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Neuropharmacology 49 (2005) 45–56
NEURO PHARMACOLOGY
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Group II and III mGluRs-mediated presynaptic inhibition of EPSCs recorded from hippocampal interneurons of CA1 stratum lacunosum moleculare

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Received 16 March 2005; received in revised form 27 April 2005; accepted 3 May 2005

Abstract

We have studied the effects of groups II and III metabotropic glutamate receptor (mGluR) activation on excitatory responses recorded from hippocampal interneurons of CA1 stratum lacunosum moleculare (SLM). Excitatory postsynaptic currents (EPSCs) evoked by stimulation of the perforant pathway were reduced either by the group II mGluR agonist LY354740 (50–100 nM, 49.1 ± 5.7% of control) or by the group III mGluR agonist L-2-amino-4-phosphonobutyric acid (L-AP4) (50 μM, 36.8 ± 4.4% of control). Both drugs significantly enhanced paired-pulse facilitation of the EPSCs. Furthermore, both 100 nM LY354740 and 50 μM L-AP4 reduced the frequency, but not the amplitude, of miniature excitatory synaptic currents (mEPSCs), recorded in the presence of 1 μM TTX and 50 μM picrotoxin, or EPSCs evoked by perforant pathway stimulation in the presence of 2.5 mM Sr^{2+}. The broad-spectrum mGluR antagonist LY341495 (10–50 μM) did not affect test EPSCs elicited 210 ms after stimulation at 100 Hz. At network level, 1–5 μM LY354740 significantly reduced the power of gamma frequency oscillations induced by 20 μM carbachol, 600 nM kainate and 5 mM K^+ in hippocampal CA1 area. Our results show powerful modulation of excitatory transmission impinging on interneurons of CA1 SLM by presynaptic group II or III mGluRs.

Keywords: Presynaptic receptor; mGluRs; Interneuron; Entorhinal cortex; Hippocampus

1. Introduction

The stratum lacunosum moleculare (SLM) of CA1 hippocampus contains the somata of several types of GABAergic interneurons, classified according to their morphology, electrophysiological properties and biochemical fingerprint (Freund and Buzsaki, 1996; Somogyi and Klausberger, 2005). These interneurons receive several glutamatergic inputs, which are likely to originate from areas both extrinsic and intrinsic to the hippocampal formation (Freund and Buzsaki, 1996). One major excitatory input is represented by the perforant pathway, which originates from layer III neurons of the entorhinal cortex. Type I synapses have been observed between axons of the perforant pathway and parvalbumin-immunoreactive dendrites of GABAergic interneurons (Desmond et al., 1994; Kiss et al., 1996). The axons of SLM interneurons project to hippocampal pyramidal neurons or to other interneurons

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doi:10.1016/j.neuropharm.2005.05.009

(Somogyi and Klausberger, 2005). Stimulation of SLM interneurons elicits in CA1 pyramidal cell a $GABA_A$ receptor-mediated unitary IPSP with slow rise time and decay, consistent with the projection of these interneurons mainly to dendritic regions of the pyramidal neuron (Vida et al., 1998). Likewise, we have recently observed a slow unitary IPSC occurring within a network of electrically and chemically connected cells formed by a specific SLM interneuron type, the so called neurogliaform cell (Price et al., 2005). Due to their strategic placement and connectivity, it is likely that SLM interneurons play a key role in modulating the input of information from the entorhinal cortex into the CA1.

Hippocampal SLM displays particularly strong immunoreactivity for mGluR2 and mGluR7a (Shigemoto et al., 1997), and although not enriched, the presence of mGluR4 and mGluR8 has also been reported (Corti et al., 2002). The activation of these mGluRs powerfully inhibits synaptic responses evoked in CA1 area by perforant pathway stimulation (Kew et al., 2001; Capogna, 2004). Furthermore, segregation of receptors at glutamatergic axon terminals in this layer of hippocampus is observed, so that mGluR7a and mGluR4 are detected at active zones, whereas mGluR2 is found at preterminal zones (Yokoi et al., 1996; Shigemoto et al., 1997; Corti et al., 2002). This segregation has functional meaning, because perforant pathway-evoked excitatory postsynaptic currents (EPSCs) recorded in CA1 pyramidal neurons are inhibited by presynaptic group II or III mGluRs via different mechanisms (Capogna, 2004). However, it is not known whether group II or III mGluRs also modulate perforant pathway-evoked EPSCs recorded from CA1 SLM interneurons, namely whether the functional expression of group II/III mGluRs at perforant pathway is target specific. Therefore, the aim of this study is to test and compare the effects of activation of group II or III mGluRs on synaptic responses recorded from SLM CA1 interneurons.

2. Methods

2.1. Preparation of slices

All procedures involving animals were performed using methods approved by the UK Home Office and according to The Animals (Scientific Procedures) Act, 1986. Juvenile Sprague–Dawley rats (13–21 days) were anaesthetized with isoflurane and decapitated. The brain was quickly removed, extra tissue trimmed away and the remaining block containing the hippocampus mounted for vibratome sectioning in ice cold artificial cerebrospinal fluid (ACSF) containing (in mM): 130 NaCl, 3.5 KCl, 2.5 $CaCl_2$, 1.5 $MgSO_4$, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 10 glucose and saturated with 95% O_2 and 5% CO_2, to which 3 mM kynurenic acid was added. Alternatively, a high sucrose/high magnesium solution containing (in mM): 87 NaCl, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$ and 7 $MgCl_2$ was also used for slice preparation. Horizontal sections containing dorsal hippocampus and entorhinal cortex were cut with a thickness of 300–320 μm (450 μm for the extracellular experiments, to better maintain the cellular network). The slices were allowed to recover in ACSF without kynurenic acid at room temperature for at least 45 min before recording.

2.2. Electrophysiology: recording and analysis

SLM interneurons were identified using a microscope (Zeiss Axioscop 2 F/S Plus, Jena, Germany) with a 40× immersion DIC objective coupled to an IR camera system (Hamamatsu, Hamamatsu-City, Japan). Slices were placed in a submerged (intracellular) or interface (extracellular) recording chamber and were constantly superfused (1–2 ml/min.) with ACSF at 30–35 °C. Patch pipettes were pulled and filled with (mM): 126 K-gluconate, 4 KCl, 4 ATP-Mg, 0.3 GTP-Na_2, 10 Na_2-phosphocreatine, and 10 HEPES, pH of 7.3, osmolarity 290 mOsmol. Biocytin was added to 1 ml aliquot of intracellular solution at 0.5% before recording. The DC resistance of the electrodes was 3–7 MΩ when filled with the pipette solution. Whole-cell patch clamp recordings were performed with an EPC9/2 amplifier (HEKA Elektronik, Lambrecht, Germany) or with an Axopatch-1D amplifier (Axon Instruments, Union City, CA), either in current clamp, to assess the firing pattern of the neurons, or in voltage-clamp at a holding potential of −70 mV. Series resistance and whole-cell capacitance were monitored and experiments were discontinued if series resistance increased by more than 25–30%. EPSCs were elicited by stimulation of the perforant pathway using either a tungsten concentric bipolar electrode or monopolar electrode inserted close to the presubiculum. Picrotoxin (50 μM) was added to the ACSF to isolate evoked EPSCs. Paired-pulse ratio was calculated by dividing the peak of the mean EPSC evoked by the second stimulus (EPSC2) with the peak of the mean EPSC evoked by the first stimulus (EPSC1). Prior to recording a cut was made between the CA3 and CA1 regions of the hippocampus to prevent indirect activation of CA1 by perforant pathway stimulation through CA3 or dentate granule cells (Yeckel and Berger, 1990). Evoked synaptic responses were analysed offline using Pulsefit (HEKA, Lambrecht, Germany) and IGOR Pro (WaveMetrics, Lake Oswego, Oregon). The analysis of mEPSCs was carried out using Mini-analysis software (Synaptosoft, Decatur, GA) as described previously (Capogna et al., 2003). For the analysis of asynchronous EPSCs recorded in the presence of

$SrCl_2$, a time window (280–290 ms) starting 15–25 ms following the onset of the stimulus to the start of the next stimulus was used to scan for events. This allowed for the evoked EPSC to completely subside. The duration of this time window was then used to calculate asynchronous event frequencies.

Statistical analysis was performed using Prism software (GraphPad, San Diego, CA), and the tests used are specified throughout the results. Unless indicated otherwise, values presented in the text and in figures represent the mean ± standard error of the mean.

Extracellular field recordings were obtained from stratum pyramidale of the CA1 area of slices, in which the CA3 was cut out, using patch pipettes filled with oxygenated ACSF. Recordings were performed using an Axopatch-1D amplifier with pClamp6 acquisition software (Axon Instruments). Offset potentials were eliminated on line and/or off line. The signals were filtered at 2 kHz and digitised at 5 kHz. Power spectra were obtained from 60 s recording periods using a Fast Fourier transform algorithm contained in Spike2 software (CED, Cambridge, UK). To quantify the data, power spectra of the last 60 s before the end of either control or drug application period were obtained and subsequently analysed. The diffusion of the drugs in slices kept at the interface chamber was slower than that obtained in submerged slices, and therefore the drugs were applied at concentrations about 10-fold higher than those used in the whole-cell patch clamp experiments. An one-sample *t*-test was used to compare the normalised peak amplitude of the power spectra in the control and during drug applications. Data are presented as mean ± standard error.

2.3. Histological processing and anatomical evaluation

Following electrophysiological recording, slices were fixed overnight by immersion in fixative containing 4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% saturated picric acid in 0.1 phosphate buffer (pH 7.4). Fixed slices were then embedded in gelatin and re-sectioned at 60 μm thickness. The recorded cells were labelled by avidin-biotinylated HRP complex (Vector Laboratories, Burlingame, CA, USA) followed by peroxidase reaction using diaminobenzidine (0.05%) as chromogen and 0.01% H_2O_2 as substrate. The sections were then dehydrated and permanently mounted on slides.

2.4. Chemical and drugs

All drugs were bath applied and reached a steady-state concentration in the chamber in <4 min. Salts used in the preparation of the internal recording solution and ACSF were obtained from either BDH or Sigma–Aldrich (St. Louis, Missouri, USA). LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid, (Tocris Cookson Ltd., Bristol, UK), was stored in frozen aliquots of 10 mM in DMSO. L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) (Tocris Cookson Ltd., Bristol, UK), was stored in frozen aliquots of 10 mM in dH_2O. Tetrodotoxin (TTX), (−)-bicuculline methochloride, carbachol, kainate and picrotoxin were purchased from Tocris Cookson (Bristol, UK).

3. Results

3.1. Anatomy and firing pattern of the recorded neurons

In the present study, neurons were recorded under visual control and I/V protocols were performed in whole-cell current clamp mode to assess the firing pattern of the recorded cells in response to depolarising rectangular current pulses. All neurons included in this study displayed membrane properties, action potential kinetics and discharge patterns consistent with features previously observed in interneurons of CA1 SLM (data not shown, see also: Lacaille and Schwartzkroin, 1988; Williams et al., 1994). In addition, all the recorded slices were histologically processed, and the neurons analysed using light microscopy (data not shown). All biocytin-filled neurons analysed were identified as interneurons in agreement with previous papers (Spruston et al., 1997; Vida et al., 1998). Light microscopic analysis revealed in our sample the presence of several distinct populations of SLM interneurons with various axonal trajectories. Since the mGluR ligands affected virtually all EPSCs recorded, no attempts were made to classify the recorded neurons in different interneuronal types.

3.2. Pharmacological activation of group II or III mGluRs presynaptically inhibits perforant pathway-evoked EPSCs

Group II or III presynaptic mGluRs have been detected at synaptic regions in SLM of hippocampus, often associated with type I synapses (Shigemoto et al., 1997; Corti et al., 2002). Consistent with these observations, the application of the selective group II mGluR agonist LY354740 or the selective group III mGluR agonist M-2-amino-4-phosphonobutyric acid (L-AP4) (Schoepp et al., 1999) reduced the EPSCs evoked by perforant pathway stimulation recorded in CA1 SLM interneurons (Fig. 1). A representative plot of the time course of the effect elicited by 50–100 nM LY354740 applied for 5 min is shown in Fig. 1A2. On average, the mean amplitude of the EPSCs during LY354740 was 49.1 ± 5.7% of the control (Fig. 1A3, two-tailed *t*-test $p \leq 0.01$, $n = 10$), and was associated with an increase

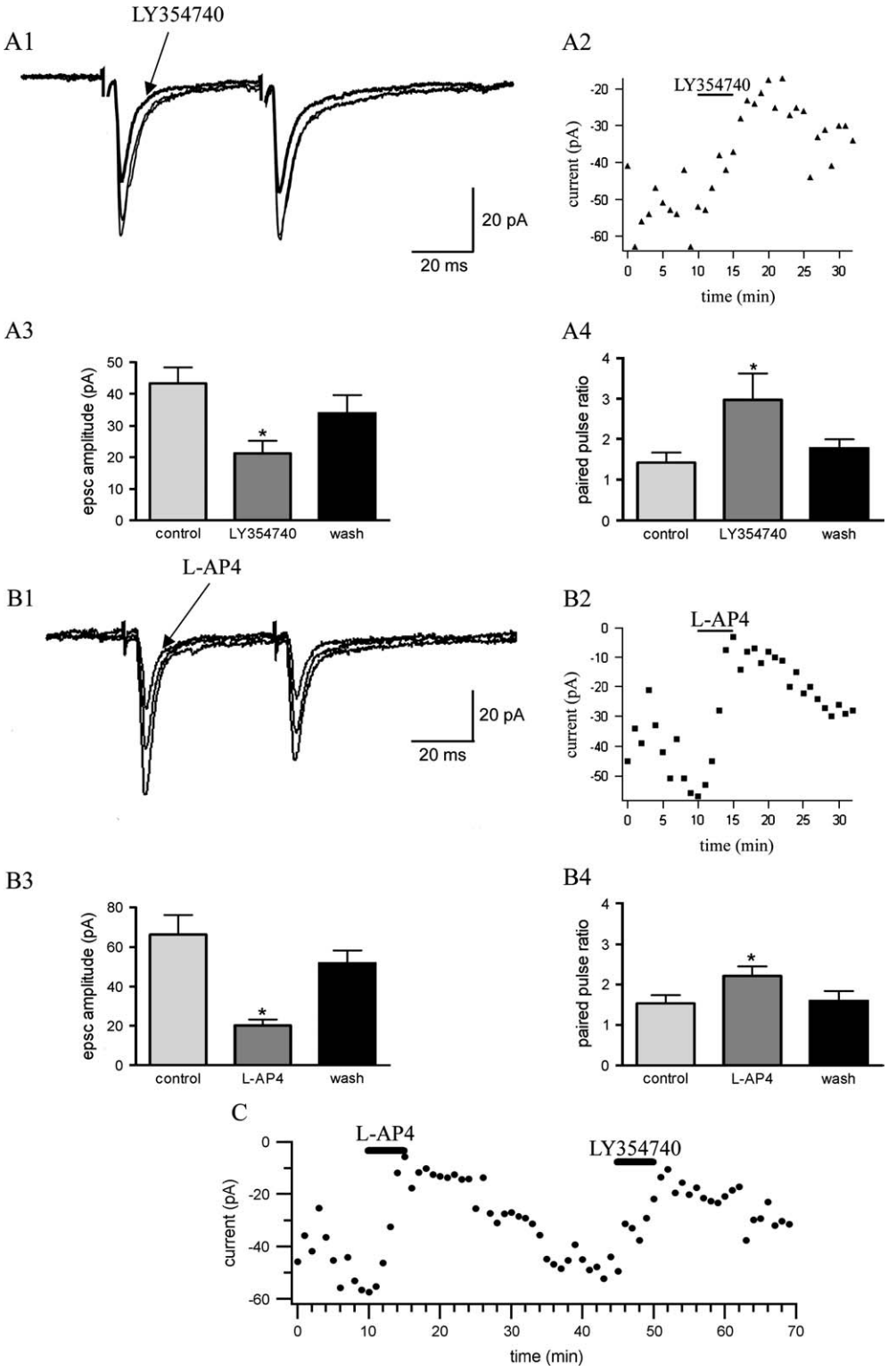

Fig. 1. Activation of group II or III mGluR reduces the perforant pathway-CA1 SLM interneuron EPSCs. (A1) EPSCs evoked by perforant pathway stimulation were reversibly inhibited by 100 nM LY354740. Each trace is an average of 10 EPSCs recorded before, during and after washing of LY354740. (A2) Representative time course of LY354740 effect. Each point is the average of 6 EPSCs evoked at 10 s intervals. (A3) Summary histogram showing the effect of LY354740 (50–100 nM) on perforant pathway-evoked EPSCs ($n = 10$). (A4) The PPR showed a significant increase during LY354740 application. (B1) L-AP4 (50 μM) reduced EPSCs evoked by perforant pathway stimulation. (B2) Representative time course of the effect of L-AP4 on perforant path-evoked EPSCs. (B3) Summary histogram showing the effect of 50 μM L-AP4 on EPSCs ($n = 17$). (B4) L-AP4 application also caused a significant increase in the PPR in 12 of 17 neurons tested. (C) L-AP4 (50 μM) reduced the peak amplitude of the evoked EPSCs and following washout of the drug, application of LY354740 (50 nM) also depressed the EPSC amplitude. Each point is the average of six EPSCs evoked at 10 s interval.

of the paired-pulse ratio (PPR) (Fig. 1A4, two-tailed t-test $p \leq 0.05$). LY354740 did not change the holding current of the interneurons clamped at −70 mV (the mean difference between the holding current during and before the drug was 0.3 ± 2.5 pA, two-tailed paired t-test $p \geq 0.9$, $n = 10$). Likewise, L-AP4 (50 μM) significantly inhibited the EPSCs in a reversible manner (Fig. 1B). Quantitatively, the mean amplitude of the EPSCs during 100 μM L-AP4 was $37 \pm 4.4\%$ of the control (Fig. 1B3, two-tailed t-test $p \leq 0.001$, $n = 12$), and was associated with an increase in the PPR (Fig. 1B4, two-tailed t-test $p \leq 0.01$). In a further five neurons there was also a significant decrease in the amplitude of the EPSC, however, this was associated with a significant decrease in the PPR towards unity (data not shown, two-tailed t-test $p \leq 0.001$). L-AP4 did not change the holding current of the cells clamped at −70 mV (the mean difference between the holding current during and before 100 μM L-AP4 was -1.6 ± 1.8 pA, two-tailed paired t-test $p \geq 0.35$, $n = 16$). The application of 20 μM DNQX always abolished the EPSCs ($n = 10$). Furthermore, when L-AP4 or LY354740 were subsequently applied after wash out of the other agonist, either drug inhibited the evoked EPSCs (Fig. 1C, $n = 6$), demonstrating that the SLM interneurons were subjected to the modulatory action of both mGluR agonists. These data suggest that pharmacological activation of presynaptic group II or III mGluRs inhibits perforant pathway-evoked EPSCs in CA1 SLM interneurons.

3.3. Does the glutamate released by the perforant pathway activate presynaptic group II or III mGluRs?

We stimulated the perforant pathway at high frequency and tested the effect of the broad-spectrum mGluRs antagonist LY341495 (10–50 μM, Kingston et al., 1998) in order to uncover an effect of mGluRs following the endogenous release of glutamate. A conditioning train involving high frequency stimulation at 100 Hz was used to mimic entorhinal-hippocampal activity at gamma frequency. The train was followed by a test stimuli applied at 210 ms after the onset of the last conditioning pulse. At the recording temperature of approximately 32 °C no significant change in the amplitude of the seven pulses delivered during the 100 Hz stimulation, or in the amplitude of the test pulse was observed following bath application of LY341495 (Fig. 2, Two-way ANOVA $p > 0.05$; $n = 4$).

3.4. LY354740 and L-AP4 inhibited the frequency, but not the amplitude, of mEPSCs

The inhibition of evoked EPSCs associated with changes in the PPR by LY354740 or L-AP4 is consistent

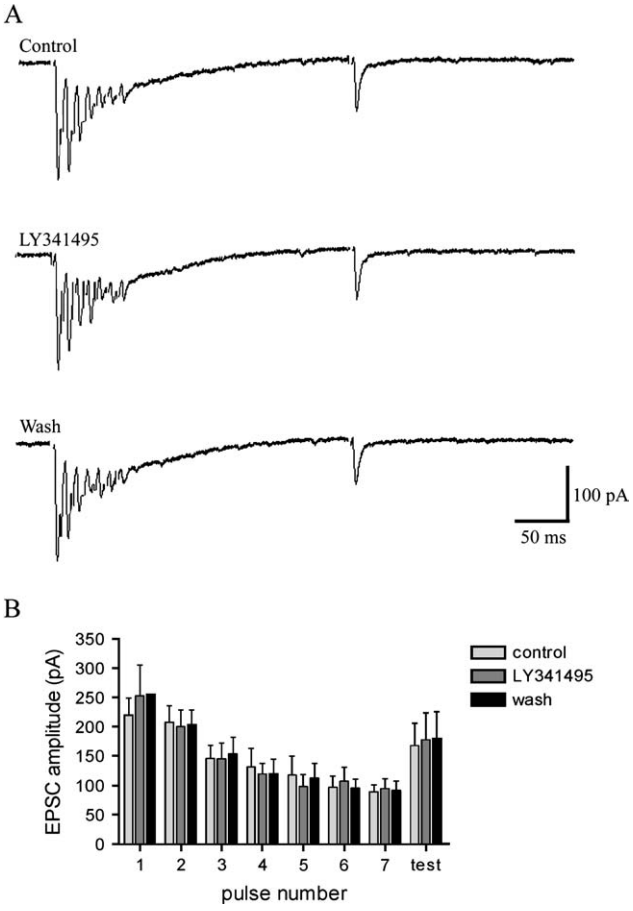

Fig. 2. The broad-spectrum mGluRs antagonist LY341495 does not change perforant pathway-evoked EPSCs during or after train stimulation. (A) Stimulation (100 Hz) followed by a test pulse was delivered before (top), during application (middle) and after washing out of 50 μM LY341495 (bottom). Each EPSC represents the average of 10 individual traces. (B) Summary plot showing no clear effect of LY341495 on the amplitude of the EPSCs evoked during the 100 Hz train or in the amplitude of the test pulse delivered 210 ms after the train stimulus ($n = 4$).

with a presynaptic activation of group II or III mGluRs, respectively. In order to further investigate this issue, we tested the agonists on spontaneous mEPSCs recorded from interneurons of CA1 SLM. In the presence of 1 μM TTX and 50 μM picrotoxin, to block action potentials and $GABA_A$ receptors, respectively, application of 100 nM LY354740 caused a significant reduction in the frequency of mEPSCs ($n = 12$, $p < 0.005$, paired t-test, Fig. 3A). On average, the mEPSC frequency was 1.01 ± 0.18 Hz in control, 0.45 ± 0.07 Hz during the application of LY354740, and 1.23 ± 0.27 Hz after washing out the drug. In contrast, the mean mEPSC amplitude was unchanged ($n = 12$, $p > 0.05$, paired t-test). Likewise, application of 50 μM L-AP4 significantly reduced the frequency, but not the mean amplitude of mEPSCs ($n = 12$, $p < 0.0005$ and $p > 0.05$, respectively, paired t-test, Fig. 3B). On average, the mEPSC frequency decreased from 1.08 ± 0.17 Hz in control to

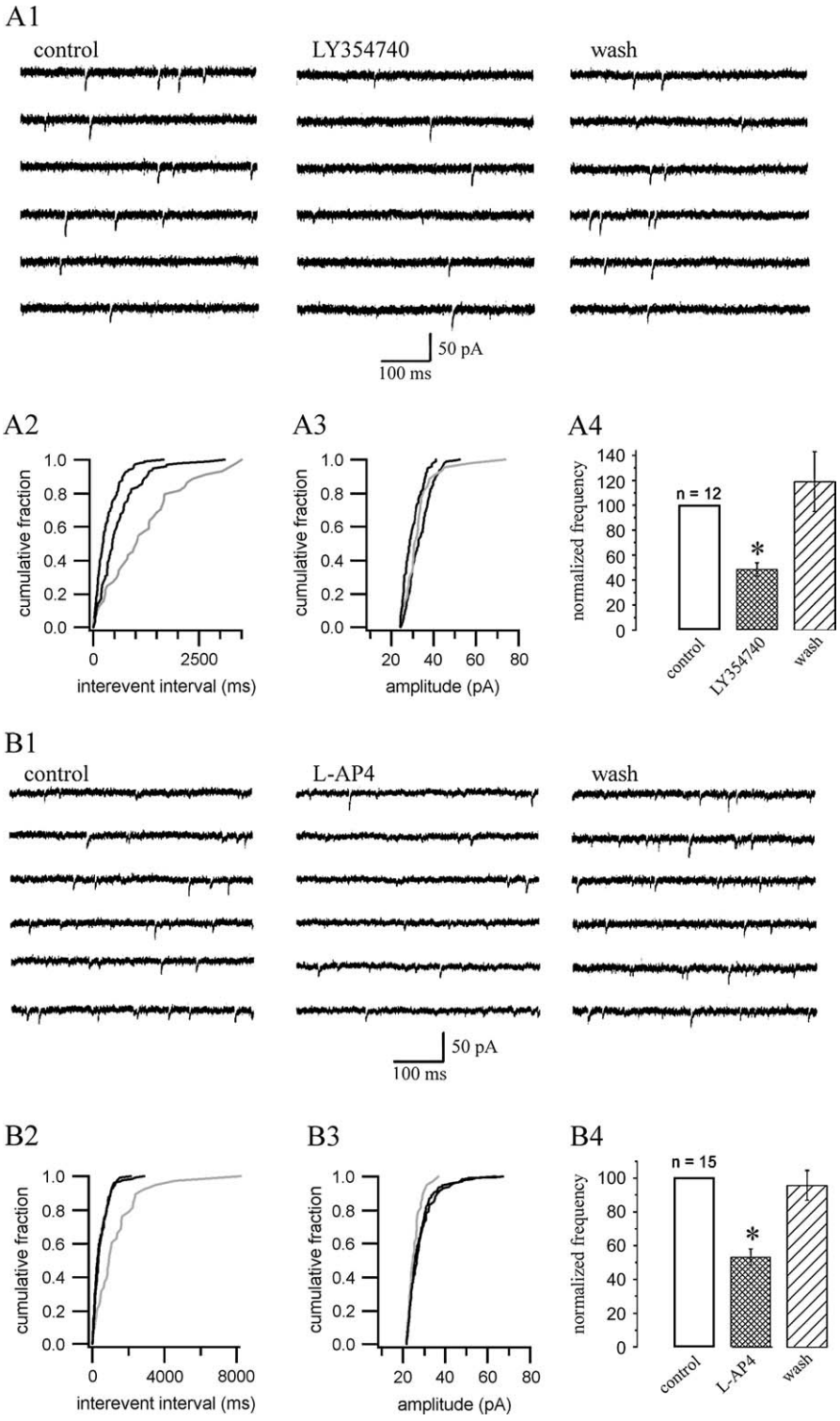

Fig. 3. LY354740 (100 nM) or L-AP4 (50 μM) reduced the frequency of mEPSCs without affecting the mean or median amplitude of mESPCs. (A1, B1) Recordings of mEPSCs showing the reversible reduction in mEPSC frequency observed during LY354740 or L-AP4 applications. (A2, B2) A significant reversible shift in the interevent interval distribution occurs ($p < 0.01$, Kolmogorov–Smirnov test) during exposure to either drug (grey line) indicative of a reduction in the event frequency (black lines: control and washout) is shown for the experiments illustrated in A1 and B1. (A3, B3) The amplitude distribution was not significantly ($p > 0.05$, Kolmogorov–Smirnov test) modified by each drug (black lines: control and washout, grey line: drug). (A4, B4) Summary graphs showing the mean ± S.E.M. reversible reduction in mEPSC frequency by LY354740 or L-AP4. All recordings were performed in 1 μM TTX and 50 μM picrotoxin.

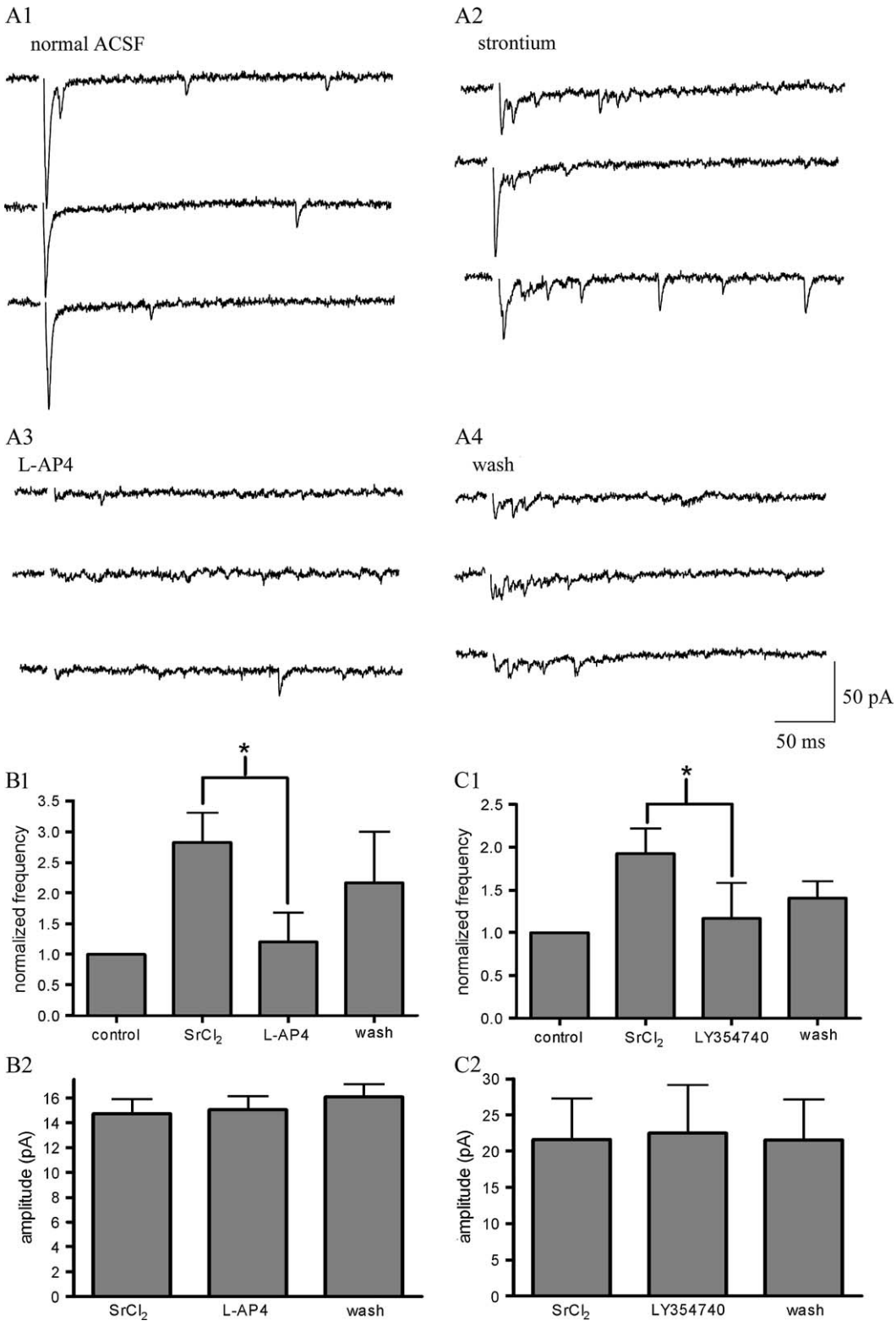

Fig. 4. Perforant pathway-evoked asynchronous EPSCs are inhibited by group II and III mGluR activation. (A) EPSCs were evoked by perforant pathway stimulation in the presence of control ASCF (A1); next $CaCl_2$ was replaced with $SrCl_2$ and this caused a marked increase in the occurrence of asynchronous, miniature-like EPSCs (A2). These asynchronous events were inhibited by 50 μM L-AP4 (A3), and recovered following washout of the drug (A4). (B1) Summary histogram illustrating the significant increase in the frequency of asynchronous EPSCs during L-AP4 application. Frequencies were normalized to the frequency of spontaneous EPSCs measured under control ACSF ($n = 4$). (B2) No changes in asynchronous EPSC amplitude were observed ($n = 4$). (C1) Likewise, 100 nM LY354740 also significantly inhibited the frequency of asynchronous EPSCs ($n = 4$), without affecting the amplitude of the events (C2).

0.59 ± 0.11 Hz during the application of L-AP4, and recovered to 1.02 ± 0.22 Hz after the washing out of the drug. Therefore, these results suggest a presynaptic inhibition of glutamate release from excitatory terminals at CA1 SLM by group II and III mGluRs.

3.5. Asynchronous EPSCs elicited in the presence of $SrCl_2$ are inhibited by groups II and III mGluR activation

There are many glutamatergic afferent inputs onto SLM interneurons, all of which may contribute to mEPSCs. Asynchronous EPSCs evoked by perforant pathway stimulation should show a decrease in their frequency of occurrence if presynaptic mGluRs are present on these terminals. In the presence of ACSF containing 2.5 mM $SrCl_2$, a large increase in the frequency of events that had similar amplitude distributions to those of mEPSCs was observed (Fig. 4). Following exposure of slices to either 50 μM L-AP4 or 100 nM LY354740 the frequency of asynchronous events was reversibly reduced (Fig. 4B1 and C1, one-tailed paired *t*-test $p < 0.05$, $n = 4$ each). In contrast, the amplitude distribution of asynchronous EPSCs was unchanged by either agonist ($p > 0.05$, paired *t*-test, Fig. 4B2, C2).

3.6. Gamma oscillations recorded in the hippocampal CA1 area are affected by LY354740

We also tested the effect of activation of presynaptic mGluRs at a network level by monitoring the electrical activity of CA1 neuronal populations.

To this purpose, we have superfused the slices with a pharmacological cocktail consisting of carbachol (20 μM), kainate (600 nM) and 5 mM extracellular K^+. The cocktail elicited extracellular field activity consisting of oscillations in the gamma frequency range that stabilised after about 1–3 h (Fig. 5). Experiments were continued when the oscillations were stable and the power spectra of the most prominent frequency reached $>25\ \mu V^2$. At steady state, the mean frequency of the oscillation was 29.65 ± 2.48 Hz, and the mean power was 86.1 ± 24.06 μV^2; TTX abolished this activity (not shown). LY354740 (1–5 μM) was bath applied to slices showing stable oscillations in the presence of the pharmacological cocktail (Fig. 5A, B). LY354740 reduced significantly the power of the oscillations in six slices to 37.47 ± 9.6 μV^2 ($p < 0.05$), without changing the frequency of the oscillations ($p > 0.05$). Application of the group II/III mGluR antagonist LY341495 (4 μM) partially reversed the effect of the agonist (Fig. 5A, B). Therefore, we conclude that pharmacological activation of group II mGluRs attenuates the synchronous activity of neuronal populations of CA1 area.

4. Discussion

In the present study, we have recorded from a heterogeneous class of interneurons of hippocampal CA1 SLM. The neuronal types we have observed are consistent with previous work that analysed the anatomy and the electrophysiology of intracellularly biocytin-filled SLM interneurons in detail (Lacaille and Schwartzkroin, 1988; Williams et al., 1994; Spruston et al., 1997; Vida et al., 1998). We have pooled all the pharmacological data, since we observed that EPSCs recorded from virtually all SLM interneurons sampled were modulated by the mGluR ligands tested.

4.1. Presynaptic inhibition of EPSCs by group II or III mGluRs

Application of the selective group II mGluR agonist LY354740 or the selective group III mGluR agonist L-AP4 reversibly depressed the EPSCs elicited by perforant pathway stimulation and recorded in CA1 SLM interneurons. In the large majority of cells tested, agonist application resulted in an increase in the PPR. However, for five neurons a decrease in PPR was observed, even though peak current amplitudes still decreased. All of these five neurons showed paired-pulse facilitation in the control, whereas the PPR was shifted towards unity during L-AP4. If the probability of release was small to start with and further decreased to such an extent that very little transmitter was released even during the second pulse, PPR would not necessarily be the best reflection of presynaptic events. Indeed these five neurons had a greater mean PPR, and mean peak current amplitude nearly a third of that seen for the 12 neurons that showed an increase in PPR following L-AP4 application. No changes in the holding current were observed, and therefore these results suggest that the ligands modulate excitatory transmission through activation of presynaptic mGluRs. This interpretation was confirmed by the action of the agonists on spontaneous, mEPSCs. We found a clear-cut decrease in the frequency, but not in the amplitude, of the events induced by either mGluRs agonist. Obviously, spontaneous mEPSCs recorded from SLM interneurons come from glutamate released from a heterogeneous pool of fibers including the Schaffer collaterals and not only from the perforant pathway. To correctly interpret our data with mEPSCs, however, it is important to keep in mind that immunoreactivity for group II mGluR is undetectable in the stratum radiatum and in CA1 pyramidal cell layer (Shigemoto et al., 1997; Tamaru et al., 2001), and that L-CCG-1, a group II mGluR agonist, does not change the Schaffer collateral-evoked EPSCs recorded in CA1 pyramidal neurons (Kawakami et al., 2003). Therefore, in spite of the widespread origin of mEPSCs, it is likely that a substantial amount of

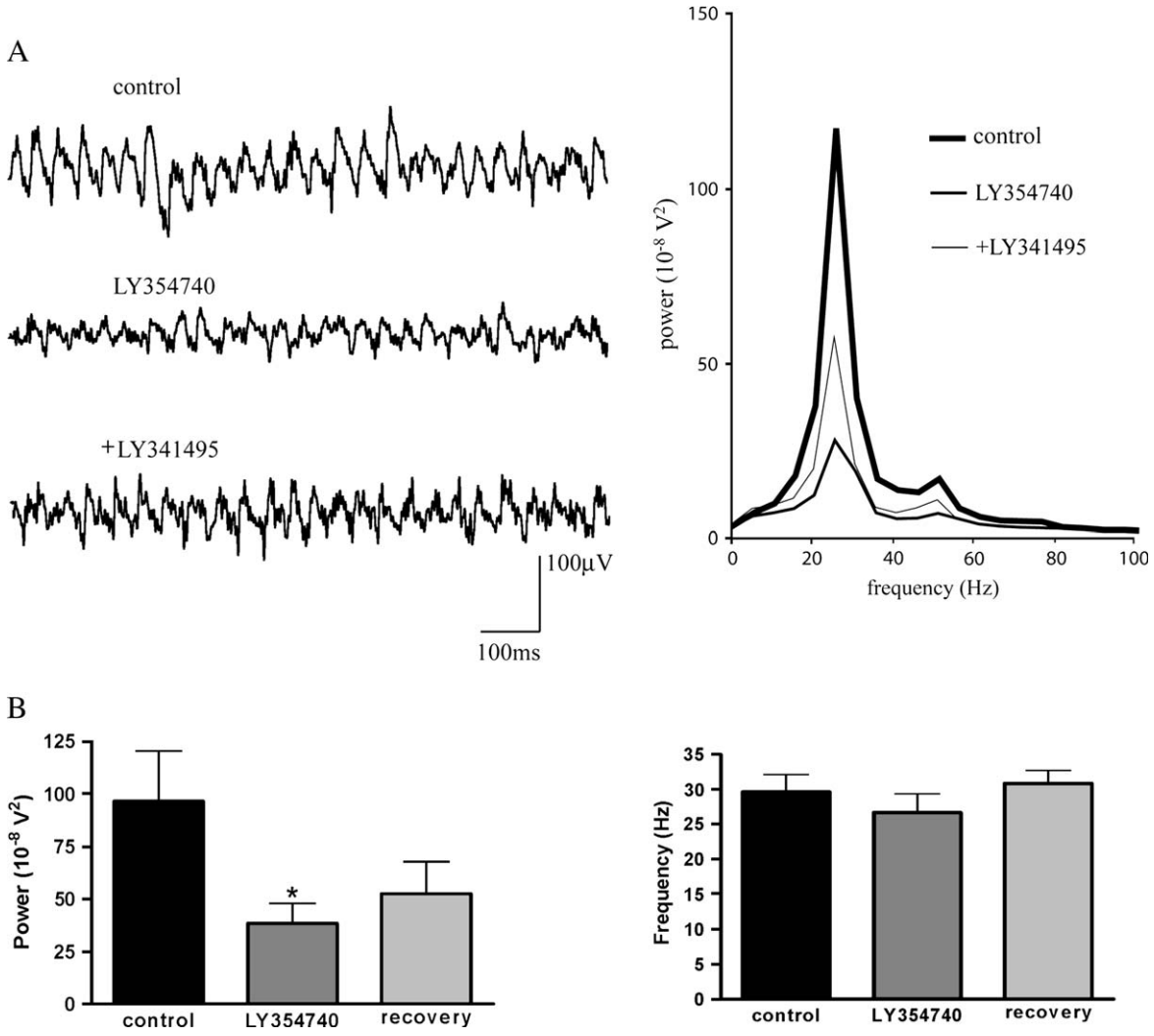

Fig. 5. LY354740 affects carbachol/kainate-induced gamma oscillations. (A) Traces of extracellular field potential recordings from stratum pyramidale of the CA1 area. Bath application of 20 μM carbachol, 600 nM kainate and 5 mM extracellular K^+ elicits oscillations in gamma frequency range. LY354740 (5 μM) reduced the power of the oscillations whereas the frequency was unaffected. The group II/III mGluR antagonist LY341495 (4 μM) partially reversed the effect of LY354740. Right panel shows power spectra of the recorded traces illustrated on the left. (B) Left graph, pooled data, the power of oscillation was significantly reduced by LY354740 ($n = 6$ control, $n = 6$ LY354740, $n = 6$ recovery ($n = 5$ with LY341495 and $n = 1$ wash out)); right graph, pooled data, the frequency of oscillation was unchanged by LY354740 ($n = 6$ control, $n = 6$ LY354740, $n = 6$ recovery ($n = 5$ with LY341495 and $n = 1$ wash out)).

mEPSCs affected by LY354740 originates from the perforant pathway. Furthermore, we have also studied Sr^{2+}-induced EPSCs occurring shortly after perforant pathway stimulation. These events, usually referred to as asynchronous/delayed release, are due to the release of neurotransmitter from the terminals that have been stimulated, and they occur with some delay from the synchronous release because either Sr^{2+} binds to a different intraterminal sensor protein than Ca^{2+} (Goda and Stevens, 1994), or because the clearance of Sr^{2+} within the terminal is slower than that for Ca^{2+} (Abdul-Ghani et al., 1996). We found that both LY354740 and L-AP4 reduced the frequency, but not the amplitude, of perforant pathway-evoked asynchronous events in the presence of Sr^{2+}. This result strongly validates the idea that mEPSCs originating from the perforant pathway are affected by both mGluR agonists.

The present results agree with recent data showing that group II and III mGluRs presynaptically inhibit EPSCs evoked by perforant pathway stimulation and recorded from CA1 pyramidal neurons (Capogna, 2004). Thus, mGluRs at perforant pathway-CA1 synapses lack target specificity, and are functionally expressed at terminals of perforant pathway making synapses with distal dendrites of CA1 pyramidal neurons as well as with SLM interneurons. A presynaptic locus of action is also consistent with previous immunocytochemical data showing the presence of immunoreactivity for mGluR2, mGluR7a and mGluR4

in the SLM of CA1 area, but not in postsynaptic elements (Shigemoto et al., 1997; Tamaru et al., 2001; Corti et al., 2002). Several other studies have previously described the inhibition by group II and/or III mGluRs of excitatory (reviewed in: Anwyl, 1999; Cartmell and Schoepp, 2000) and also inhibitory synapses (Semyanov and Kullmann, 2000; Kogo et al., 2004) in the hippocampus.

4.2. mGluRs types and mechanisms involved in the effects observed

Which mGluRs are activated in the present experiments? LY354740 is a potent and very selective agonist (up to 1 μM) at mGluR2 and 3 with an EC_{50} of about 10–50 nM in the rat cortex, hippocampus and striatum, and 10 or 30 nM in cells expressing recombinant mGluR2 or mGluR3, respectively (Schoepp et al., 1999). The concentration used in this study, therefore, was about 2–10 fold higher than the EC_{50}, in order to compensate for the drop in the effective concentration of the drug at synapses within the slice during its application. Thus, it is almost sure that LY354740 in our experiments selectively activated mGluR2/3 only. Since a very weak immunostaining by an mGluR3 antibody was found in CA1 SLM of mGluR2 knock-out (Tamaru et al., 2001), compared to wild type preparation (Shigemoto et al., 1997), it is likely that the effect we observed is mediated by mGluR2. Further experiments with specific knock-out mGluRs mice will be necessary for more accurate identification of the receptor type involved.

L-AP4 is the most potent and selective agonist for group III mGluR, including mGluR4, 6, 7 and 8, but, unfortunately, it is not selective among individual group III mGluR subtypes (Schoepp et al., 1999). Although the effective concentration of L-AP4 on mGluR7a in situ at the synapses is not known, at human mGluRs expressed in a mammalian cell line, the EC_{50}s of L-AP4, for the inhibition of forskolin stimulated cAMP production, were 0.32, 175 and 0.061 μM, for mGluR4, 7, and 8, respectively (Wu et al., 1998). If these values were applicable to presynaptic mGluRs, the involvement of group III mGluR subtypes other than mGluR7 (and also mGluR6 that is undetectable in the hippocampus (Shigemoto and Mizuno, 2000)) would be expected from the actions induced by 50 μM L-AP4.

By which presynaptic mechanism do mGluRs exert presynaptic inhibition at the synapses studied? We have not addressed this question specifically in the present experiments. Interestingly, segregation of group II and III mGluRs has been observed at glutamatergic axon terminals of SLM hippocampus, so that mGluR7a and mGluR4 are detected at active zones, whereas mGluR2 are found at preterminal zones (Shigemoto et al., 1997; Corti et al., 2002). Recently it was discovered that this segregation has functional relevance (Capogna, 2004). Specifically, group III mGluRs reduce the release of glutamate and the perforant pathway-EPSCs in CA1 pyramids through a G-protein-mediated inhibition of presynaptic N-type Ca^{2+} channels. In contrast, the inhibition mediated by group II mGluRs acts via the activation of dendrotoxin-sensitive K^+ channels, and this action is under the control of protein kinase A. Regarding the latter effect the data reported by Capogna (2004) are also compatible with group II also targeting N- and P/Q type Ca^{2+} channels. It could be that similar mechanisms are also involved in the modulation of perforant pathway-SLM interneuron synapses. The fact that in the present experiments we observed a stronger effect of L-AP4 on evoked EPSCs compared to mEPSCs frequency is consistent with a direct action of group III mGluRs on presynaptic Ca^{2+} channels (for discussion of drugs modulation of evoked and spontaneous release see also: Thompson et al., 1993). It is also important to note that recent data suggest that the pool of vesicles underlying spontaneous transmitter release can be different from that involved in evoked release (Sara et al., 2005).

4.3. Lack of endogenous activation of presynaptic mGluRs

Activation of presynaptic mGluRs by endogenous release of glutamate was examined with high frequency stimulation to produce a sustained release of neurotransmitter before and after the application of LY341495, a broad-spectrum mGluRs antagonist effective for group II and III mGluRs, to displace glutamate binding to the receptors. High frequency stimulation was used, to mimic gamma activity, which can be recorded in vivo in the entorhinal/hippocampal circuitry (Chrobak et al., 2000). In the present experiments, however, we have observed that LY341495 did not substantially change the EPSCs elicited during or shortly after train stimulation. This result renders unlikely that synaptically released glutamate tonically activates presynaptic mGluRs to a significant extent at the synapses studied, at least under our experimental conditions. Our data agree with some (Turner and Salt, 1999; Capogna, 2004), but not all previous studies. For example, Losonczy et al. (2003) detects a substantial increase of EPSCs induced by LY341495 recorded from hippocampal interneurons of stratum oriens–alveus. Furthermore, extracellular analysis of field potentials in CA1 or dentate gyrus of rat or mice elicited by train stimulation of the perforant pathway also shows some tonic synaptic activation of group II/III mGluRs (Kew et al., 2001, 2002). We feel that many factors can account for detecting or failing to observe tonic synaptic activation of group II/III mGluRs. These factors include: different synapses studied, extracellular versus intracellular recordings and therefore the number of

synapses monitored, technical differences, such as the plane used to cut the slices, the depth within the slices of the synapses stimulated by ambient glutamate, and the different recording temperature employed. It could also be that other sources of glutamate, like glial cells, or other excitatory fibres, originating from the amygdaloid complex (Pikkarainen et al., 1999), the nucleus reuniens (Wouterlood et al., 1990), and/or the inferotemporal cortex (Yukie and Iwai, 1988), present in the SLM area, activate the mGluRs at perforant pathway-SLM interneuron synapses.

4.4. Actions of presynaptic mGluRs at network level

Previous studies have shown that carbachol, kainate or group I mGluRs agonist can induce field potential oscillatory activity in vitro which might mimic some aspects of behaviour-contingent network oscillations in vivo (Traub et al., 2004). We were able to evoke persistent synchronised extracellular field activity in the CA1 area by co-application of carbachol and kainate, since carbachol alone was not effective in doing so, as previously reported (Fisahn et al., 1998). We have not investigated the exact contribution of CA1 pyramids, SLM interneurons, CA3 and entorhinal input in the oscillatory activity we have observed. However, based on similar oscillations in CA3 area, it is likely that they reflect an integrated activity of excitatory and inhibitory synapses (Mann et al., 2005). Oscillatory activity in the gamma frequency range is critically dependent on the rhythmic synchronous output of populations of interneurons in this area of hippocampus (Traub et al., 2004). Application of LY354740 caused a significant decrease in the power but not in the frequency of the oscillations recorded in CA1 area. It is difficult to identify the location of group II mGluRs responsible for the modulation of population activity in CA1, since the potential involvement of several types of synapses in the hippocampus, entorhinal cortex and subiculum in the generation and/or maintenance of the oscillations. However, it is likely that group II mGluRs which are highly enriched at the perforant pathway (Shigemoto et al., 1997), play some role. In this respect, we also found that group II mGluRs inhibit perforant path-EPSCs recorded from CA1 pyramids (Capogna, 2004) as well as from CA1 SLM interneurons (present study). These interneurons are known to generate feed forward inhibition to CA1 pyramids (Vida et al., 1998). Thus, the first effect by perforant pathway-associated group II mGluRs should produce an inhibition and/or desynchronisation of firing of CA1 pyramids, whereas the latter should cause disinhibition of CA1 pyramids. Since we have observed a decrease in the amplitude of CA1 oscillations by LY354740, it appears that the net effect of activation of perforant pathway-associated group II mGluRs is to reduce the synchronous output of CA1. We have not tested the action of L-AP4 on the population activity since group III mGluRs are even more widely expressed than group II mGluRs in CA1 area, including at Schaffer collaterals (i.e., Gereau and Conn, 1995). Thus, the potential L-AP4-induced modulation of oscillations would have been of even more uncertain relevance for the direct entorhinal-CA1 hippocampal circuits. In conclusion, our data suggest that activation of group II mGluRs powerfully inhibit the excitability of neuronal populations of CA1 area.

Acknowledgements

This work was supported by the Medical Research Council, U.K. We thank Romana Hauer for technical help and Eli Lilly and Co., Indianapolis, IN, USA, for supplying LY354740.

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