Synaptic Depolarizing GABA Response in Adults Is Excitatory and Proconvulsive When GABA\(_B\) Receptors Are Blocked

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Submitted 29 September 2004; accepted in final form 2 December 2004


In the hippocampus and neocortex, GABA-mediated inhibitory synaptic transmission is widely considered to be the mechanism by which glutamate-mediated excitation is kept under control; thus a popular model of epilepsy is one in which GABAergic transmission is blocked (e.g., Dingerline and Gjerstad 1980). However, experiments done in tissue taken from temporal lobe epilepsy patients indicate that the interictal-like epileptiform activity recorded in that tissue can be blocked by either glutamate antagonists or GABA\(_A\) antagonists, suggesting that GABA transmission is also involved in promoting epilepsy (Cohen et al. 2002; Köhling et al. 1998). In the 4-aminopyridine (4-AP) model of temporal lobe epilepsy in the rodent hippocampal slice (Rutecki et al. 1987), epileptiform events occur spontaneously in the presence of intact GABAergic transmission. In this model, giant GABA-mediated postsynaptic potentials (GPSPs) directly precede, and seem to initiate, the epileptiform discharges (Avoli et al. 1996a). The GPSPs result from synchronous burst-firing in a group of interneurons (Lamsa and Taira 2003; Michelson and Wong 1991, 1994; Müller and Misgeld 1990). This synchronous firing is due to GABA-mediated recurrent excitation and gap junctions among interneurons (Lamsa and Kaila 1997; Michelson and Wong 1991, 1994; Perkins 2002; Traub et al. 2001; Yang and Michelson 2001). The giant GABA-mediated postsynaptic current (GPSC; the voltage-clamp version of the GPSP) recorded from the adult CA3 pyramidal cell in 4-AP is a triphasic event composed of an early, outward GABA\(_A\)-mediated Cl\(^-\) current, an inward GABA-mediated HCO\(_3^-\)/Cl\(^-\) current (depolarizing GABA; GABA\(_B\);), and a late, outward GABA\(_B\)-mediated K\(^+\) current (Perkins and Wong 1996). We hypothesize that the epileptiform discharges seen in 4-AP are triggered by the depolarizing component of the GPSP and that an enhancement of the depolarizing component should increase epileptiform activity.

We propose that GABA\(_B\) receptors exert control over the postsynaptic depolarizing GABA response in adult pyramidal cells. The first hypothesized form of control is at the postsynaptic pyramidal cell: block of GABA\(_B\) receptors and depolarizing components of the GABA-mediated postsynaptic potential may overlap in time (Thallmann 1988a); this overlap would be expected to increase the net excitation of the pyramidal cell and thus enhance the ability of the GPSP to trigger epileptiform discharges. The second hypothesized form of control is upstream at the presynaptic interneuron: block of GABA\(_B\)-receptor-mediated K\(^+\) currents on the cell body and dendrites, or block of GABA\(_B\) autoreceptors on axon terminals, may increase GABA release and thus enhance the depolarizing.
GABA component of the GPSP. Interestingly, loss of GABA_B receptor function has been demonstrated in tissue from both rodents (Mangan and Lothman 1996) and humans (Deisz 1999) with temporal lobe epilepsy.

METHODS

Slice preparation

Experiments were done in hippocampal brain slices from adult (180–440 g) guinea pigs. Guinea pigs were anesthetized with halothane and decapitated with a guillotine. One hippocampus was removed and the middle third selected for slicing. Transverse slices (300 μm) were cut in oxygenated, ice-cold solution (same as the extracellular solution detailed in the following text, except 8 mM MgCl_2 and 0.5 mM CaCl_2) using a vibratome (Technical Products International, St. Louis, MO). Slices were transferred to the holding chamber (Gibb and Edwards 1987) where they were maintained in extracellular solution continuously perfused with 95% O_2-5% CO_2 gas at 31–32°C for 1 h. At 1 h, the holding chamber containing the slices was removed from the heated water bath and allowed to cool down to room temperature. Slices were maintained in the holding chamber until ready to record, at which time one or two slices were placed in the recording chamber. Recording chamber used for microelectrode and “blind” whole cell recording was purchased from Fine Science Tools (Foster City, CA), and the recording chamber used for cell-attached recordings was a modified RC-29 chamber purchased from Harvard Apparatus (Holliston, MA).

Extracellular solution

The extracellular solution contained (in mM) 125 NaCl, 2.5 KCl, 1.6 MgCl_2, 2.0 CaCl_2, and 11 D-glucose. During recording the solution included 4-AP (50 μM), which blocks a subset of K⁺ channels in axons (Coetzee et al. 1999; Storm 1988) and increases transmitter release (Buckel and Haas 1982). Except where stated otherwise, the recording solution also contained the AMPA/kainate ionotropic glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) or CNQX disodium (10 μM) or 2,3-dioxo-6-nitro-1,2,3,4-tetrahydronbenzof[1]quinoxaline-7-sulfonamide disodium salt (NBQX disodium, 10 μM) and the N-methyl-D-aspartate (NMDA) receptor antagonist 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 20 μM) or D-(-)-2-amino-5-phosphonoic acid (d-AP5, 50 μM). The GABA_B receptor antagonist (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl] phenylmethyl) phosphinic acid (CGP 55845A, 1–2 μM; gift from Ciba-Geigy, Basel, Switzerland, or purchased from Tocris Cookson, Ellsville, MO) was added when noted; CGP 55845A (CGP) blocks both presynaptic and postsynaptic GABA_B receptors at 1 μM (Davies et al. 1993). The GABA_A antagonist gabazine (SR 95531; 3 or 9 μM) or bicuculline methiodide (30–50 μM) was added as noted. The glutamate antagonists and the gabazine were purchased from Sigma-Aldrich. Other chemicals were purchased from Sigma-Aldrich.

Whole cell recording

Slices recorded from with whole cell electrodes were submerged during recording in solution perfused with 95% O_2-5% CO_2 gas at 31°C. Electrophysiological recordings were carried out in the whole cell voltage-clamp configuration (Hamill et al. 1981) on CA3 pyramidal cells using a List EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany) and pClamp software (Axon Instruments, Union City, CA). Whole cell electrode resistances ranged from 3 to 7 MΩ when filled with intracellular recording solution. For the whole cell recordings, seals were established using the patch-slice method of Blanton et al. (1989). No series resistance or slow capacitance compensation was used during the experiment.

The recording pipette solution contained (in mM) 49 KHC_2O_3, 78 K gluconate, 5 NaCl, 2 CsCl, 5 KCl, 10 HEPEs, 2 EGTA, 0.5 N-(2,6-dimethylphenylcarbamoylmethyl)-triethylammonium bromide (QX-314; Calbiochem, La Jolla, CA), 1 or 4 Mg-ATP, and 0.3–0.5 mM GTP-Na or GTP-Li. The pH of the solution was 7.7 when equilibrated with 95% O_2-5% CO_2 gas (see Perkins and Wong 1996). A higher than normal [HCO_3] in the pipette shifts the reversal potential of the GABA_B component of the GPSC in the depolarizing direction significantly more than it does that of the early GABA_A component (Perkins and Wong 1996). A higher than normal [HCO_3] in the pipette was used here to make the reversal potential of the depolarizing component of the GPSC further from the reversal potential of the early GABA_A component. This manipulation, along with holding the cell near the reversal potential of the GABA_A component, facilitated the comparison of the GABA_A timecourse with that of the GABA_B (RESULTS).

Intracellular QX-314 blocks voltage-dependent sodium currents (Connors and Prince 1982) at 0.5 mM. In some experiments, 10 mM QX-314 was used to block the GABA_B component of the GPSC (Perkins and Wong 1996; see also Nathan et al. 1990). Note that adding 10 mM QX-314 to the pipette solution adds 10 mM of the GABA_A channel-permeant ion bromide (Bormann et al. 1987). At 10 mM, QX-314 also blocks the hypopolarization-activated inward current I_h (Perkins and Wong 1995) and the K⁺-dependent, nonsynaptic depolarization that can follow and merge with the synaptic depolarizing GABA response triggered by a stimulus train (Smirnov et al. 1999). Usually the recording pipette solution contained alkaline phosphatase (500–550 DEA units/ml; Sigma P6772) to prevent or reduce the development of inward leak current (Q. X. Chen, unpublished observation), thus stabilizing the input resistance (R_i). [Alkaline phosphatase did not reduce the GABA conductance; this finding is in contrast to the reduction in GABA conductance seen by Chen et al. (1990) in acutely dissociated neurons using exogenous GABA.] The charging current response to a 5-mV hyperpolarizing voltage step (ΔV) was recorded in all cells and periodically retested during the experiment. The access resistance (R_a) was estimated using the equation R_a = ΔV/Δi. Membrane potentials have not been corrected for series resistance error.

Intracellular microelectrode recording and simultaneous extracellular field recordings

Slices were placed on a net in the recording chamber at an interface between humidified 95% O_2-5% CO_2 gas and solution perfused with 95% O_2-5% CO_2 gas at 33°C. Microelectrode current-clamp recordings from CA3 pyramidal cells were carried out using a high-impedance microelectrode amplifier (IE-210; Warner Instruments, Hamden, CT) and pClamp software. Microelectrode impedance was 30–80 MΩ when filled with 3 M potassium acetate. Only recordings with a resting potential of −55 mV or more negative, overshooting action potentials, and an input impedance of ≥18 MΩ were accepted. Simultaneous extracellular field recordings were performed in some experiments. The glass recording electrode was placed in the CA3.
pyramidal cell body layer or CA3 stratum radiatum. Electrode resistance was 0.3–1.2 MΩ when filled with 150 mM NaCl. Voltage recordings were made using a high-impedance microelectrode amplifier (IE-210; Warner Instruments) at 50 times gain. Recordings were further amplified, AC-coupled, and filtered with a 20-Hz low-pass filter using a Gould signal conditioner.

**Cell-attached recording and simultaneous extracellular field recording**

Slices recorded from in cell-attached mode were submerged during recording in solution perfused with 95% O₂-5% CO₂ gas at 31°C. Cell-attached recordings of action potential currents were made on visualized CA3 pyramidal cells using a List EPC-7 patch-clamp amplifier (List Electronic) and pClamp software. Cells were visualized using a Nikon upright compound microscope equipped with a ×40 water-immersion objective and IR/DIC optics. Cell-attached recording electrodes were filled with 150 mM NaCl and had a resistance of 4–7 MΩ. Seal (25 MΩ to >5 GΩ) between recording pipette and cell membrane was obtained by applying suction to the electrode. Action potential currents were recorded in “search” mode on the EPC-7 amplifier, which maintains an average 0-pA holding current. Recordings with action potential currents <50 pA in magnitude were discarded.

Simultaneous field recordings were made with glass electrodes placed in CA3 stratum lacunosum-moleculare. Field electrodes were filled with 150 mM NaCl and had a resistance of 1–2 MΩ. Voltage was recorded with a second List EPC-7 amplifier and amplified and filtered with an LPF-100B (Warner Instruments).

**Stimulation**

A bipolar stimulating electrode was made of twisted Teflon-coated platinum-iridium wire (50-µm bare diameter). The electrode was placed in the hilus. A single, brief (50 μs) current pulse was used to evoke a GPSC when indicated.

**Data analysis**

All data in the text are expressed as means ± SD (Curran-Everett and Benos 2004). The n is the number of cells. Unless otherwise noted, each cell was from a different slice. Throughout the paper, unless otherwise noted, paired t-test was used for statistical analysis (SigmaPlot), using the recording from the same cell prior to drug as its own control. P < 0.05 was considered significant. Precise P values, rather than P < values, are reported as per the new American Physiological Society guidelines (Curran-Everett and Benos 2004). In one section, percentage change in conductance ([new conductance/original conductance/original conductance] is reported; however, the raw data were used for the paired t-test. When counting the number of action potentials triggered by the GPSP (Fig. 1), action potentials were counted from the start of the depolarizing component until the cell repolarized. In the experiments testing the effect of CGP alone, only slices which were subsequently recorded from in 4-AP and seen to express epileptiform activity in 4-AP were included in the analysis.

**RESULTS**

The first experiments (Figs. 1–6) were done in the presence of ionotropic glutamate receptor antagonists and 4-AP to examine GPSPs or GPSCs in the absence of epileptiform activity. The last experiments (Fig. 7) were done in the presence of 4-AP with glutamatergic transmission intact to study epileptiform activity. Portions of this work have appeared in abstract form (Perkins et al. 2003).

FIG. 1. Depolarizing GABA component of giant GABA-mediated postsynaptic potential (GPSP) triggered action potentials in CA3 pyramidal cells in the presence of GABA<sub>B</sub> receptor antagonist. A: spontaneous GPSP recorded at different membrane potentials in control solution containing 4-aminopyridine (4-AP) and ionotropic glutamate receptor antagonists. The GPSP appears as a triphasic hyperpolarizing-depolarizing-hyperpolarizing synaptic event. Current was injected to change the cell’s membrane potential. Spontaneous GPSPs were lined up for display, and the traces were offset for display. B: GPSP recorded in the presence of 4-AP and ionotropic glutamate receptor antagonists before (left) and after (right) addition of the GABA<sub>B</sub> antagonist (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethylamine-2-hydroxypropyl](phenylmethyl) phosphinic acid (CGP 55845A) to bath solution. Intracellular microelectrode recording: resting potential −62 mV. Different cell from cell in A. C: mean percentage of GPSPs with ≥1 action potential (AP) triggered by the depolarizing component of the GPSP before (control) and after CGP 55845A (n = 4, P = 0.0001). D: mean number of action potentials triggered by the depolarizing component of the GPSP before (control) and after CGP 55845A (n = 4, P = 0.003). Error bars are SD. *, significantly different from control.

**Depolarizing GABA component triggered action potentials in the presence of GABA<sub>B</sub> receptor antagonist**

Microelectrode current-clamp recordings from CA3 pyramidal cells were done in 4-AP and blockers of ionotropic glutameric transmission. The typical resting membrane potential
of these cells was between −60 and −65 mV. Rhythmic, spontaneous GPSPs occurred at a rate of one every 15–25 s. Figure 1A shows the GPSP recorded at several different membrane potentials in a typical CA3 pyramidal cell. As has been previously reported (Michelson and Wong 1991), the GPSP is made up of three components: an early hyperpolarization, a depolarization, and a late hyperpolarization. These components correspond to the GABA_A, depolarizing GABA, and GABA_B components of the GPSC recorded in voltage clamp (Perkins and Wong 1996). In the recording shown in Fig. 1A, the cell stops firing action potentials during the GPSP, demonstrating that the triphasic GPSP is usually inhibitory. In the −74- and the −62-mV traces, the depolarizing GABA component of the GPSP depolarizes the cell above baseline potential. Notice that the depolarizing GABA component is still present as a depolarizing inflection in the −59- and −53-mV traces (n = 6 cells in 5 slices).

In control solution, which contains 4-AP and ionotropic glutamate antagonists, most GPSPs showed no action potentials (APs) during the depolarizing component of the GPSP. Some cells showed an action potential during the early hyperpolarizing component of the GPSP, as has been reported by others (Traub et al. 2001). The GABA_B receptor antagonist CGP was washed in while maintaining the intracellular recording (Fig. 1B). Hyperpolarizing or depolarizing current was delivered if needed as needed to keep the baseline membrane potential between −60 and −65 mV as the experiment progressed. Rhythmic, spontaneous GPSPs continued to occur in the presence of CGP, although the average interval between GPSPs increased from 20 ± 4 to 27 ± 5 s (n = 4, P = 0.004). As predicted, CGP dramatically increased the mean percentage of GPSPs that had at least one action potential during the depolarizing component of the GPSP (Fig. 1C). CGP also significantly increased the average number of APs triggered by the depolarizing component of the GPSP (Fig. 1D). Three of four cells had at least one GPSP that triggered ≥10 APs in CGP. Recordings obtained from new cells in the same slices revealed that washout of CGP resulted in a return to an average of 0 ± 0 APs/GPSP (n = 3).

**Cell-attached recordings confirm that CA3 pyramidal cells are excited during GPSP in CGP 55845A**

To ensure that the intracellular recording was not distorting the natural firing behavior of the cell, cell-attached recordings from visualized CA3 pyramidal cells were also made (Fig. 2). Simultaneous field potentials were recorded from CA3 s. lacunosum-moleculare. These recordings were done in 4-AP and glutamate receptor antagonists before and after the addition of CGP. The first 200 ms of the field GPSP was considered to represent the hyperpolarizing GABA_A component of the intracellular GPSP. The remainder of the field GPSP will be referred to here as the “later phase” of the field GPSP. Four of eight cells did not fire during the first 200 ms of any GPSPs and the other four fired zero to three spikes during the first 200 ms of the field GPSPs.

Before the addition of CGP, the CA3 cells fired a mean of 1 ± 2 spikes during the later phase of the field GPSP (n = 8). After the addition of CGP, the CA3 pyramidal cells fired a cluster of spikes (mean of 24 ± 12 spikes) during the later phase of the field GPSPs. Data for the individual cells can be

![Figure 1](http://jn.physiology.org/)

**A** control

**B** after CGP 55845A

**C** control

**D** after CGP 55845A

**FIG. 2.** Simultaneous cell-attached and field recordings demonstrated that CGP 55845A increases the excitation mediated by the depolarizing GABA component of the GPSP. A–C: recordings in 4-aminopyridine (4-AP) and ionotropic glutamate receptor antagonists. Top: a cell-attached recording from a CA3 pyramidal cell; bottom: a simultaneous field recording from CA3 s. lacunosum-moleculare. Vertical lines in the cell-attached traces are action potential currents. *, the GPSPs that are expanded in C. A: before the addition of CGP 55845A, no clusters of action potentials occurred during the field GPSPs. B: after the addition of CGP 55845A, clusters of action potentials occurred during the field GPSPs. C: expanded time scale showing that after the addition of CGP 55845A, a cluster of action potentials occurred during the later phase of the field GPSP. D: the number of action potentials during the field GPSP increased with the addition of CGP 55845A. Graph shows the mean number of action potentials during the later phase of the GPSP before (control) and after CGP 55845A for 8 cells in 8 different slices. Mean of the means was 1 ± 2 in control increasing to 24 ± 12 with the addition of CGP 55845A (P = 0.0005).
seen in Fig. 2D. Before CGP the mean percentage of GPSPs which had at least one spike was 30 ± 38% (n = 8). After CGP 100% of all GPSPs recorded showed spikes during the late phase of the GPSP (mean = 100%, SD 0, n = 8). Both the increase in the mean number of action potentials during the later phase of the GPSP (P = 0.0005) and the increase in the percentage of GPSPs that had at least one spike during the later phase (P = 0.001) were highly significant. This finding confirms that CA3 pyramidal cells are excited during the later, depolarizing phase of the GPSP in the presence of CGP.

Addition of GABA_A antagonist allowed comparison of time course of GABA_B and GABA_D components of GPSC

In this group of experiments, GPSCs were recorded from CA3 pyramidal cells in whole cell voltage-clamp in 4-AP and blockers of ionotropic glutameric transmission (Perkins and Wong 1996). To investigate whether the postsynaptic GABA_D and GABA_B components of the GPSC overlap, the GPSC was recorded before and after addition of gabazine. Gabazine blocks the GABA_A and GABA_D components of the GPSC but not the GABA_B component.

GPSCs were evoked at an interval (25–40 s) that was 10–20 s shorter than the natural interval of spontaneous GPSCs in the submerged slices. The stimulating Electrode was in the hilus rather than nearby the recording electrode (cf. Perkins 1999) so that the immediate presynaptic axons would not be directly stimulated by the external stimulus. In addition, the recording was made at a holding potential just positive to GABA_A reversal potential so that the amplitude of the early GABA_A component of the event would be relatively small. As the gabazine washed in, the GPSC amplitude decreased and the delay between the stimulus and the onset of the GPSC increased. This was followed by an inability of the stimulus to trigger a response for several minutes. Spontaneous GPSCs also stopped for several minutes before resuming at a reduced rate (see also Michelson and Wong 1994). After several minutes, the stimulus once again evoked GPSCs, but these were monophasic (Fig. 3B). These monophasic events are blocked by CGP (Perkins and Wong 1996), indicating that they are GABA_B-mediated.

An overlay of the triphasic GPSC and the GABA_B-mediated monophasic GPSC (Fig. 3C) shows that the falling phase of the responses overlapped nicely but that there was a prominent delay (234 ± 57 ms) between the onset of the triphasic GPSC and the onset of the GABA_B-mediated monophasic GPSC (n = 3). Figure 3D shows the subtracted trace A − B, which is the biphasic GPSC composed of outward GABA_A component and inward GABA_D component, overlaid with trace B, which is the GABA_B component. This overlay illustrates that the postsynaptic GABA_D and GABA_B responses overlapped substantially.
in time, with the peak of the GABA_B response occurring at nearly the same time as the peak of the GABA_D response ($n = 3$).

Peak conductance of spontaneous GABA_B-mediated monophasic GPSC

The conductance of the spontaneous GABA_B-mediated monophasic GPSC was measured in 4-AP, glutamate antagonists, and the GABA_A antagonist bicuculline or gabazine. The peak conductance of spontaneous events was measured by commanding 5-mV hyperpolarizing steps repeatedly during the baseline and throughout the spontaneous GABA_B-mediated monophasic GPSC. The baseline input resistance [$R_{\text{in(base)}}$] and the input resistance during the peak amplitude of the event [$R_{\text{in(peak)}}$] were determined as outlined in METHODS, and peak GABA_B-mediated conductance [$g_{\text{GABA(B)}}$] was determined by the equation: $g_{\text{GABA(B)}} = 1/R_{\text{in(peak)}} - 1/R_{\text{in(base)}}$. Conductance measurements included in the analysis were made in neurons that had been recorded from for $< 20$ min. The GABA_B-mediated conductance measured at or near the peak of the monophasic GPSC was $13 \pm 5$ nS ($n = 8$), which indicates that the membrane conductance was approximately doubled when the GABA_B receptor-activated channels were open. (Baseline conductance, $1/R_{\text{in(base)}}$, measured in same recordings was $14 \pm 7$ nS; $n = 8$).

Addition of GABA_B antagonist confirmed delayed onset of GABA_B component of GPSC

To further investigate the apparent delay between the onset of the GPSC and the onset of the GABA_B component, GPSCs were recorded before and after the addition of the GABA_B antagonist CGP, and then the traces were subtracted (Fig. 4). GPSCs were evoked as above and were recorded from CA3 pyramidal cells in whole cell voltage-clamp. CGP blocked the GABA_B component of the GPSC, leaving a biphasic GABA_A-GABA_D GPSC (Fig. 4B). Subtraction of the traces recorded before and after CGP revealed the current which was blocked by the GABA_B antagonist (plus the inverse of any current induced by GABA_B antagonist, see following text.) Comparison of Fig. 4A and C revealed a delay between the onset of the GPSC and the onset of the GABA_B component of the GPSC (delay of $331 \pm 32$ ms, $n = 3$), in confirmation of the data presented in Fig. 3. In several cells, 5-mV voltage steps were commanded during the early part of the GPSC to measure conductance. GABA-mediated conductance ($g_{\text{GABA}}$) was determined by the equation: $g_{\text{GABA}} = 1/R_{\text{in(during GPSC)}} - 1/R_{\text{in(base)}}$. There was no consistent increase or decrease in the $g_{\text{GABA}}$ measured at times before 385 ms with addition of CGP. Note that the test steps subtracted nearly perfectly in Fig. 4C. Test steps delivered at points between 55 and 115 ms after the onset of the GPSC gave a $g_{\text{GABA}}$ in CGP of $441 \pm 177$ nS (which was a change of $-11 \pm 52\%$, not significant, $P = 0.3; n = 5$.) Test steps delivered at points between 360 and 385 ms after the onset of the GPSC gave a $g_{\text{GABA}}$ in CGP of $294 \pm 177$ nS (which was a change of $-13 \pm 52\%$, not significant, $P = 0.3; n = 5$). Although CGP did not increase the conductance at times before 385 ms, a comparison of the biphasic GPSC in Fig. 3D with the biphasic GPSC recorded in the presence of CGP (Fig. 4B) suggests that it may have increased the duration of the GABA_D component of the GPSC (see following text for further experiments addressing this point).

Increase in conductance late in the GPSC with addition of CGP 55845A

The effect of CGP on the excitatory nature of the depolarizing GABA component of the GPSP (Figs. 1 and 2) could be due solely to a postsynaptic effect of removing an overlapping inhibitory GABA_B response or could be due also to a presynaptic effect of increased or prolonged transmitter release. If the effect was solely postsynaptic, one would expect a small decrease in conductance late in the GPSC as the GABA_B
component was blocked. If the effect was also presynaptic, one would expect an increase in conductance late in the GPSC. Five-millivolt voltage steps were applied at two or three points during the evoked GPSC to measure the conductance. Figure 5 shows the evoked GPSC before and after the addition of CGP. In agreement with Fig. 4, the test steps before and after CGP revealed little change in conductance during the GABA_A component and little change during the apparent peak of the GABA_D component; however, at the latest conductance checkpoint, the current response to the test step increased by 70%, indicating an increase in \( g_{\text{GABA}} \) late in the event. Test steps delivered at points 675–800 ms after the onset of the GPSC revealed an increase in \( g_{\text{GABA}} \) after CGP of 183 ± 119% to 29 ± 15 nS (\( n = 4 \), \( P = 0.015 \)).

**CGP 55845A increased the duration of biphasic spontaneous GPSCs**

To further investigate an apparent effect of CGP on GABA release, spontaneous biphasic GPSCs in which the GABA_B component had already been blocked from inside the cell were recorded before and after CGP. The GABA_B component was blocked from inside the cell using 10 mM QX-314 in the recording pipette (Perkins and Wong 1996). After the GABA_B component was completely blocked with intracellular QX-314, CGP was added to the bath solution (Fig. 6A). In three of three cells, the duration of the GABA_D component of the spontaneous biphasic GPSCs increased significantly with the addition of CGP (Fig. 6B; \( t \)-test on each cell separately). The mean GABA_D duration increased from 875 ± 215 to 1,883 ± 542 ms (\( P = 0.038 \), \( n = 3 \)). The mean peak amplitude of the GABA_D component also increased with the addition of CGP (\( P = 0.034 \)), and the mean interval between GPSCs increased as well (from 52 ± 3 to 68 ± 5 s; \( P = 0.029 \)). The increase in amplitude was probably due in part to the increase in mean interval; the shortest preceding intervals were associated with the smallest GABA_D components. GPSCs with similar preceding intervals were chosen for the comparison shown in Fig. 6B. To ensure that the CGP was increasing the duration of the GABA_D component and not adding some new component to the event, such as a K⁺-mediated current, the reversal potential of the GABA_D component of the GPSC was measured in CGP by recording GPSCs at potentials on both sides of reversal. The GABA_D component reversed at −46 ± 3 mV (Fig. 6C; \( n = 3 \)).

**Blocking GABA_B receptors increased epileptiform activity in 4-AP**

The following experiments were done in 4-AP in the presence of intact glutamatergic transmission to study the relation of GPSPs to epileptiform activity. In this condition, some GPSPs were followed by one or more afterdischarges. Adding CGP to block GABA_B receptors increased epileptiform activity (Fig. 7). The percentage of GPSPs followed by one or more afterdischarges increased from 32 ± 30% before CGP to 99 ± 2% after CGP (Fig. 7C; \( P = 0.000001 \), \( n = 16 \)). The afterdischarges per GPSP increased from 1 ± 1 to 6 ± 2 (\( P = 0.00000001 \), \( n = 16 \)). Data for individual slices are shown in Fig. 7D. CGP also caused an increase in the interval between GPSPs from 15 ± 3 to 20 ± 4 s (\( P = 0.000008 \), \( n = 16 \)). That being the case, the total number of afterdischarges occurring in a 10-min period before and after CGP was also compared. In slices exposed to 4-AP for 1 h before the addition of CGP, CGP caused a significant increase in the number of afterdischarges per 10 min period from 46 ± 47 before CGP to 168 ± 80 after CGP (\( P = 0.00005 \), \( n = 9 \)). To control for time in 4-AP, a group of slices was exposed to 4-AP for 2 h before the addition of CGP. The number of afterdischarges per 10-min period increased from 49 ± 57 before CGP to 183 ± 111 after CGP (\( P = 0.005 \), \( n = 7 \)) in the 2-h group. There was no significant difference in the number of afterdischarges before \( P = 0.9 \) or after \( P = 0.7 \) CGP between the 1- and 2-h groups.

To measure the change in the number of afterdischarges per epileptiform event with the addition of CGP, the analysis was repeated excluding GPSPs that had no associated afterdischarges. The average number of afterdischarges per epileptiform event increased from 4 ± 3 before CGP to 6 ± 3 after CGP (\( P = 0.001 \), \( n = 14 \)). (Two cells had no afterdischarges before CGP and thus were not included in the analysis.) The maximum number of afterdischarges after any single GPSP in
each slice increased from 5 ± 5 before CGP to 9 ± 3 after CGP (P = 0.0005, n = 16).

In the absence of 4-AP, exposure of slices to CGP for 30 min to 1 h did not induce epileptiform activity (26 slices from 17 guinea pigs; field recordings; glutamatergic transmission intact). All slices subsequently developed epileptiform activity when exposed to 4-AP. This finding is in agreement with data from rat neocortical slices (Badran et al. 1997; Sutor and Luhmann 1998); however, see Uusisaari et al. (2002).

**DISCUSSION**

The whole cell voltage-clamp data presented here definitively show for the first time that the depolarizing GABA and GABA_B components of the GPSC peak at nearly the same time, allowing the GABA_B-mediated hyperpolarization to suppress the excitation mediated by the depolarizing GABA response. The data presented here also show that the likelihood of a pyramidal cell firing action potentials during the depolarizing component of the GPSP increased dramatically in the presence of the GABA_B receptor antagonist CGP. This enhanced excitation mediated by the depolarizing component of the GPSP in CGP increased the likelihood of a GPSP triggering an epileptiform event to 99% and significantly increased the number of afterdischarges per epileptiform event. The data indicate that the GABA_B receptor antagonist increased the excitatory effect of the depolarizing GABA component both via a block of the overlapping postsynaptic GABA_B-mediated potential on the pyramidal cell and via an increase in the duration of the depolarizing GABA response. The data presented here support a model in which the depolarizing GABA component of the GPSP triggers each epileptiform event recorded in the presence of 4-AP.

**Postsynaptic interaction of depolarizing GABA and GABA_B**

Our whole cell voltage-clamp recordings of GPSCs before and after gabazine (Fig. 3) reveal that the timecourse of the GABA_A and GABA_B components of the GPSC overlap substantially and that their peaks occur at nearly the same time. This postsynaptic timing promotes GABA_B-component control over the excitation mediated by the depolarizing GABA component. The GABA_B conductance is small compared with that of the GABA_A (RESULTS); however, the driving force is large. The calculated GABA_B reversal potential is −104 mV with an extracellular K^+ concentration ([K^+]o) of 2.5 mM. We hypothesize that the strong hyperpolarizing effect of the GABA_B component of the GPSP usually prevents the depolarizing GABA component from eliciting an action potential in the postsynaptic neuron.

**Depolarizing GABA response in adults**

In immature brain, the GABA response is depolarizing because of a high-standing intracellular [Cl^-] due to the late expression of the KCC2 K^+/Cl^- cotransporter (Rivera et al. 1999; Zhang et al. 1991). In contrast, the synaptic depolarizing
GABA response in adult hippocampus is typically part of a biphasic hyperpolarizing-depolarizing response (Alger and Nicoll 1982) and has been variously attributed to Cl\(^-\) accumulation due either to GABA-mediated Cl\(^-\) influx or to a reduction in KCC2 action caused by a build-up of extracellular K\(^+\), to a nonsynaptic K\(^+\) current, to a bicarbonate conductance, or to some combination thereof (Grover et al. 1993; Isomura et al. 2003; Kaila et al. 1997; Lamsa and Kaila 1997; Perkins 1999; Perkins and Wong 1996, 1997; Smirnov et al. 1999; Staley et al. 1995). In fact, the adult “depolarizing GABAs” may not all be the same; different mechanisms may underlie the depolarizing GABA responses seen under different conditions. Work done in this lab (Perkins 1999; Perkins and Wong 1996, 1997) indicates that the GABA\(_D\) component of the GPSC recorded in 4-AP is a bicarbonate/Cl\(^-\) conductance.

**Effect of CGP 55845A on transmitter release**

CGP caused an increase in the duration and late conductance of the depolarizing component of the GPSC (Figs. 5 and 6). These results suggest that CGP caused an increase in the duration of GABA release. We propose that CGP caused an increase in transmitter release either by acting at autoreceptors on the presynaptic terminals or by acting upstream at the interneuron cell body or dendrites.

Synaptically released GABA can act at autoreceptors to reduce transmitter release (Deisz and Prince 1989; McCarren and Alger 1985). CGP block of autoreceptors has been shown to reduce paired-pulse depression—the phenomenon in which the second of two evoked inhibitory postsynaptic potentials is smaller than the first (Davies and Collingridge 1993; Davies et al. 1990). Block of GABA\(_B\) autoreceptors has also been hypothesized to underlie the increased depolarizing GABA response recorded in response to a “theta burst” stimulation protocol in the presence of GABA\(_B\) receptor antagonist (Cobb et al. 1999). Applying this autoreceptor scenario to the 4-AP model, GABA released during the early part of the GPSP would bind to GABA\(_B\) autoreceptors and limit the amount of GABA released during the later phases of the GPSP. CGP would block this effect, which would increase the conductance and duration of the GPSP. It has been shown that CGP-sensitive paired-pulse depression occurs with intervals as short as 20 ms and is maximal at ~250 ms (Davies and Collingridge 1993). If the autoreceptor scenario is the correct explanation for the effect of CGP on GPSC duration, why was the conductance at test points before 385 ms unaffected by CGP (Fig. 5)? One possibility is that postsynaptic GABA receptors are saturated during the early but not the later part of the GPSC.

Instead we favor the hypothesis that CGP is working to increase the duration of GABA release by acting on the interneuron cell body or dendrites rather than on the terminals. GABA release in response to a spike invading the axon terminal may be already increased so much by the 4-AP that a block of GABA\(_B\) autoreceptors would not cause substantial additional increase; evidence of maximization of transmitter...
release by 4-AP in hippocampus has been presented by others (Klapstein and Colmers 1992; Varma et al. 2002). An alternative to greater GABA release per spike is more spikes invading the axon terminal. Interneurons fire bursts of action potentials in 4-AP due to synaptic excitation mediated by the depolarizing GABA response (Michelson and Wong 1991). CGP would be expected to block any overlapping GABA<sub>B</sub>-mediated hyperpolarization on the interneuron (as seen here in the pyramidal cell) and thus may increase the number of action potentials per burst. Why in this scenario would the depolarizing GABA component of the GPSC be preferentially enhanced? The explanation may be that interneurons fire a longer train of action potentials in CGP but not at a higher frequency. This change in firing behavior in CGP would increase the duration of the GPSP in the pyramidal cell but not the early conductance. Alternatively, as hypothesized in an earlier paper (Perkins 1999), separate groups of presynaptic interneurons may mediate the depolarizing and hyperpolarizing GABA responses on the pyramidal cell. It is possible that CGP may increase firing preferentially in the subset of interneurons which mediate the depolarizing GABA response in pyramidal cells. This hypothesis predicts that one would find a larger postsynaptic GABA<sub>B</sub> response on those “GABA<sub>D</sub> interneurons.”

Depolarizing GABA and [K<sup>+</sup>]<sub>o</sub>

Other laboratories have measured a rise in extracellular [K<sup>+</sup>]<sub>o</sub> that accompanies the GPSP in 4-AP (Avoli et al. 1996b; Lamsa and Kaila 1997). It has been hypothesized that the rise in [K<sup>+</sup>]<sub>o</sub> is responsible for the triggering of afterdischarges in 4-AP (Avoli et al. 1996a). In accordance with this hypothesis, Avoli and colleagues have measured a larger rise in [K<sup>+</sup>]<sub>o</sub> and enhanced epileptiform activity, in the presence of CGP in juvenile rats (Motalli et al. 2002).

The increased pyramidal cell firing seen here during the GPSP in CGP would certainly be expected to cause a rise in [K<sup>+</sup>]<sub>o</sub>. The experiments presented here do not exclude the possibility of the reverse sequence—that a rise in [K<sup>+</sup>]<sub>o</sub> may play a role in causing pyramidal cell firing during the GPSP. However, our voltage-clamp experiments indicate that the depolarization during the GPSP is associated with an increase in conductance, which a depolarization mediated by a rise in [K<sup>+</sup>]<sub>o</sub> would not be. In addition, we show that the extended depolarizing GABA response seen in CGP is reversed easily at about −46 mV (Fig. 6), which would not be the case for a K<sup>+</sup>-mediated current caused by a rise in [K<sup>+</sup>]<sub>o</sub> (e.g., Kaila et al. 1997).

GABA<sub>B</sub> receptor block, depolarizing GABA, and epileptiform activity in slices

It has been shown previously that a GPSP precedes each afterdischarge event in slices exposed to 4-AP (Avoli et al. 1996a). Our data support a preeminent role for the depolarizing GABA component of the GPSP in particular in initiating the epileptiform discharges. Our data (Figs. 1, 2, and 7) suggest that if the depolarizing GABA component of the GPSP is not sufficiently depolarizing to cause action potentials in at least some of the CA3 pyramidal cells, then no afterdischarges accompany the GPSP. In solution containing 4-AP but no CGP, 9 of 16 slices showed an average of <1 afterdischarge per GPSP and 2 of those 9 slices had no afterdischarges at all. We attribute this lack of epileptiform activity to the fact that in the presence of an intact, overlapping GABA<sub>B</sub>-mediated hyperpolarization, the depolarizing GABA component of the GPSP was often unable to depolarize pyramidal cells sufficiently to trigger spikes: cell-attached recordings revealed that five of eight cells had an average of 0 action potentials per depolarizing GABA component in solution containing 4-AP but no CGP. Blocking GABA<sub>B</sub> receptors dramatically increased the percentage of GPSPs in which the depolarizing component triggered action potentials (Figs. 1 and 2) and dramatically increased the percentage of GPSPs which were followed by at least one afterdischarge when glutamatergic transmission was intact (Fig. 7).

The mean number of afterdischarges per epileptiform event was also increased by CGP. CGP may lengthen the epileptiform event either by blocking the depressive effect of GABA on glutamate release, which is mediated by GABA<sub>B</sub> receptors on glutamatergic terminals (Isaacson et al. 1993), or by a reduction in postsynaptic GABA<sub>B</sub>-mediated inhibition (Dutar and Nicoll 1988; Newberry and Nicoll 1985; Thalmann 1988b). One or both of these mechanisms have been suggested to be involved in the enhancement of epileptiform discharges caused by GABA<sub>B</sub> receptor antagonist in other models of epileptiform activity (Badran et al. 1997; Hussáz and Merlin 2004; McLean et al. 1996; Scanziani et al. 1994; Sutor and Luhmann 1998). Alternatively, we hypothesize that the increase in the number of afterdischarges in CGP seen here is due to the prolonged depolarization of pyramidal cells mediated by the enhanced depolarizing GABA component of the GPSP. The GABA<sub>D</sub> component of the spontaneous GPSC was up to 2 s long in CGP, which is comparable to the duration of the longer epileptiform discharges seen in CGP when glutamatergic transmission was intact.

We favor a model in which the depolarizing component of the GPSP triggers an epileptiform event by synchronously exciting CA3 pyramidal cells. The pyramidal cells would then use glutamatergic transmission to excite one another to create the afterdischarges. We further propose that the length of the GABA-mediated depolarization of pyramidal cells plays a role in determining the length of the epileptiform event.

Loss of GABA<sub>B</sub> receptor function, depolarizing GABA, and epilepsy

Block of GABA<sub>B</sub> receptors alone, without the addition of 4-AP, did not cause epileptiform events in our experiments. We attribute this result to the fact that the brain has been cut into slices. GABA<sub>B</sub> receptor knockout mice have epilepsy, but slices from these mice do not show epileptiform activity without the addition of 4-AP or some other convulsant (Brown et al. 2003). Likewise, areas of brain expressing seizure discharges in humans do not necessarily show epileptiform events when excised and cut into slices (Hwa et al. 1991; Schwartzkroin et al. 1983). Studies examining epileptiform activity in human tissue from epilepsy patients typically use 4-AP, bicuculline, or low Mg<sup>2+</sup> to elicit the activity (e.g., Avoli et al. 1987; D’Antuono et al. 2004; Tasker et al. 1992). It is known that the firing activity of neurons is lower in in vitro slice preparations than it is in vivo (Fellous et al. 2003); 4-AP may promote epileptiform activity in part by increasing the basal level of spiking (Traub et al. 2001).
On the other hand, in vivo experiments show that loss of GABA_B receptor function can cause epilepsy. GABA_B subunit knockout mice have clonic and tonic-clonic seizures (Prosser et al. 2001; Schuler et al. 2001). In addition, microinjection of GABA_B receptor antagonist into the hippocampus can induce focal seizures in adult rats (Vergnes et al. 1997), and intraperitoneal administration of GABA_B antagonist can induce tonic-clonic seizures in mice (Badran et al. 1997). Future data show that epilepsies induced in ways other than via direct block of GABA_B receptors can be associated with loss of GABA_A receptor function. For example, pyramidal cells in hippocampal slices taken from rats with kindling-induced chronic temporal lobe epilepsy have neither presynaptic autoreceptor nor postsynaptic GABA_B receptor function (Mangan and Lothman 1996). In addition, brain tissue removed in surgery from persons with intractable seizures also has markedly reduced presynaptic autoreceptor (Deisz 1999; D’Antuono et al. 2004) and postsynaptic (Deisz 1999) GABA_B receptor function. Based on the experiments presented here, we hypothesize that loss of GABA_A receptor function leads to epilepsy because of a loss of control over excitation mediated by the depolarizing GABA response. Loss of GABA_A autoreceptor function would allow sustained GABA release in response to a train of presynaptic spikes (similar to the effect of 4-AP at the axon terminal), generating a prolonged depolarizing GABA response in the pyramidal cells. Loss of the postsynaptic GABA_A-mediated hyperpolarization on the pyramidal cell would allow the overlapping depolarizing GABA response to excite the cell and trigger afterdischarges. In fact, in recent studies from two different groups, pyramidal cells in brain tissue taken from persons with intractable temporal lobe epilepsy showed depolarizing, excitatory GABAergic events (Cohen et al. 2002; Deisz 2002; Deisz et al. 1998). During the interictal events recorded in one of those studies (Cohen et al. 2002), interneurons fired a burst of action potentials, and a subset of pyramidal cells experienced a GABA-mediated synaptic depolarization which triggered action potentials. This electrophysiology in tissue from epileptic human brain is reminiscent of the excitatory GSPPs that we see here in guinea pig hippocampal slices exposed to 4-AP and CGP.

In conclusion, we show here that loss of GABA_A receptor-mediated control of the depolarizing GABA response can lead to increased GABA-mediated excitation of the pyramidal cells and increased epileptiform activity.

ACKNOWLEDGMENTS

The authors thank S. Young, R. Bianchi, and L. Merlin for helpful discussion.

GRANTS

This project was sponsored by the Epilepsy Foundation and by National Institute of Neurological Disorders and Stroke Grant NS-047435 to K. L. Perkins.

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J Neurophysiol • VOL 93 • MAY 2005 • www.jn.org


