Excitatory synaptic potentials dependent on metabotropic glutamate receptor activation in guinea-pig hippocampal pyramidal cells

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1. Intracellular and extracellular recordings of CA1 and CA3 neurones were performed in guinea-pig hippocampal slices to examine synaptic activities dependent on metabotropic glutamate receptors (mGluRs).

2. Long burst activities were elicited by 4-aminopyridine in the presence of ionotropic glutamate receptor and $GABA_A$ receptor blockers (6-cyano-7-nitroquinoxaline-2,3-dione and 3-(*RS*-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, and picrotoxin). Long bursts were also elicited by α-dendrotoxin.

3. Long bursts consisted of a 5–25 s depolarization with overriding action potentials and occurred rhythmically at intervals ranging from 1 to 20 min. Long bursts were generated in a population of CA3 neurones and the synchronized output elicited long bursts in CA1 cells. Depolarizing potentials underlying long bursts in CA1 cells had a reversal potential of $-14{\cdot}8 \pm 5{\cdot}1$ mV.

4. Long burst-associated depolarizations in CA1 neurones were suppressed by local application of L-(+)-2-amino-3-phosphonopropionic acid (L-AP3) and of the phenylglycine derivatives (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), *S*-4-carboxyphenylglycine (*S*-4CPG) and *S*-4-carboxy-3-hydroxyphenylglycine (*S*-4C3HPG). (−)-MCPG or atropine application did not affect the long burst-associated depolarization.

5. Bath perfusion of (+)-MCPG (0·5 mM), *S*-4CPG (0·5 mM), *S*-4C3HPG (0·5 mM) or L-AP3 (1 mM) blocked the occurrence of long bursts.

6. The results suggest that the long burst-associated depolarizations are synaptic potentials dependent on mGluR activation. Activation of mGluRs may also be involved in the generation of synchronized long bursts in the CA3 region.

Hippocampal CA3 pyramidal cells make synaptic contacts on neighbouring cells and CA1 neurones (Li, Somogyi, Ylinen & Buzsáki, 1994). The neurotransmitter released at these synapses is likely to be glutamate or a related amino acid (Nadler, Vaca, White, Lynch & Cotman, 1976). Glutamate binds to two major classes of receptors: ionotropic GluRs (iGluRs) and metabotropic GluRs (mGluRs).

The effects of iGluR activation have been extensively analysed and consist of excitatory responses mediated by AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) and NMDA (*N*-methyl-D-aspartate) receptors (Mayer & Westbrook, 1987). In contrast, less is known of the responses mediated by mGluR activation. Application of mGluR agonists excites central neurones by a number of mechanisms (Schoepp & Conn, 1993). These include depolarization through suppression of potassium conductances (Stratton, Worley & Baraban, 1989; Charpak, Gähwiler, Do & Knöpfel, 1990), depression of spike accomodation and after-hyperpolarization (Stratton *et al.* 1989; Charpak *et al.* 1990; Chinestra, Aniksztejn, Diabira & Ben-Ari, 1993; Miles & Poncer, 1993), induction of burst firing (Zheng & Gallagher, 1991), potentiation of an after-depolarization (Zheng & Gallagher, 1992), and enhancement of the NMDA response (Aniksztejn, Bregestovski & Ben-Ari, 1991). Recently, induction of inward currents associated with conductance increase has also been reported (Staub, Vranesic & Knöpfel, 1992; Crépel, Aniksztejn, Ben-Ari & Hammond, 1994; Guérineau, Bossu, Gähwiler & Gerber, 1995; Linden, Smeyne & Connor, 1994; Zheng & Gallagher, 1995). MGluR agonists also depress synaptic transmission through a presynaptic action (Baskys & Malenka, 1991). The suppression of Ca^{2+} currents (Lester & Jahr, 1990) at the axon terminals could be involved in this effect (Schoepp & Conn, 1993). Multiple intracellular messenger pathways

have been identified as targets of mGluR agonists, including the phosphoinositides (Sladeczek, Pin, Recasens, Bockaert & Weiss, 1985), the phospholipase D and the cyclic nucleotides (see for review Schoepp & Conn, 1993). Such a variety of effects is paralleled by the existence of multiple subtypes of mGluRs (Nakanishi, 1994).

At present, the involvement of mGluR-mediated responses in the signalling processes within the hippocampus remains unclear. MGluR-mediated responses were induced by afferent stimulation in a number of preparations (Charpak & Gähwiler, 1991; Glaum & Miller, 1992; Miles & Poncer, 1993; Batchelor, Madge & Garthwaite, 1994). In all instances, high frequency or intensity of stimulation was required to elicit detectable responses. This finding is consistent with the relatively low affinity of mGluRs for glutamate in brain slices compared with that of iGluRs (Schoepp *et al.* 1990). It is also possible that strong stimulations are required because mGluRs are located at the edge of the synapses in the postsynaptic membrane or at some distance from synaptic junctions as suggested by recent immunocytochemical data in the hippocampus and cerebellum (Baude *et al.* 1993). These data raise the question whether spontaneous activity within the hippocampus can reach a sufficient level for the activation of mGluR-mediated responses.

In this study we asked whether mGluRs may play a role in generating excitatory synaptic potentials in hippocampal neurones under conditions of increased spontaneous glutamate release and decreased inhibition. We observed long duration bursts in CA3 neurones which persisted in the presence of ionotropic glutamate receptor blockers. These bursts were synchronized in CA3 cells and occurred rhythmically at intervals of minutes. The bursts elicited long depolarizations in CA1 pyramidal cells. The results suggest that the long depolarizations are synaptic events dependent on mGluR activation. Furthermore, the generation of synchronized bursts in CA3 neurones also appeared to be dependent on mGluR activation. Part of this study has been presented in abstract form (Bianchi & Wong, 1993, 1994*b*).

METHODS

Slice preparation

Guinea-pigs (3–5 weeks old) were anaesthetized with halothane. Their brains were quickly removed and submerged in cold (0–4 °C) artificial cerebrospinal fluid (ACSF). The ventral hippocampus was dissected out and sectioned in 350–500 μm-thick slices. The slices were placed in an interface chamber and continuously perfused with ACSF warmed at 34·0 ± 0·5 °C and gassed with 95% O_2–5% CO_2 (pH 7·4). The composition of the ACSF was (mM): 124 NaCl; 26 $NaHCO_3$; 5 KCl; 2 $CaCl_2$; 1·6 $MgCl_2$; 10 D-glucose. The low Ca^{2+}–high Mg^{2+} solution contained 0·5 mM $CaCl_2$ and 8 mM $MgCl_2$. A period of at least an hour passed before starting the experiment. In some experiments slices were cut with a razor blade to separate the CA3 region from the CA1 and the dentate gyrus regions (see drawing in Fig. 2).

Electrophysiological recordings

Electrophysiological recordings were performed in the CA1 and CA3 regions with glass pipettes driven by micromanipulators under stereoscope visual guidance. Intracellular microelectrodes were filled with potassium acetate (2 M; 40–80 MΩ). Extracellular recordings were performed either with NaCl-filled pipettes (2 M; 4–10 MΩ) or with low-impedance potassium acetate electrodes (10–30 MΩ) withdrawn from cells. In the majority of the experiments dual intracellular or intra- and extracellular recordings were carried out. The closest distance between the tips of the electrodes was about 200 μm. The electrical responses were recorded in current-clamp mode with a two-channel high-impedance amplifier (Axoclamp-2A; Axon Instruments, Foster City, CA, USA). The recordings were displayed on an oscilloscope (BK-Precision 2160, Chicago, IL, USA) and written on a chart recorder (TA240; Gould Instruments, Valley View, OH, USA), or digitized and stored on videotape for off-line analysis. The pCLAMP software (Axon Instruments) was used to acquire and analyse the data. In dual recordings capacitive transients associated with spikes in the other cell were subtracted by means of an electronic device. A digital stimulator (PG4000; Neuro Data Instruments, New York) was used to time the intracellular current pulses. Square-wave current pulses (100–300 ms; ± 0·1–1 nA; 0·2–1 Hz) were injected to monitor the passive and active properties of the neurones. Input resistance was calculated as the ratio of the voltage amplitude response to the current pulse injected and the current pulse intensity. Elicitation of EPSPs to test the effectiveness of the blockers (see below) was obtained with a tungsten bipolar electrode connected to a constant current stimulator (S.I.U. 90; Neuro Data Instruments). Single electric shocks (100 μs; 0·1–1 mA) were delivered to the hilus or the Schaffer collateral system.

Pharmacological agents

The following substances were added to the perfusing solution from freshly prepared stock solutions: 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10–40 μM), AMPA–KA receptor antagonist; 3-(*RS*-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10–40 μM), competitive NMDA receptor antagonist; MK-801 (100 μM), non-competitive NMDA receptor antagonist; kynurenic acid (2 mM), broad spectrum iGluR antagonist; picrotoxin (PTX, 50 μM), $GABA_A$ receptor antagonist; CGP 36742 (500 μM), $GABA_B$ receptor antagonist; tetrodotoxin (TTX, 1 μM), Na^+ channel blocker; 4-aminopyridine (4-AP, 70 μM) and α-dendrotoxin (α-DTX, 1 μM), blockers of the transient K^+ current I_D; L(+)-2-amino-3-phosphonopropionic acid (L-AP3, 1 mM), non-competitive mGluR antagonist; (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG, 0·5–1 mM), competitive mGluR receptor antagonist; (−)-α-methyl-4-carboxyphenylglycine ((−)-MCPG, 1 mM), inactive isomer of MCPG; *S*-4-carboxyphenylglycine (*S*-4CPG, 0·5 mM) and *S*-4-carboxy-3-hydroxyphenylglycine (*S*-4C3HPG, 0·5 mM), antagonists at mGluR1 and mGluR5 subtypes with agonistic action at mGluR2 and mGluR3 subtypes. A two-way input to the recording chamber allowed switching between different solutions. *N*-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314; 50 mM) was added to the potassoim acetate electrode filling solution for intracellular injection. In some experiments QX-314 was added to a mixture of potassium acetate (1 M) and caesium acetate (1 M). Local application of the phenylglycine derivatives, L-AP3 and atropine was performed with a glass pipette lowered on the slice by means of a third manipulator (see drawing in Fig. 4). Preliminary

experiments showed that the following parameters were optimal for selective and localized effect of the blockers: the pipette tip diameter was 20–100 μm; the pipette was lowered on the stratum radiatum for 0·5–5 min; the drug in the pipette was dissolved in perfusing solution at ten times the effective bath concentration. Acetylcholine chloride (1 mM) was pressure-applied in the stratum radiatum of the CA1 region near the recording electrode by means of a Picospritzer II (General Valve Corp., Fairfield, NJ, USA). CNQX, CPP, L-AP3 and the phenylglycine derivatives were obtained from Tocris Neuramin (Bristol, UK); MK-801 and QX-314 from RBI (Matick, MA, USA); α-DTX from Alomone Labs Ltd (Jerusalem, Israel). CGP 36742 was provided by Ciba-Geigy (Basel, Switzerland). All the other drugs were purchased from Sigma.

To test whether the concentrations of CNQX and CPP routinely used in these experiments were effective in blocking the ionotropic glutamate receptor-mediated responses, we monitored the EPSPs evoked either by hilar stimulation in CA3 pyramidal cells ($n = 3$) or by collateral Schaffer fibre stimulation in CA1 neurones ($n = 3$), before and after addition of CNQX and CPP (10 μM each) to the ACSF. In all cases the EPSPs recorded in ACSF were completely blocked 20–30 min after addition of the CNQX and CPP to the perfusate, even at stimulation intensities four times greater than those required to elicit responses in ACSF.

The solution used in most experiments, containing CNQX and CPP (10 μM each), PTX (50 μM) and 4-AP (70 μM), is referred to as 'control solution'.

RESULTS

The data in this study were collected from recordings in CA3 ($n = 251$ intracellular and 23 extracellular) and in CA1 ($n = 124$ intracellular and 15 extracellular) regions of 142 hippocampal slices isolated from 97 guinea-pigs. The neurones had stable resting membrane potential in the range of −52 to −75 mV, membrane input resistance of 25–54 MΩ and overshooting action potentials.

Long bursts and epileptiform bursts in CA3 pyramidal cells

In the presence of ionotropic glutamate receptor antagonists and picrotoxin, 4-aminopyridine elicited phases of bursting in CA3 pyramidal cells. Each phase consisted of a depolarization lasting 8–10 s with overriding action potentials and it is referred to as 'long burst' (Fig. 1*A* and *B*, top traces). Long bursts occurred rhythmically at intervals of 2·8–3 min (Fig. 1*A*, top trace) and they were observed in 82% of the slices tested ($n = 108$). Overall, the long burst duration was 5–25 s ($n = 83$). The interval between long bursts varied in different slices (range, 1–20 min; $n = 83$), but it was relatively constant in the same slice throughout the recording period (0·3–3 h). Often an individual long burst was preceded by 1–5 brief bursts of 50–150 ms of duration (see Fig. 1*B*, top trace).

The long bursts were compared with the bursts recorded in CA3 pyramidal cells in the presence of convulsants. The latter events are referred to as epileptiform bursts because of their synchronous occurrence and resemblance with the interictal bursts recorded in epileptic foci *in vivo* (Prince, 1978). Epileptiform bursts were observed when PTX alone (50 μM) was present in the perfusing solution ($n = 24$; Fig. 1*A* and *B*, bottom traces). The time course of long bursts and epileptiform bursts showed significant differences. Both the duration of an individual long burst and the interval between consecutive long bursts were ~10–100 times longer than those of epileptiform bursts (Fig. 1). In addition, long bursts were recorded in saturating concentrations of ionotropic glutamate receptor antagonists, whereas epileptiform bursts were completely blocked by such agents ($n = 4$). In the presence of these antagonists (and when 4-AP was not present), the electrical stimulation of the hilus did not induce any fast EPSPs in CA3 neurones, even at four times the stimulation intensity sufficient to induced maximal size fast EPSPs in saline perfusing solution. In addition, once long bursts were recorded their time course was also not affected when (i) CNQX and CPP concentrations were both raised up to 40 μM ($n = 3$), (ii) MK-801 (100 μM), a non-competitive NMDA receptor antagonist, was added to the control solution ($n = 3$), and (iii) kynurenic acid (2 mM), a broad spectrum iGluR antagonist, was added to the control solution ($n = 3$).

Synchronized IPSPs mediated by $GABA_B$ receptors were also observed under the above described experimental conditions as reported in a previous study (Michelson & Wong, 1994). Long bursts were not noticed in the former study probably because of the difference in the time scale used for data analysis. A prolonged period of unperturbed recordings (i.e. no injected current nor applied stimuli) is required for a clear expression of the long bursts.

Simultaneous intracellular recordings invariably showed long bursts occurring in pairs of CA3 pyramidal cells ($n = 49$ dual recordings; not shown). The long burst interval was 298 ± 35 s ($n = 30$ slices) and the onset delay between long bursts recorded in two cells was at most 1·98 s. The long bursts generated in CA3 cells were considered simultaneous because the onset delay was much shorter than the interburst interval (less than 1 : 100). The frequency of the long bursts did not change at different membrane potentials.

Induction of long bursts by α-dendrotoxin

At concentrations of 100 μM, 4-aminopyridine (4-AP) was reported to block mainly the D-type potassium current (Wu & Barish, 1992). We tested whether a more specific blocker of this current, α-dendrotoxin (α-DTX, 1 μM), could elicit the long bursts.

4-Aminopyridine was first applied to demonstrate long burst occurrence. After washing out 4-AP, the long bursts disappeared. Subsequent addition of α-DTX reversibly induced long bursts in the same slices ($n = 3$ out of 3; not shown).

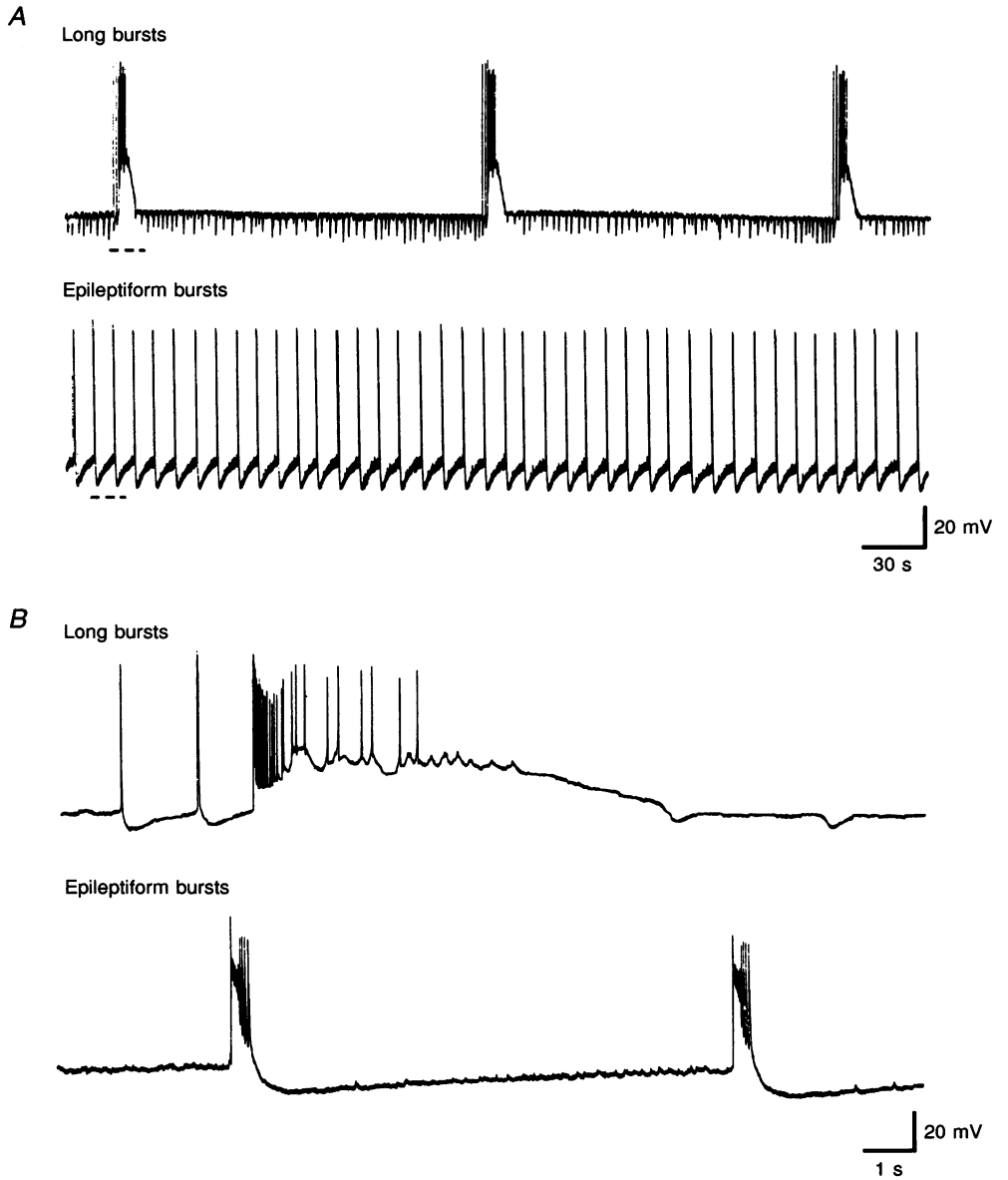

Figure 1. Long bursts and epileptiform bursts in CA3 pyramidal cells

A, intracellular current-clamp recordings from CA3 pyramidal cells in different experiments. Spontaneous excitatory potentials (long bursts) of 8–10 s duration and occurring at 2·8–3 min intervals were recorded in the presence of CNQX, CPP, PTX and 4-AP (see 'control solution' in Methods). The downward deflections are the large spontaneous IPSPs dependent on $GABA_B$ receptor activation typically observed in these experimental conditions as previously reported (Michelson & Wong, 1994). Membrane potential −64 mV. In 83 slices the mean duration of each long burst was 12·01 ± 0·45 s (mean ± S.E.M.; range, 5–25 s) and their mean frequency was 0·0039 ± 0·0002 Hz (range, 0·0009–0·0135 Hz). Another CA3 pyramidal cell showed much briefer and more frequent bursts (epileptiform bursts) when only PTX was added to the perfusing ACSF. Membrane potential −63 mV. In 24 slices, the mean duration of each epileptiform burst was 0·40 ± 0·02 s (range, 0·22–0·60 s) and the mean frequency was 0·1022 ± 0·0057 Hz (range, 0·0701–0·2000 Hz). *B*, portions of the recordings in *A* (dashed lines) are shown on a faster time scale. The long burst was characterized by a depolarizing envelope with overriding action potentials. In this case two brief (50–150 ms) bursts preceded the long burst. Note that the duration of the long burst was ~17–23 times longer than that of the epileptiform bursts.

Long bursts in the CA1 region of the hippocampal slice

Long bursts were recorded also in CA1 pyramidal cells ($n = 124$; Fig. 2*A*). In simultaneous recordings from the CA3 and CA1 regions ($n = 94$), each long burst in the CA1 region followed the one recorded in CA3 by 0·1–2 s. Occasionally, this delay was observed to be as long as 2–5 s. Other CA3–CA1 dual recordings were performed in preparations where the CA3 region had been separated from the rest of the slice ($n = 4$; see drawing in Fig. 2*B*). In these instances, long bursts were recorded in the isolated CA3 region, but they have not been observed in the isolated CA1 region (Fig. 2*B*).

Figure 2*C* shows that long bursts in both CA1 and CA3 regions were associated with deflections in the extracellular field potentials, consistent with the assumption that the long bursts were simultaneously generated in a population of neurones.

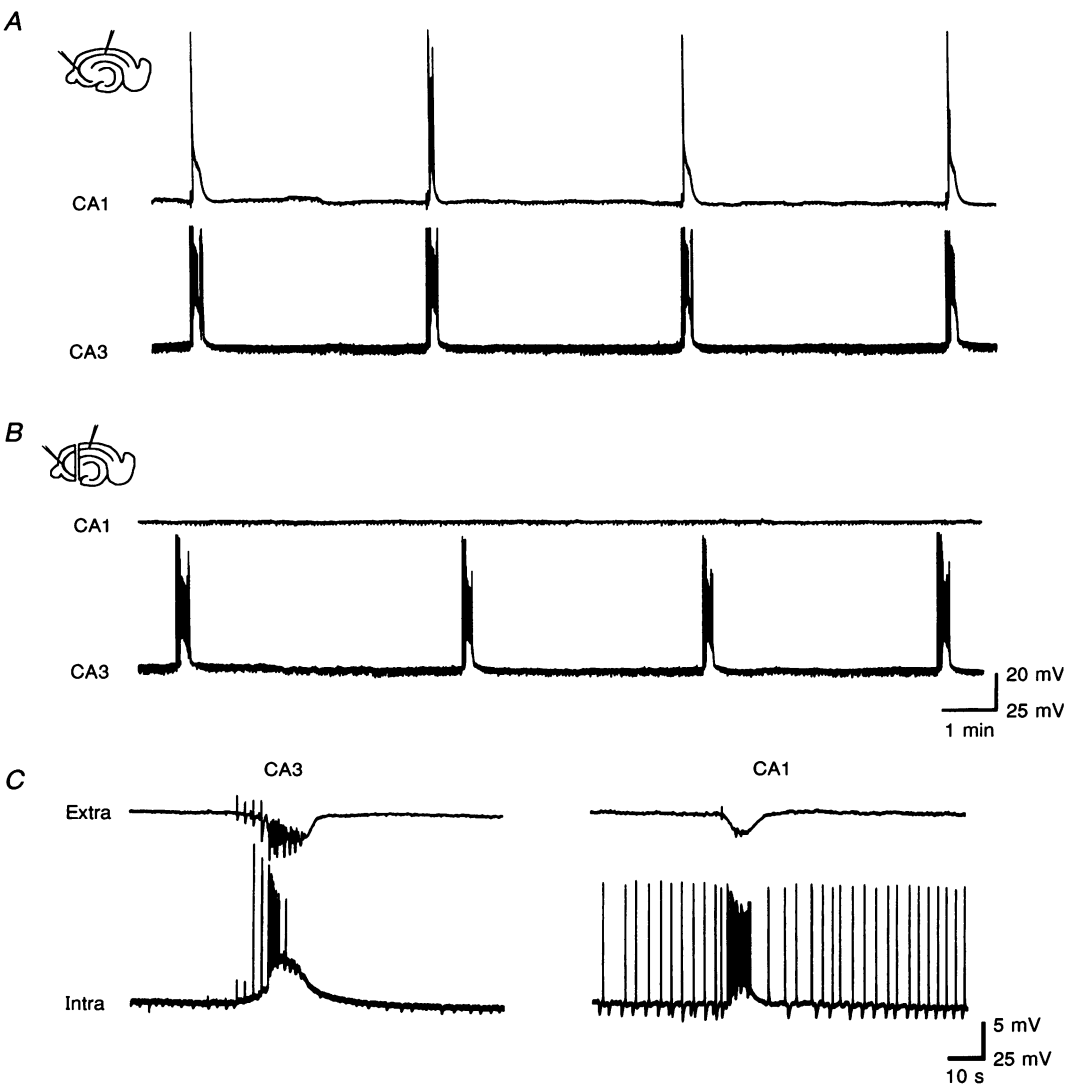

Figure 2. Effects of isolating the CA3 and CA1 regions on the long bursts

A, the drawing indicates the recording sites in an intact slice. Simultaneous recording from CA1 and CA3 pyramidal cells during long burst activity. Long bursts were recorded also in CA1 neurones and they were concomitant to the long bursts of CA3 pyramidal cells. Membrane potentials: CA1, −75 mV; CA3, −70 mV. *B*, the drawing indicates the recording sites and the level of the razor blade cut performed to isolate the CA3 from the CA1 region. Recording from two other neurones of the same slice as in *A* after the cut. Long bursts continued to occur in the CA3 neurone, while no long burst activity was observed in the CA1 neurone. Membrane potentials: CA1, −73 mV; CA3, −71 mV. *C*, simultaneous extracellular (upper traces) and intracellular (lower traces) recordings from the CA3 (left record) and CA1 (right record) regions. Both extracellular recordings were obtained in the stratum pyramidale. Similar field recordings were recorded in the presence of α-DTX. Membrane potentials: CA3, −72 mV; CA1, −66 mV.

When fast action potentials were blocked in a CA1 pyramidal cell by injection of either constant hyperpolarizing current or QX-314 (50 mM), a depolarization was observed in the CA1 neurone associated with a long burst in a CA3 pyramidal cell (Fig. 3*A*).

Properties of the depolarization associated with the long burst

CA1 pyramidal cells were recorded with electrodes filled with QX-314 (50 mM) and Cs^+ (1 mM) to study the

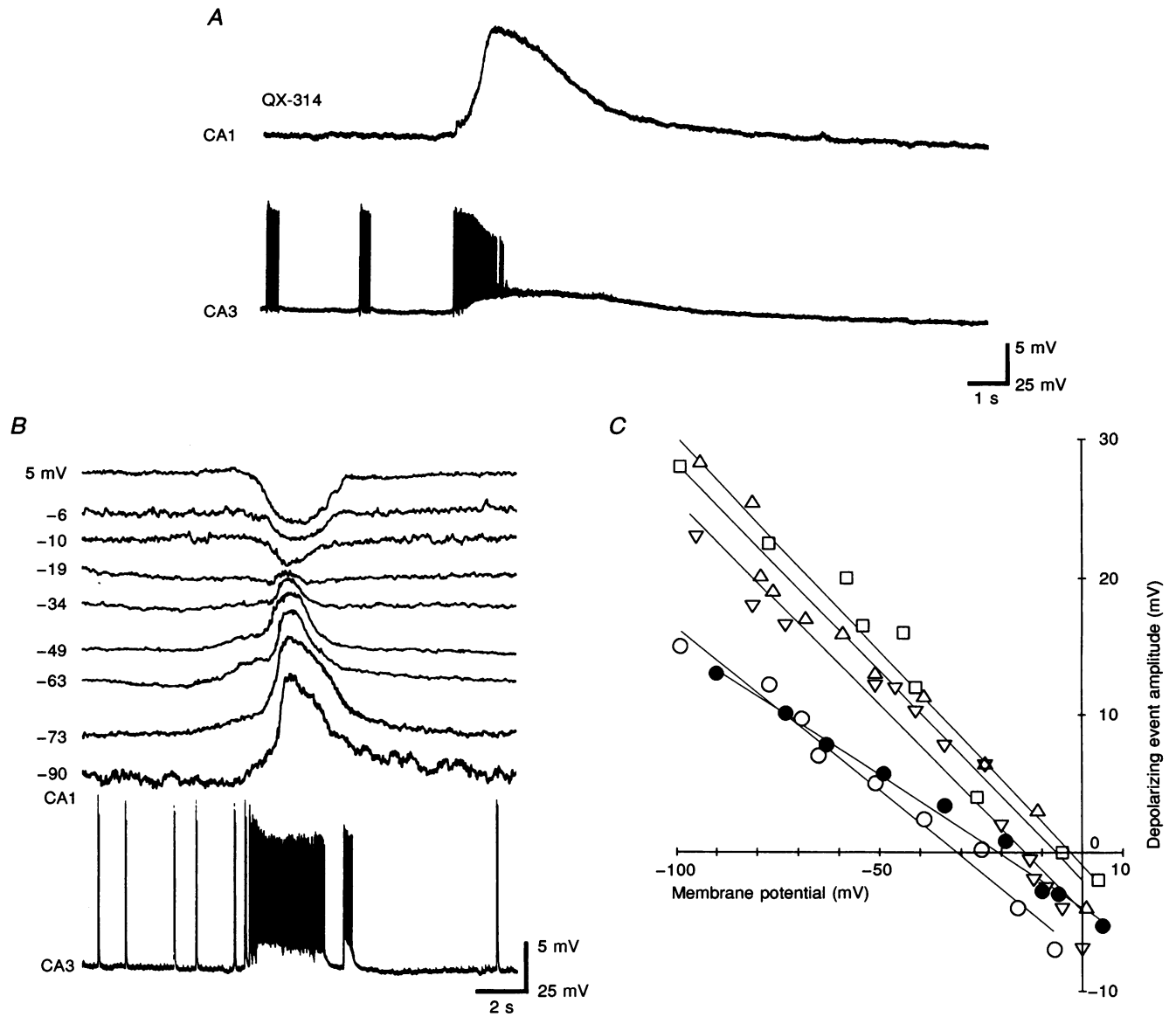

Figure 3. Depolarization in a CA1 neurone associated with a long burst in a CA3 pyramidal cell

A, dual intracellular recording of CA1 and CA3 neurones. QX-314 (50 mM) in the recording electrode blocked the fast Na^+-mediated action potential in the CA1 neurone. The control solution contained CGP 36742 (500 μM), a $GABA_B$ receptor antagonist, to block the spontaneous large IPSPs (see also Michelson & Wong, 1994). Note that a large depolarization in the CA1 neurone was associated with the long burst recorded in the CA3 pyramidal cell. Membrane potentials: CA1, −60 mV; CA3, −68 mV. *B*, reversal potential for the long burst-associated depolarization. QX-314 and Cs^+ (1 M) were present in the recording electrode of the CA1 neurone. CGP 36742 was present in the bath. Constant current of different intensities was injected into the CA1 neurone to bring the membrane potential at the indicated values during subsequent long burst-associated depolarizations. Long bursts were simultaneously recorded in a CA3 neurone (membrane potential, −67 mV). Only the long burst associated with the depolarization at −90 mV is shown. *C*, plots of the long burst-associated depolarization peak amplitudes *vs.* the membrane potential at which they were measured in five neurones. ●, values from the example in *B*. Linear fits of the ●, ○, ▽, △ and □ plots yielded regression coefficients of 0·995, 0·989, 0·979, 0·983 and 0·980, and *x*-axis intercepts at −20·5, −30·8, −13·9, −6·3 and −2·6 mV, respectively.

properties of the long burst-associated depolarization. Long bursts were simultaneously recorded in CA3 pyramidal cells. In the example of Fig. 3*B*, the long burst-associated depolarization reversed polarity at membrane potential close to −20 mV. The amplitude of the event shows a monotonic linear relationship with the membrane potential (Fig. 3*C*, filled circles; regression coefficient $r = 0{\cdot}995$). Similar data were obtained from four other cells (Fig. 3*C*,

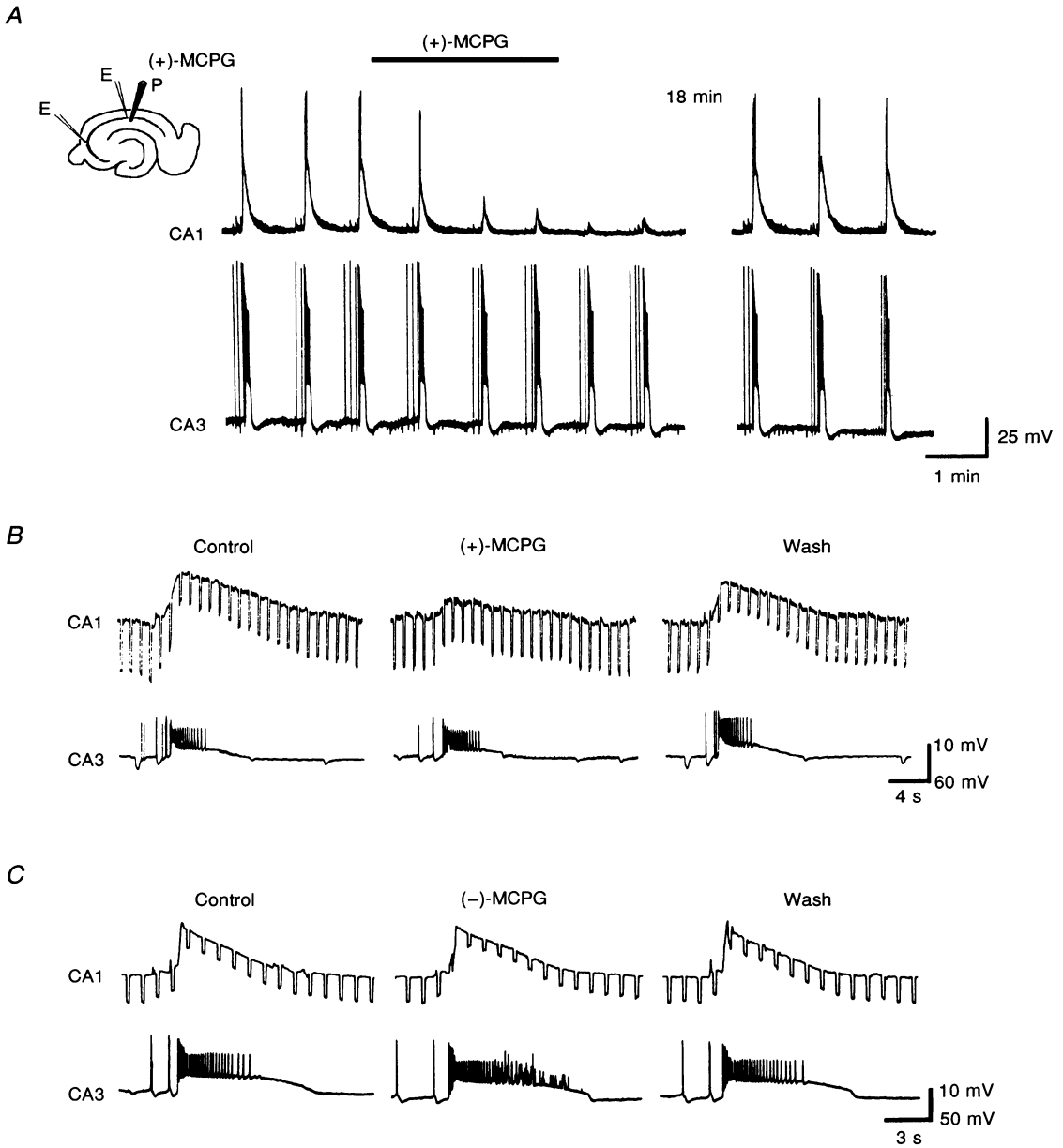

Figure 4. (+)-MCPG, but not (−)-MCPG, depressed the long bursts and the associated depolarizations in CA1 neurones

A, dual intracellular recording in the CA1 and CA3 regions during the long burst activity. The drawing indicates the recording sites (E) and the placement of the pipette (P) containing (+)-MCPG (10 mM). (+)-MCPG was locally applied to the CA1 region during the period indicated by the bar. Recording after 18 min shows the recovery of long bursts in CA1. Membrane potentials: CA1, −63 mV; CA3, −65 mV. *B*, dual intracellular CA1–CA3 recording. QX-314 was present in the CA1 recording electrode and current pulses (200 ms, −0·4 nA, 1 Hz) were injected in the CA1 neurone. The long burst events are shown before (Control), during ((+)-MCPG) and 28 min after (Wash) the local application of (+)-MCPG to the CA1 region. (+)-MCPG reversibly depressed the amplitude and the associated input resistance decrease. Membrane potentials: CA1, −69 mV; CA3, −65 mV. *C*, similar experiment to that in *B* except that (−)-MCPG was applied. Intracellular current pulses: 200 ms, −0·3 nA, 1 Hz. Membrane potentials: CA1, −68 mV; CA3, −72 mV.

open symbols). In five cells, the mean reversal potential for the depolarizing event associated with the long burst was −14·8 ± 5·1 mV (range, from −2 to −31 mV).

The membrane conductance change associated with the long burst was monitored through current pulse injection. The peak of the long burst-associated depolarization was accompanied by an input resistance decrease relative to the baseline (see Figs 4*B* and *C*, 5*A* and *C*, and 6*A*). In a total of forty-four neurones, this input resistance decrease was 5–55% (24·2 ± 1·9%). A similar relative decrease in input resistance was observed at different membrane potentials (see Fig. 5*A*, Control).

Effects of phenylglycine derivatives and L-AP3 on the long bursts and the associated depolarizations in CA1 neurones

During the long burst, glutamate is expected to be released from pyramidal cell axon terminals. We asked whether postsynaptic mGluRs were activated and were involved in sustaining the long burst. The effects of local application of a specific mGluR antagonist, (+)-MCPG, were tested on the long bursts and on the long burst-associated depolarizations recorded in CA1 neurones (Fig. 4*A* and *B*).

Local application of (+)-MCPG (see Methods) in the CA1 region strongly and reversibly suppressed the long bursts in the CA1 neurone, while the long bursts recorded in the CA3 pyramidal cell remained unaffected ($n = 4$; Fig. 4*A*). The threshold for elicitation of action potentials by current injection in the CA1 neurone was not affected during (+)-MCPG application (not shown). Furthermore, (+)-MCPG did not affect the spontaneous bursting during the epileptiform activity. Local application of (+)-MCPG in the CA1 region reversibly reduced the amplitude of the long burst-associated depolarization and the relative conductance change in the CA1 neurone (Fig. 4*B*, CA1). The suppressive effect of (+)-MCPG on the depolarizing event was observed in eight other slices tested (Fig. 7). Previous studies show that while (+)-MCPG is a broad spectrum blocker of mGluRs, (−)-MCPG is ineffective at mGluRs (Hayashi *et al.* 1994). Figure 4*C* shows that local application of (−)-MCPG, applied in the same manner as (+)-MCPG, did not affect the amplitude of the long burst-associated depolarization (see also Fig. 7).

In a separate series of experiments, (+)-MCPG was tested on the iGluR-mediated giant excitatory synaptic potentials that sustain the picrotoxin-induced bursts (Johnston &

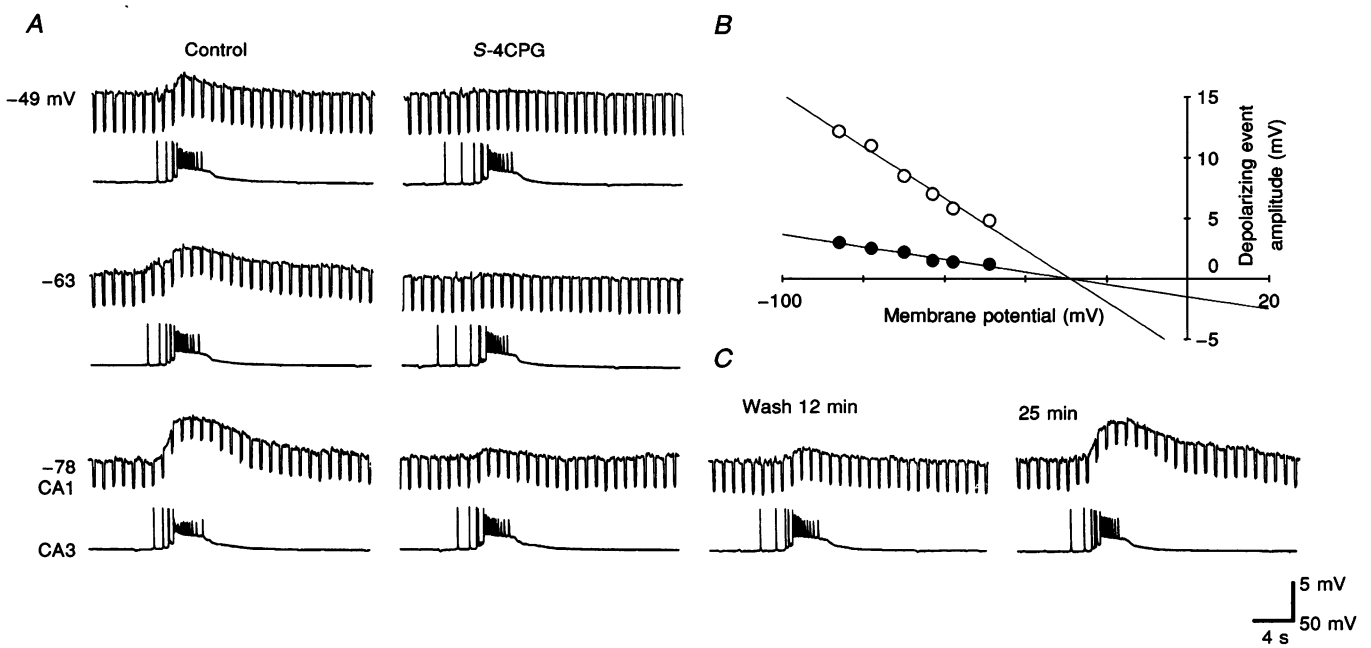

Figure 5. *S*-4CPG depressed the long burst-associated depolarization in a CA1 neurone

In *A* and *C*, dual intracellular recording of the same CA1 and CA3 neurones. *A*, the cell membrane potential of the CA1 neurone was set to three different levels as indicated and the depolarizing events were recorded at these potentials before (left column) and after (right column) local application of *S*-4CPG (10 mM). Transient downward deflections were caused by hyperpolarizing pulses (200 ms, −0·2 nA, 1 Hz). *B*, plots of the amplitude of the depolarizing event at different membrane potentials before (○) and after (●) the application of *S*-4CPG. Linear fits intercept the *x*-axis at −29·0 mV ($r = 0·990$) and −29·2 mV ($r = 0·977$) for the ○ and ● plots, respectively. *C*, recovery of the amplitude of the depolarizing event upon washout of *S*-4CPG. Membrane potential of the CA3 cell: −66 mV.

Brown, 1981; Miles, Wong & Traub, 1984). We uncovered the giant EPSP by the inclusion of QX-314 in the recording pipette of the CA1 neurones. Local application of (+)-MCPG did not affect the peak amplitude of the picrotoxin-induced giant EPSP (not shown). The peak amplitude of the event was 36·0 ± 5·4 mV before and 35·5 ± 5·5 mV after (+)-MCPG ($n = 6$).

S-4CPG and *S*-4C3HPG are phenylglycine derivatives with antagonistic action on mGluR1 and mGluR5 subtypes and agonistic effect on mGluR2 and mGluR3 subtypes (Hayashi *et al.* 1994). We tested the effects of these compounds on the long burst-associated depolarization. Figure 5*A* shows the depolarizing event recorded in a CA1 neurone at three different membrane potentials. The amplitude of the depolarizing event in relation to the membrane potential is plotted in Fig. 5*B*. Local application of *S*-4CPG close to the recording site in the CA1 region suppressed the depolarizing event at all the membrane potentials tested (Figs 5*A* and *B* and 7). The suppression of the depolarizing event by *S*-4CPG was reversible as demonstrated in Fig. 5*C*, which shows the recovery of the event upon washout of the agent. Similar suppressive actions were observed with *S*-4C3HPG (Fig. 7).

As with the phenylglycine derivatives, local application of L-AP3 in the CA3 region also suppressed the long burst-associated depolarization (Fig. 7).

Atropine did not affect the long burst-associated depolarization

Acetylcholine (ACh) produces depolarization and burst discharge in hippocampal pyramidal cells (Benardo & Prince, 1982). Pressure-applied ACh produced prolonged depolarizations which were blocked by local application of atropine (10 μM; $n = 3$; Fig. 6*B*). In other slices, atropine applied in the same manner and concentration did not change the amplitude nor the time course of the depolarization associated with the long burst ($n = 5$; Figs 6*A* and 7). In contrast to atropine action, local application of (+)-MCPG (10 mM) in the CA1 region did not affect the excitatory response to ACh ($n = 4$).

Effects of low extracellular [Ca^{2+}] and TTX on the long burst activity

Since the long burst-associated depolarization in the CA1 region appeared to depend on the synaptic transmission between CA3 and CA1 neurones, we tested whether synaptic transmission interactions were also required in the CA3 region to generate the long bursts. Experimental manipulations that suppress synaptic transmission were performed during the long burst activity recorded in the CA3 region.

The long bursts were completely and reversibly blocked by a low Ca^{2+} (0·5 mM)–high Mg^{2+} (8 mM) solution ($n = 3$; not shown). This solution did not affect the membrane potential,

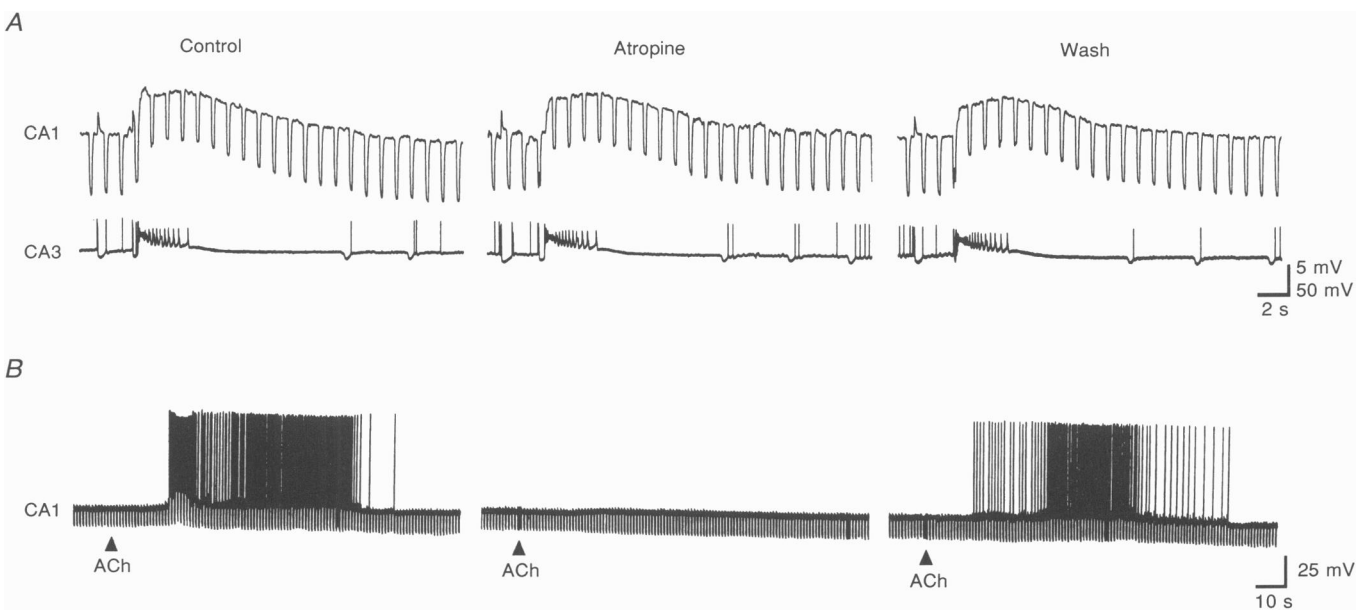

Figure 6. Atropine did not affect the long burst-associated depolarization

A, the depolarizing events in CA1 associated with the long bursts in CA3 were recorded. Local application of atropine (10 μM) in the CA1 region did not produce a noticeable effect on the event. Intracellular current pulses: 200 ms, −0·2 nA, 1 Hz. Membrane potentials: CA1, −78 mV; CA3, −56 mV. *B*, in another slice, pressure application of acetylcholine (ACh, 1 mM, 20 psi, 50 ms) produced a phase of rapid action potential discharge in a CA1 neurone (left record). Application of atropine (10 μM) in the CA1 region abolished the discharge induced by ACh (middle record) in a reversible manner (right record). Intracellular current pulses: 200 ms, −0·5 nA, 1 Hz. Membrane potential: −61 mV.

the membrane input resistance, nor the firing threshold. Previously, it has been shown that chemical synaptic transmission is blocked in this medium (Schwartzkroin & Prince, 1978). TTX (1 μM) in the perfusing solution also blocked completely the long bursts as well as the fast action potentials and any spontaneous synaptic potential ($n = 3$; not shown).

Effects of bath application or local application of phenylglycine derivatives and L-AP3 on the CA3 region during the long burst activity

In the CA1 region the long burst-associated depolarization required the activation of mGluRs. Thus, we asked whether the generation of long bursts in the CA3 region was also dependent on mGluR activation.

Bath application of (+)-MCPG (1 mM) reversibly blocked the long bursts in 5 out of 5 slices (Fig. 8*A*, open symbols). The frequency of the long bursts gradually slowed down before the blockade. At 0·5 mM, (+)-MCPG reversibly blocked the long bursts in 5 out of 7 slices. In the remaining 2 slices long bursts were still recorded after 40 min of perfusion with (+)-MCPG, although their frequencies were slightly decreased compared with the controls. In additional experiments (+)-MCPG was locally applied in the CA3 region during dual CA3-CA1 recordings. All applications resulted in a complete and reversible blockade of the long bursts in both regions ($n = 6$; not shown). L-AP3 (1 mM) suppressed long burst activities in a similar manner (Fig. 8*A*, filled symbols). Bath perfusion with (−)-MCPG (1 mM) did not affect the long burst activity ($n = 3$; not shown).

S-4CPG (0·5 mM) and *S*-4C3HPG (0·5 mM) also blocked the occurrence of long bursts. However, with these two agents we consistently observed an increase in frequency of the event before a subsequent decrease leading to the blockade (Fig. 8*B*).

DISCUSSION

Prolonged synaptic depolarizations that persisted in saturating concentrations of ionotropic glutamate receptor blockers were recorded in CA1 neurones. The results suggest that the synaptic depolarizations were dependent on metabotropic glutamate receptor activation. MGluR-dependent synaptic events may also be involved in the generation of rhythmic long bursts occurring in the CA3 neuronal population.

Origin and properties of the synaptic depolarizations in CA1 neurones

4-Aminopyridine elicited long bursts in both CA3 and CA1 neurons. Data from isolated CA1 and CA3 slices suggest that the long bursts were generated in the CA3 population and the events recorded in the CA1 region were driven by the synchronized output from the CA3 population. Long bursts in CA1 neurones were sustained by long synaptic depolarizations. Their existence in ionotropic glutamate receptor blockers implies the non-involvement of the

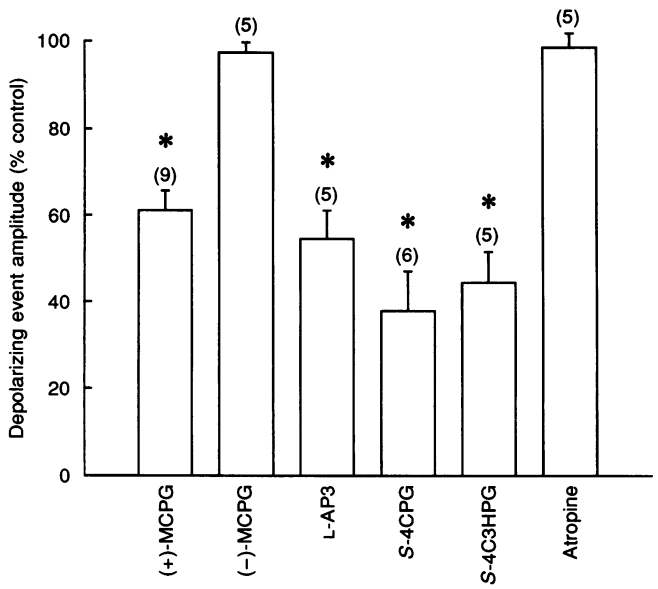

Figure 7. Summary histogram of the effects of the agents tested on the long burst-associated depolarization

The agents were all applied locally via a pipette in the CA1 region close to the intracellular recording electrode at the following concentrations (mM): (+)-MCPG, 10; (−)-MCPG, 10; L-AP3, 10; *S*-4CPG, 10; *S*-4C3HPG, 10; atropine, 0·01. The number in parenthesis above each bar indicates the number of cells for each experiment. Asterisks indicate significant differences compared with controls with $P < 0·05$ by Student's paired t test.

ionotropic glutamate receptors. Two observations suggest that the blockers indeed eliminated the contribution of ionotropic glutamate receptor-dependent activities during the long burst generation. First, the blockers completely suppressed fast synaptic events elicited in control extracellular perfusate. Second, after long bursts had occurred, increasing the concentration of ionotropic glutamate receptor blockers (CNQX and CPP to 40 μM) and addition of other ionotropic glutamate receptor antagonists (kynurenic acid and MK-801) did not alter the time course of the long bursts nor the associated long synaptic depolarizations.

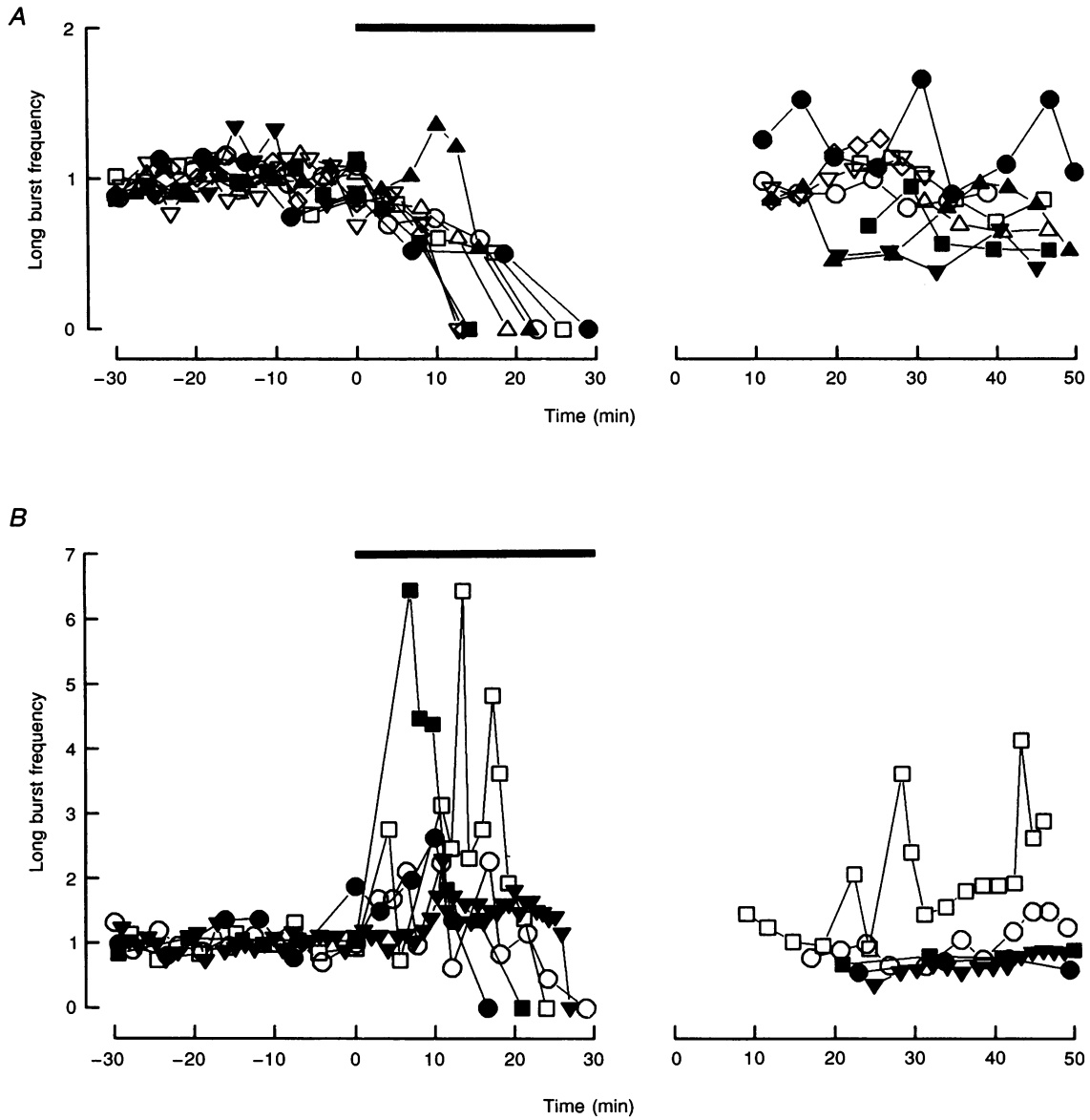

Figure 8. Effects of bath application of phenylglycine derivatives and L-AP3 on the long burst activity

The occurrence of a long burst is indicated by a symbol and the instantaneous frequency was calculated from the interval to the next long burst. The interval was measured from the onset of one long burst to the onset of the next one. Frequencies were normalized to the mean value of the 30 min control period for each slice. The agents were added to the perfusing solution at time 0 of the left graphs at concentrations of (mM): (+)-MCPG, 1; L-AP3, 1; *S*-4CPG, 0·5; *S*-4C3HPG, 0·5. The perfusion time was 30–50 min in different experiments. The 0 values on the *y*-axis indicate the last long bursts recorded over more than 20 min. Time 0 in the right graphs indicates the beginning of drug washout. The filled bar represents the duration of application of (+)-MCPG (open symbols) and L-AP3 (filled symbols) in *A* and *S*-4C3HPG (open symbols) and *S*-4CPG (filled symbols) in *B*. *A*, (+)-MCPG and L-AP3 gradually slowed down the frequency before blocking the events. *B*, *S*-4CPG and *S*-4C3HPG caused a transient increase in the frequency before the slowing down and eventual blockade of the events. The right graphs show that the blockades were reversible.

(+)-MCPG suppressed the long burst-associated synaptic depolarization, but it did not affect the epileptiform giant EPSP, indicating that the long synaptic depolarization is dependent on mGluR activation. (+)-MCPG was reported as an effective mGluR antagonist in the concentration range of 0·5–1 mM (Bashir *et al.* 1993; Guérineau, Gähwiler & Gerber, 1994). The suppression of the long burst-associated depolarization by (+)-MCPG was not complete. Since the antagonistic efficacy of (+)-MCPG depends on receptor subtypes (Hayashi *et al.* 1994), one interpretation of the incomplete suppression is that the synaptic depolarization in CA1 neurones results from the activation of either a mGluR subtype that is not completely blocked by (+)-MCPG or more than one mGluR subtype with different sensitivities to (+)-MCPG. For example, Chinestra *et al.* (1993) showed that some responses induced by mGluR agonists were not antagonized by (+)-MCPG in CA1 neurones. Alternatively, the incomplete blockade of the long burst-associated depolarization may be due to the inadequate delivery of (+)-MCPG to the membrane sites. The local concentration of (+)-MCPG may not have reached a sufficient level or the diffusion of the agent after application was not extensive enough to reach all active synaptic sites in the somato-dendritic membrane. All these factors could set an upper limit on the extent to which the depolarizing event could be suppressed by the focal application of (+)-MCPG. The specificity of (+)-MCPG is supported by additional observations that (−)-MCPG produced no changes in the long burst-associated depolarization. The effectiveness of *S*-4CPG and *S*-4C3HPG suggests that the generation of the long burst-associated depolarization may be dependent on mGluR1/5 and not on mGluR2/3, since the agents have antagonistic and agonistic actions, respectively, at these two groups of mGluR subtypes (Hayashi *et al.* 1994). The suppressive action of the phenylglycine derivatives together with the blocking effect of L-AP3 are all consistent with the suggestion that the long burst-associated depolarizations are dependent on mGluR activation.

Our results also show that the synaptic depolarizations underlying the long bursts were associated with a decrease in input resistance. In addition to excitatory responses involving decrease in potassium conductances (Charpak *et al.* 1990), recent data show that application of the mGluR agonist 1*S*,3*R*-ACPD induced inward currents associated with conductance increase (Staub *et al.* 1992; Crépel *et al.* 1994; Linden *et al.* 1994; Guérineau *et al.* 1995; Zheng & Gallagher, 1995).

Generation of the long bursts

Because the long bursts were generated in CA3 cells and passively driven in CA1 neurones, pharmacological manipulation of the events in the CA1 region did not alter the frequency nor the time course of the long bursts in CA3 cells. The results suggest that glutamate released by the CA3 Schaffer collateral axons activated mGluRs in CA1 neurones and produced synaptic depolarizations. It is possible that recurrent axons among CA3 cells also elicit mGluR-dependent excitations to produce synchronized discharges of the CA3 population. In this study we have not attempted to elucidate the mechanism involved in the synchronization of CA3 cells during the long burst. However, the results do suggest that generation of the long bursts is at least in part dependent on mGluR activation, since phenylglycine derivatives and L-AP3 abolished the long bursts (Fig. 8).

4-Aminopyridine and picrotoxin were two additional agents required for an activation of long bursts. Since 4-AP blocks transient potassium currents in hippocampal cells, we speculate that a block of the transient currents may be the necessary condition for the elicitation of long bursts. In addition, the low level of 4-AP applied points to the blockade of the D-current as a prerequisite for long burst induction, because at low doses (100 μM) 4-AP completely blocks the D-current and does not suppress the A-current (Wu & Barish, 1992). The finding that long bursts could also be elicited by a more specific blocker of the D-current (α-dendrotoxin) supports the hypothesis that long bursts were elicited when the D-current was suppressed. Additional studies are required to both confirm and define more precisely the effect of the blockade of the D-current. One question arises: are the long bursts elicited when D-currents are generally depressed over the entire neuronal surface membrane or only when the D-current is suppressed at strategic sites such as the axon terminals or postsynaptic dendrites?

A previous study emphasized the stronger influence of the mGluR-dependent synaptic excitation onto hippocampal GABAergic inhibitory cells than pyramidal cells (Miles & Poncer, 1993). It is conceivable that in the absence of picrotoxin, the net effect of mGluR activation is an overall inhibition of pyramidal cell activities and that blockade of inhibition is necessary to allow the expression of long bursts. When 4-AP alone was present in the bath, robust iGluR-dependent depolarizations and synchronized bursts occurred (Rutecki, Lebeda & Johnston, 1987). It is likely that these bursts would occlude the long bursts since the former occur at a higher frequency. Ionotropic GluR blockers allowed the isolated occurrence and the observation of long bursts.

We have recently demonstrated, in a septohippocampal slice preparation, that activation of the cholinergic input induced rhythmic bursts with a time course similar to that of the long bursts reported here (Bianchi & Wong, 1994*a*). In the present study we show that atropine does not affect long burst-associated depolarizations (Fig. 6*A*). On a number of occasions, long bursts faded over time during the experiment. Invariably, upon the addition of carbachol to the perfusing solution long bursts reappeared. Thus, the cholinergic input may interact with the local glutamatergic synapses in the hippocampus to facilitate long burst

production. Additional studies are required to understand how neurotransmitters can interact to facilitate or inhibit long burst occurrence.

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