Dynamic Modulation of Excitation and Inhibition During Stimulation at Gamma and Beta Frequencies in the CA1 Hippocampal Region

ENRICO BRACCI, MARTIN VREUGDENHIL, STEPHEN P. HACK, AND JOHN G. R. JEFFERYS

Department of Neurophysiology, Division of Neuroscience, The Medical School, The University of Birmingham, Birmingham B15 2TT, United Kingdom

Received 18 December 2000; accepted in final form 13 March 2001

INTRODUCTION

The hippocampal slice provides several models for the study of fast oscillations. In particular, CA1 neurons can generate rhythmic activity at gamma (30–100 Hz) and beta (10–30 Hz) frequencies following chemical or electric stimulation (Bracci et al. 1999; Fisahn et al. 1998; Whittington et al. 1995, 1997a). Mathematical models of this activity (Traub et al. 1996a,b; Wang and Buzsáki 1996; Whittington et al. 1997a) are based on the generation of rhythmic, synchronous synaptic potentials, which are thought to play an essential role in timing collective neuronal firing. These models have not yet included realistic activity-dependent, short-term modifications of synaptic events (Arai and Lynch 1996; Davies and Collingridge 1993, 1996; Davies et al. 1990). However, these modifications are very likely to take place and might affect dramatically synaptic communication during fast oscillations. The picture is complex, involving at least four different receptor classes [N-methyl-D-aspartate (NMDA), AMPA, GABAA, and GABAB]. The use-dependent changes of responses mediated by each of these receptors have been investigated (Arai and Lynch 1996; Davies and Collingridge 1993, 1996; Davies et al. 1990; Debanne et al. 1996), commonly in pharmacological isolation and with low-intensity stimuli delivered in pairs or at fixed frequencies. However, the way in which glutamatergic and GABAergic responses interact during synchronous oscillatory activity is difficult to predict from these data. Summation of postsynaptic potentials can be highly nonlinear due to factors such as shunting effects on synapses at particular electrotonic locations (Andersen et al. 1980b; Harris et al. 1992) and activation of presynaptic receptors (Cobb et al. 1999; Cohen et al. 1992; Colmers et al. 1988; Davies and Collingridge 1993; Davies et al. 1990). Furthermore, even for a single receptor class, strong nonlinear summation of responses may arise from receptor saturation (Liu et al. 1999), concentration-dependent rate of transmitter clearance from synaptic cleft (Clements 1996; Otis et al. 1996; Roepstorff and Lambert 1994; Scanziani et al. 1997), activation of extrasynaptic receptors (Pham et al. 1998; Rusakov and Kullmann 1998), and changes in transmembrane ionic concentration gradients (Schwartzkroin et al. 1998; Staley et al. 1995).

A practicable experimental approach consists in studying a particular pattern of synaptic activation that is specifically relevant to a certain phenomenon, as done by Davies and Collingridge (1996) to investigate the interaction between GABA and glutamate receptors during priming protocols that enhance LTP expression in the CA1 region. During fast oscillations in vitro, a large number of CA1 pyramidal neurons and interneurons fire simultaneously on each cycle (Traub et al. 1996b; Whittington et al. 1997a).
Therefore CA1 neurons receive rhythmic, simultaneous inhibitory and excitatory inputs from many other presynaptic CA1 neurons. To cast light on the dynamic changes accompanying this repetitive synaptic activation, we used a protocol comprising a train of stimuli at 40 Hz followed by one at 10 Hz to mimic the frequency pattern observed (Bracci et al. 1999; Traub et al. 1999; Whittington et al. 1997b). Stimulation was applied in the CA1 region to activate local pyramidal neurons and interneurons. The responses evoked by this experimental protocol were not dependent on cholinergic or metabotropic glutamate receptors (as shown by application of specific antagonists), thus allowing a dissection of the effects of GABAergic and ionotropic glutamatergic events from those due to slower changes of membrane properties. Activation at gamma and beta frequency was found to produce a profound short-term alteration of the balance between excitation and inhibition within the local network.

METHODS

Transverse hippocampal slices (400 µm), were prepared from young male Sprague-Dawley rats (90–120 g; anesthetized with ketamine and medetomidine). Slices were maintained (and experiments were performed) at 32–36°C in gassed (5% CO2 -95% O2) artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 26 NaHCO3, 2 CaCl2, 3 KCl, 1.25 NaH2PO4, 1 MgCl2, and 10 glucose. Whole cell recordings were obtained from visualized pyramidal neurons; slices were submerged in a chamber mounted on an Olympus XY212 upright microscope with a ×40 water-immersion objective and differential interference contrast optics. Patch pipettes contained (in mM) 145 potassium-glucuronate, 10 KCl, 10 HEPES, 2 NaCl, 2 Mg-ATP, and 0.1 EGTA, pH = 7.25 (adjusted with KOH). GTP was omitted to minimize the effects of G-protein-dependent processes. A stimulating electrode (consisting of a glass pipette filled with ACSF) was placed in the stratum pyramidale of the CA1 region. Tight-seal whole cell recordings were obtained from visualized neurons located within 50 µm from the stimulation site. The stimulation protocol consisted of two consecutive trains, one at 40 Hz (for 1 s) followed by another at 10 Hz (for 2–3 s). Pulse duration was 0.1 ms. A single stimulation was usually delivered 1 s before and 1 s after this protocol to monitor the changes in the evoked response.

Modulation of the evoked postsynaptic responses, and of the associated slow depolarization (see results), were observed when the stimulation intensity was sufficiently strong. The stimulation intensity was routinely set at 1.5 times the minimum required to elicit a slow depolarizing response during the 40/10-Hz stimulation (defined as threshold; 10–30 V, 0.1-ms duration).

The protocol was repeated every 5 min to allow a complete recovery to control conditions. Whole cell patch-clamp recordings were made using an Axoclamp 2B amplifier under current-clamp conditions in bridge mode. Data were stored and analyzed using SIGNAL software (CED, Cambridge, UK). Statistical significance was assessed by one-way ANOVA (SigmaPlot, SPSS). Data are expressed as means ± SD.

Staley and Proctor (1999) have shown that depolarizing GABA responses mainly arise from dendrites, where surface to volume ratio is larger than in the soma. These authors used a standard electric circuit model of the dendrite to simulate chloride accumulation taking place after focal GABA application or tetanic stimulation. We used a similar model to simulate the voltage-dependent behavior observed in the present study. The model included the following features: membrane potential was determined by the equilibrium condition of the currents flowing through a set of conductive pathways (each characterized by its reversal potential determined by the correspondent ionic gradient) and the externally injected current; the GABA A receptor mediated conductance consisted of two different electrochemical pathways, representing the bicarbonate permeability and the chloride permeability of these receptors, respectively; conductance ratio of these pathways was fixed and equal to 0.25; intracellular chloride concentration (and thus chloride reversal potential calculated through the Goldman equation) was dynamically determined by chloride flow-through GABA conductances, by chloride extrusion through a potassium-chloride cotransporter and by a constant chloride leak into the cell; transport rate (v) of the cotransporter was determined by the Lineweaver-Burke equation as a function of intracellular chloride concentration [Ci],

\[
\frac{1}{v} = \frac{K_D}{[Cl]} + \frac{1}{v_{max}}
\]

where \(K_D\) (15 mM) is intracellular chloride concentration at which extrusion is half of the maximum rate, \(v_{max}\) (5 mM s \(^{-1}\)); and dendrite volume was 75 µm\(^3\), initial dendritic chloride concentration was 2 mM, and bicarbonate reversal potential \(E_{HCO_3}^{\text{bic}}\) was assumed to be constant as a result of fast enzymatic homeostasis, with its value (~14.3 mV) determined by intracellular and extracellular concentrations (15 and 26 mM, respectively). The effects of individual stimuli were not considered; rather, a continuous function of time representing the time course of \(GABA_A\) conductance responsible for the slow waveform was found empirically to provide a good match of the experimental data obtained at resting membrane potential (Fig. 9E). This function was expressed as a sum of three functions of time \(t\)

\[
\sum_{i=1}^{n} \frac{a_i}{1 + e^{-(t - t_i)}} + d_i
\]

and attained a maximum value of 40 nS (Staley and Proctor 1999).

RESULTS

Single shock stimulation delivered to the pyramidal layer gave usually rise to a typical EPSP-IPSP sequence in pyramidal neurons (current-clamped between ~60 and ~55 mV, close to their physiological resting potential; Fig. 1A). The evoked IPSP was mainly mediated by GABA A receptors with little or no contribution from GABA B receptors, as indicated by its monoexponential decay time course and its insensitivity to application of the GABA A antagonist CGP 55845A (1 µM; see Fig. 6). This was due, at least in part, to GTP-free intracellular solution, used here to minimize metabotropic receptor influence on the postsynaptic cell (Pham et al. 1998; Saugstad et al. 1998). In a small number of cells \(n = 4\), it was possible to elicit a purely hyperpolarizing response with a single stimulus delivered to the stratum pyramidale (Fig. 1B). During 40-Hz stimulation, IPSPs fused together and could not be detected individually as also was apparent in the presence of ionotropic glutamate receptor blockers (see Fig. 7). The signals observed during the 40/10-Hz protocols are therefore better described in terms of the evoked EPSPs, which remained individually discernible, and of a slower polarizing waveform on which these EPSPs were superimposed. Provided the stimulation intensity was strong enough, this slow waveform comprised: an initial hyperpolarization resulting from fused IPSPs during the early phase of 40-Hz stimulation and a subsequent depolarization (developing either during the late phase of 40-Hz stimulation, as in the example of Fig. 1A, or during the early phase of 10-Hz stimulation, as in the example of Fig. 1B). The minimum stimulation intensity able to elicit a slow depolarization during the 40/10-Hz protocol was defined as thresh-
old \((T, \text{ see METHODS})\). In 55 of 87 neurons tested, a late hyperpolarization was also present, starting either during the late phase of 10-Hz stimulation or just after it and persisting for 3–10 s after the end of the stimulation protocol (as in the examples of Fig. 1, A and B). On average, the amplitude of these responses (for cells kept between −55 and −60 mV) was 9 ± 5 mV for the early hyperpolarization, 12 ± 6 mV for the slow depolarization, and 5 ± 4 mV for the late hyperpolarization (calculated only for the group of cells in which such hyperpolarization was observed). When pyramidal neurons were hyperpolarized by current injection to levels more negative than the GABA_A IPSP reversal potential \((-77 ± 5 \text{ mV}, n = 12)\), the early hyperpolarization was converted into a depolarization while the late hyperpolarization (if present) was depressed or abolished.

The EPSPs elicited by the second to fourth pulses of 40-Hz stimulation (during the early hyperpolarizing phase of the envelope) were larger in amplitude than those elicited by a single pulse (by 29 ± 8%, \(P < 0.001\)). This observation cannot be explained solely by the increased driving force for EPSP during the early hyperpolarization because in some cases, the EPSP elicited by the second to fourth pulses at 40 Hz attained a more positive peak than the one elicited by a single pulse (not shown); EPSP half-width during the first eight stimuli at 40 Hz was not significantly different from single-stimulus response, showing that each response was still curtailed by an IPSP. These data are consistent with the observations of Davies and Collingridge (1996), who reported paired-pulse potentiation of AMPA receptor-mediated EPSPs with 20-ms interval (50 Hz) and paired-pulse depression of <40% for GABA_A IPSPs with 25-ms interval (40 Hz)

In the cells in which a pure IPSP was evoked by a single stimulus, the 40/10-Hz protocol still elicited a slow waveform similar to the one observed in the other cases, and EPSPs appeared during the 40-Hz train, starting from the second stimulus (Fig. 1Bii).

In both groups of cells, the amplitude of the evoked EPSPs progressively decreased from the peak amplitude reached by the third to fifth stimulus at 40 Hz. The amplitude of the EPSPs evoked by the 30th-40th stimuli at 40 Hz was on average 21 ± 19% of the maximal one, elicited by the 2nd-4th stimuli \((P < 0.001)\). This decrease could not be attributed to concomitant changes in membrane potential as is clear from comparison of responses elicited at similar potentials but at different times during the early hyperpolarization (Fig. 1, Aii and Bii). On the other hand, the half-width of EPSPs evoked by 30th-40th stimuli at 40 Hz was slightly \((12 ± 7\%)\), but significantly \((P < 0.05)\), larger than for a single-stimulus response.

During 10-Hz stimulation, the amplitude and the half-width of the EPSPs gradually increased to levels well above those observed with a single pulse or during 40-Hz stimulation. Hyperpolarizing IPSPs were not detectable during the 10-Hz train. The EPSP potentiation (and the absence of IPSPs) persisted for several seconds after the 40/10-Hz protocol; a single stimulus delivered 1 s after the protocol elicited a monophasic depolarizing response, whose amplitude, duration and time-to-peak were significantly \((P < 0.001)\) larger than those of the largest EPSPs observed during 40-Hz stimulation (Fig. 2, A and B). Furthermore, a multi-peak shape was often observed in the responses elicited during the 10-Hz protocol or 1 s after it (Fig. 2A). These data suggest that the gradual increase of EPSP during 10-Hz stimulation was due to progressive recruitment of

![FIG. 1. Effects of 40/10-Hz stimulation in CA1 pyramidal neurons. A: a single stimulus delivered to the pyramidal layer elicited a typical excitatory-inhibitory postsynaptic potential (EPSP-IPSP) sequence (i, see also enlarged trace on bottom panel). During 40-Hz stimulation (ii), each stimulus elicited an individually identifiable EPSP, while IPSPs were fused together, giving rise to a hyperpolarizing envelope which later turned into a slow depolarization accompanied by decreased EPSP amplitude (iii). Ten-hertz stimulation was characterized by a gradual repolarization and a gradual increase in EPSP amplitude and duration, which persisted 1 s after the end of the train (iv), when an afterhyperpolarization was also present. B: in this cell, a single stimulus delivered to the pyramidal layer elicited a purely hyperpolarizing response (i). Forty-hertz stimulation evoked a hyperpolarizing envelope with clearly visible evoked EPSP of decreasing amplitude (ii). A slow depolarization developed at the end of the 40-Hz train, and, as in A, the cell repolarized during 10-Hz stimulation to levels more negative than rest and EPSPs increased (iii), a feature preserved 1 s after the end of the train (iv). In this and the following figures, the membrane potential at which neurons were current-clamped before the stimuli are indicated near the traces.](http://jn.physiology.org/issue/v493/i11/fig1.jpg)
were not present with the same stimulation before the 40/10-Hz protocol (Fig. 3). Thus modulation of the evoked postsynaptic potentials is physiologically relevant being able to convert subthreshold signals into superthreshold ones.

When monitored with 1-Hz stimulation, evoked PSPs recovered to their prestimulation shape (comprising an EPSP-IPSP sequence) within 30–45 s from the end of the 40/10-Hz protocol. More persistent changes in the EPSP amplitude were not observed, suggesting that the present protocol did not cause long term potentiation of the evoked responses.

These data show that at the end of the 40/10-Hz protocol the local network was strongly biased toward excitation (apparent in the vast increase of polysynaptic EPSPs and in the absence of hyperpolarizing IPSPs). One of the factors affecting postsynaptic responses is the membrane input resistance, therefore we tested the changes induced by 1 s of 40-Hz stimulation. As shown in Fig. 4, membrane resistance collapsed to ~20% at the end of 40-Hz stimulation, i.e., when a large reduction in EPSP amplitude was observed (on average, by 77 ± 11%). Membrane resistance gradually recovered to control value either in the absence of stimuli (Fig. 4) or during 10-Hz stimulation (not shown). Recovery to 90% of control value was complete within 1.6 ± 0.6 s from the end of 40-Hz stimulation (n = 4) in the absence of stimuli. This drop in input resistance can account for the decrease in EPSP amplitude observed during the late part of the 40-Hz train and the first part of the 10-Hz train.

The temporal link between the changes in EPSPs and the different phases of the slow polarizing waveform led us to suspect that the cellular mechanisms responsible for the generation of such a slow signal could also be involved in EPSP modulation. The slow waveform and the evoked potentials were not affected by the metabotropic glutamate receptor (mGluR) antagonist MCPG (1 mM; n = 5) or by the muscarinic acetylcholine receptor antagonist atropine (1 μM; n = 4; data not shown). Slow hyperpolarizing-depolarizing waveforms are typical of responses to the sustained presence of GABA, which can be elicited in CA1 pyramidal neurons by several protocols, including high-frequency stimulation

previously silent polysynaptic pathways. The large (>300%) increase in EPSP half-width at the end of 10-Hz stimulation could not be merely attributed to the absence of a curtailing IPSP because this phenomenon is expected to produce an increase in half-width <90% even for >70% depression of the IPSP (Davies and Collingridge 1996). No significant differences were found in EPSP modulation between cells in which the late hyperpolarization was present and those in which this event was absent.

In 34% of cells tested, facilitated EPSPs elicited after the 40/10 Hz were large enough to generate action potentials that

![FIG. 2. Changes in evoked EPSPs during the 40/10-Hz protocol. A: comparison of time course of EPSPs evoked at the beginning of 40-Hz train (gray) and 1 s after the end of the 10-Hz train (black). Responses are scaled in amplitude (see calibration bars). Note the multiphase appearance of the response evoked after the protocol. B: quantification of amplitude, time to peak, and half-width observed at the beginning of the 40-Hz train (the EPSP with largest amplitude was selected for analysis in each cell) and 1 s after the end of the 10-Hz train. Asterisks denote statistical significance (P < 0.001; n = 22).]

![FIG. 3. Changes in evoked PSPs induced by the 40/10-Hz protocol. A: synaptic responses evoked in a pyramidal neuron at 1 Hz before the 40/10-Hz protocol consisted of a typical EPSP-IPSP sequence and did not elicit action potentials. B: 1 s after the 40/10-Hz protocol, synaptic responses to the same stimulation consisted were purely depolarizing, and evoked an action potential. Calibration bars apply to both A and B.]

![FIG. 4. Decrease in input resistance induced by 40-Hz stimulation. A: input resistance was monitored with current pulses (~30 pA for 200 ms) before (left) and after (right) the end of a 40-Hz train in a pyramidal neuron (prestimulation membrane potential = −85 mV). Average percentage changes in input resistance (with respect to the value observed before the trains) are plotted vs. time from the end of the 40-Hz train in B (plot is time-aligned with the trace in A).]
After 10-Hz stimulation, a rapid repolarization to baseline, followed by a hyperpolarization (Fig. 5C), was observed. These changes in the slow waveform observed during the 40/10-Hz protocol in control ACSF and in the presence of bicuculline. A: single stimulus (arrows) was delivered 1 s before and 1 s after the 40/10-Hz protocol (prestimulation membrane potential = −75 mV). Despite the hyperpolarization of the cell after the trains, the evoked EPSP was slightly depressed after the 40/10-Hz protocols, in marked contrast with persistent potentiation in controls (Figs. 1 and 2).

The induction of the slow depolarizing waveform and the associated modulation of EPSPs was strongly facilitated so that the minimum stimulation intensity required to elicit a slow depolarization during the 40/10-Hz protocol was considerably reduced (by 57 ± 21%; P < 0.001). Thus low stimulation intensities, which elicited no depolarizing responses and gave rise to little and inconsistent dynamic modulation of EPSPs in control solution, were sufficient to elicit a slow depolarization in the presence of CGP 55845A as illustrated in the example of Fig. 6. If the slow waveform ended in a late hyperpolarization in control solution, this was not abolished by CGP 55845A (Fig. 6). The use of whole cell recordings without intracellular

In other words, the duration of EPSPs evoked in control solution after the stimulation protocol was similar to the one observed with a single pulse in the presence of bicuculline or picrotoxin, and in the presence of these agents, no further broadening of evoked EPSPs could be induced by the stimulation trains.

These data suggest that GABA_A receptor-mediated effects were mainly responsible for the slow envelope and for the dynamic changes in the evoked EPSPs. Since GABA release is controlled by presynaptic GABA_B receptor (Davies and Collingridge 1993), we tested the effects of a selective antagonist of this receptor. In the presence of CGP 55845A (1 μM, n = 4), the induction of the slow depolarizing waveform and the associated modulation of EPSPs was strongly facilitated so that the minimum stimulation intensity required to elicit a slow depolarization during the 40/10-Hz protocol was considerably reduced (by 57 ± 21%; P < 0.001). Thus low stimulation intensities, which elicited no depolarizing responses and gave rise to little and inconsistent dynamic modulation of EPSPs in control solution, were sufficient to elicit a slow depolarization in the presence of CGP 55845A as illustrated in the example of Fig. 6. If the slow waveform ended in a late hyperpolarization in control solution, this was not abolished by CGP 55845A (Fig. 6).

In control solution, application of the 40/10-Hz protocol at 0.5 times T stimulation intensity elicited no slow depolarization and little EPSP modulation (responses evoked 1 s before and after the protocol are indicated by ↓). B: in the presence of CGP 55845A, the same stimulation intensity produced a slow depolarization and a stronger EPSP modulation; under these conditions, a single stimulus delivered 1 s after the end of the 10-Hz train elicited a purely depolarizing response that was large enough to evoke an action potential. C: in the presence of CGP 55845A, stimulation at 1.5 times T (routinely used in control solution, see METHODS) elicited a much larger slow depolarization characterized by irregular firing activity. A single stimulus delivered after 1 s from the end of the 10-Hz train elicited a large EPSP accompanied by a burst of action potentials.
GTP virtually abolished the effect of postsynaptic GABA<sub>B</sub> receptor activation (as mentioned in the preceding text). We conclude that other cellular mechanisms must be responsible for the observed afterhyperpolarization but did not investigate this further because the afterhyperpolarization did not appear to be linked to EPSP modulation. When stimulation intensity was adequately reduced in the presence of CGP 55845A, EPSP modulation during the 40/10-Hz protocol was in all respects similar to those observed in control solution with stronger stimulation intensities (see METHODS for a description of the intensities used). On the other hand, when the stimulation intensity was similar to that previously used in control solution, the 40/10-Hz protocol elicited a paroxysmal slow depolarization associated with intense firing activity, which often prevented the identification of individual evoked EPSPs (Fig. 6C). Under these conditions, a single shock applied 1 s after the end of the 40/10-Hz protocol elicited a large EPSP accompanied by a burst of two to four action potentials (Fig. 6C). These results are consistent with recent reports on the role of presynaptic GABA<sub>B</sub> receptor in modulating depolarizing GABA effects (Manuel and Davies 1998). In the presence of GABA<sub>B</sub> antagonist, the negative-feedback of GABA release is impaired and much more GABA is released for the same stimulation (Cobb et al. 1999). As a consequence, depolarizing GABA effects, which require large levels of transmitter release to be expressed (Staley et al. 1995), are strongly facilitated.

To gain further insight into the cellular mechanisms mediated by GABA<sub>A</sub> receptors during 40/10-Hz activation, we performed experiments in which the other transmitters involved in this phenomenon were pharmacologically blocked. This was accomplished by simultaneous bath-application of the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzof[1]quinoxaline (NBQX) (50 µM), the NMDA receptor antagonist d-AP5 (50 µM), and the GABA<sub>B</sub> antagonist CGP 55845A (1 µM). In the presence of these agents, polysynaptic IPSPs are blocked and fewer GABAergic terminals are activated by a given stimulation intensity; on the other hand, as described in the preceding text, GABA release from terminals activated directly by the stimulus is facilitated by block of GABA<sub>B</sub> presynaptic receptors (Bracci et al. 1999; Davies and Collingridge 1993). Under these conditions, it was possible to adjust the stimulation intensity to evoke a slow hyperpolarizing—depolarizing waveform of similar amplitude to that in control solution. This was accomplished by changing the stimulation intensity by a factor of 0.7–2.5 with respect to control solution. In the presence of these agents, the slow depolarization was accompanied by a decrease in input resistance similar in extent and time course to that observed in control solution (not shown), showing that such a decrease was largely due to activation of GABA<sub>A</sub> conductances.

The cellular mechanisms of depolarizing GABA effects are controversial. Intradendritic chloride accumulation (Staley and Proctor 1999; Staley et al. 1995) and extracellular potassium accumulation (Kaila et al. 1997) appear to play different roles under different experimental conditions. We performed experiments to identify the main mechanism operating under the present conditions. Extracellular potassium accumulation is produced by the local CA1 network following tetanic stimulation at 100 Hz (Kaila et al. 1997). The time course of this phenomenon should not be strongly affected by manipulations of the membrane potential of the recorded neuron. On the other hand, transmembrane chloride movements are expected to depend on the membrane potential of the individual neuron. Whole cell recording technique allowed us to manipulate the membrane potential of the current-clamped cell reliably over a large range of values while the 40/10-Hz protocol was applied. Typical results of these experiments are shown in Fig. 7. When the cell was current-clamped at −50 mV, a single shock elicited a hyperpolarizing IPSP; a hyperpolarizing—depolarizing sequence was observed during the 40/10-Hz protocol, as in control solution. Phasic responses were markedly attenuated or absent during the 10-Hz train; a single stimulus applied 1 s after the end of 10-Hz train (at a membrane potential similar to the preprotocol one) elicited little or no response. When the neuron was held at −95 mV, a single stimulus evoked a depolarizing IPSP. During the 40–Hz train, the cell underwent a brisk depolarization resulting from summed depolarizing IPSPs, followed by a slower one, which gradually recovered to baseline during the 10-Hz train. Phasic depolarizing IPSPs were absent during the first 3—10 stimuli at 10 Hz but were clearly detectable with the subsequent stimuli. A single stimulus applied 1 s after the end of the 10-Hz train elicited a depolarizing IPSP characterized by smaller amplitude (on average by 29 ± 19%, n = 4, P < 0.001) than the one elicited before the 40/10-Hz protocol. An important feature of this phenomenon was that in all the cells tested (n = 5), the slow depolarization reached its peak value at different times under
the two conditions. The peak was attained significantly ($P < 0.001$) later (670 ± 210 ms after the end of 40-Hz train) when the cell was depolarized (between −40 and −50 mV) than when it was hyperpolarized (between −90 and −100 mV; peak 47 ± 67 ms after the end of 40-Hz train). When the cell was kept close to IPSP reversal potential, 40-Hz stimulation evoked a slow monophasic depolarization that persisted during the early phase of 10-Hz stimulation and then progressively declined to baseline (Fig. 7B); during the late phase of 10-Hz stimulation, phasic depolarizing IPSPs were apparent. Similarly, a single stimulus delivered after 1 s after the 10-Hz train elicited a clearly discernible depolarizing response (Fig. 7B). These findings suggested that major changes in GABA$_A$ receptor driving force occurred during the 40/10-Hz protocol and that the modifications were sensitive to the state of polarization of the neuron membrane. The observation that the kinetics of the slow waveform depended on the membrane potential of the recorded cell suggested that a network-mediated extracellular accumulation of potassium was not the major factor responsible for these events.

To acquire more direct information on potassium accumulation, we performed whole cell recordings from glial cells ($n = 3$) located in the pyramidal layer during the 40/10-Hz protocol. A typical example of these experiments is shown in Fig. 8. When stimulation intensity was at threshold for the observation of the slow potential envelope (and the associated modulation of EPSPs) in local pyramidal neurons, the glial cell (kept without injected current at −68 mV) displayed a 4-mV depolarization during the 40-Hz train. A further small depolarization (<1 mV) was observed during the 10-Hz train, followed by a slow repolarization to baseline, which took several seconds. When the stimulation intensity was increased to 1.5 times $T$, a larger depolarization was elicited by the 40-Hz train, and the cell continued to depolarize (by >2 mV) during the 10-Hz train. The time course of this behavior was clearly different from that observed in an adjacent pyramidal neuron (and typical of this kind of cell). In this cell, the depolarization observed during the 40-Hz train was followed (both with 1 or 1.5 times $T$ stimulation) by an immediate repolarization during the 10-Hz train. Furthermore, despite the expected greater sensitivity of glial cells to increases in extracellular potassium (Lothman and Somjen 1975), the depolarization observed in the neuron during the 40-Hz train was more than double that in the glial cell. These results suggested that the 40/10-Hz protocol did cause a local increase in extracellular potassium, but this phenomenon could not be considered as the main cause of the slow depolarization observed in pyramidal neurons.

To test whether the experimental observations could be explained in terms of dendritic intracellular chloride accumulation, we performed numerical simulations using a standard electrical model similar to the one used by Staley and Proctor (1999) (Fig. 9A; see also METHODS). The results of the simulations are shown in Fig. 9, B–F. Reversal potential for chloride ($E_{Cl}$) and for GABA$_A$ receptor ($E_{GABA}$, depending on chloride and bicarbonate transmembrane gradients through the Goldman-Hodgkin-Katz equation) and membrane potential ($E_m$) are plotted versus time (Fig. 9, B–D). The time course of GABA$_A$ conductance ($g_{GABA}$) (same for all simulations) is shown in Fig. 9F. Constant amounts of positive or negative current were injected in the model dendrite to reproduce the experimental conditions of Fig. 7.

When current injection was such that at rest $E_m = E_{GABA}$ (Fig. 9B), intracellular chloride accumulation caused a depolarizing shift of $E_{GABA}$. In agreement with experimental observations (see Fig. 7B), this shift was such that $E_{GABA}$ remained more positive than $E_m$ for several s after closure of $g_{GABA}$. When the dendrite was kept at −50 mV by positive current injection (Fig. 9C), a hyperpolarizing-depolarizing sequence was present with the slow depolarization peaking at 1,670 ms. When the cell was hyperpolarized to −95 mV (Fig. 9D), a slow, monophasic depolarization was present. This depolarization peaked much earlier (at 1,185 ms) than the one observed with positive current injection. The cause of this difference in time course can be understood as follows. When the cell was depolarized by current injection, the initial driving force for chloride was >60 mV and caused a massive chloride influx during $g_{GABA}$ activation. This resulted in a large increase in intracellular chloride (plotted in Fig. 9E) and a consequent depolarizing shift of $E_{Cl}$ and $E_{GABA}$. As expected from thermodynamical considerations, $E_{Cl}$ remained more negative than $E_m$ during the period in which $g_{GABA}$ increased (Perkins and Wong 1997). However, $E_{GABA}$ became more positive than $E_m$ after ~750 ms. At the time when $g_{GABA}$ peaked, $E_m - E_{Cl} = 2.7$ mV and a substantial chloride influx persisted for 1 s after this peak. Thus intracellular chloride continued to increase during this period (Fig. 9E) and $E_{Cl}$ and $E_{GABA}$ continued to depolarize (Fig. 9C). As a result, despite $g_{GABA}$ decreasing, the product $g_{GABA} \times E_{GABA}$ (which is the variable term in the
expression for $E_m$) continued to rise for several hundreds of ms after $g_{\text{GABA}}$ peak. Thus the cell continued to depolarize despite the ongoing decrease of $g_{\text{GABA}}$. On the other hand, at hyperpolarized potentials internal chloride accumulation was much more limited (Fig. 9E) because the initial driving force for chloride was much smaller (−15 mV). At the time when $g_{\text{GABA}}$ peaked, the driving force for chloride was <1 mV. Thus the rate of chloride accumulation was too small for the increase in $E_{\text{GABA}}$ to counterbalance the decrease in $g_{\text{GABA}}$. As a consequence, $E_m$ soon started to decrease, giving rise to an earlier repolarization (and an earlier recovery of the intracellular chloride transient). It should be noted that the results of these simulations depend on the characteristics of the potassium-chloride cotransporter (see METHODS). In particular, if the simulations depend on the characteristics of the potassium-chloride cotransporter (see METHODS), Extracellular chloride concentration (140 mM) and the transmembrane gradients of the other ions were assumed to be constant throughout the simulations. While $E_m$ was variable, $E_{\text{HCO}_3}$ was assumed to be constant (−14.3 mV), resulting from an intracellular concentration of 15 mM and an extracellular concentration of 26 mM. Simulation results are shown in B–D, where $E_m$, $E_{\text{Cl}}$, and $g_{\text{GABA}}$ are plotted vs. time and in E, where the intracellular chloride concentrations for the simulations of C ([$\text{Cl}]_{\text{depol.}}$) and D ([$\text{Cl}]_{\text{hyperpol.}}$) are plotted. In each simulation a different, fixed amount of current was injected into the cell. The time course of the total $G_{\text{ABA}}$ conductance $g_{\text{GABA}}$ (the same for all simulations) is shown in F (see METHODS). Activation of $G_{\text{ABA}}$ started at time 0. B: the current injection was such as to make $E_m$ equal to $E_{\text{GABA}}$ at the beginning of $g_{\text{GABA}}$ activation. Note the gradual development of a persistent negative driving force for $G_{\text{ABA}}$, conductance. C: the cell was depolarized to −50 mV before GABA conductance was activated. D: the cell was hyperpolarized to −95 mV. The vertical dashed line marked $i$ indicates the time when $E_m$ attains its most depolarized value in the simulation of D, and that marked $ii$ the time when $E_m$ attains its most depolarized value in the simulation of the C. Note that the maximum depol- arization is attained earlier in the case in which the cell is hyperpolarized.

These data show that intracellular chloride accumulation can account for the differences in the slow depolarization kinetics observed at different membrane potentials. The simulations also account for the time course of the driving force for $G_{\text{ABA}}$ receptor mediated potentials (i.e., $E_m - E_{\text{GABA}}$) observed experimentally with different levels of membrane polarization: when the cell is depolarized (Fig. 9C), this driving force (which is >30 mV at rest) reverses during the rising phase of the slow depolarization, and persists at levels <5 mV (in absolute value) for several s after the peak of the slow depolarization. Such a small value can account for the persistent absence of detectable postsynaptic potentials observed experimentally during 10-Hz stimuli and with a single stimulus delivered 1 s after the end of the 40/10-Hz protocol during block of fast glutamateric transmission (Fig. 7A). On the other hand, when the cell is hyperpolarized the driving force $E_m - E_{\text{GABA}}$ (which remains negative all the time) becomes >10 mV (in absolute value) soon after the depolarization peak. This observation thus explains the earlier reappearance of depolarizing IPSPs observed experimentally when negative current is injected into the cell (Fig. 7A).

DISCUSSION

In this study, we used stimulation of the pyramidal layer at gamma and beta frequency to mimic the pattern of activation of excitatory and inhibitory synapses observed in CA1 during fast oscillations in vitro (Bracci et al. 1999; Whittington et al. 1997b). Individual IPSPs were not discernible during this protocol; they merged to create a biphasic hyperpolarizing-depolarizing waveform on which evoked EPSPs were superimposed. The EPSPs underwent a strong dynamic modulation during repetitive stimulation, resulting in a large polysynaptic facilitation at the end of the protocol. Changes in synaptic
inhibition, rather than in the efficacy of excitatory synapses, were responsible for this phenomenon. Intracellular chloride accumulation and the resulting depolarizing shift in GABA<sub>\text{A} \text{ergic}
</sub> receptor reversal potential turned out to be the main cellular determinant of decreased efficacy of inhibitory processes during gamma and beta frequency activation.

This study used electrical stimulation to recruit both monosynaptic and polysynaptic postsynaptic potentials. This was done because summation of IPSPs and EPSPs involves a number of nonlinear processes that complicate prediction of the effects of simultaneous activation of a large number of synapses. During fast oscillations induced by tetanic stimulation in the CA1 region, a large number of pyramidal neurons and interneurons fired simultaneously on each cycle (Traub et al. 1996b; Whittington et al. 1997a). Therefore a large number of inhibitory and excitatory synapses are repetitively activated on a given pyramidal neuron. Understanding the dynamic changes induced by these events is essential to understand the operation of the rhythmonic network.

During the first two to four stimuli at 40 Hz, evoked EPSPs increased in amplitude. They then strongly decreased, concomitantly with a slow depolarization and with a large decrease in input resistance. While the early increase in EPSP amplitude can be attributed to a genuine potentiation of excitatory synapses (Davies and Collingridge 1996), the late decrease in amplitude is attributable to decreased input resistance. This decrease in amplitude was accompanied by a moderate increase in EPSP half-width, which was likely due to the absence of a curtailing IPSP because a similar increase has been observed for monosynaptic EPSPs as a result of paired-pulse depression of GABA<sub>\text{A} \text{ergic}
</sub> potentials (Davies and Collingridge 1996). When stimulation frequency was switched to 10 Hz, a progressive, large increase in EPSPs amplitude and duration was observed. This is attributable to recovery of pyramidal neuron input resistance and persistent decreased efficacy of synaptic inhibition, also manifest from the absence of hyperpolarizing IPSPs. Under these conditions, polysynaptic EPSPs were strongly facilitated as shown by their irregular, multi-peak shape and by the more than threefold increase in their half-width. In support of this view, GABA<sub>\text{A} \text{ergic}
</sub> antagonists abolished the EPSP increase-decrease-increase sequence. These agents also unmasked a moderate use-dependent depression of EPSPs at the end of the 40/10-Hz protocol, showing that excitatory synapses did not undergo a direct potentiation under these conditions. The observation that GABA<sub>\text{A} \text{ergic}
</sub> antagonists also converted the slow biphasic waveform into a paroxysmal burst during 40-Hz stimulation, followed by a rapid repolarization during 10-Hz stimulation, led to the conclusions that the slow waveform seen under control conditions was due to depolarizing GABA effects and that it was causally related to the associated modulation of the EPSPs. The present experimental protocol did not cause long-term changes in synaptic efficacy either in control solution or in the other pharmacological conditions, presumably due to the features of the trains applied and the position of the stimulating electrode.

Although depolarizing GABA effects have been observed under several experimental conditions in the hippocampus (Andersen et al. 1980a; Avoli and Perreault 1987; Cherubini et al. 1991; Grover et al. 1993; Kaila et al. 1997; Lambert et al. 1991; Lamsa and Kaila 1997; Manuel and Davies 1998; Smirnov et al. 1999; Staley and Proctor 1999; Staley et al. 1995), the cellular mechanisms underlying this phenomenon remain controversial. Staley and Proctor (1999) pointed out intracellular chloride accumulation as the main factor for depolarizations induced by exogenous GABA application or tetanic stimulation (200 Hz for 50 ms), while Kaila et al. (1997) identified extracellular potassium accumulation as the sustaining mechanism of high-frequency (100 Hz for 400 ms) stimulation induced depolarizations.

Our experiments addressed this issue in a different experimental condition in which depolarizing GABA effects arise from repetitive activation of inhibitory synapses at 40 Hz. The experiments in the presence of ionotropic glutamate and GABA<sub>B</sub> receptor blockers allowed a pharmacological isolation of GABA<sub>A</sub>-mediated effects. Under these conditions, GABA release was reduced by suppression of polysynaptic IPSPs (Davies et al. 1990), but it was favored by removal of GABA<sub>B</sub> receptor mediated presynaptic inhibition. By adjusting the stimulation intensity, it was possible to elicit a slow depolarization of similar amplitude to that in control solution and to study the mechanisms underlying this event. If the slow depolarization was sustained by extracellular potassium accumulation, its time course would not be affected by manipulations of the recorded neuron membrane potential. Under the present conditions, however, the kinetics of the slow depolarization depended on the state of polarization of the membrane. Recordings from current-clamped glial cells, used to monitor potassium-mediated depolarization (Kaila et al. 1997), revealed that the 40/10-Hz protocol produced a measurable potassium accumulation, but the time course of this phenomenon was much slower than the depolarization observed in pyramidal neurons. Thus even though extracellular potassium accumulation directly depolarizes neurons and can affect E<sub>Cl</sub> by altering intracellular chloride (Thompson and Gahwiler 1989), it is unlikely to be the main factor responsible for the slow depolarization observed in the present experiments. Similarly, the increase in polysynaptic EPSPs does not seem to be mainly due to potassium mediated facilitation of transmitter release because it lasted much longer than the extracellular potassium accumulation.

Numerical simulations, which included intracellular chloride changes, accounted for the voltage dependence of the slow depolarization and of the GABA<sub>A</sub> receptor driving force (E<sub>m</sub> − E<sub>GABA</sub>) observed during the 40/10-Hz protocol. Experimentally, this driving force was monitored through responses to 10-Hz pulses. These experiments revealed that when the cell was between −60 and −40 mV, GABA<sub>A</sub> driving force (positive at rest) became very small (i.e., did not give rise to detectable postsynaptic potentials) during the whole 10-Hz train and remained so for several seconds after it; conversely, when the cell was hyperpolarized to −95 mV, the (negative) GABA<sub>A</sub> driving force recovered much earlier, so that already after 500 ms of stimuli at 10-Hz GABAergic potentials were clearly detectable. In the simulations, this behavior could be explained by considering the mixed permeability of GABA<sub>A</sub> receptor to chloride and bicarbonate (Staley et al. 1995) and assuming stability of transmembrane bicarbonate gradients (Staley and Proctor 1999; Staley et al. 1995). One of the key features of the simulations was that the initial driving force for chloride (E<sub>m</sub> − E<sub>Cl</sub>) was much larger in the more depolarized conditions and remained so during the activation of the GABA conductance, thus allowing a more prolonged chloride accu-
mulation and delaying both the peak of the slow depolarization and the return of \( E_{\text{GABA}} \) to control values. We conclude that, under the present conditions, intracellular chloride accumulation (rather than extracellular potassium accumulation) was the main determinant of the GABAergic depolarization and the associated EPSP modulation.

Whether depolarizing GABA effects are functionally excitatory or inhibitory under different conditions is a matter for debate. Depolarizing GABA effects induced by tetanic stimulation of CA1 in the presence of the GABA uptake inhibitor tiagabine are accompanied by a functional reduction of evoked EPSPs (Jackson et al. 1999). Under the conditions of the present study, depolarizing GABA effects caused a clear disinhibition of the CA1 network, favoring polysynaptic EPSPs and converting subthreshold evoked PSPs into superthreshold inhibitory currents. As in the case of depolarizing GABA potentials induced by tetanic stimulation (Bracci et al. 1999), presynaptic GABA \(_{\text{B}}\) receptors exert an important negative feedback by limiting the amount of GABA released and therefore the extent of the GABAergic depolarization.

The present results set some constraints on the models of fast oscillations in CA1, which often rely on synchronous, rhythmic IPSPs to explain rhythmogenesis (Fisahn et al. 1998; Jefferys et al. 1996; Traub et al. 1996a; Whittington et al. 1995). When a large number of inhibitory synapses impinging on pyramidal neurons are activated simultaneously at gamma frequency, a rapid collapse of chloride gradients takes place, resulting in a loss of inhibitory efficacy. Stimuli were applied to the pyramidal layer, presumably activating local interneurons and pyramidal neurons. This method does not allow, however, a precise control over the class of interneurons excited, either directly or synaptically. Other studies have shown that the GABAergic synapses giving rise to depolarizing effects tend to be located on the dendrites rather than the soma of pyramidal neurons (Lambert et al. 1991; Staley and Proctor 1999). It is possible that, if only a particular subgroup of interneurons (possibly impinging on the soma of pyramidal neurons) is rhythmically active, less intracellular chloride accumulation might take place, thus preserving the inhibitory efficacy of the IPSPs. This may be the case of gamma oscillations induced by focal application of mGluR agonists (Traub et al. 1996a; Whittington et al. 1995), where a much more limited number of interneurons and even fewer pyramidal neurons are actively involved in the oscillation than during tetanically induced oscillations.

Intense mental activity may trigger epileptic discharges (Matsuoka et al. 2000). Gamma oscillations have been linked to cognitive functions (Hoffeld 1995; Singer 1999). These two observations lead to the intriguing hypothesis that one possible mechanism of this physiopathological transition might involve intracellular chloride accumulation induced by repetitive activation of inhibitory neurons at gamma frequency, resulting in a transient disinhibition of the local networks and therefore in a decreased threshold for epileptic discharges (Köhling et al. 2000).

This work was supported by Wellcome Trust.

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