Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices

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1. Cell-attached and whole-cell recordings from interneurons localized in the stratum radiatum of the CA3 subfield (SR-CA3) of neonatal (postnatal days 2–5) rat hippocampal slices were performed to study their activity during the generation of GABAergic giant depolarizing potentials (GDPs) in CA3 pyramidal cells.

2. Dual recordings revealed that during the generation of GDPs in CA3 pyramidal cells, the interneurons fire bursts of spikes, on average 4·5 ± 1·4 spikes per burst (cell-attached mode). These bursts were induced by periodical large inward currents (interneuronal GDPs) recorded in whole-cell mode.

3. Interneuronal GDPs revealed typical features of polysynaptic neuronal network-driven events: they were blocked by TTX and by high divalent cation medium and they could be evoked in an all-or-none manner by electrical stimulation in different regions of the hippocampus. The network elements required for the generation of GDPs are present in local CA3 circuits since spontaneous GDPs were present in the isolated CA3 subfield of the hippocampal slice.

4. Interneuronal GDPs were mediated by $GABA_A$ and glutamate receptors, since: (i) their reversal potential strongly depended on $[Cl^-]_i$; (ii) at the reversal potential of $GABA_A$ postsynaptic currents an inward component of GDPs was composed of events with the same kinetics as α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor-mediated EPSCs; and (iii) once $GABA_A$ receptors were blocked intracellularly by dialysis with F^-–MgATP-free solution, the remaining component of interneuronal GDPs reversed near 0 mV and rectified at membrane potentials more negative than −20 mV, suggesting an important contribution of NMDA receptors in addition to AMPA receptors.

5. In cell-attached recordings from interneurons, electrical stimulation in the stratum radiatum evoked a burst of spikes that corresponded to evoked GDPs. Pharmacological study of this response revealed that excitation of SR-CA3 interneurons during GDPs is determined by the co-operative depolarizing actions mediated by $GABA_A$ and glutamate (AMPA and NMDA) receptors. Interestingly, after blockade of AMPA receptors, $GABA_A$ receptor-mediated depolarization enabled the activation of NMDA receptors presumably via attenuation of their voltage-dependent magnesium block.

6. It is concluded that synchronous activation of SR-CA3 interneurons during generation of GDPs is mediated synaptically and is determined by the co-operation of (i) excitatory GABAergic connections between interneurons and (ii) glutamatergic connections to interneurons originating presumably from the pyramidal cells.

Synchronous inhibition of the pyramidal cells by GABAergic interneurons has been recently shown to be an important mechanism of the synchronization of neuronal activity in adult hippocampus (Cobb, Buhl, Halasy, Paulsen & Somogy, 1995; Whittington, Traub & Jefferys, 1995; Ylinen, Soltész, Bragin, Penttonen, Sik & Buzsaki, 1995*b*). In the hippocampus of neonatal rats, interneurons provide a different type of control of activity of the pyramidal cells to that in the adult, since GABA acting via the $GABA_A$ receptor operates as an excitatory transmitter at the early stages of development (for review, see Cherubini, Gaiarsa & Ben-Ari, 1991). The temporal window when GABA exerts excitatory effects coincides with a particular pattern of synchronous activity of the pyramidal cells of CA3/CA4 subfields,

characterized by periodical network-driven events, so-called giant depolarizing potentials (GDPs) (Ben-Ari, Cherubini, Corradetti & Gaiarsa, 1989; Xie & Smart, 1991; Strata, Sciancalepore & Cherubini, 1995). Since GDPs were shown to be mediated by $GABA_A$ receptors (Ben-Ari *et al.* 1989; Xie & Smart, 1991), the GABAergic interneuron network must play a crucial role in their generation.

However, the mechanisms of generation of GDPs remain unclear because the behaviour of interneurons during GDPs has not been studied. The principal question is whether and how the interneurons are synchronously activated during the generation of GDPs. In previous studies, an important role has been suggested for recurrent glutamatergic inputs to interneurons from pyramidal cells, since antagonists of glutamate receptors efficiently blocked GDPs (Ben-Ari *et al.* 1989; Gaiarsa, Corradetti, Cherubini & Ben-Ari, 1991; Xie, Hider & Smart, 1994; Strata *et al.* 1995). Interestingly, NMDA receptors have been shown to make an important contribution to the generation of GABAergic polysynaptic activity in the neonatal hippocampus (Corradetti, Gaiarsa & Ben-Ari, 1988; McLean, Rovira, Ben-Ari & Gaiarsa, 1995).

In the present paper, we have studied mechanisms of synchronization of activity of interneurons localized in the stratum radiatum of the CA3 subfield (SR-CA3) of neonatal hippocampal slices during GDP generation in CA3 pyramidal cells. We found that activation of interneurons is determined by the co-operation of activities of two types of synaptic connections: (i) excitatory GABAergic connections between interneurons and (ii) glutamatergic connections to interneurons originating presumably from the pyramidal cells. Part of the present work has been reported in abstract form (Khazipov, Gaiarsa, McLean, Konig & Ben-Ari, 1995; Khazipov, Khalilov, Leinekugel, Miles & Ben-Ari, 1996).

METHODS

Slice preparation

Experiments were performed on hippocampal slices obtained from 2- to 5-day-old male Wistar rats. Slices were prepared as described previously (Ben-Ari *et al.* 1989). Animals were anaesthetized by intraperitoneal injection of pentobarbitone (0·02 ml of 6% solution). After killing the rat by decapitation, the brain was rapidly removed and placed in oxygenated, ice-cooled artificial cerebrospinal fluid (ACSF) (mM: 126 NaCl, 3·5 KCl, 2·0 $CaCl_2$, 1·3 $MgCl_2$, 25 $NaHCO_3$, 1·2 NaH_2PO_4 and 11 glucose). Hippocampal transverse slices (400–600 μm) were cut with a McIlwain tissue chopper and kept in oxygenated (95% O_2–5% CO_2; pH 7·3) ACSF at room temperature (20–22 °C), at least 1 h before use. Individual slices were then transferred to the recording chamber where they were fully submerged and superfused with oxygenated ACSF at 30–32 °C, at a rate of 2–3 ml min^{-1}.

Electrophysiological recordings

Recordings were performed using the patch-clamp technique in the cell-attached and whole-cell configurations using Axopatch 200 (Axon Instruments), ROK-3 (Puschino, Russia) and EPC-9 (List-Medical) patch-clamp amplifiers. Cells were recorded either 'blindly' with subsequent morphological identification using biocytin labelling (1%) or under visual control using an Axioscope Karl Zeiss microscope (×40 water-immersion objective lens, ×400 magnification). Microelectrodes had a resistance of 7–10 MΩ. Internal solutions of the following compositions were used (mM): (1) 135 potassium gluconate, 2 $MgCl_2$, 0·1 $CaCl_2$, 1 EGTA, 2 Na_2ATP and 10 Hepes; pH 7·25 ($[Cl^-]_i = 4·2$ mM); (2) 125 potassium gluconate, 15 NaCl, 1 $CaCl_2$, 1 MgATP, 10 EGTA and 10 Hepes; pH 7·25 ($[Cl^-]_i =$ 17 mM); (3) 140 CsCl, 1 $CaCl_2$, 10 EGTA, 10 Hepes and 2 MgATP; pH 7·25 ($[Cl^-]_i = 142$ mM); and (4) 140 CsF, 1 $CaCl_2$, 10 Hepes and 10 EGTA; pH 7·3. The osmolarity of the internal solutions was 270–280 mmol l^{-1}. Biocytin (1%) was routinely added to the pipette solution for morphological analysis. In some experiments 2(triethylamino)-*N*-(2,6-dimethylphenyl) acetamide (QX314; 1 mM) was added to the internal pipette solution to block sodium-dependent action potentials. Slices were stimulated by a bipolar electrode placed in the different areas of the hippocampus and the fascia dentata; stimulation parameters were 30–80 V amplitude and 10–30 μs duration, delivered at a frequency of 0·02–0·05 Hz. Series resistance was compensated, when necessary, at the level of 50–70%.

Histological processing

After electrophysiological recording, slices were placed between two millipore filters and immersed overnight at 4 °C in a fixative solution of 2·5% paraformaldehyde and 1·25% glutaraldehyde in 0·12 M sodium phosphate buffer (pH 7·3). Following fixation, slices were rinsed several times in the phosphate buffer for 1·5 h and immersed overnight at 4 °C in a cryoprotective solution of 30% sucrose in the same buffer. To increase the penetration of the reagents used for biocytin detection, slices were quickly frozen on dry ice and thawed in phosphate buffer. Slices were then rinsed in 0·05 M Tris-buffered saline (TBS; pH 7·4) containing 0·3% Triton X-100 for 30 min and incubated overnight at 4 °C in an avidin–biotin–peroxidase solution prepared in TBS according to the manufacturer's recommendations (Vectastain Elite ABC, Vector Laboratories, Burlingame, CA, USA). After a 30 min wash in TBS, and a 10 min rinse in Tris buffer (TB; pH 7·6) slices were processed for 15 min in 0·06% 3,3′-diaminobenzidine tetrahydrochloride and 0·01% hydrogen peroxide diluted in TB. The slices were then rinsed in TB for 30 min, mounted on gelatin-coated slides, dehydrated and coverslipped with Permount. After light microscopic analysis of the biocytin-filled cells, identified interneurons were drawn with the aid of a ×40 or a ×100 oil-immersion objective attached to a Leitz microscope. Identification of interneurons was based on the location of their soma within the stratum radiatum, the non-pyramidal shape of the soma and the organization of the dendritic and axonal arborizations (Ben-Ari, Tseeb, Raggozzino, Khazipov & Gaiarsa, 1994).

Solutions and drugs

Biocytin and tetrodotoxin were purchased from Sigma and isoguvacine, bicuculline, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and APV (D-2-amino-5-phospho-pentanoate) from Tocris Neuramin.

Data analysis

Synaptic currents and agonist-evoked responses were acquired on a DAT tape-recorder (Biologic, France) and into the memory of an 80486 personal computer using a Labmaster interface (USA). Data were then analysed using Acquis (Gérard Sadoc, France) and pCLAMP 5.1 (Axon Instruments, USA) software. AxoTape (Axon Instruments) and SE04 (S. F. Traynelis) programs were used for the acquisition and analysis of spontaneous events.

Statistical analysis

Group measurements were expressed as means ± S.E.M. Statistical significance of differences between means was assessed using Student's t test, with the aid of statistical software StatView™ SE+Graphics (Abacus Concepts, France), and the Wilcoxon ranked sign test was employed for analysis of paired data. The level of significance was set at $P < 0.05$.

RESULTS

GDPs in SR-CA3 interneurons are network-driven events

Spontaneous activity in SR-CA3 interneurons recorded in whole-cell configuration was characterized by monosynaptic currents and periodic giant inward currents which appeared with a frequency of 6·2 ± 2·1 min^{-1} ($n = 81$). To keep with established terminology these currents hereafter will be referred to as interneuronal giant depolarizing potentials (GDPs; Ben-Ari *et al.* 1989; Xie & Smart, 1991; Strata *et al.* 1995), although most of the present study was performed in a voltage-clamp mode.

The following observations suggest that interneuronal GDPs, as pyramidal cell GDPs (Ben-Ari *et al.* 1989), are network-driven synaptic events (Johnston & Brown, 1984): (i) they were abolished by TTX (1 μM) (Fig. 1*A*); (ii) they were blocked by superfusion with a solution containing high concentrations of divalent cations (6 mM Mg^{2+} and 4 mM Ca^{2+}), known to block preferentially polysynaptic activity (Berry & Penthreath, 1976) (Fig. 1*B*); (iii) they could be evoked by electrical stimulation in different areas of the hippocampus and fascia dentata in an all-or-none manner, and the latency decreased with increase of stimulus intensity while the amplitude did not change (Fig. 1*C*); evoked interneuronal GDPs were blocked by TTX and high divalent cation medium, as were spontaneous events; (iv) the frequency of spontaneous GDPs was not dependent on the membrane potential of the recording cell (Fig. 1*D*); (v) they could not be triggered by short (30–50 ms) depolarizing current pulses injected through the recording electrode; and (vi) they were synchronous in intracellular and extracellular recordings.

Finally, dual recordings revealed that during the generation of GDPs in the pyramidal cell, interneurons fired a burst of action potentials (from 2 to 19, on average 4·5 ± 1·4 action potentials per burst; $n = 11$) when recorded in cell-attached configuration (Fig. 2*A*). When interneurons were recorded in whole-cell configuration, synchronous GDPs were recorded in all pairs studied (Fig. 2*B*; $n = 11$). There was no systematic trend in onset latencies of GDPs between pyramidal cells and interneurons (the latency varied from −24 ± 4 to 67 ± 17 ms delay of pyramidal cell from interneuron; Fig. 2*C*). A given type of cell did not systematically precede the other (mean delay, 2 ± 8 ms; $n = 11$ pairs). This may be due to the relatively small number of pairs recorded.

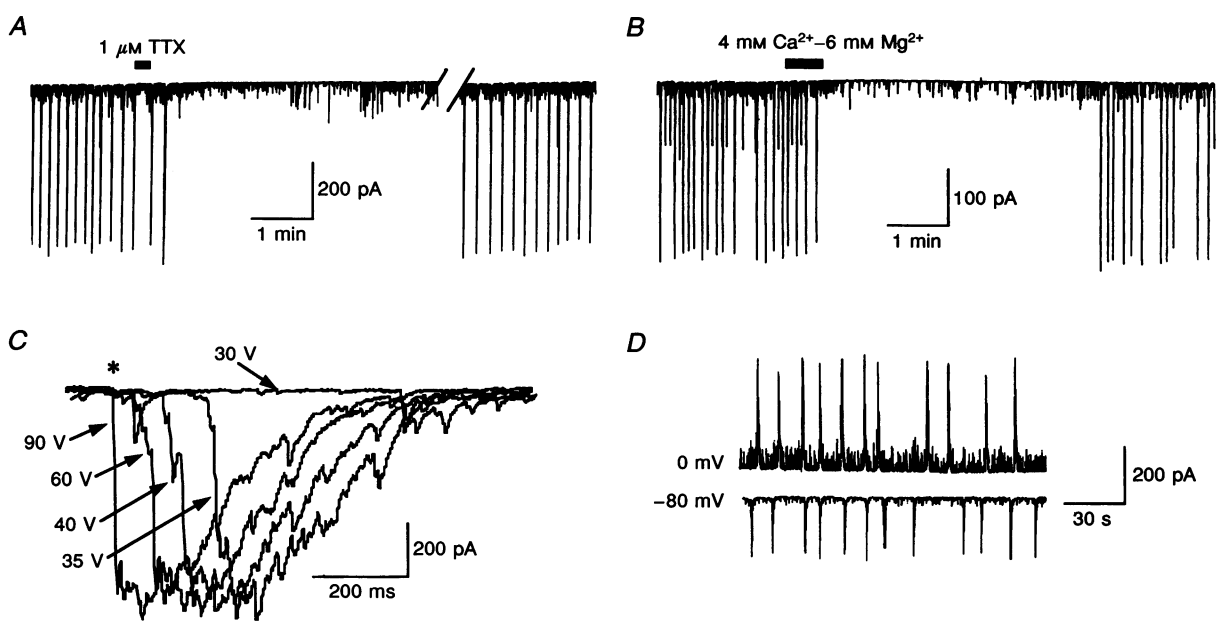

Figure 1. GDPs in SR-CA3 interneurons are polysynaptic network-driven events

A, spontaneous GDPs are blocked by brief bath application of tetrodotoxin (TTX, 1 μM) and recover after 15 min of wash. *B*, high divalent cation medium (4 mM Ca^{2+}–6 mM Mg^{2+}) reversibly blocks spontaneous GDPs. *C*, GDPs can be evoked in SR-CA3 interneurons by electrical stimulation (*) in an all-or-none manner. Note that the latency of the GDP decreases but amplitude does not change as the stimulus intensity is increased. *D*, frequency of GDPs is independent of holding potential. *A–C*, whole-cell recordings with CsCl pipette solution (3); holding potential −80 mV. *D*, whole-cell recordings with potassium gluconate solution (1).

Nevertheless, this is in keeping with the observation that both GABA and glutamate contribute to GDPs (Ben-Ari *et al.* 1989; Gaiarsa *et al.* 1991; Xie *et al.* 1994; Strata *et al.* 1995). The generation of network-driven GDPs therefore probably results from a complex interaction between the population of interneurons and pyramidal cells.

To study whether GDPs are present in interneurons from other regions of the hippocampus, we recorded from interneurons located in the hilus ($n = 5$), stratum oriens of CA3 ($n = 2$), stratum oriens of CA1 ($n = 2$) and stratum lacunosum-moleculare of CA1 ($n = 3$). In all these cells, we observed GDPs that were similar to those recorded in the CA3 stratum radiatum interneurons.

Nature of GDPs in SR-CA3 interneurons

Two types of synaptic inputs, GABAergic and glutamatergic, could contribute to the generation of GDPs in SR-CA3 interneurons. The contribution of a $GABA_A$ receptor-activated Cl^- conductance was estimated from the dependence of the charge–voltage relationships of GDPs on $[Cl^-]_i$.

The reversal potential of interneuronal GDPs strongly depended on the $[Cl^-]_i$: decrease of $[Cl^-]$ in the pipette solution from 142 to 17 and 4·2 mM (solutions 3, 2 and 1, respectively) induced a negative shift of the reversal potential of GDPs after about 10 min of dialysis from 2 ± 4 mV ($n = 5$) to -32 ± 3 mV ($n = 4$) and -58 ± 5 mV ($n = 6$), respectively (Fig. 3). Thus, a Cl^- conductance clearly contributes to GDPs in SR-CA3 interneurons. However, for the recordings with low $[Cl^-]_i$ solutions, the reversal potentials of GDPs were more positive than those predicted by the Nernst equation (−54 and −91 mV for solutions 2 and 1, respectively) suggesting that, in addition to a Cl^- conductance, some other conductance(s) with a more positive reversal potential, could also contribute to interneuronal GDPs.

Indeed, after dialysis with a solution containing low $[Cl^-]_i$ (4·2 mM, solution 1), when GDPs were recorded at the reversal potential of $GABA_A$ postsynaptic currents (PSCs), a clear inward component of the GDPs was revealed ($n = 6$). Single events that composed this inward component in some cases could be clearly resolved and the kinetics of these

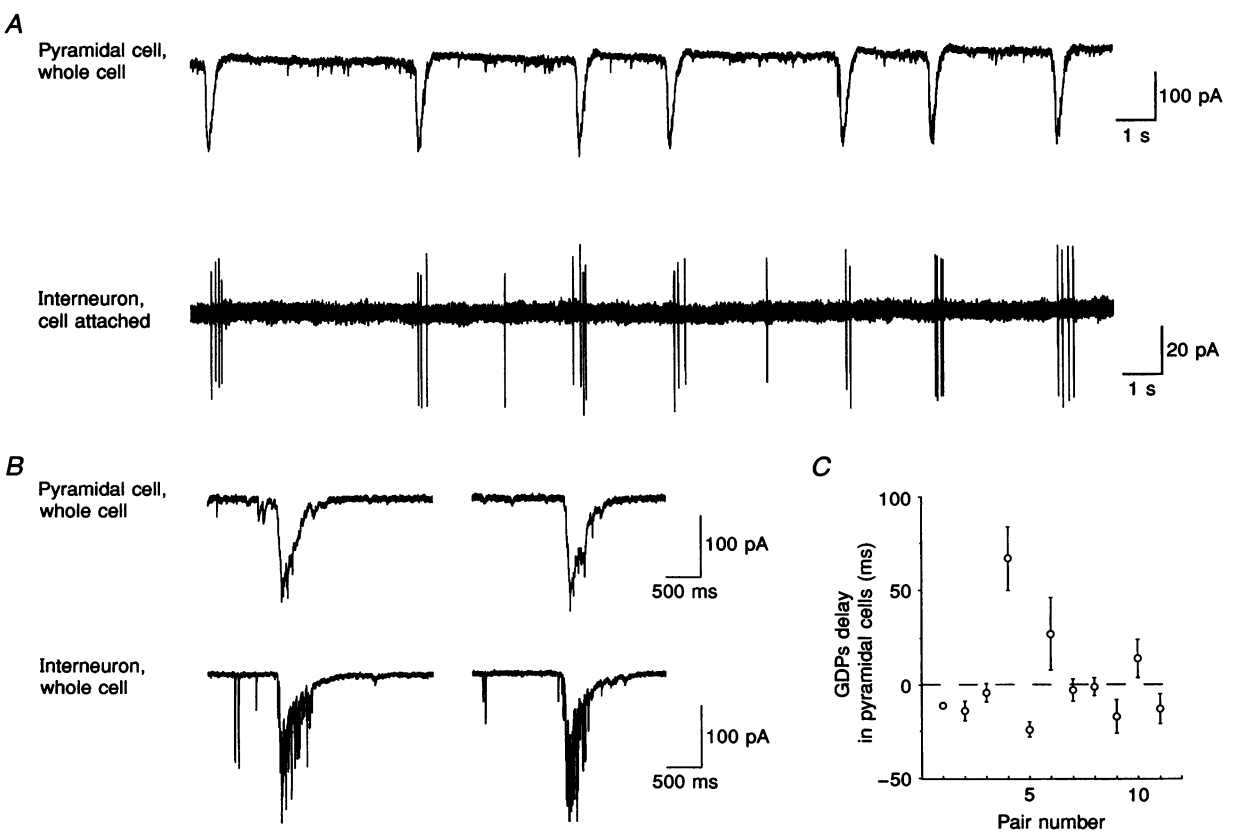

Figure 2. GDPs in SR-CA3 interneurons are synchronous with GDPs in CA3 pyramidal cells

A, dual recordings of CA3 pyramidal cell in whole-cell mode (upper trace) and SR-CA3 interneuron in cell-attached mode (lower trace). Note that bursts of action potentials in the interneuron are synchronous with GDPs in the pyramidal cell. *B*, dual whole-cell recordings of CA3 pyramidal cell and SR-CA3 interneuron. Note synchronous generation of GDPs in simultaneously recorded cells. *C*, latency between onset of GDPs in pyramidal cells and simultaneously recorded interneurons. Each point represents one pair: SR-CA3 interneuron–CA3 pyramidal cell ($n = 11$ pairs). Recordings with potassium gluconate pipette solution (1). In whole-cell recordings cells were kept at −80 mV; cell-attached recordings were performed in 'track' mode.

events were fast (decay time constant, $\tau = 2{\cdot}6 \pm 0{\cdot}3$ ms; $n = 6$) (Fig. 4) and very similar to those of spontaneous α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) EPSCs ($\tau = 2{\cdot}4 \pm 0{\cdot}2$ ms, $n = 9$). Application of the AMPA receptor antagonist CNQX (10 μM) completely blocked this component ($n = 4$; not shown), further suggesting that a synaptic glutamate receptor-mediated component may contribute to GDPs in SR-CA3-interneurons. An NMDA receptor-mediated component of EPSCs in these experiments could not be detected presumably because recordings were performed at a hyperpolarized potential (about −70 mV) at which the NMDA receptor-mediated component is blocked by Mg^{2+}.

Another approach to study the conductances activated during GDPs in interneurons is based on a selective intracellular blockade of $GABA_A$ receptor-mediated currents. In control experiments, prolonged (> 1 h) whole-cell dialysis with an internal solution (solution 4) containing the poorly permeable F^- as the main anion (Bormann, Hamill & Sakmann, 1987), and without Mg^{2+} and ATP to promote run-down of the $GABA_A$ conductance (Stelzer, Kay & Wong, 1988), completely blocked both $GABA_A$ receptor-mediated PSCs and responses to the $GABA_A$ agonist isoguvacine, whereas AMPA EPSCs were not modified and NMDA EPSCs were only slightly reduced (by 20%) (R. Khazipov, I. Khalilov & X. Leinekugel, unpublished observations).

At a holding potential of −80 mV, the charge passing during GDPs was reduced in stratum radiatum interneurons after 1 h of dialysis with F^-–MgATP-free solution from -42 ± 15 to -14 ± 6 pC ($n = 5$; $P < 0{\cdot}05$) (Fig. 5). A study of the charge–voltage relationships of GDPs revealed a shift in the reversal potential of GDPs from -28 ± 5 mV in control to 0 ± 3 mV ($n = 5$) after more than 1 h of dialysis. The dialysis-resistant component rectified at membrane potentials more negative than −20 mV suggesting a significant contribution of NMDA receptors at depolarized potentials. Assuming that the dialysis-resistant component was mediated by glutamate receptors, subtraction of the charge of the dialysis-resistant component from the total charge of GDPs recorded immediately after penetrating to whole cell

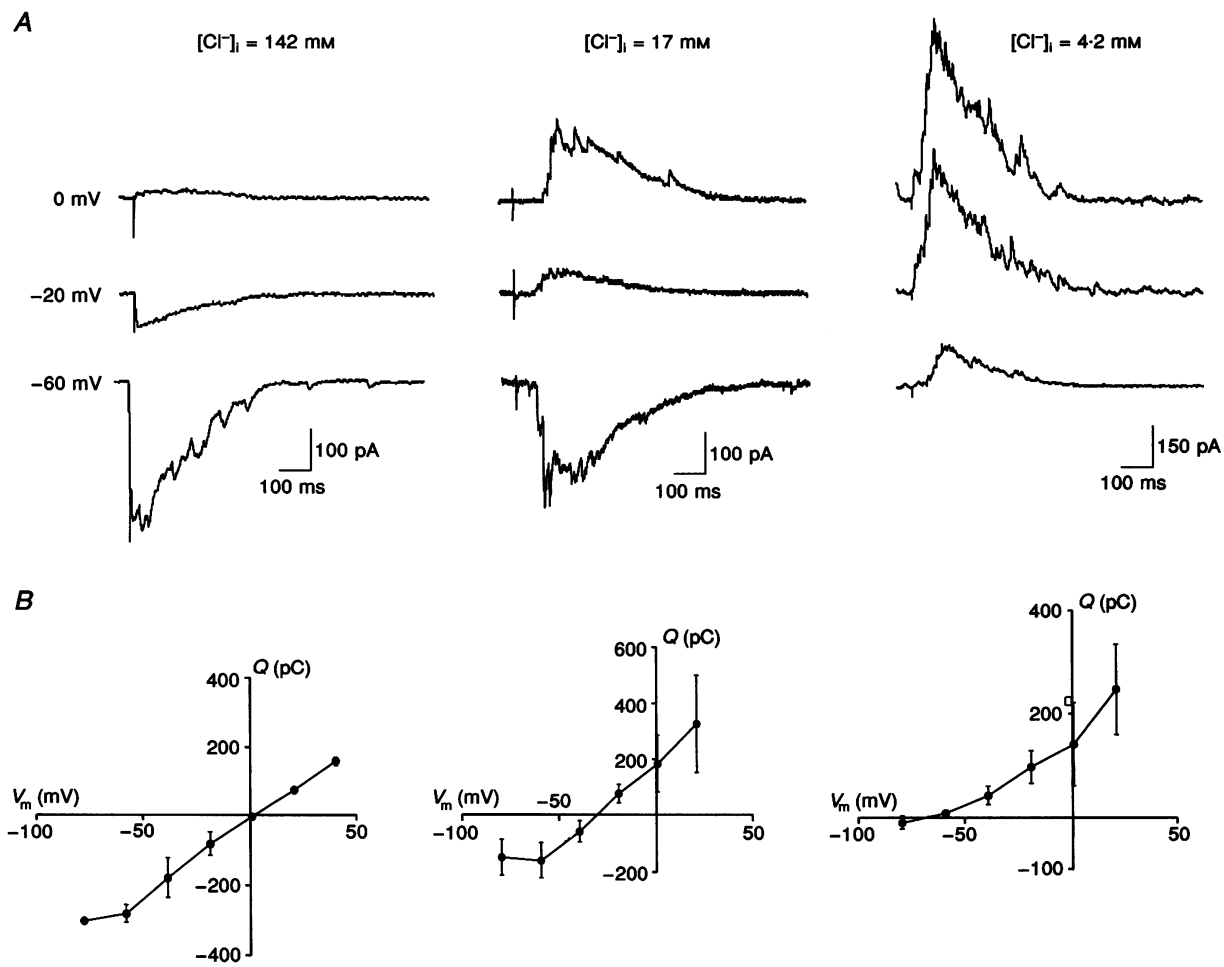

Figure 3. GDPs in SR-CA3 interneurons are dependent on $[Cl^-]_i$

A, traces of GDPs recorded at different membrane potentials (0, −20 and −60 mV) and after 10 min of dialysis with pipette solutions containing different concentrations of $[Cl^-]_i$ (142, 17 and 4·2 mM). *B*, charge–voltage relationships of GDPs with the different $[Cl^-]_i$. Note that the reversal potential shifts to negative values as $[Cl^-]_i$ is decreased.

gives an estimation of a 'pure' GABA receptor-mediated component of GDPs, although probably underestimated because of early dialysis. The reversal potential of this 'pure' GABA component of GDPs was −48 mV (Fig. 5*C*), similar to the values of reversal of GABA PSCs in neonatal pyramidal neurons (Ben-Ari *et al.* 1989).

These results suggest that $GABA_A$ and glutamate receptors are activated during GDPs in SR-CA3 interneurons. To estimate the functional contribution of different receptors to the excitation of interneurons during GDPs, we studied the pharmacology of the evoked responses recorded in the cell-attached configuration (Fig. 6). Although the cell-attached configuration has limitations for pharmacological analysis of GDPs in comparison with whole-cell recordings (since (i) only changes above spike threshold will be observed and (ii) the synaptic component of the response cannot be studied in separation by clamping voltage-gated conductances) this non-invasive approach was chosen because it should not change the intracellular Cl^- concentration of the cells, i.e. it does not affect Cl^--dependent $GABA_A$ receptor-mediated responses. In control, electrical stimulation evoked bursts of action potentials, equivalent to evoked GDPs recorded in whole-cell configuration (cf. Fig. 2) with, on average, 4·1 ± 0·7 action potentials per burst (Fig. 6*Aa* and *Ba*, $n = 8$). Blockade of AMPA receptors by CNQX (10 μM) reduced the response to 3·7 ± 0·5 action potentials ($n = 8$); further addition of APV (50 μM) to block NMDA receptors strongly reduced the response to 1·4 ± 0·2 spikes ($n = 8$). The latter response was completely blocked by further addition of bicuculline (10 μM). In the presence of CNQX and bicuculline, electrical stimulation failed to generate spikes in interneurons (0 spikes, $n = 4$), suggesting that NMDA receptors alone cannot provide excitation of interneurons, presumably because of blockade of NMDA channels by Mg^{2+} at the resting membrane potential. Indeed, in whole-cell recordings, polysynaptic excitatory responses could be evoked in the presence of bicuculline and CNQX following perfusion with nominally Mg^{2+}-free external solution ($n = 5$; Fig. 6*C*). Application of bicuculline (10 μM) alone blocked evoked GDPs in interneurons as has been reported previously in pyramidal cells (Ben-Ari *et al.* 1989; Xie & Smart, 1991; Strata *et al.* 1995). However, evoked glutamatergic polysynaptic bursts could often be observed further suggesting that AMPA receptors can also contribute to the excitation of interneurons during GDPs. Thus, the results of the pharmacological study suggest that a co-operative depolarizing action of $GABA_A$ and glutamate (AMPA and NMDA) receptors contributes to excitation of neonatal interneurons during GDPs.

GDPs in the disconnected CA3 subfield

To examine whether GDPs can be generated locally in the CA3 region of the hippocampus or whether they require the activity of neurons in other regions, the CA3 region was completely isolated by a knife cut from the fascia dentata and CA1. Spontaneous GDPs could be recorded from CA3 pyramidal cells following the surgical isolation of CA3 in all slices, with a frequency of 5·6 ± 0·8 min^{-1} ($n = 5$). Therefore, a local population of pyramidal cells and interneurons within the CA3 region can generate GDPs and they do not necessarily depend on a pacemaker activity outside CA3.

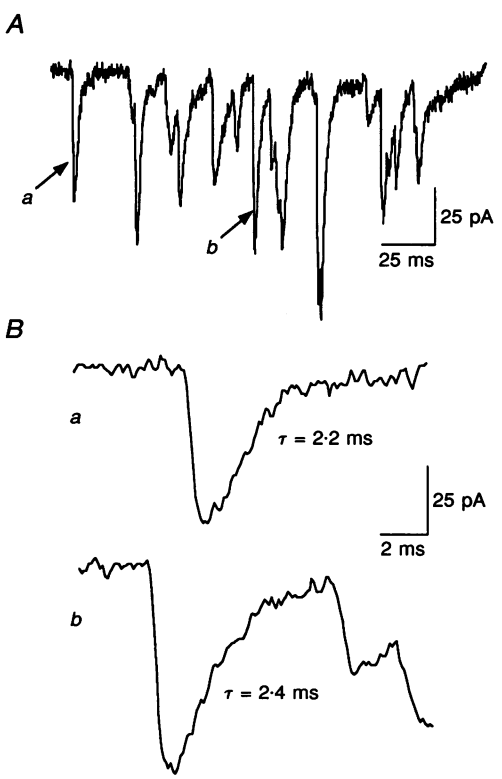

Figure 4. Glutamate component of GDP recorded at the $GABA_A$ reversal potential

A, trace of GDP recorded at the reversal potential of $GABA_A$ postsynaptic currents (here, −65 mV). Most of the events composing the GDP overlap; however, two of them (*a* and *b*) can be resolved separately. *B*, the decay time constant (τ) of these events is very similar to that of AMPA EPSCs. Recording with potassium gluconate solution (1).

DISCUSSION

GABAergic GDPs in CA3 pyramidal cells are polysynaptic, network-driven events presumably resulting from the synchronous discharge of GABAergic interneurons (Ben-Ari *et al.* 1989; Xie & Smart, 1991; Strata *et al.* 1995). In the present study we provide direct evidence for this hypothesis by recording from SR-CA3 interneurons: indeed, SR-CA3 interneurons fired bursts of action potentials during GDPs in CA3 pyramidal cells. Since GDPs were observed in interneurons and pyramidal cells from other hippocampal subfields, they probably represent a property of all populations of neurons in the neonatal hippocampal slice.

The principal aim of the present work was to understand the mechanisms determining the synchronous excitation of interneurons during GDPs. Although non-synaptic mechanisms (Jefferys, 1995) and other neurotransmitters can participate, our results suggest that GABAergic and glutamatergic synaptic connections are involved in the synchronization of interneuron discharge. A contribution of $GABA_A$ receptor-activated currents was concluded from (i) the dependence of the reversal potential of GDPs on $[Cl^-]_i$ and (ii) the reduction of GDP conductance after intracellular blockade of $GABA_A$ receptor-mediated currents. A contribution of glutamate receptors to the generation of GDPs was concluded from (i) the similar kinetics and CNQX sensitivity of the events recorded during GDPs and AMPA EPSCs when recorded at the reversal potential of GABA PSCs and (ii) once $GABA_A$ receptors were blocked intracellularly by dialysis with F^-–MgATP-free solution, the remaining component of interneuronal GDPs reversed near 0 mV and had a negative slope at membrane potentials more negative than −20 mV, suggesting an important contribution

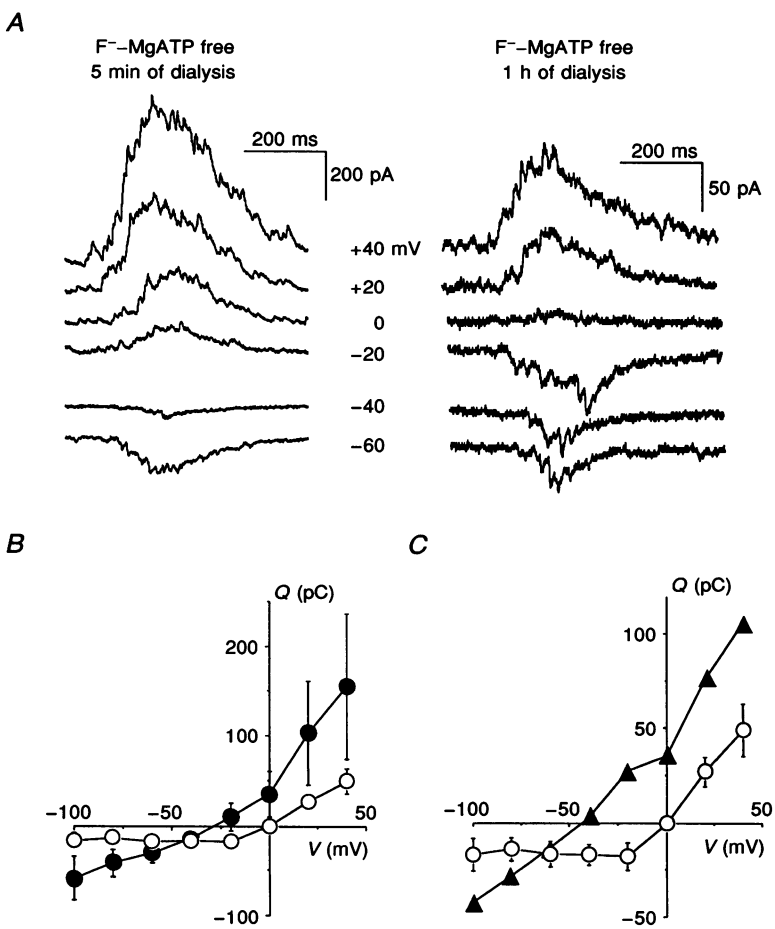

Figure 5. Intracellular blockade of $GABA_A$ reveals the glutamate component of GDPs in SR-CA3 interneurons

A, traces of GDPs recorded immediately after entry to whole-cell mode (left) and after 1 h (right) of dialysis with F^-–MgATP-free solution (4) at different membrane potentials. Such dialysis in control completely blocked $GABA_A$ currents with minor effects on glutamatergic responses (see text). Note reduction of GDPs after dialysis. *B*, charge–voltage relationships of GDPs at the beginning (5 min of dialysis, ●) and after 1 h of dialysis (○). *C*, charge–voltage relationships of dialysis-resistant, presumably glutamatergic (1 h of dialysis, ○), and dialysis-sensitive, presumably GABAergic (5 min to 1 h of dialysis, ▲), components of GDPs.

of NMDA receptors. These results suggest that synchronization of interneuron activity leads to a GDP that involves two types of synaptic connections: (i) excitatory GABAergic connections between interneurons and (ii) glutamatergic synaptic input to interneurons that presumably originates from CA3 pyramidal cells.

Results of pharmacological experiments suggested that $GABA_A$ and glutamate receptors are involved in the excitation of interneurons during GDPs. Depolarizing and excitatory effects of GABA have been reported in neurons from different regions of immature brain including spinal cord (Reichling, Kyrozis, Wang & MacDermott, 1994; Serafini, Valeyev, Barker & Poulter, 1995), hypothalamus (Chen, Trombley & van den Pol, 1996) cerebellum (Connor, Tseng & Hockberger, 1987), neocortex (Yuste & Katz, 1991) and hippocampal CA3 pyramidal cells (Ben-Ari *et al.* 1989). Using non-invasive cell-attached recordings, which should not change the intracellular chloride concentration, we observed that the $GABA_A$ receptor-mediated synaptic response can also trigger action potentials in neonatal SR-CA3 interneurons (Fig. 6*A c* and *d*). These results are in agreement with the observation that synaptically released GABA increases $[Ca^{2+}]_i$ in neonatal CA3 interneurons via activation of voltage-gated calcium channels (Leinekugel, Tseeb, Ben-Ari & Bregestovski, 1995). The depolarizing effect of GABA probably results from the reversed chloride gradient because of the delayed maturation of a chloride extrusion system (Misgeld, Deisz, Dodt & Lux, 1986; Hara, Inoue, Yasukura, Ohniski, Mikama & Inagaki, 1992; Serafini *et al.* 1995). Involvement of HCO_3^- permeability of $GABA_A$ receptors in the depolarizing effect of GABA, which has been observed in adult hippocampus (Staley, Soldo & Proctor, 1995), is unlikely in the neonates since GDPs are present and isoguvacine still increases $[Ca^{2+}]_i$ in HCO_3^--free Hepes buffer (X. Leinekugel, unpublished observations).

Within the glutamate ionotropic receptor family, both AMPA and NMDA receptors were found to contribute to GDP generation in interneurons. Interestingly, the depolarization that is necessary to activate NMDA receptors was provided in neonatal interneurons not only by AMPA receptors, as in the adult, but also by $GABA_A$ receptors. Indeed, the response mediated by $GABA_A$ and NMDA receptors in combination (in CNQX, 3·7 spikes) was larger than a simple sum of responses mediated by these receptors after pharmacological isolation (1·4 and 0 spikes in CNQX + APV and in CNQX + bicuculline, respectively). Therefore we suggest that a co-operative interaction occurs between $GABA_A$ and NMDA receptors during GDP generation. As a primary

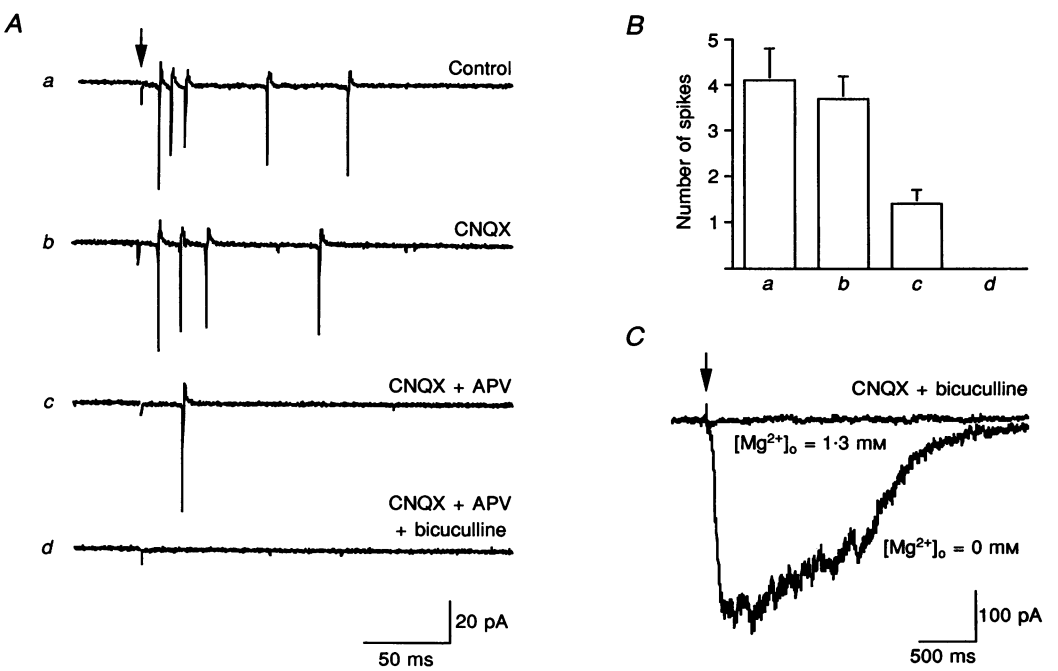

Figure 6. $GABA_A$ and glutamate receptors provide the excitatory drive to SR-CA3 interneurons during GDP

A, traces of responses evoked in SR-CA3 interneurons by electrical stimulation (arrow) in stratum radiatum: control (*a*); in the presence of CNQX (10 μM) (*b*); in the presence of CNQX (10 μM) and APV (50 μM) (*c*); and in the presence of CNQX (10 μM), APV (50 μM) and bicuculline (10 μM) (*d*). Cell-attached recordings with potassium gluconate solution (1) in 'track' mode. *B*, average of results of experiments as in *A* from 8 cells. *C*, whole-cell recordings of responses evoked by electrical stimulation (arrow) in the presence of CNQX (10 μM) and bicuculline (10 μM) in control and after perfusion with nominally Mg^{2+}-free external solution. Recording with CsCl pipette solution (3); holding potential, −60 mV.

mechanism for this co-operation we suggest that the depolarization generated by the activation of $GABA_A$ receptors as well as that due to voltage-gated sodium (Fig. 6*A c*) and calcium channels (Leinekugel *et al.* 1995) activated by the GABA-dependent depolarization is sufficient to activate NMDA channels by the attenuation of their voltage-dependent Mg^{2+} block. Indeed, we have recently observed that activation of the $GABA_A$ receptor attenuates the voltage-dependent Mg^{2+} block of NMDA channels recorded in neonatal SR-CA3 interneurons in cell-attached mode and that this effect is due to $GABA_A$ receptor-mediated depolarization (Ben-Ari *et al.* 1996). Activated NMDA receptors in turn will provide further depolarization and excitation of interneurons that would lead to an increased release of GABA.

Although synaptically activated AMPA receptors contribute to interneuronal GDPs (Fig. 4), they play a relatively minor role in excitation of interneurons in comparison with NMDA receptors (Fig. 6). The reasons for the preferential contribution of NMDA receptors to the excitation of the interneurons during GDPs in comparison with AMPA receptors remain unclear. We propose that several factors can be involved, including: (i) the time course of GDPs is in the range of hundreds of milliseconds – long enough to provide expression of NMDA receptor activity during a whole time course of NMDA EPSCs; (ii) slow decay kinetics of NMDA EPSCs in neonatal SR-CA3 interneurons (Khazipov *et al.* 1995); and (iii) postsynaptic $GABA_B$ receptor-mediated responses that efficiently inhibit the activity of NMDA receptors in adults are poorly expressed in neonates (Fukuda, Mody & Prince, 1993; Gaiarsa, Tseeb & Ben-Ari, 1995). Finally, one cannot exclude that pure NMDA receptor-based glutamatergic synapses are present in neonatal SR-CA3 interneurons, as has been reported for CA1(Durand, Kovalchuk & Konnerth, 1996) and CA3 (Ben-Ari *et al.* 1989) hippocampal pyramidal cells.

Synchronous neuronal activity reminiscent of hippocampal GDPs has been reported in various structures of developing brain, including spinal cord (Spitzer, 1994), neocortex (Yuste, Nelson, Rubin & Katz, 1995) and retina (Feller, Wellis, Stellwagen, Werblin & Schatz, 1996). Although the underlying mechanisms may differ, synchronous activity seems to represent a fundamental property of the developing neuronal networks. The physiological role of GDPs is presently unknown. In keeping with morphogenic and trophic actions of GABA and glutamate (Constantine-Paton, Cline & Debski, 1990; Ben-Ari *et al.* 1994) we propose that GDPs can represent a physiological pattern of activity that may regulate neuronal growth and establishment of synaptic connections in immature hippocampus. Recently we found that GDPs trigger transient increases in postsynaptic $[Ca^{2+}]_i$ in interneurons and pyramidal cells (Leinekugel, Medina, Khalilov, Ben-Ari & Khazipov, 1996). Since these $[Ca^{2+}]_i$ transients are synchronous with the activity of presynaptic afferents, they can provide Hebbian modulation of developing synapses (Cash, Zucker & Poo, 1996; Durand *et al.* 1996) and may be involved in the activity-dependent formation of the hippocampal network. In addition, GDPs might be regarded as neonatal precursors of physiological sharp wave bursts observed in the adult hippocampus (Ylinen *et al.* 1995*a*).

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