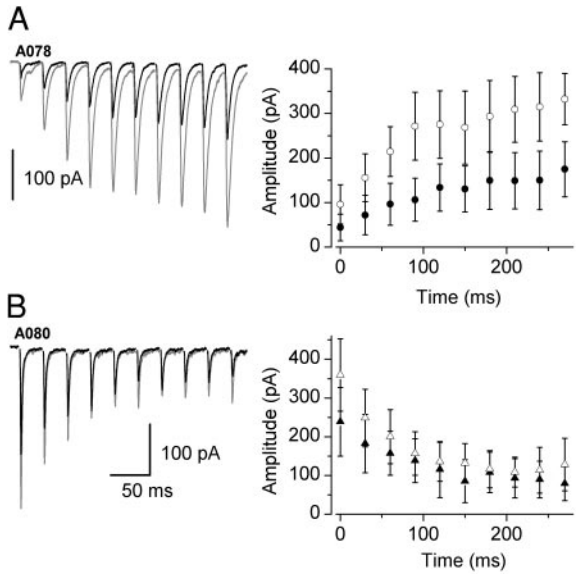

FIG. 3. Effect of 1 μM LY341495 on short-term plasticity of eEPSCs recorded in O-LM (*A*) and basket (*B*) cells. *A* and *B*: averaged whole-cell voltage-clamp traces of 10 EPSCs evoked at 33 Hz in control solution (black trace) and in 1 μM LY341495 (gray trace). The mean ± SD of individual EPSC amplitudes (*right*) illustrates that the application of 1μM LY341495 resulted in a larger increase of the EPSCs in this O-LM cell compared with this basket cell.

increase of the second and subsequent responses was not significantly ($P > 0.05$ Mann-Whitney *U* test) larger than that of the first response, indicating that the released transmitter did not significantly result in further mGluR activation. However, instead of an increase, there was a significant decline (1st 3 responses vs. last 3 responses of the train; unpaired *t*-test, $P < 0.01$) in the relative amplitude increase throughout the train (Fig. 4*B*) in those O-LM cells that showed a significant LY341495 effect. This may be explained by an earlier depletion of the readily releasable pool of vesicles during the drug period.

It is also apparent from the plot in Fig. 4 that the relative increase in the amplitude of eEPSCs by LY341495 is much larger in O-LM than in basket cells. The relative increase in the amplitude of the first eEPSC in all O-LM cells was 110 ± 123%, whereas in all basket cells it was only 20 ± 25% ($P < 0.05$, Mann-Whitney *U* test; Fig. 4*A*). The mean relative increase of the EPSC amplitudes was also larger in O-LM (83 ± 92%) than in basket cells (21 ± 24%; $P < 0.01$, Mann-Whitney *U* test; Fig. 4*A*). When we compared the relative amplitude increase in those O-LM and basket cells in which a significant LY341495 effect was observed (Fig. 4*B*), an even more robust difference was found. The enhancement of first EPSC amplitude in these O-LM cells was 216 ± 102% and that in the corresponding basket cells was only 32 ± 30% ($P < 0.01$, Mann-Whitney *U* test). Similarly to the relative increase of the first peak, the mean relative increase throughout the whole train was also much larger in O-LM (178 ± 92%) than in basket (38 ± 20%) cells ($P < 0.01$ Mann-Whitney *U* test). These data, taken together, indicate that the extent of reduction of EPSCs by persistently active mGluRs depends on the postsynaptic cell type. To elucidate the type of mGluRs involved in the reduction of transmitter release at these glutamatergic terminals, we applied mGluR subtype-selective agonists and an antagonist.

Identification of mGluR subtypes involved in the regulation of transmitter release

Because of the lack of potent mGluR subtype-specific antagonists, we have performed complex series of experiments with group II- and III-specific agonists to characterize the mGluR subtype(s) involved in the regulation of glutamate release from these hippocampal axon terminals. First, 3 μM L-AP4, an agonist of mGluR4/6/8 at this concentration (Conn and Pin 1997), was used. As mGluR6 has not been found in the hippocampus (Nomura et al. 1994), in the following part of the paper we will disregard this receptor subtype as a possible candidate in hippocampal axons, and L-AP4 at 3 μM concentration will be referred to as mGluR4/8-selective agonist. The application of 3 μM L-AP4 resulted in no change in the amplitude of eEPSCs in the majority of recorded cells (8 of 10 interneurons, e.g., Fig. 5, *B* and *C*), including two O-LM, two basket, and three O-Bi cells (Table 2) in agreement with the low expression of these two mGluR subtypes in the s. oriens/alveus of the CA1 area (Corti et al. 2002; Shigemoto et al. 1997). However, in two O-LM cells, we found a relatively small (~25%), but significant reduction of the eEPSCs by 3 μM L-AP4 (Fig. 5*A*), indicating that at some axon terminals contacting O-LM cells mGluR4/8 are present, as found recently using immunocytochemistry (Corti et al. 2002; Dalezios et al. 2001).

Next, we applied a much higher concentration of L-AP4 (600 μM) to test the involvement of mGluR7 (EC_{50} for L-AP4: 160 or 252 μM) (Conn and Pin 1997; Okamoto et al. 1994) in these axon terminals. We found a consistent and pronounced reduction of EPSC amplitudes by 600 μM L-AP4 in every recorded interneuron ($n = 11$, Fig. 6 and Table 2), including four O-LM, two basket, and four O-Bi cells. Surprisingly, the effect of 600 μM L-AP4 was not larger on eEPSC amplitudes recorded in O-LM cells (60 ± 16% reduction of the 1st EPSC)

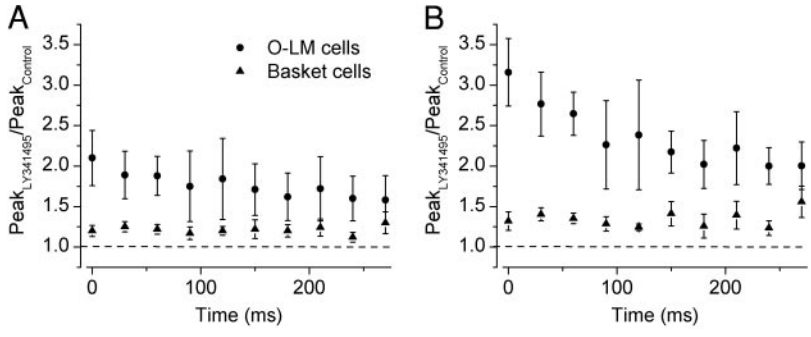

FIG. 4. Summary of the effect of 1μM LY341495 in all O-LM and basket cells (*A*) and in those cells in which significant effect was observed (*B*). The relative change in EPSC amplitude caused by the application of 1 μM LY341495 is much larger in O-LM compared with basket cells. Interestingly, the relative change in EPSCs at a given time point did not increase significantly during the train of stimuli in either cell type, indicating that the released transmitter did not significantly increase mGluR activation during the stimulus train. Data are expressed as means ± SE (*A*: $n = 13$ O-LM cells for the 1st 3 responses, $n = 5$ for the rest of the responses; $n = 14$ basket cells for the 1st 3 responses, $n = 8$ for the rest of the responses; *B*: $n = 6$ O-LM cells for the 1st 3 responses, $n = 3$ for the rest of the responses; $n = 7$ basket cells for the 1st 3 responses, $n = 4$ for the rest of the responses).

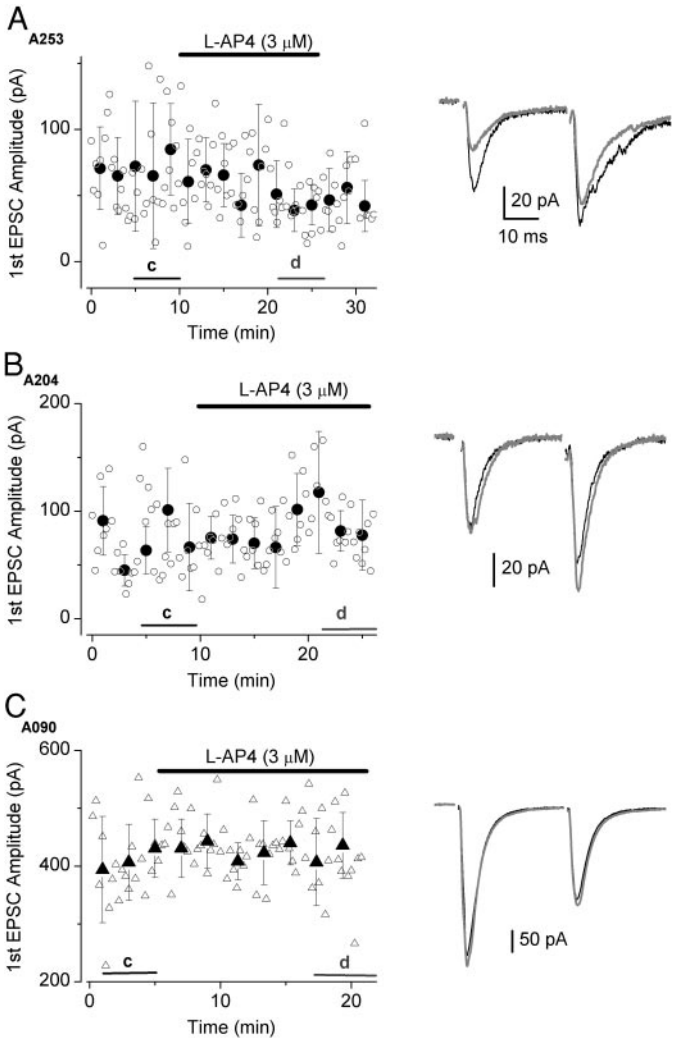

FIG. 5. Effect of 3 μM L(+)-2-amino-4-phosphonobutyric acid (L-AP4) on eEPSCs recorded in O-LM (*A* and *B*) and basket (*C*) cells. The amplitude of the 1st EPSC (small open symbols) of the stimulus train is superimposed on the mean ± SD (filled symbols) of the same data calculated every 2 min. The bars labeled with c and d indicate the control and drug application periods, respectively, which are compared (Mann-Whitney *U* test). *Right*: averaged traces of the synaptic responses to the 1st 2 stimuli in control (black traces) and in L-AP4 (gray traces). *A* and *B*: the application of 3 μM L-AP4 resulted in either a significant reduction (A, $P < 0.01$, $n = 20$ responses each) or no change (*B*) of the eEPSCs recorded in O-LM cells. *C*: no significant change in the amplitude of the eEPSCs was observed in basket cells after the application of 3 μM L-AP4.

than in the rest of the cells (66 ± 18%), although axon terminals making synapses on O-LM cells contain a much higher density of presynaptic mGluR7 than those contacting other interneuron types (Losonczy et al. 2002; Shigemoto et al. 1996). This effect of L-AP4 is similar to that produced by 10 or 50 μM concentration of the drug on unidentified hippocampal interneurons (Scanziani et al. 1998).

In the third series of agonist experiments, we tested the presence of group II mGluRs on glutamatergic axons with 1 μM DCG-IV (Conn and Pin 1997). A significant change in the amplitude of eEPSCs was observed in only 4 of 15 interneurons (Fig. 7 and Table 2). In two of three O-LM cells, 1 μM DCG-IV reduced the first eEPSC amplitude by 21 and 55% (e.g., Fig. 7*A*). In two of seven basket cells, the application of DCG-IV resulted in a significant change in EPSC amplitudes; in one cell, it increased and in the other one, it decreased the postsynaptic responses (Fig. 7*C* and Table 2). From our agonist experiments, it seems that mGluR2/3/4/7/8 are all candidates as presynaptic receptors at some axon terminals making synapses onto hippocampal interneurons. The only mGluR subtype that seems to be present at every axon terminal is mGluR7.

Because 1 μM LY341495 very likely did not antagonize mGluR4, all other group II/III mGluR subtypes (mGluR2/3/7/8) remained as possible candidates for mediating the effect of 1 μM LY341495. We made another attempt to distinguish between mGluR2/3/8 and mGluR7 by applying LY341495 at a concentration of 200 nM (Kingston et al. 1998). In three of three recorded basket cells, 200 nM LY341495 significantly increased the amplitude of the eEPSCs (mean increase for 3

TABLE 2. *Effects of mGluR agonists on the amplitude of eEPSCs*

Cell ID	Cell Type	Peak 1_{Drug}/ Peak 1_{Contr}	Peak 2_{Drug}/ Peak 2_{Contr}	Peak 3_{Drug}/ Peak 3_{Contr}
3 μM L-AP4				
A094	O-LM	0.76	1.25	1.24
A204	O-LM	1.05	1.16	1.13
A243	O-LM	0.92	**0.75**	**0.79**
A253	O-LM	**0.63**	0.83	0.93
A093	Basket	1.20	1.05	0.81
A090	Basket	1.07	1.05	1.16
A092	O-Bi	0.88	0.89	0.84
A098	O-Bi	0.95	1.26	0.98
A089	O-Bi	1.08	1.11	1.09
A097	non classif.	0.89	0.87	0.98
600 μM L-AP4				
A100	O-LM	**0.32**	**0.64**	**0.50**
A224	O-LM	**0.21**	**0.14**	**0.13**
A204	O-LM	**0.58**	0.77	0.78
A253	O-LM	**0.47**	**0.63**	**0.58**
A099	Basket	**0.30**	**0.37**	**0.29**
A111	Basket	**0.29**	**0.27**	**0.27**
A092	O-Bi	**0.09**	**0.05**	**0.28**
A089	O-Bi	**0.69**	**0.59**	**0.62**
A103	O-Bi	**0.28**	**0.46**	**0.54**
A116	O-Bi	**0.33**	**0.23**	**0.25**
A097	non classif.	**0.43**	**0.34**	**0.44**
1 μM DCG-IV				
A112	O-LM	0.97	0.98	0.88
A113	O-LM	**0.45**	**0.50**	**0.58**
A119	O-LM	**0.79**	0.93	0.87
A106	Basket	1.03	0.97	0.92
A108	Basket	0.90	0.83	0.69
A111	Basket	0.94	0.99	0.95
A117	Basket	**3.33**	**3.31**	**2.55**
A120	Basket	**0.16**	**0.23**	**0.41**
A126	Basket	1.24	1.21	1.23
A127	Basket	1.05	1.13	1.05
A114	O-Bi	0.88	0.84	0.94
A115	O-Bi	0.91	0.98	0.91
A116	O-Bi	1.24	1.02	0.98
A122	non classif.	1.00	1.15	0.90
A128	non classif.	1.22	1.16	1.08

The bold numbers in the $Peak_{Drug}/Peak_{Contr}$ columns indicate significant change in the amplitude of EPSCs ($P < 0.01$ with the Mann-Whitney *U* test). The soma of most cells was in the CA1 area; two were at its border with the CA3 area (*A103*, *A127*), and one in the subiculum (*A120*). Detailed presentation of the anatomical data is in Losonczy et al. (2002). mGluR, metabotropic glutamate receptor; O-LM and O-Bi, oriens-lacunosum moleculare and bistratified; L-AP4, L(+)-2-amino-4-phosphonobutyric acid; DCG-IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine.

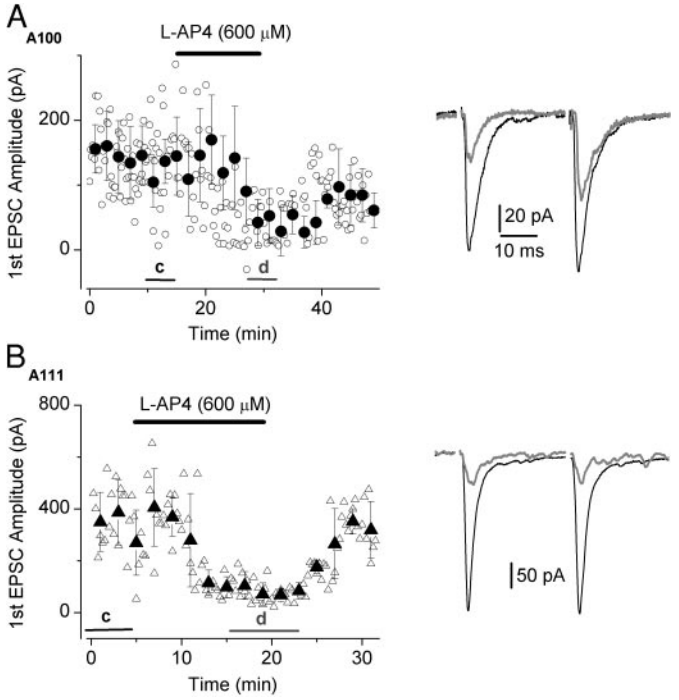

FIG. 6. Reduction of eEPSC amplitudes by 600 μM L-AP4 in both O-LM (*A*) and basket (*B*) cells. *Left*: the amplitude of the 1st EPSCs (small open symbols) and superimposed are the mean ± SD (filled symbols) calculated every 2 min. Bars c and d indicate the control and drug periods, respectively. During these periods, EPSCs evoked by the 1st 2 stimuli were averaged and are displayed (*right;* control, black traces; L-AP4, gray traces).

stimuli: 26 ± 8%). The magnitude of this increase is comparable to that obtained with 1 μM LY341495 (mean increase for the1st 3 stimuli: 36 ± 19%). In one identified O-LM cell, 200 nM LY341495 increased the amplitude of the eEPSCs by 215% (averaged for all 3 stimuli) with a 223% increase of the first eEPSC. These results are consistent with mGluR2/3/8 being responsible for the persistent reduction of glutamate release from these hippocampal axon terminals.

DISCUSSION

Our results support the hypothesis that persistently active mGluRs reduce the probability of transmitter release in vitro from axon terminals making synapses onto some hippocampal GABAergic interneurons. The magnitude of the reduction of EPSCs is cell-type specific; excitatory synaptic inputs to O-LM cells are approximately seven times more affected than those to basket cells. Glutamate released by 10 stimuli at 33 Hz did not appear to increase mGluR activation further. The results obtained using mGluR subtype-selective agonists and antagonist point to mGluR2/3/8 being responsible for the cell-type selective, persistent regulation of glutamatergic postsynaptic responses.

Considering a presynaptic mechanism of action, glutamate released from an axon terminal could activate presynaptic mGluRs present at the same terminal (Takahashi et al. 1996; von Gersdorff et al. 1997) or could activate receptors at neighboring terminals following its diffusion out from the synaptic cleft (Dube and Marshall 2000; Mitchell and Silver 2000; Semyanov and Kullmann 2000). In the case of group II mGluRs, which are not concentrated in the synaptic active

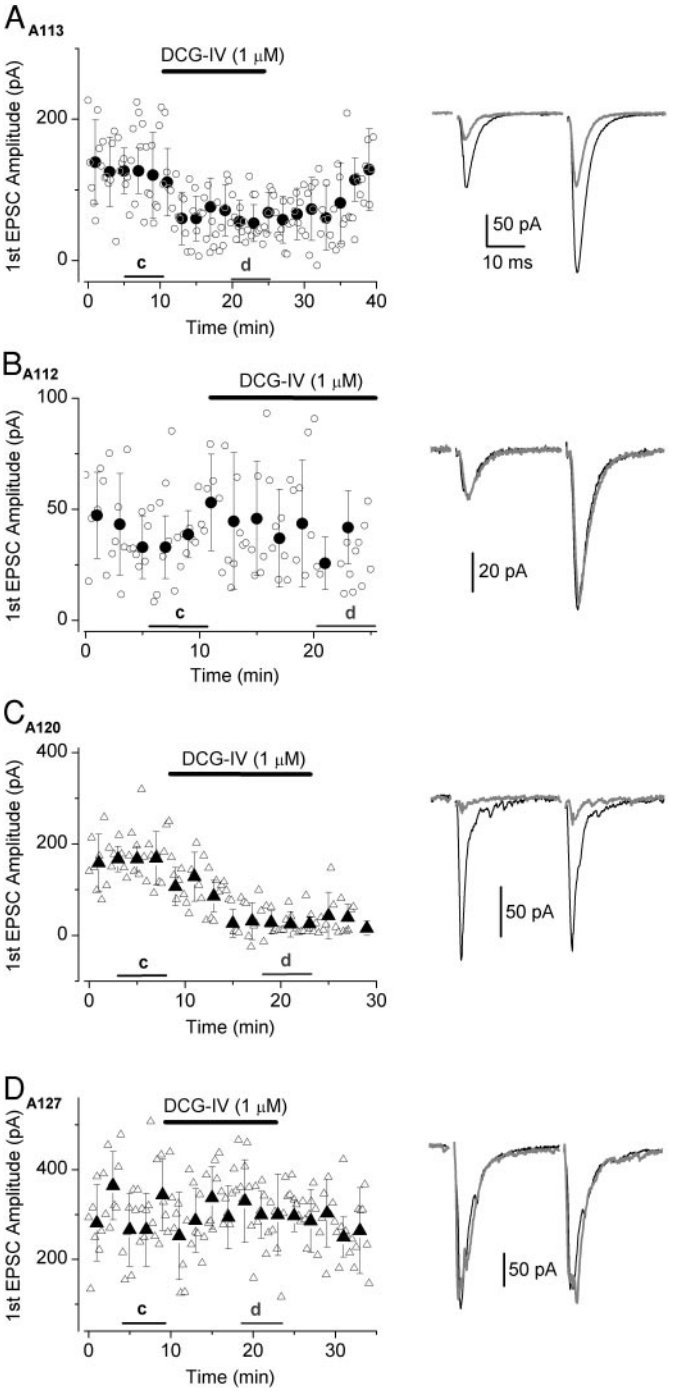

FIG. 7. Heterogeneous effect of 1 μM (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) on EPSCs in identified O-LM (*A* and *B*) and basket (*C* and *D*) cells. *Left*: the amplitudes of the 1st EPSCs during the recordings (small open symbols) and superimposed are the mean ± SD (filled symbols) calculated every 2 min. Bars c and d indicate the control and drug periods, respectively. *Right*: EPSCs evoked by the 1st 2 stimuli in control conditions and during the application of 1 μM DCG-IV were averaged (black traces, control; gray traces, DCG-IV). *A*: the application of 1 μM DCG-IV resulted in a reversible reduction in the amplitude of the eEPSCs in this O-LM cell. *B*: the selective group II mGluR agonist had no significant effect in this O-LM cell. *C*: eEPSCs in this basket cell showed a large reduction after the application of 1 μM DCG-IV. *D*: in this cell, as in the majority of basket cells, DCG-IV had no significant effect on the amplitude of the eEPSCs.

zone but are mainly present on the preterminal axon and on the nonsynaptic part of the terminal (Lujan et al. 1997; Shigemoto et al. 1997), glutamate has to diffuse out from the synaptic cleft to activate them (Scanziani et al. 1997). Group III mGluRs are concentrated in the presynaptic specialization (Shigemoto et al. 1996, 1997), and therefore it is tempting to speculate that they will be activated by glutamate released in the same synapse. Thus the activation of the presynaptic group III receptors would reflect the activity of a single presynaptic neuron. However, the presence of group III mGluRs in the presynaptic active zone of GABAergic terminals in the islands of Calleja (Kinoshita et al. 1998), in the isocortex (Dalezios et al. 2001, 2002), and also in the hippocampus (Somogyi et al. 1999) suggests that receptors in the presynaptic active zone are not necessarily activated by glutamate release at the same synapse.

The level of glutamate reaching receptors following its diffusion outside the synaptic cleft (spillover) could reflect the activity of all those neurons, whose axons are in the vicinity of the receptors. Depending on the details of transmitter diffusion, uptake, and the geometry of the neuropil, the activity of probably no more than a handful of cells could be monitored in this way. The activity of the whole network would be monitored best by receptors sensitive enough to be activated by low concentrations of ambient glutamate in the extracellular space. Provided the activation of such receptors follows the fluctuations in the extracellular glutamate concentration, they would constitute a highly efficient mechanism for monitoring the activity of a large population of nerve cells. Such persistently active mGluRs have been reported in the hypothalamus (Oliet et al. 2001; Schrader and Tasker 1997), where the glial coverage of synapses is reduced during lactation and, as a consequence, the ambient glutamate concentration increases around the synapses, resulting in a presynaptic mGluR activation. Furthermore, it has been shown that (RS)-α-methyl-4-phosphonophenylglycine (MPPG), a group III receptor antagonist, can produce a variable increase of some cortical EPSPs (Jin and Daw 1998), pointing to a steady-state activation of the presynaptic group III mGluRs. In the present study, some hippocampal postsynaptic responses were also found to be under the control of persistently active mGluRs. Furthermore, the rigorous identification of the postsynaptic GABAergic interneurons allowed us to examine how the persistent regulation of release depends on the type of target cell. Glutamatergic inputs to O-LM cells are significantly more depressed by persistently active mGluRs than those to basket cells; inputs to O-Bi cells showed a wide range of increase in eEPSC amplitude following the application of the mGluR antagonist. As mentioned in the preceding text, persistent mGluR activation suggests that this mechanism monitors the overall activity of the hippocampal network and possibly adjusts the probability of glutamate release in a cell-type-specific manner. This may have a specific effect on the hippocampal network. We hypothesize that, in periods when a large population of pyramidal cells is active, the excitatory drive to O-LM cells, cells that innervate distal pyramidal dendrites, is preferentially scaled back to an as yet undetermined level, allowing the sustained activation of these cells and an optimal interaction of GABA released by them with the entorhinal input to pyramidal cells.

The cell-type specific difference in the regulation of glutamate release parallels the known difference in the amount of presynaptic mGluR7a at these axon terminals (Shigemoto et al. 1996). However, it seems from our experiments with low concentrations of LY341495 that mGluR7a is probably not responsible for this action, but instead mGluR2/3/8 mediate it. This is consistent with the low efficacy of glutamate at mGluR7a and the much higher efficacy at mGluR2/3/8 (Conn and Pin 1997). It is also noteworthy that the exogenously applied 600 μM L-AP4 (mGluR4/7/8 agonist at this concentration) reduces the postsynaptic responses to a similar extent in O-LM and basket cells despite the large difference in the mGluR7a density in the corresponding innervating terminals. It remains to be determined if the presence of other potential splice variants of mGluR7 (Schulz et al. 2002) may explain these results.

The results also show that, although persistently active mGluRs control excitatory responses, their activation plays a minor role in the short-term plasticity of the excitatory inputs. In other words, facilitating EPSCs remained facilitating in O-LM cells, and depressing EPSCs in basket cells remained depressing following the application of an mGluR antagonist. Similarly, a minor contribution of presynaptic mGluR activation to the short-term depression of EPSCs was found at the calyx of Held (von Gersdorff et al. 1997). Depression at these synapses may involve the depletion of the readily releasable pool of vesicles (Dobrunz and Stevens 1997; Stevens and Wesseling 1999), whereas the temporal summation of free Ca^{2+} in the terminals may underlie facilitation, as shown at cortical glutamatergic synapses onto neocortical bitufted cells (Rozov et al. 2001), the homologous cell type to hippocampal O-LM cell studied here. Furthermore, Sansig et al. (2001) have reported a slowing in the recovery from paired-pulse facilitation of EPSPs in bitufted cells of mice lacking mGluR7, indicating the involvement of mGluRs in the dynamic properties of the synapses. These data, taken together, are consistent with distinct mGluR subtypes being able to differentially modulate the dynamic properties of synapses.

Following the analysis of the relative increase in the EPSC amplitudes by LY341495 throughout the train of stimuli, we concluded that glutamate released by 10 stimuli at 33 Hz did not cause further detectable mGluR activation. This may be explained by a full occupancy of mGluRs by the ambient glutamate in the extracellular space. Alternatively, a low occupancy of mGluRs by ambient glutamate is also consistent with this finding if the synaptically released glutamate transients are too fast to be "sensed" by these receptors. The lack of knowledge of microscopic binding and unbinding rates of glutamate to these mGluRs prevents us to test these possibilities with modeling.

One intriguing finding is that a significant effect of 1 μM LY341495 was observed in only approximately half of the recorded cells, and this ratio was more or less the same for all cell types. There are at least two main possible reasons for this variability. First, we may have activated presynaptic fibers of different origin with differential mGluR expression. There are at least two major glutamatergic inputs to the s. oriens/alveus in the CA1 area, the local collaterals of the CA1 pyramidal cells and the Schaffer collateral/commissural input from CA3 pyramidal cells. At least one type of the interneurons, the O-LM cell, receives its main excitatory innervation from local pyramidal cells in the CA1 area (Blasco-Ibanez and Freund 1995), but even this type of cell may receive additional gluta-

matergic innervation from other sources. A glutamatergic input also arrives from the entorhinal cortex (Deller et al. 1996), and although numerically minor, it preferentially innervates interneurons rather than pyramidal cells (J. T. Sanz, E. H. Buhl, and P. Somogyi, unpublished observation). It is also possible that even within a single population of presynaptic input there is a heterogeneous distribution of presynaptic mGluRs. In both cases, the effect or lack of effect of the antagonist would reflect whether a certain mGluR was present or not in the stimulated axon. The second possibility is that although the receptors are present on the stimulated axon, they are not activated by the ambient level of transmitter. This could be due to some technical conditions of the slice preparation (e.g., synapses too close to the surface) or to a genuine, functionally relevant difference in the ambient glutamate level. Our experiments with 3 μM L-AP4 and 1 μM DCG-IV are consistent with the first possibility, namely that not every fiber contains mGluR2/3/8. Whether the differential source of the presynaptic glutamatergic axons corresponds to the expressed mGluR subtype or whether a differential mGluR expression is also found within a single population of presynaptic fiber remains to be determined.

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