Use-Dependent Shift From Inhibitory to Excitatory GABA<sub>A</sub> Receptor Action in SP-O Interneurons in the Rat Hippocampal CA3 Area

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Lamsa, Karri and Tomi Taira. Use-dependent shift from inhibitory to excitatory GABA<sub>A</sub> receptor action in SP-O interneurons in the rat hippocampal CA3 area. J Neurophysiol 90: 1983–1995, 2003. First published May 15, 2003; 10.1152/jn.00060.2003. Cortical inhibitory interneurons set the pace of synchronous neuronal oscillations implicated in synaptic plasticity and various cognitive functions. The hyperpolarizing nature of inhibitory postsynaptic potentials (IPSPs) in interneurons has been considered crucial for the generation of oscillations at β (15–30 Hz) and γ (30–100 Hz) frequency. Hippocampal basket cells and axo-axonic cells in stratum pyramidale-orientes (S-PO) play a central role in the synchronization of the local interneuronal network as well as in pacing of glutamatergic principal cell firing. A lack of conventional forms of plasticity in excitatory synapses onto interneurons facilitates their function as stable neuronal oscillators. We have used gramicidin-perforated and whole cell clamp recordings to study properties of GABAA<sub>R</sub>-mediated transmission in CA3 SP-O interneurons and in CA3 pyramidal cells in rat hippocampal slices during electrical 5- to 100-Hz stimulation and during spontaneous activity. We show that GABAergic synapses onto SP-O interneurons can easily switch their mode from inhibitory to excitatory during heightened activity. This is based on a depolarizing shift in the GABA<sub>A</sub> reversal potential (<em>E<sub>GABA-A</sub></em>), which is much faster and more pronounced in interneurons than in pyramidal cells. We also found that the shift in interneuronal function was frequency dependent, being most prominent at 20- to 40-Hz activation of the GABAergic synapses. After 40-Hz tetanic stimulation (100 pulses), GABA<sub>A</sub> responses remained depolarizing for ~45 s in the interneurons, promoting bursting in the GABAergic network. Hyperpolarizing <em>E<sub>GABA-A</sub></em> was restored >60 s after the stimulus train. Similar but spontaneous GABAergic bursting was induced by application of 4-aminopyridine (100 μM) to slices. A shift to depolarizing IPSPs by the GABA<sub>A</sub>R permeant weak acid anion formate provoked interneuronal population bursting, supporting the role of GABAergic excitation in burst generation. Furthermore, depolarizing GABAergic potentials and synchronous interneuronal bursting were enhanced by pentobarbital (100 μM), a positive allosteric modulator of GABA<sub>A</sub>Rs, and were blocked by picrotoxin (100 μM). Intriguingly, GABAergic bursts displayed short (<1 s) oscillations at 15–40 Hz, even though only depolarizing GABA<sub>A</sub> responses were seen in the SP-O interneurons. This β-γ rhythmity in the interneuron network was dependent on electrotonic coupling, and was abolished by blockade of gap junctions with carbamoylcholine (200 μM). Results here implicate the rapid activity-dependent degradation of hyperpolarizing IPSPs in SP-O interneurons in setting the temporal limits for a given interneuron to participate in β-γ oscillations synchronized by GABAergic synapses. Furthermore, they imply that mutual GABAergic excitation provided by interneurons may be an integral part in the function of neuronal networks. We suggest that the use-dependent change in <em>E<sub>GABA-A</sub></em> could represent a form of short-term plasticity in interneurons promoting coherent and sustained activation of local GABAergic networks.

INTRODUCTION

Fast network oscillations at 15–100 Hz are thought to be central for the timing and coordination of neural activity in the brain and have been implicated in various cognitive functions (for review see Jefferys et al. 1996). The generation of fast oscillations relies on tonic excitation of the interneuron network and synchronizing connections between interneurons. In the hippocampus, GABAergic interneurons in CA1-CA3 stratum pyramidale-orientes (S-PO) have a central role in the generation of these oscillations (Freund and Buzsaki 1996).

Hyperpolarizing GABA<sub>A</sub> receptor-mediated signaling in interneurons is thought to be essential for the generation of oscillations (Cobb et al. 1995; Traub et al. 1996b; Whittington et al. 1995). Furthermore, it has been recently shown that in the neocortex gap junctions may also participate in the synchronization of fast oscillations (Hormuzdi et al. 2001; McBain and Fisahn 2001; Tamas et al. 2000; Traub et al. 2001; but see Venance et al. 2000). According to recent views, the source of the interneuronal excitation is not critical for the oscillations to be generated, yet it is likely that in the hippocampus glutamatergic or cholinergic inputs provide the major synaptic excitatory drive via activation of either ionotropic or metabotropic receptors (Fisahn et al. 1998; Palva et al. 2000; Whittington et al. 1995; but see Bracci et al. 1999). GABA is the main inhibitory neurotransmitter in the brain, although it is now known that GABA<sub>A</sub>R activation can also provide excitation in hippocampal interneurons and pyramidal cell dendrites during heightened GABA release (Forti and Michelson 1998; Kaila et al. 1997; Michelson and Wong 1991; Staley et al. 1995; Taira et al. 1997). Studies on slices from various areas of CNS suggest that GABAergic excitation could drive population discharges in interneuronal networks (Bazhenov et al. 1999; Chub and O’Donovan 2001; Lamsa et al. 2000; Leinekugel et al. 1997; Michelson and Wong 1991; Xie and Smart 1991). However, changes of <em>E<sub>GABA-A</sub></em> occurring in individual interneurons during neuronal activity, and their relevance to the behavior of the interneuron network, have remained unclear.

So far, the ionic mechanisms underlying GABA-driven in-
terneuron population bursts in the adult hippocampus have been studied using extracellular ion-selective measurements as well as field potential recordings or GABAergic responses in pyramidal cells as an indicator of interneuronal activity (Kaila et al. 1997; Lamsa and Kaila 1997; Perkins 1999). By using gramicidin perforated-patch recordings from SP-O interneurons we show here that the activity-induced changes of GABA_A reversal potential (E_{GABA_A}) result in relatively fast degradation of the hyperpolarizing inhibitory postsynaptic potentials (IPSPs) and set the stage for a rapid use-dependent shift from inhibitory to excitatory GABA action. Change in IPSP polarity is most efficiently induced by β-γ-frequency activity patterns. Manipulations of E_{GABA_A} also directly show that the shift from hyperpolarizing to depolarizing GABA_A action drives the local interneuron network to massive population bursting. Intriguingly, we found that in the presence of depolarizing GABAergic IPSPs the interneuron network can display <1 s synchronous 15- to 40-Hz oscillations entrained by gap junctions, emphasizing the role of gap junctions in the generation of synchronous β-γ rhythms among hippocampal interneurons.

**METHODS**

**Preparation and solutions**

Eighteen- to 28-day-old Wistar rats were decapitated under deep pentobarbital (30–40 mg/kg) anesthesia. Transverse slices (400–500 μm) were cut from the hippocampi using a McIlwain tissue chopper. Experiments were carried out in a fast-perfused (3 ml/min) submerged chamber (0.3 ml) at 32°C. Standard solution contained (in mM) 124 NaCl, 3.0 KCl, 2.0 CaCl_2, 2.5 NaHCO_3, 1.1 NaH_2PO_4, 2.0 MgSO_4, and 10 glucose (pH 7.4, equilibrated with 95% O_2-5% CO_2).

**Drugs**

6-cyano-7-nitroquinazoline-2,3-dione (CNQX, 20 μM; Tocris Cookson) and t-2-amino-5-phosphonopentanoic acid (AP-5, 40 μM; Tocris Cookson) were present in the standard solution in all experiments. 4-aminopyridine (4-AP, 100 μM; Sigma), carbenoxolone (100–200 μM; Sigma), CGP55845 (1 μM; Tocris Cookson), 1-octanol (500 μM), pentobarbital sodium (PB, 100 μM; Sigma), and picrotoxin (PTX, 100 μM; Sigma) were bath applied. Twenty to 30 mM NaCl in standard solution was replaced with equimolar Na-formate or -propionate as described previously (Lamsa and Kaila 1997).

**Electrophysiology**

Whole cell and gramicidin perforated-patch recordings were obtained from neurons in CA3 s. pyramidale and in s. oriens using an axoclamp 2A amplifier (Axon Instruments). Only cells with resting membrane potential more negative than −56 mV were accepted. Patch pipettes had a resistance of 6–9 MΩ when filled with (in mM) 125–135 K-glucuronate, 10 HEPES, 5 EGTA, 2 Ca(OH)_2, 2 Mg-ATP, 1–10 KCl, and 2 QX-314 (pH 7.0 with NaOH). For perforated-patch recordings, gramicidin (Sigma) stock solution was prepared by dissolving 100 mg/ml in dimethyl sulfoxide (DMSO) (see Lamsa et al. 2000). Gramicidin was diluted in the filling solution to give a final concentration of 100 μg/ml. The tip of the filaments was filled with gramicidin-free pipette solution. The gramicidin-perforated resistance stabilized close to 100 MΩ. A liquid junction potential correction (−3 mV) was performed for all membrane potential values reported (Neher 1992). Cell input resistances were measured by −0.02 nA current steps in current clamp or were calculated from the clamping current required for a 5-mV hyperpolarizing step (for 10 ms every 20 ms) in voltage clamp. Conventional 5–10 MΩ glass capillary electrodes (filled with 150 mM NaCl) were used for field potential recordings. The tip of the bipolar stimulus electrode was positioned within 0.5 mm of the recording site in SP-O. Stimulus intensity (14–20 V) was set slightly supramaximal to a single-pulse IPSP. Stimulation trains (40–100 pulses, 5–100 Hz, pulse duration: 0.1 ms) were given at 2- to 3-min intervals.

**Data analysis**

Signals were tape recorded with a TEAC SR-31 recorder and digitized off-line at 4 kHz using a National Instruments AT-MIO-16-E-2 A/D board and Lab View software (National Instruments). Action potential peaks and spontaneous GABAergic PSPs were determined with a peak detection algorithm (Lab View) and verified visually. Before the PSP analysis, traces were low-pass filtered at 1 kHz. Only PSPs with an amplitude greater than three times the noise SD and with a decay time constant >20 ms were included in the analysis. The time-frequency (TF) representations of the GABA_A current bursts were computed by convoluting the signal with a Gabor wavelet: $h(t,f) = kexp(-t^2/2 + imx)$, $x = 2\pi ft/m$, where time and frequency are denoted by $t$ and $f$, $m = 6$, $i$ is the imaginary unit, and $k$ is the constant used to normalize the wavelet to unit area (Sinkkonen et al. 1995). Before averaging, TF representations of the bursts were aligned according to the ends of the bursts. The burst was considered to end when the signal amplitude (in the TF domain) fell below three times preburst SD (computed for each frequency band over the 1 s preceding the burst). For each burst, the mean amplitude of preburst noise was subtracted from each frequency band. The integrated current carried by spontaneous PSCs was calculated using LabView software. Data are given as means ± SE if not stated otherwise. Care and use of animals conformed to the guidelines of the Helsinki University Animal Care Committee.

**RESULTS**

We studied the properties of rat hippocampal interneurons in CA3 SP-O using gramicidin perforated-patch and whole cell recordings. The ionotropic glutamate receptor antagonists CNQX (20 μM) and AP-5 (40 μM) were present in the extracellular solution in all experiments. We distinguished interneurons from pyramidal cells by their distinct action potential firing in response to a depolarizing pulse. Cells in s. pyramidale and s. oriens showing high-frequency discharges (spike interval close to 5 ms) with little adaptation on constant depolarization (1 s) were identified as interneurons (hereafter referred to as SP-O interneurons) (see Buhl et al. 1994, 1996; Freund and Buzsaki 1996; Lacaille and Williams 1990). Most neurons in s. pyramidale showed a maximal firing frequency around 100 Hz and strong spike-frequency adaptation. These were classified as pyramidal cells. Data are illustrated in Fig. 1A.

**Effect of tetanic stimulation on the polarity of GABA_A-mediated PSPs in SP-O interneurons and in pyramidal cells**

We first investigated monosynaptic stimulus-evoked IPSPs in SP-O interneurons and in pyramidal cells employing the gramicidin perforated-patch-clamp technique. This method permits the measurement of cell membrane potential without altering the cytoplasmic anion content and consequently the reversal potential of GABA_A receptor-mediated responses.
FIG. 1. Use-dependent shift in $E_{\text{GABA}}$ in stratum pyramidale-oriens (SP-O) interneurons vs. pyramidal cells during afferent stimulation. Traces are gramicidin-perforated membrane potential recordings in the presence of glutamate receptor blockers 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX; 20 μM) and t-2-amino-5-phonophonopentaric acid (AP-5; 40 μM) and GABA$_B$ receptor antagonist CGP55845 (1 μM). A: SP-O interneurons (top), unlike pyramidal cells (bottom), show fast action potential firing (spike interval close to 5 ms) and little adaptation of discharge on constant (1 s) depolarization. Both SP-O interneurons and pyramidal cells show a hyperpolarizing reversal potential in response to single pulse-evoked GABA$_A$ receptor (GABA$_A$R)-mediated potentials. Timing of electrical stimulus is indicated (△). Resting membrane potentials −62 and −66 mV, respectively. Bar scaling 40 mV/250 ms (left) and 5 mV/500 ms (right). B1: stimulation at ≥ 10 Hz (40 pulses) generates a shift of the GABA$_A$ response from hyperpolarizing to depolarizing in SP-O interneurons ($n = 4$, mean ± SE). Hyperpolarizing GABA$_A$R-mediated potentials switch to depolarizing most readily at 20- and 40-Hz stimulation ($n = 12$ trains in 4 cells). Postsynaptic potential (PSP) amplitudes at 40 Hz differ significantly from other stimulation frequencies by the 10th pulse (ANOVA; *$P < 0.05$). At the 20th, 30th, and 40th pulse, both 20- and 40-Hz responses differ significantly from other frequencies (**$P < 0.01$, ***$P < 0.005$). B2: sample responses at 40- and 10-Hz stimulation (100 pulses). Bar scaling 20 mV/10 mV, respectively. B3: blockade of the GABAergic potentials with PiTX (100 μM). Bar scaling 10 mV/10 mV. C1: in the pyramidal cells, inhibitory PSPs (IPSPs) remain hyperpolarizing throughout the stimulation trains. At the 100 Hz 40 pulse stimulation, PSP amplitude is significantly more hyperpolarizing than at other stimulation frequencies (*$P < 0.05$). C2: hyperpolarizing IPSPs at 40 and 10 Hz. Bar scaling 5 mV.
The reversal potential for single pulse-evoked IPSPs was $-4.8 \pm 1.0$ mV relative to resting membrane potential ($E_m = -60.5 \pm 0.8$ mV, $n = 8$) in SP-O interneurons and $-8.1 \pm 0.9$ mV in pyramidal cells ($E_m = -64.6 \pm 0.8$ mV, $n = 12$) (Buhl et al. 1994, 1996; Rivera et al. 1999; Thompson and Gähwiler 1989). Input resistances for these neurons were $172.3 \pm 12.0$ and $54.2 \pm 4.9$ MΩ, respectively. Rupture of the gramicidin-perforated patch followed by recording (15 min) in the whole cell configuration with low chloride (1 mM) filling solution shifted IPSP reversal potential to $-88.2$ mV in the interneurons ($n = 2$) and to $-86.0 \pm 3.7$ mV in the pyramidal cells ($n = 12$). Next, we studied the effects of high-frequency stimulation on IPSPs in interneurons and pyramidal cells (Fig. 1, B and C). The experiments were carried out in the presence of 1 μM CGP55845, which blocks pre- as well as postsynaptic GABA<sub>B</sub> receptors. This was to avoid GABA<sub>B</sub> autoreceptor induced depression of IPSCs. Furthermore, the glutamate receptor antagonists (CNQX, 20 μM and AP-5, 40 μM) would block modulation of GABA-release via presynaptic kainate receptors, which could be qualitatively different at varying stimulation frequencies (see Min et al. 1999). Trains of 40–100 pulses were delivered at various frequencies (5, 10, 20, 40, and 100 Hz). Stimulus intensity was set supramaximal for a monosynaptic single-shock IPSP. With 5 Hz (40 pulses) stimulation, only hyperpolarizing GABA<sub>A</sub>R-mediated potentials were generated in both cell types. In contrast, when higher stimulus frequencies were used, depolarizing GABAergic potentials were generated in interneurons. Maximal shift was seen at 40-Hz stimulation, where initially 4.6 ± 0.3 mV hyperpolarizing GABA<sub>A</sub>R-mediated PSPs turned to 6.3 ± 1.0 mV depolarizing (40th pulse, $n = 4$ cells). The PSPs were blocked by PiTX (100 μM, $n = 3/3$). At 10, 20, and 100 Hz, GABAergic potentials were 1.1 ± 0.2, 4.8 ± 2.5, and 1.8 ± 0.9 mV depolarizing, respectively (40th pulse, $n = 4$ cells). In contrast to our observations in SP-O interneurons, the GABA<sub>A</sub>R-mediated responses in pyramidal cells to stimuli (40 pulses) remained hyperpolarizing at all frequencies. On 5-, 10-, 20-, 40-, and 100-Hz tetanic stimulation the IPSPs at 40th pulse were $-3.0 \pm 0.3$, $-3.2 \pm 0.3$, $-2.7 \pm 0.5$, $-3.7 \pm 0.4$, and $-5.0 \pm 0.4$ mV, respectively ($n = 10$ cells).

**Tetanic high-frequency stimulation is followed by GABA<sub>A</sub>R-mediated excitation in the SP-O interneurons and sustained GABAergic network activity**

We found that 40-Hz tetanic stimulation (100 pulses) was followed by spontaneous PSPs, which were depolarizing (see Fig. 2A) in interneurons (4 cells) but were hyperpolarizing in the pyramidal cells (10 cells). Furthermore, single-pulse electrical stimuli elicited depolarizing PSPs in the interneurons until 45.3 ± 4.3 s ($n = 4$ cells) after the train. The depolarizing PSPs had a decay time constant (68.0 ± 11.2 ms) similar to hyperpolarizing IPSPs (hIPSPs) before the train (62.5 ± 12.2 ms, $n = 30$ in 3 cells, Fig. 2B) and were blocked by PiTX (100 μM, $n = 3$). Importantly, 3–5 s after the train a single shock generated an excitatory polysynaptic GABAergic response, which had duration <500 ms. hIPSPs were fully restored >60 s after the train stimulus. These data are shown in Figs. 2, B and C.

**Periodic occurrence of hyperpolarizing and depolarizing GABAergic PSPs in SP-O interneurons during 4-AP-promoted spontaneous activity**

As described previously, exposure to 4-aminopyridine (4-AP, 100 μM) induced spontaneous interneuron population

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**FIG. 2.** High-frequency tetanic stimulation is followed by enhanced GABAergic network activity and excitatory GABA<sub>A</sub>R-mediated potentials in the interneurons. A: spontaneous GABA<sub>A</sub>R-mediated potentials in an SP-O interneuron following a 40-Hz 100-pulse train. Specimen traces 3 s, 5 s, (bar: 3 mV/500 ms) and 10 s after train (bar: 1 mV/500 ms). B: responses to single-shock stimuli at various time points after the 40-Hz (100 pulse) train show the recovery of hyperpolarizing GABA<sub>A</sub>R-mediated potential in interneurons. Hyperpolarizing GABA<sub>A</sub>R-mediated responses are restored 60–80 s after the train. 0 indicates resting membrane potential. Inset: normalized PSPs show similar decay time constants ($\tau$). C: single shock-elicited GABA<sub>A</sub>R-mediated potentials 5, 20, 40, and 80 s after the 40-Hz (100 pulse) train (bar scaling: 10 mV/250 ms). Electrical stimulus is indicated (•).
bursts, which appeared in field potential recordings as negative deflections lasting a few hundred milliseconds (mean interval: 38 ± 6 s, n = 43 slices). These events occur in the presence of glutamate receptor blockers and are abolished by GABA-A receptors (e.g., Lamsa and Kaila 1997; Perreault and Avoli 1992), confirming their GABA-A dependence. Simultaneous field potential measurements and cell-attached recordings in SP-O interneurons showed that the field potential deflection was accompanied by a burst of action potentials (14 ± 4) in the interneurons (n = 12 cells, 60 bursts).

We performed a series of whole cell recordings in SP-O interneurons with low Cl− (1–8 mM) and QX-314 (2 mM) in the pipette (see METHODS). The whole cell clamp recordings showed that there was continuous occurrence of the GABA-A-mediated PSCs between the population bursts and that the PSC frequency gradually increased toward a population burst. The frequency of postsynaptic GABA-A currents was lowest 5–10 s after the population burst (68 ± 5% of the baseline, n = 36 bursts in 3 slices), whereupon it gradually increased toward an onset of the next population burst, being highest (204 ± 12% of the baseline) at 5 to 0 s prior to the burst (Fig. 3, D and E). The occurrence of GABA-A currents was calculated from 5-s periods, and the baseline (mean occurrence ± SE = 4.4 ± 1.6 Hz) was taken 20 to 15 s before each individual burst. Only burst intervals >30 s were used for the analysis.

We next wanted to study the GABA-A reversal potential (E_GABA-A) in SP-O interneurons with gramicidin perforated recordings during this spontaneous activity. We analyzed the amplitude of spontaneous PSPs in the interneurons (see METHODS). PSPs with amplitudes greater than three times the SD of the noise were included in the analysis. The analysis may have excluded small-amplitude deflections, and a peak amplitude detection algorithm could not be used when the cell was firing. Data were collected from 60 bursts (from 6 cells) to obtain a sufficient number of PSPs. One-second bins were used for temporal pooling of PSPs. The data showed that the spontaneous unitary GABA-A-mediated PSPs were hyperpolarizing (1.2 ± 0.1 mV, n = 60 in 6 cells) between the field potential population bursts (see Fig. 3A, see 4, A and B). However, in gramicidin perforated-patch recordings, hyperpolarizing PSPs were never observed <3 s before the bursts, indicating that there was a gradual positive shift in the GABAergic PSPs prior to the population bursts. During the burst the GABA-A-mediated potential was 5.2 ± 1.3 mV depolarizing (n = 30 in 10 cells). Hyperpolarizing IPSPs were restored only >10 s after the population bursts (Fig. 3A). Consequently, hIPSPs were never seen in these cells when the burst interval was less than 10 s. The decay time constant of the spontaneous unitary hIPSPs (53.5 ± 4.2 ms) was similar to that of depolarizing IPSPs (dIPSPs; 59.0 ± 8.2 ms, n = 60, 6 cells; Fig. 3B).

Rupture of the patched membrane and perfusion of the cells with the low Cl− (1 mM, 10 min) filling solution in the whole cell configuration switched the PSPs to hyperpolarizations. Thus before, during, and after the burst, only hIPSPs were seen. Maximal hyperpolarization (13.3 ± 1.1 mV) was generated during the population burst (n = 18 in 6 cells, Fig. 3C). The PSPs were blocked by PiTX (100 μM, n = 3/3). The data are shown in Fig. 3, D and E.

**Effect of E_GABA-A shift on synchronization of interneuronal network activity**

We then wanted to know how changes in interneuronal E_GABA-A affect interneuronal population bursting. We studied this question in two ways using the 4-AP model: 1) we investigated the direct effect of a change in GABA-A reversal potential on interneuronal excitation. This was achieved by generating a positive shift in E_GABA-A by replacing 20 mM extracellular NaCl with Na-formate (see Lamsa and Kaila 1997; Mason et al. 1990). 2) We exposed interneurons to pentobarbital (PB, 100 μM), a positive allosteric modulator of GABA-A receptors that has been shown both to promote interneuron population bursting (Lamsa and Kaila 1997) and to enhance depolarizing GABAergic potential generated in pyramidal cell dendrites (Alger and Nicoll 1982; Autere et al. 1999; Manuel and Davies 1998; Staley 1992).

Formate anions show high permeability across GABA-A channels (0.5 vs. chloride) (Bormann et al. 1987), and equilibration of formate across cell membranes should cause a ~10-mV positive shift in the GABA-A reversal potential. Gramicidin perforated-patch recordings showed that all IPSPs gradually shifted from hyperpolarizing to depolarizing in the presence of formate (20 min). The amplitudes of the GABA-A-mediated depolarizations during the population bursts increased from 4.7 ± 1.7 to 9.5 ± 2.3 mV (P < 0.005, paired t-test, n = 15 bursts in 3 cells). The effect of formate is shown in Fig. 4A. Formate application also leads to more frequent interneuron population bursts (rate of occurrence 154 ± 14% in comparison to control; P < 0.005, paired t-test, n = 6, see Fig. 4C).

Another weak-acid anion, propionate, was used as a control to verify that the shift in E_GABA-A in SP-O interneurons and subsequent increase in spontaneous bursting were indeed produced by the equilibration of formate and the consequent positive shift in E_GABA-A. Propionate is virtually impermeant across GABA-A channels (<0.02 vs. chloride) (Bormann et al. 1987) and a transmembrane propionate gradient therefore has no direct effect on GABA-A. On exposure to Na-propionate (20 mM, 20–30 min), there was a slight decrease in the network-driven GABAergic depolarization (from 5.7 ± 1.5 to 5.1 ± 1.0 mV; n = 10 bursts, P < 0.05, paired t-test, n = 10 bursts in 3 cells). This was accompanied by a decrease in the synchronous burst occurrence to 81 ± 5% (P < 0.05, paired t-test, n = 6, see Fig. 4C). In contrast to formate application, hyperpolarizing IPSPs were seen between the bursts in the presence of propionate.

PB (100 μM) enhanced the positive shift of GABA-A-mediated potentials in SP-O interneurons. We observed that GABAergic depolarizations during the bursts increased concomitantly (from 4.4 ± 1.2 to 7.2 ± 1.0 mV, P < 0.005, paired t-test, n = 15 bursts in 3 cells) with the increase in input conductance (from 42 ± 4 to 73 ± 10 nS, P < 0.005, n = 3 cells, paired t-test). We did not observe hyperpolarizing IPSPs between the bursts in the interneurons after exposure to PB. This might be explained by the larger depolarizing potentials generated by GABA during the bursts as well as by a substantial shortening of the burst interval, making restoration of hyperpolarizing values between the bursts less likely. Application of PB was accompanied by an increase in interneuron
population burst occurrence to 221 ± 18% (P < 0.005, paired t-test, n = 6). The effect of PB is illustrated in Fig. 4, B and C.

Gap junctions in synchronization of the interneuronal activity

We analyzed the temporal patterns of network-driven GABA$_A$R-mediated inputs to individual CA3 neurons during 4-AP-promoted spontaneous activity. Whole cell voltage-clamp recordings revealed that short (<1 s) periods of rhythmic GABA$_A$R-mediated activity occurred during interneuron population bursts. Time-frequency (TF) analysis of whole cell voltage-clamp data revealed that prominent GABA$_A$R currents were generated at $\beta$ frequencies (15–30 Hz) during the bursts.
These oscillations were 440 ± 40 ms in duration (n = 30 bursts in 6 slices; range: 200–700 ms). The relatively narrow frequency band seen in the averaged TF plot demonstrates the stability of the oscillations. Furthermore, a smaller amplitude γ oscillation (30–40 Hz) was frequently observed during the β oscillations. To determine whether the oscillating GABAergic currents were single PSCs or population events, we first analyzed the kinetics of the currents (Fig. 5B). Unlike the unitary IPSCs, time-to-peak values of the GABA A R-mediated currents during the oscillations were slow (4.8 ± 0.5 ms, n = 120 in 6 cells) and variable (range from 2.3 to 8.7 ms). For the unitary currents, the corresponding time-to-peak value was 2.0 ± 0.1 ms varying between 1.0 and 2.8 ms (cf. Agmon et al. 1996). Furthermore, the rising phases of the oscillating currents were jagged, suggesting that they were composed of several separate IPSCs. In addition, the increase in the input conductance during the oscillating GABA A currents (38 ± 5 nS) was 10-fold when compared with that seen during unitary GABAergic responses (4 ± 2 nS) between the population bursts (n = 3 cells, see METHODS). Taken together, these data indicate that the oscillat-
An interneuron network showing depolarising GABA<sub>A</sub>-R-mediated potentials displays short oscillation bursts (<1 s) with β (15–30 Hz) and γ (30–100 Hz) frequencies. Bursts are in the presence of glutamate receptor antagonists and 4-AP.

A: analysis of the GABA<sub>A</sub>-R-mediated currents in whole cell voltage clamped CA3 neurons shows rhythmicity in the synchronous GABAergic activity. Above: averaged time-frequency representation of 23 bursts demonstrates the presence and stability of approximately 20 Hz and approximately 40 Hz oscillations. Time and frequency are on the x- and y-axes, respectively. The signal amplitude (pA) is color-coded according to the scale on the right side of the figure. Note the characteristic decrease in oscillation frequency toward the end of the event. Below: (Top) a typical burst (time-scale as above). Bottom three traces: band-pass filtered traces on an expanded time-scale (filtering as indicated) illustrate the joint occurrence of approximately 40 Hz and approximately 20 Hz oscillations.

B: rhythmic synaptic events are compound currents, arising from simultaneous activation of several presynaptic GABAergic interneurons. GABA<sub>A</sub>-mediated, PSCs during the burst; the oscillating GABA<sub>A</sub>-R-mediated currents are large, up to 10 times bigger from those seen between the bursts. The currents are slow and variable and they often have a complex waveform. GABA<sub>A</sub>-mediated PSCs between the bursts; the monoexponential form, stability of kinetics and small amplitude characterize the unitary PSC.

C: rhythmicity in the GABAergic activity is sensitive to gap junction blockers. Averaged normalized power spectra of the GABA<sub>A</sub>-R-mediated activity during synchronous bursts (500 ms) under control conditions (black line) and following the application of carbenoxolone (200 µM, >10 min, gray line). Dotted lines indicate ± SE in both conditions. Note the absence of narrow-band rhythmicity in the presence of carbenoxolone. Insets: barrages of GABA<sub>A</sub>-R-mediated currents during interneuronal
GABAergic excitation in SP-O interneurons

Population bursts in control conditions and following exposure to carbonoxolone. Bar scaling 500 pA / 250 ms. D: the gap junction blocker carbonoxolone (200 μM) inhibits generation of interneuronal population bursts. A combined fp and cell-attached recording shows that the bursts are partially restored by 20 mM formate. Bar scaling 300 μV and 50 pA / 30 s. E: inhibition of GABAergic population bursts by carbonoxolone. (mean ± SE, P < 0.005, paired t-test), and re-establishment of the bursting activity by formate (20–30 mM; n = 6).

DISCUSSION

Use-dependent positive shift of GABA_A reversal potential in the interneurons versus pyramidal cells

The major finding of this work is the substantial difference in the use-dependent depolarizing shift in E_{GABA_A} between SP-O interneurons and pyramidal cells. While the activity-induced positive shift in E_{GABA_A} is more pronounced in interneurons, targeted stimulation of GABAergic synapses in s. radiatum can produce GABAergic depolarization and excitation in pyramidal cells as well (Alger and Nicoll 1982; Autere et al. 1999; Grover et al. 1993; Kaila et al. 1997; Staley et al. 1995; Taira et al. 1997). However, activation of GABAergic synapses in s. pyramidale, s. oriens, or alveus elicits almost solely hyperpolarizing responses in these neurons (Alger and Nicoll 1982; Jackson et al. 1999). It is apparent that the depolarizing and excitatory effect of GABA in pyramidal cells during s. radiatum stimulation results from selective activation of the GABAergic synapses on their apical dendrites (Grover et al. 1993; Jackson et al. 1999; Staley et al. 1995; see also Alger and Nicoll 1982). If GABAergic synapses on both dendrites and the somatic area are activated, the shift in E_{GABA_A} in principal cells is much weaker or nonexistent (see Lamsa and Kaila 1997; Michelson and Wong 1991; Perreault and Avoli 1992). In contrast, the activity-induced positive shift in E_{GABA_A} is rapid in CA3 SP-O interneurons under similar conditions, and results promptly in postsynaptic excitation.

hIPSPs have been proposed to be a prerequisite for synchronous 15- to 100-Hz oscillations of neuronal networks (McBain et al. 1999; Traub et al. 1996a; Whittington et al. 1995). In the hippocampus, SP-O interneurons have a central role in the generation and maintenance of these oscillations. We show here that when stimulated at β-γ frequencies (particularly at 20–40 Hz), GABAergic input to SP-O interneurons induced a rapid use-dependent erosion of hIPSPs and eventually a switch to depolarizing GABA_A receptor-mediated responses. Given the presumed dependency of synchronous β-γ oscillations on hyperpolarizing IPSPs, their rapid plasticity may have important implications in the oscillatory properties of hippocampal networks. In particular, we propose that the fast erosion of the hyperpolarizing IPSPs may set the temporal limits of a given interneuron to follow synaptically mediated synchronization during β and γ frequency oscillations. It also highlights the importance of taking into account the dynamic properties of inhibitory synapses in models of oscillatory neural networks.

Generation of excitatory GABA_A responses and synchronization of the interneurons

Spontaneous activity of interneurons maintains a tonic mutual inhibition in the interneuronal network via hyperpolarizing IPSPs (see Häusser and Clark 1997). This can be seen as a frequent occurrence of spontaneous hIPSPs in the interneurons (e.g., see Figs. 3A and 4, A and B). It has been recently shown that even miniature hIPSPs modulate action potential firing of small high-impedance interneurons (Carter and Regehr 2003).

We found that the generation of excitatory GABA_A-mediated responses in SP-O interneurons was determined mainly by two postsynaptic factors: first, by changes in the postsynaptic GABA_A-mediated conductance as demonstrated by depolarizing shifts of the GABA_A response on application of pentobarbital, a positive allosteric modulator of GABA_A Rs. A similar effect of PB on activity-induced GABAergic depolarization in pyramidal cell dendrites has previously been shown (Alger and Nicoll 1982; Autere et al. 1999; Manuel and Davies 1998; see also Staley 1992). The second factor was shifts in the transmembrane ion gradients responsible for E_{GABA_A}; this was shown here experimentally by using formate to alter the intracellular anion load (see Lamsa and Kaila 1997). Weak-acid anions have previously been used in brain slices to study changes in E_{GABA_A} (Kaila et al. 1993; Kulik et al. 2000; Lamsa and Kaila 1997; see Grover et al. 1993; Kaila et al. 1997; Perkins 1999; Staley et al. 1995). As shown by the experiments with formate, the set point and dynamics of the GABA_A reversal potential are critical factors in determining the behavior of the CA3 interneuronal network.
The gramicidin perforated recordings from the interneurons showed that there was already a shift in the polarity of GABAergic PSPs before the spontaneous population bursts. Whole cell recordings showed a strong increase in the postsynaptic GABA_{A}R-mediated current in these cells prior to the burst. We suggest that the increased GABA_{A} conductance in the interneurons would gradually lead to a dissipation of the negative $E_{\text{GABA-A}}$ and thus degradation of hIPSPs in these cells (see e.g., Takeuchi and Takeuchi 1966). Consequently, we propose the following explanation for the periodical generation of massive interneuron population bursts. The hyperpolarizing nature of $E_{\text{GABA-A}}$ is easily degraded in interneurons when the postsynaptic GABA_{A}R activity in these cells increases up to a certain level. The mutual inhibition between interneurons is attenuated, which further promotes the activity in the GABAergic network. A collapse of the mutual inhibition in the interneuron network, and the fact that even small-amplitude depolarizing GABAergic PSPs can have an excitatory effect in these cells would eventually result in a generation of a massive interneuron population burst. It has recently been shown that GABA is easily accumulated onto interneuron-interneuron synapses because the GABA uptake system in these cells is much weaker than in the principal cells (Semyanov et al. 2003). This may partially explain why interneurons are susceptible to the use-dependent shift of $E_{\text{GABA-A}}$. After the population burst, GABAergic activity in the cells was strongly suppressed. During the low activity period, hyperpolarizing IPSPs reappeared in these cells. This was due to the fact that restoration of a hyperpolarizing $E_{\text{GABA-A}}$ is more efficient when membrane Cl$^{-}$ conductance is small (e.g., Thompson et al. 1988). Notably, a similar kind of periodic fluctuation of GABAergic activity and a postburst depression of the activity are seen in spontaneous GABAergic activity in the neonatal rat hippocampus (see O’Donovan 1999).

In the newborn rat hippocampus, GABA_{A} responses are known to be solely depolarizing, and spontaneous interneuronal population bursts are regularly seen in area CA3 (Ben-Ari et al. 1989; Khazipov et al. 1997; Lamsa et al. 2000; see also O’Donovan 1999). Furthermore, it was also recently shown that in embryonic spinal cord neurons, activity-induced redistribution of transmembrane Cl$^{-}$ is an important mechanism in modulating the postsynaptic effect of GABA_{A}R activation during spontaneous network activity (Chub and O’Donovan 2001).

Self-sustained $\beta$ and $\gamma$ oscillations in interneuronal networks

Synaptically driven synchronous 15- to 100-Hz oscillations are critically dependent on the IPSC/P decay time constant, hyperpolarizing IPSC amplitude, and the nature of the excitatory inputs to interneurons (Jefferys et al. 1996). As shown here, in the SP-O interneurons the rapid change of $E_{\text{GABA-A}}$ resulted in the first in erosion of hyperpolarizing IPSC/Ps and eventually generated autoexcitation sufficient to drive massive population bursts in the interneuronal network. During these population bursts, the CA3 GABAergic network was transiently synchronized to stereotypical $\sim$500 ms joint $\sim$20- and $\sim$40-Hz oscillations, which decreased in frequency toward the end of the event. Interestingly, $\gamma$ oscillations may lead to synchronous $\beta$ oscillations in the hippocampal network, provided that sufficient fast excitation (by pyramidal cells) is transiently coupled to interneurons (Kopell et al. 2000; Whitington et al. 1997). Here, the $\gamma-\beta$ oscillations were driven solely by the GABA_{A}Rs. Data here indicate that physiologically relevant $\beta-\gamma$ oscillation periods ($\sim$500 ms) can occur even in the absence of hyperpolarizing IPSPs and glutamatergic excitation in the interneurons. Yet the synchronization is then apparently limited to the electrically coupled interneuron networks connected by gap junctions. This would explain the fact that short $\gamma$ and high-frequency (>100 Hz) oscillations can occur in CA3 network during the first postnatal days, when GABA_{A} responses in both interneurons and pyramidal cells are depolarizing (Lamsa et al. 2000; Palva et al. 2000; see also Lahtinen et al. 2002). It has been recently shown that only morphologically similar types of interneurons are interconnected by electrical synapses (Fukuda and Kosaka 2000; Galarreta and Hestrin 1999; Gibson et al. 1999). Thus erosion of hIPSPs could effectively diminish synchronization between different interneuron subclasses, thereby providing a mechanism also for spatial control of interneuronal oscillations and inhibitory efficacy.

Metabotropic glutamatergic and cholinergic transmission are known to be important modulators of the interneuron network activity in the hippocampus, and these two mechanisms have been shown to drive synchronous $\gamma$ oscillations in the interneuron network under certain conditions (Cobb et al. 1995; Fisahn et al. 1998; Whitington et al. 1995). However, during short synchronous bursting periods when the postsynaptic conductance is mainly mediated by GABA_{A}Rs, the GABAergic excitation can override the relatively low-conductance postsynaptic metabotropic mechanisms. In this situation, modulation of interneuronal bursting could still effectively occur via presynaptic regulation of GABA release in interneurons by metabotropic glutamate receptors (Semyanov and Kullmann 2000). Interestingly, a similar kind of GABA_{A}R-mediated $\beta-\gamma$ frequency activity during 4-AP-induced population bursts can be seen in some previous studies (see Perkins 1999; e.g., Fig. 8).

Apart from the shift in $E_{\text{GABA-A}}$, passive membrane properties such as input resistance and time and length constants are also likely to change rapidly in interneurons during increased activity. Thus under conditions when the IPSP decay and IPSP reversal potential are unstable, gap-junction mediated coupling may become a critical mechanism for maintaining temporal synchrony in the local interneuron network (see Galarreta and Hestrin 1999; Gibson et al. 1999; Skinner et al. 1999; Szabadics et al. 2001; Velazquez and Carlen 2000).

Mechanisms of $E_{\text{GABA-A}}$ lability in interneurons

It is well established that the activity-induced GABA_{A}R-mediated depolarization generated in pyramidal cell dendrites is augmented by barbiturates, and that the shift is attributable to availability of HCO$_3$ as well as to extracellular accumulation of K$^+$ (Alger and Nicoll 1982; Autere et al. 1999; Kaila et al. 1997; Staley et al. 1995; Taira et al. 1997). In terms of HCO$_3$-dependency, K$^+$ and Cl$^{-}$ redistribution, and the effects of PB, it is likely that the mechanisms underlying the depolarizing shift of $E_{\text{GABA-A}}$ are the same in pyramidal cells and in SP-O interneurons (see Autere et al. 1999; Kaila et al. 1997; Lamsa and Kaila 1997). Possible explanations for the quantitative difference in the shift in the IPSPs could be, 1) a higher...
permeability of GABA_A receptors to HCO_3^{-} in interneurons (see Perkins 1999), 2) location of GABA_A receptors in interneurons on cellular structures having larger surface area/volume ratio and thus higher susceptibility to disruption of transmembrane ionic gradients, 3) differences in carbonic anhydrase activity and thus HCO_3^{-} availability in these two cell types, and 4) differences in density of transmembrane anion transporters or in phosphorylation cascades that control the transporters. However, all ionotropic GABA receptor channels found so far have similar HCO_3^{-} versus Cl^{-} permeability ratios (ranging between 0.2 and 0.3). A comparison of the data on inhibitory terminal density on interneurons and pyramidal cells does not support the hypothesis of a differential localization of GABAergic synapses (Gulyas et al. 1999; Megias et al. 2001). We therefore suggest that the difference in Cl^{-}/HCO_3^{-} homeostasis between pyramidal cells and SP-O interneurons depends either on differences in carbonic anhydrase activity in these two cell types or on differences in transmembrane anion transporters. It is known that interneuronal population bursts are effectively blocked by membrane permeant carbonic anhydrase inhibitors, thus emphasizing the importance of enzyme-catalyzed intracellular HCO_3^{-} production in GABAergic excitation between interneurons (Lamsa and Kaila 1997). Intriguingly, it also has been shown in different experimental models that interneuronal population bursting is accompanied by considerable fluctuation of extracellular [K^+] (Kaila et al. 1997; Lamsa and Kaila 1997; Lamsa et al. 2000). In turn, activity-induced changes in extracellular [K^+] might alter transmembrane Cl^{-} transport, e.g., via KCl co-transport (Rivera et al. 1999; Thompson and Gähwiler 1989). It would thus be interesting to see whether a similar change in E_{GABA_A} could be induced by GABA application onto interneurons, where a GABA concentration increase is not accompanied by extracellular K^+ accumulation.

Implications for interneuronal plasticity

GABAergic interneurons seem to lack conventional forms of synaptic plasticity (e.g., N-methyl-D-aspartate-dependent long-term potentiation). This may be important for their function as stable neuronal oscillators (McBain et al. 1999). However, there may be alternative forms of plasticity in excitatory synapses onto interneurons. A type of AMPAR abundant in hippocampal interneurons (Jonas and Burnashev 1995) is blocked by internal polyamines in a use-dependent manner (Rozov et al. 1998), repetitive activation may thus lead to a brief (<5 s) facilitation of interneuronal excitatory PSCs (EPSCs). Analogously, the use-dependent shift in E_{GABA_A} and the consequent postsynaptic excitation reported here could be seen as a form of short-term synaptic plasticity in interneurons required for the rapid and coherent recruitment of local GABAergic networks. The dramatic differences in the effect of the shift in E_{GABA_A} between interneurons and pyramidal cells implies that this effect is cell type-specific. In addition, the GABAergic interneuronal excitation described here will effectively come into play during heightened neuronal activity, thus providing another mechanism to increase interneuronal output and inhibitory efficacy in principal cells.

A frequency-dependent shift in E_{GABA_A} has also been suggested to contribute to pyramidal cell plasticity (Davies and Collingridge 1993; Staley et al. 1995; Taira et al. 1997). In principal cells, however, the GABA_A channel-mediated anion shifts result in much less extensive depolarization (Lamsa and Kaila 1997; see also Voipo and Kaila 2000), and their role may be in assisting NMDAR activation (Cobb et al. 1999; Staley et al. 1995; Taira et al. 1997) rather than being a plasticity mechanism per se.

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DISCLOSURES

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