GHB Depresses Fast Excitatory and Inhibitory Synaptic Transmission via $GABA_B$ Receptors in Mouse Neocortical Neurons

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Gamma-hydroxybutyrate (GHB) is a drug of abuse which induces sedation and euphoria. However, overdoses can severely depress the level of consciousness or can be fatal especially when combined with other substances. Studies have suggested that the GHB-effects are mediated via actions on thalamocortical pathways and local neocortical circuits, although the effect of GHB at the level of single neocortical neurons is not clear. Using whole-cell patch-clamp recordings, we studied the effects of GHB on neocortical neurons in brain slices from 12- to 33-day-old mice. We found that GHB depressed the frequency and amplitude of GABAergic and glutamatergic spontaneous inhibitory and excitatory post-synaptic currents (IPSCs and EPSCs) driven by presynaptic action potential firing, while the amplitude and frequency of Ca^{2+} entry-independent miniature IPSCs were not affected. Using minimal stimulation, GHB reduced the probability of release at inhibitory synapses onto neocortical layer 2/3 pyramidal cells. Also, GHB directly hyperpolarized layer 2/3 non-pyramidal cells by up to 11 mV and inhibited action potential firing. All these effects of GHB were mediated via $GABA_B$-receptors. In conclusion, GHB activates both pre- and postsynaptic $GABA_B$-receptors in neocortical neurons participating in fast synaptic transmission, leading to a powerful depression of neocortical network activity. We propose that $GABA_B$-receptor antagonists may be useful in the treatment of acute GHB intoxication.

Introduction

Gamma-hydroxybutyrate (GHB) is a naturally occurring substance in the brain, where it is synthesized locally from gamma-aminobutyric acid (GABA) (Bernasconi *et al.*, 1999). Due to its profound effects in the central nervous system, GHB has been used pharmacologically as an anesthetic agent (Kleinschmidt *et al.*, 1999), in the treatment of alcoholism (Gallimberti *et al.*, 1989) and opioid dependency (Rosen *et al.*, 1996), and in sleep disorders (Mamelak *et al.*, 1986).

Recently, GHB and its precursor gamma-butyrolactone (GBL) have become popular drugs of abuse due to their ability to induce euphoria, hallucinations, sedation and relaxation (Galloway *et al.*, 1997; Bernasconi *et al.*, 1999). Pharmacodynamically, GHB has a narrow concentration window within which the desired effects are obtained and aggravating side effects are absent. Thus, during abuse, when the GHB concentration is not monitored, toxic effects can easily become manifest as headache, vomiting, agitation, myoclonus and seizures, or as a severe depression of consciousness, and of cardio-respiratory function (Ingels *et al.*, 2000). Although most patients recover quickly with no apparent sequelae, fatalities occasionally occur (Timby *et al.*, 2000), primarily because there are currently no well-established antidotes.

As a research tool, GHB induces epileptic absence seizures in rats and mice (Aizawa *et al.*, 1997; Hu *et al.*, 2000). Studies have suggested that GHB modulates transmitter release and neuronal excitability in thalamic (Liu *et al.*, 1992; Emri *et al.*, 1996), neocortical (Hu *et al.*, 2000) and hippocampal (Xie and Smart, 1992a,b) regions in the brain. It was suggested that GHB acts via $GABA_B$-receptors, although the existence of distinct GHB-receptors have also been proposed (Bernasconi *et al.*, 1999). Nevertheless, in many cases the actions of GHB on GABAergic (Xie and Smart, 1992a; Hu *et al.*, 2000), glutamatergic (Xie and Smart, 1992a,b; Berton *et al.*, 1999) and dopaminergic (Engberg and Nissbrandt, 1993; Madden and Johnson, 1998; Erhardt *et al.*, 1998) neurons are blocked by $GABA_B$-receptor antagonists.

The actions of GHB have not been studied at the level of single neurons in the neocortex. Therefore we used whole-cell patch-clamp recordings to examine its effects on membrane properties and synaptic transmission in neocortical and hippocampal neurons in mouse brain slices. We determined that GHB, by acting on pre- and postsynaptic $GABA_B$-receptors (Mott *et al.*, 1999), profoundly depresses neuronal activity and fast synaptic transmission in the neocortex, especially under conditions with a high level of action potential (AP) firing.

Materials and Methods

Slice Preparation and Electrophysiological Recordings

Twelve- to 33-day-old C57Black6 mice [P12–P33, 22.6 ± 0.6 days (mean ± SEM), 49 mice; for neocortical layer 2/3 cells: P12–P33, 22.3 ± 0.8 days, 37 mice, n = 62 cells; for hippocampal CA1/CA3 pyramidal cells: P20–P29, 23.5 ± 0.8 days, 10 mice, n = 17 cells; for dentate granule cells: P15–P30, 22.9 ± 1.2 days, 10 mice, n = 12 cells] were anesthetized with halothane before decapitation, in accordance with the guidelines of the UCLA Office for Protection of Research Subjects. The brains were removed and placed into an ice-cold artificial cerebrospinal fluid (aCSF) containing (mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 D-glucose, pH 7.3, when bubbled with 95% O_2 and 5% CO_2. In several experiments, L-ascorbic acid (1 mM) and pyruvic acid (1 mM) were added to the extracellular solutions to improve slice viability. The brain was glued to a platform, and 350-μm-thick coronal slices were cut with a Leica VT1000S vibratome. The slices were stored at room temperature in bubbled aCSF until transferred to the recording chamber.

During recordings, the slices were continuously perfused with bubbled aCSF at 30–32°C. Somatic recordings were made from visually identified neurons (Zeiss Axioscope infrared differential interference contrast (IR-DIC) videomicroscopy, 40× water immersion objective) with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Patch electrodes were pulled (Narishige PP-83, Tokyo) from borosilicate glass (o.d. 1.5 mm, i.d. 1.10 mm; Garner, Claremont, CA) and were filled with a solution containing (in mM): 140 CsCl, 2 $MgCl_2$, 10 HEPES, and titrated to a pH of 7.2 with CsOH (osmolarity 275–290 mOsm, Wescor 5520 osmometer). Voltage-clamp recordings were made at a holding potential (V_h) of –70 mV, unless otherwise stated. Where inhibitory postsynaptic currents (IPSCs) were recorded without kynurenic acid, the electrode solution contained (in mM): 135 Cs-gluconate, 10 CsCl, 5 TEA, 0.1 EGTA, 15 HEPES, pH 7.2 with CsOH. For current-clamp recordings the electrode contained (in mM): 135 K-methylsulfate, 10 KCl, 2 $MgCl_2$, 0.2 Tris–GTP, 2 MgATP, 10 HEPES, pH 7.2, with KOH. The resistance of the electrodes was between 3 and 6 MΩ when filled with solution. The series resistance

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and whole-cell capacitance were monitored repeatedly during the experiment, and recordings were discontinued if the series resistance increased by >50%. The series resistance was always compensated by 70–85% using lag values of 7–8 μs. For minimal stimulation (Nusser *et al.*, 1998), bipolar wires were inserted in an aCSF-filled theta glass (o.d. 2 mm) pulled to a tip size of ~2 μm and positioned 15–30 μm from the soma. For these experiments, the Ca^{2+} and Mg^{2+} concentrations were altered to 1 and 3 mM, respect- ively, to reduce the probability of release. Current pulses (20–40 μs duration) of increasing intensity were applied until IPSCs eventually appeared. IPSCs had a typical mean amplitude of ~50 pA (including failures) and did not increase further with larger stimulus intensities.

Data Analysis

Recordings were low-pass filtered (8-pole Bessel, Brownlee 210A) at 3 kHz (1 kHz for minimal stimulation) and digitized on-line at 20 kHz using a PCI-MIO 16E-4 data acquisition board (National Instruments, Austin, TX). Spontaneous synaptic events were detected in 30 s epochs with amplitude- (typical threshold 6–8 pA) and kinetics-based criteria using custom-written LabView 5.1 based software (National Instruments) running on a Pentium III IBM/AT compatible computer. Traces were imported into a custom-written analysis program, where currents and voltages were analyzed and averaged, and amplitudes and kinetics were measured. Paired and unpaired *t*-tests were performed in Microsoft Excel (v. 2000), while Kolmogorov–Smirnov (KS) tests were done using Stastitica (v. 5.1, StatSoft). Data are expressed as means ± SEM, with *n* indicating the number of cells.

Solutions and Drugs

GHB, picrotoxin, kainic acid, kynurenic acid, L-ascorbic acid and pyruvic acid were purchased from Sigma, while tetrodotoxin was from Calbiochem (La Jolla, CA). As an Na^+ salt, GHB (up to 10 mM) had no effect on the osmolarity of the extracellular solutions. The $GABA_B$-receptor antagonists CGP 56999A and CGP 56433 were kindly provided by Dr Wolfgang Froestl (Novartis, Basel, Switzerland). These CGP compounds are highly selective $GABA_B$-receptor antagonists, and have proven to be efficacious in brain slices at the concentrations used here (Pozza *et al.*, 1999).

Results

GHB Depresses the Frequency and Amplitude of Spontaneous GABAergic IPSCs in Neocortical Neurons via $GABA_B$-receptors

Spontaneous IPSCs (sIPSCs) were recorded in the presence of the glutamate receptor antagonist kynurenic acid (3 mM) in Cl^- loaded layer 2/3 pyramidal cells of the sensorimotor cortex (Fig. 1*A*). sIPSCs appeared as rapidly rising inward currents (10–90% rise-time = 685 ± 70 μs) with a frequency (*f*) of 14.4 ± 2.1 Hz and mean amplitudes of 45.9 ± 4.3 pA (*n* = 15). The sIPSCs were blocked by picrotoxin (50 μM, *n* = 3, not shown), indicating that they are Cl^- currents mediated via $GABA_A$-receptors. Bath perfusion of GHB (10 mM, Fig. 1*A*) reduced *f* of sIPSCs by 47.8 ± 5.3% (*n* = 5, $P < 0.001$) and IPSC amplitudes by 20.3 ± 8.7% ($P = 0.08$). Amplitude distributions were significantly different in control and GHB ($P < 0.005$, KS tests), and showed that the drug preferentially inhibited the largest IPSCs. No changes in the kinetics of the sIPSCs were observed (Fig. 1*B*, right traces). The $GABA_B$-receptor antagonists CGP 56433 or CGP 56999A (both at 2 μM) completely blocked the effects of GHB on sIPSCs (Fig. 1*A*). *f* returned to 102.4 ± 2.3% of the control level (*n* = 4) and the effects of GHB on amplitude distributions were reversed (Fig. 1*B*, left panel), causing a slight enhancement of the prevalence of the largest IPSCs in three-quarters of the cells. The time-dependent effect of GHB and CGP 56433 on *f* are illustrated in Figure 1*C*, showing that both drugs exerted full effects on *f* following ~10 min perfusion. CGP

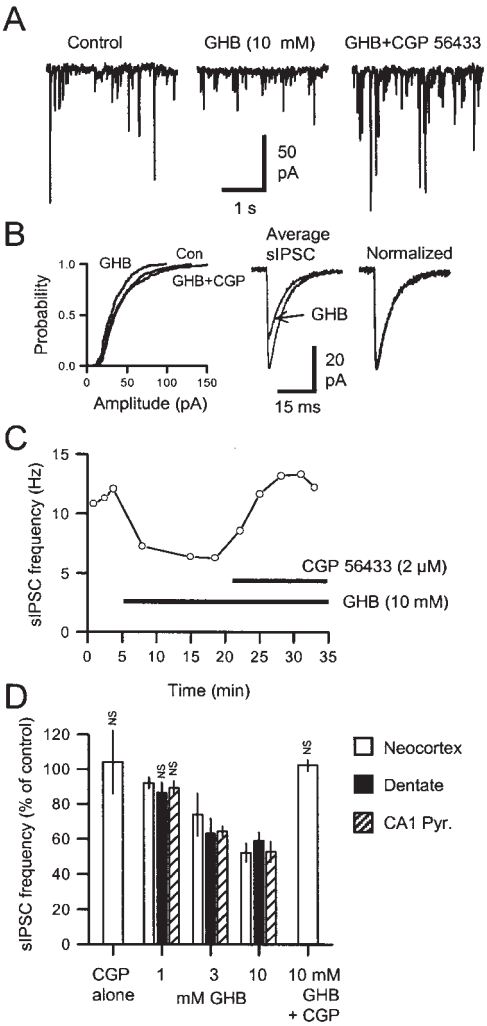

Figure 1. GHB depresses spontaneous IPSCs in neocortical pyramidal cells via $GABA_B$-receptors. (*A*) Spontaneous $GABA_A$-mediated IPSCs (sIPSCs) in a neocortical layer 2/3 pyramidal cell. sIPSCs occurred at a frequency (*f*) of 10.9 Hz with amplitudes of 41.0 ± 1.8 pA. GHB (10 mM) reduced *f* to 6.9 Hz and the amplitudes to 33.9 ± 1.2 pA. The $GABA_B$-antagonist CGP 56433 (2 μM) reversed these effects, increasing *f* to 12.4 Hz and the amplitudes to 45.6 ± 1.5 pA. (*B*) Amplitude distributions (from the cell in *A*) showing that GHB preferentially depressed the largest sIPSCs. Right: Averages of 100 sIPSCs from control and GHB. There were no differences in the IPSC kinetics. (*C*) sIPSCs frequency in a pyramidal cell, where GHB reduced *f* by ~50%, while CGP 56433 reversed the GHB-effect. (*D*) Histogram summarizing the effects of GHB and CGP on *f* of sIPSCs in layer 2/3 neocortical pyramidal cells (neocortex; open bars). *f* is also shown for dentate gyrus granule cells (closed bars) and CA1 pyramidal cells (hatched bars). Except where indicated (NS: non-significant), GHB depressed the sIPSC frequency at all concentrations ($P < 0.05$) similarly in all cell types.

56999A alone had no effects on the IPSCs in three other cells ($P > 0.05$). The summarized effects of GHB at 1, 3 and 10 mM on *f* in neocortical cells are shown in Figure 1*D* (open bars). We also measured the effect of GHB on sIPSC *f* in Cl^- loaded dentate gyrus granule cells (V_h = –80 mV, *n* = 12) and hippocampal CA1 pyramidal cells (V_h = –70 mV, *n* = 15), where GHB acted with a similar concentration-profile as in the neocortex (Fig. 1*D*).

Comparison of the Effect of GHB during High- and Low-frequency Neuronal Activity

In order to increase AP firing of GABAergic interneurons, kynurenic acid was withdrawn from the bath solution and 700–900 nM kainate was added. Layer 2/3 pyramidal cells were

recorded with Cs-gluconate pipettes and held at + 5 to + 7 mV. Under these conditions, sIPSCs appear as outward currents with minimal contamination by sEPSCs. The *f* of sIPSCs was 24.5 ± 3.0 Hz (n = 5, not shown) and GHB (10 mM) depressed *f* strongly, by 73.3 ± 9.3% ($P < 0.005$), while the amplitudes changed from 28.0 ± 2.7 to 20.6 ± 2.0 pA ($P < 0.01$).

In another set of experiments, miniature IPSCs (mIPSCs, not shown) were recorded in Cl^- loaded pyramidal cells in the presence of kynurenic acid (3 mM). mIPSCs were isolated using extracellular perfusion of either TTX, a sodium channel blocker, or Cd^{2+}, a Ca^{2+} channel blocker. In TTX (1 μM), mIPSCs occurred at 5.0 ± 1.2 Hz (n = 4) and GHB (10 mM) reduced the mIPSC frequency by 31.3 ± 5.5% (to 3.6 ± 1 Hz, $P < 0.05$). mIPSCs amplitudes did not change (35.3 ± 3.5 pA vs. 33.5 ± 1.9 pA, $P > 0.05$). Amplitude distributions were not different in ¾ cells ($P > 0.05$, KS tests) while the fourth cell showed a small decrease in amplitudes. 10–90% rise-times (740 ± 144 vs. 726 ± 158 μs) and τ_{decay} were not affected by GHB ($P > 0.05$). When mIPSCs were recorded in the presence of $CdCl_2$ (50–100 μM) to block presynaptic Ca^{2+} entry, the effect of GHB on *f* was abolished (*f* = 88.8 ± 15% of control, $P > 0.05$, n = 6), and amplitudes (109 ± 6% of control, $P > 0.05$) and kinetics were still unaffected by GHB.

GHB Hyperpolarizes Neocortical Non-pyramidal Cells via $GABA_B$-receptors

Next, we examined the postsynaptic effects of GHB in neocortical layer 2/3 non-pyramidal cells in current-clamp recordings. Cells with a small, round soma and lacking an apical dendrite were chosen under IR-DIC visualization. Using K-methylsulfate pipettes, the membrane potential (V_{mem}) was –70.2 ± 4.5 mV (range –52 to –91 mV, n = 9). GHB (10 mM) was tested on six of these presumed non-pyramidal cells. Every 8–10 s, a depolarizing current was injected for 500 ms to evoke repetitive AP firing (Fig. 2). Four neurons showed a fast-spiking firing pattern typical for GABAergic neurons (e.g. the cell in Fig. 2*A*), while two others were regular spiking non-pyramidal cells. GHB caused a hyperpolarization in all cells tested by up to 11 mV (mean 4.8 ± 1.5 mV, $P < 0.05$), and reduced the number of APs in response to current injection. The effects on V_{mem} and AP firing reversed slowly upon washing (Fig. 2*C*, left panel). In the presence of CGP 56999A or CGP 56433 (2 μM), GHB did not significantly change V_{mem}, where the hyperpolarization was reduced to 0.2 ± 0.3 mV (Fig. 2*C*, right panel and *D*, n = 4; two fast-spiking and two regular spiking non-pyramidal cells tested).

GHB Lowers the Probability of GABA-release via Presynaptic $GABA_B$-receptors in Neocortical Neurons

To test the effect of GHB at GABAergic terminals, IPSCs were evoked using perisomatic minimal stimulation in a total of 10 neocortical layer 2/3 pyramidal cells (Fig. 3). Mini-trains consisting of five pulses at 5 Hz evoked five IPSCs, termed $eIPSC_{1\text{-}5}$. $eIPSC_1$ had a mean amplitude of 50.6 ± 8.2 pA, while subsequent eIPSCs in the train displayed tetanic depression, leading to an $eIPSC_5$:$eIPSC_1$ ratio of 0.85 ± 0.1 (n = 5). GHB (10 mM) increased the number of failures on the first pulses in the train (Fig. 3*A*), thus reducing the mean amplitude of $eIPSC_1$ to 21.6 ± 7 pA (i.e. by 56.6 ± 11%, n = 4, $P < 0.01$). Later eIPSCs in the train were less affected by GHB which led to an increase in the $eIPSC_5$:$eIPSC_1$ ratio to 1.50 ± 0.2 ($P < 0.05$). CGP 56999A (2 μM) antagonized the effect of GHB (Fig. 3*B*,*C*) such that $eIPSC_1$ was not significantly depressed (from 43.4 ± 15 to 39.9 ± 14 pA, i.e. to 91 ± 5.5% of control, n = 3, $P > 0.05$). Also, CGP

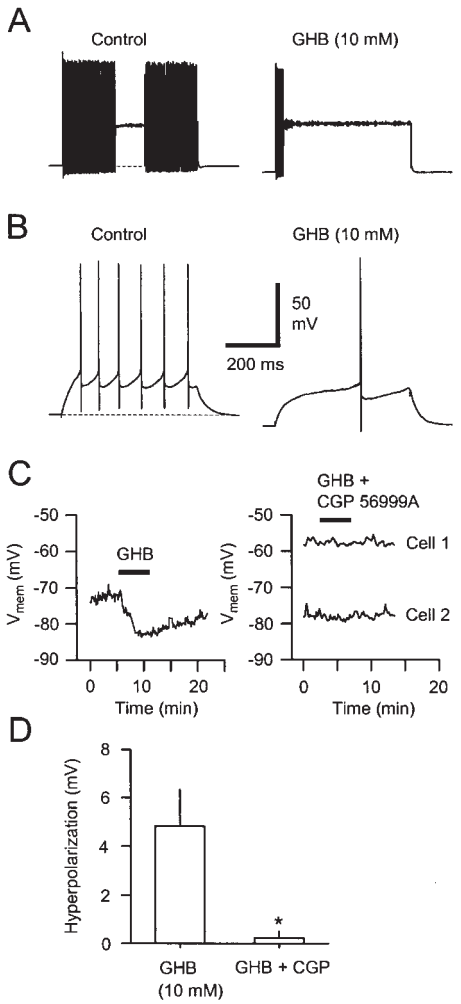

Figure 2. GHB hyperpolarizes neocortical non-pyramidal cells via $GABA_B$-receptors. (*A*) Current-clamp recording from a neocortical non-pyramidal cell (V_{mem} = –55 mV). A depolarizing current was injected every 8–10 s that showed that the neuron was fast-spiking. GHB (10 mM) hyperpolarized the neuron by 6 mV and strongly reduced the AP firing. (*B*) Recording from another non-pyramidal cell resting at –73 mV. The AP firing showed mild frequency accommodation. GHB also hyperpolarized this cell and reduced the AP firing. (*C*) Left: V_{mem} from the cell in *B*. GHB (10 mM) hyperpolarized the cell by 10.5 mV, and the effect reversed slowly upon washing. Right: V_{mem} from two other non-pyramidal cells (termed 1 and 2) where GHB was perfused with CGP 56999A (2 μM), which completely blocked the hyperpolarization. (*D*) Histogram summarizing the effect on neocortical non-pyramidal cells of GHB (10 mM) and GHB (10 mM) + CGP 56999A on V_{mem}. The hyperpolarization by GHB was 4.8 ± 1.5 mV (n = 6), compared with 0.2 ± 0.3 mV in the presence of CGP 56999A (n = 4, $P < 0.01$).

blocked the effects of GHB on short-term plasticity, where $eIPSC_5$:$eIPSC_1$ was 0.73 ± 0.04 (Fig. 3*E*). In control experiments, baclofen (5 μM) depressed $eIPSC_1$ by 76.0% (from 61.4 to 17.3 pA, mean of two cells) and turned synaptic depression towards facilitation in both cells, leading to a change in $eIPSC_4$:$eIPSC_1$ from to 0.68 to 1.19 and in $eIPSC_5$:$eIPSC_1$ from to 0.83 to 1.00. The effects of GHB, baclofen and CGP on $eIPSC_1$ are summarized in Figure 3*D*, while the changes in short-term synaptic plasticity are shown in Figure 3*E*.

Spontaneous EPSCs are Depressed by GHB via $GABA_B$-receptors

We also tested whether fast glutamatergic spontaneous EPSCs (sEPSCs) were affected by GHB. sEPSCs were recorded in

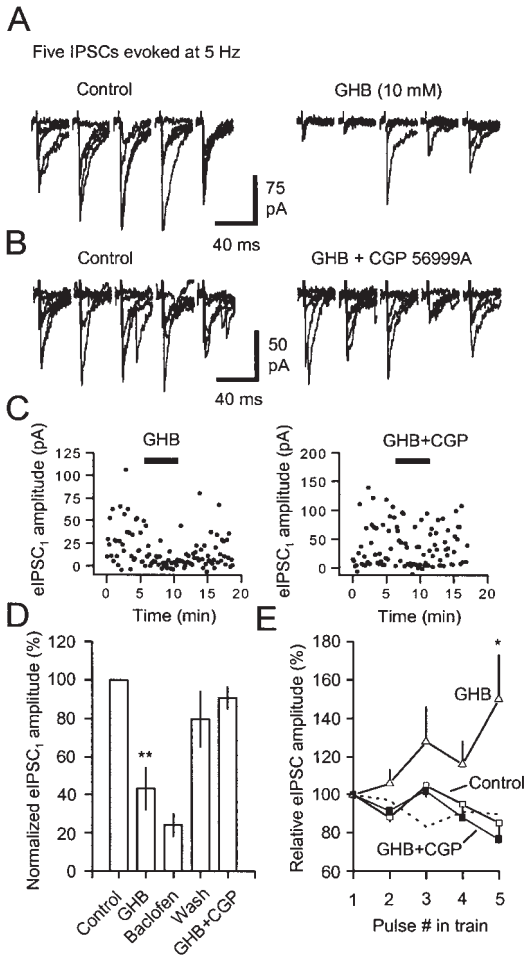

Figure 3. GHB reduces the probability of release at neocortical GABAergic synapses onto layer 2/3 pyramidal cells. (*A*) Evoked IPSCs (eIPSCs) in neocortical layer 2/3 pyramidal cells using minimal stimulation. Five pulses at 5 Hz evoked IPSCs termed $eIPSC_{1–5}$. Five consecutive trains are superimposed. In control, eIPSCs showed a large variability and occasional failures. GHB (10 mM) increased the failure rate, especially on pulse 1 and 2. (*B*) In the presence of CGP 56999A (2 μM), the effect of GHB on eIPSCs was blocked. (*C*) Graphs showing the amplitude of $eIPSC_1$ during the course of the experiment. Left: GHB (10 mM) depressed $eIPSC_1$ reversibly. Right: In CGP 56999A, the GHB effect on $eIPSC_1$ was nearly blocked. (*D*) Histogram showing the effects of GHB, baclofen (5 μM) and GHB + CGP 56999A on the amplitude of $eIPSC_1$. GHB reduced $eIPSC_1$ by 56.7 ± 11% ($n = 5$; $^{**}P < 0.01$), but only 9.2 ± 5.6% in the presence of CGP 56999A ($n = 3$). (*E*) To illustrate the train-induced changes in IPSCs amplitudes, $eIPSC_{2–5}$ were normalized to $eIPSC_1$ in each solution. For each cell, data were collected from 15–30 sweeps in control (open squares, $n = 4$), GHB (open triangles, $n = 4$), GHB + CGP (closed squares, $n = 3$) and washing (stippled line, $n = 4$) solutions. In control, the $eIPSC_5$:$eIPSC_1$ ratio was 0.85 ± 0.1 ($P > 0.05$), while it was 1.5 ± 0.2 in GHB ($^{*}P < 0.05$). CGP 56999A blocked the effects of GHB on short-term plasticity.

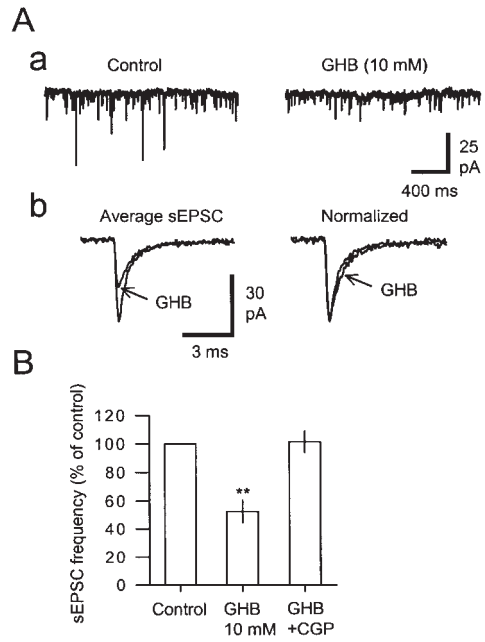

Figure 4. GHB depresses spontaneous EPSCs in neocortical neurons. (*A*) *a*. Spontaneous EPSCs (sEPSCs) in a neocortical layer 2/3 non-pyramidal cell. In control solution containing 50 μM picrotoxin, sEPSC occurred with a frequency of 22.8 Hz and a mean amplitude of 40 pA. GHB (10 mM) lowered the sEPSC frequency to 14.2 Hz and reduced their amplitudes. *b*. Averages of 50 sEPSCs showed that GHB reduced the mean amplitude from 40 to 23 pA. Right: There were no major differences in the sEPSC kinetics between control and GHB. (*B*) Histogram showing the normalized sEPSC frequency in control (100%), GHB (10 mM) and GHB + CGP 56999A (2 μM). The sEPSC frequency decreased to 52.4 ± 8% in GHB ($n = 6$, $P < 0.01$); this effect was reversed by CGP 56999A (101.5 ± 7.4%, $n = 3$).

neocortical layer 2/3 non-pyramidal cells (Fig. 4A) in the presence of picrotoxin (50 μM). sEPSCs occurred at a frequency of 5.6 ± 3.0 Hz, with mean amplitudes of 18.2 ± 2.4 pA (n = 7). GHB depressed f by 47.6 ± 7.8% (to 3.4 ± 2.0 Hz, P < 0.05) and the amplitude by 14 ± 6.2% (P < 0.05), with no significant effect the sEPSC kinetics (Fig. 4*Ab*). Again, the effect of GHB was blocked by the $GABA_B$-antagonists, whereupon f returned to 101.5 ± 7.4% of the control level (Fig. 4*B*). In CA3 pyramidal cells (n = 2), sEPSCs were depressed by GHB (10 mM) similarly as in the neocortex (not shown).

Discussion

According to our results, GHB reduces neuronal excitability and synaptic activity in neocortical and hippocampal neurons. In short, GHB hyperpolarized layer 2/3 neurons of the neocortex and inhibited AP firing, and GHB depressed afferent synaptic input onto layer 2/3 neocortical pyramidal and non-pyramidal cells, hippocampal CA1 and CA3 pyramidal cells, and dentate gyrus granule cells. These effects of GHB were mediated entirely via $GABA_B$-receptors.

At GABAergic connections onto neocortical layer 2/3 pyramidal cells, GHB depressed the probability of release from proximally located boutons activated by minimal stimulation. This conclusion was based on the fact that GHB increased the apparent proportion of failures in response to stimulation, and turned a slight synaptic depression into facilitation (Thomson, 2000). Furthermore, during spontaneous activity, where the pyramidal cells receive a mixture of AP-driven IPSCs and miniature events, GHB depressed the frequency of synaptic currents by ~50%. When the spontaneous activity was increased using kainate (Mody, 1998) to depolarize other neurons in the slice and increase spontaneous presynaptic firing to shift the mixture of events towards AP-driven IPSCs, GHB's effect was greater. This is probably because GHB depressed AP-initiation at the soma, and eventually increased failure of GABA-release at the synapses. Conversely, Ca^{2+}-entry independent spontaneous release of GABA which occurs at a lower frequency, was much more resistant to GHB.

The blocking effect of $GABA_B$-receptor antagonists points to an activation of $GABA_B$-receptors by GHB (Lorente *et al.*, 2000). $GABA_B$-receptors (Misgeld *et al.*, 1995) are present both pre- and postsynaptically in rat and mouse neocortical neurons (Fukuda *et al.*, 1993; Badran *et al.*, 1997; Deisz, 1999), and $GABA_B$-receptor

activation affects synaptic activity similarly to GHB. This effect is most likely to be mediated via an increase in somatodendritic K^+ conductances (Takigawa and Alzheimer, 1999) and a depression of transmitter release at the nerve terminals (Fukuda *et al.*, 1993). In support of this, $GABA_B$-agonist and GHB-binding overlap in the neocortical layers I–III, in contrast to the thalamus and cerebellum, where GHB-binding is less prominent or absent (Mathivet *et al.*, 1997). Thus, we can confirm the hypothesis (Hu *et al.*, 2000) that GHB at the level of single neocortical neurons activates pre- and postsynaptic $GABA_B$-receptors to depress neuronal network activity. However, in contrast to previous reports using microdialysis (Hu *et al.*, 2000), we found that neocortical glutamate release was also depressed by GHB, since the frequency of sEPSCs was reduced by ~50% by GHB.

The concentration threshold for the effects of GHB was near 1 mM, which is close to that found earlier in electrophysiological studies in tegmental dopaminergic neurons (Madden and Johnson, 1998), hippocampal pyramidal cells (Xie and Smart, 1992a) and at recombinant $GABA_B$-receptors where GHB acts as a partial agonist (Lingenhoehl *et al.*, 2000). Furthermore, in the rodent GHB absence epilepsy model, electroencephalographic spike-and-wave seizure activity starts when GHB exceeds ~250 μM in the brain (Snead, 1991). GHB is water-soluble and passes freely across the blood–brain barrier. In humans, sedation is achieved at plasma concentrations close to 1 mM (Hoes *et al.*, 1980), while plasma concentrations of 3 mM induces moderate anesthesia (Kleinschmidt *et al.*, 1999). Furthermore, for abuse purposes (Galloway *et al.*, 1997) subjects can consume tens of grams of GHB (10 g Na-GHB is equivalent to 80 mmol), which would similarly give rise to millimolar concentrations in the volume of distribution for GHB. Since the GHB concentrations found to be effective in our study are similar to those that generate behavioral effects *in vivo*, the effects reported here are probably relevant to understanding conditions such as human abuse or anesthesia, or GHB-induced rodent absence seizures (Banerjee *et al.*, 1993).

In summary, GHB in millimolar concentrations causes a general depression of neuronal firing and fast synaptic transmission mediated entirely via $GABA_B$-receptors. The net effect on neocortical activity will be determined by the resulting dynamic balance between inhibitory and excitatory mechanisms. The resemblances between GHB and $GABA_B$-agonists at the cellular level are likely to reflect the effects in humans, where both substances induce sedation, dizziness, agitation, vomiting, respiratory and cardiovascular depression and coma (Korsgaard, 1976; Bernasconi *et al.*, 1999). Therefore, based on our present findings, $GABA_B$-antagonists may be beneficial in acute GHB-intoxication.

Notes

K.J. is a Glaxo/Wellcome scholar. This work was supported by NIH grant NS 30549 to I.M. We thank Dr W. Froestl for kindly providing CGP 56999A and CGP 56433.

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References

Aizawa M, Ito Y, Fukuda H (1997) Pharmacological profiles of generalized absence seizures in lethargic, stargazer and gamma-hydroxybutyrate-treated model mice. Neurosci Res 29:17–25.

Badran S, Schmutz M, Olpe HR (1997) Comparative *in vivo* and *in vitro* studies with the potent $GABA_B$ receptor antagonist, CGP 56999A. Eur J Pharmacol 333:135–142.

Banerjee PK, Hirsch E, Snead 3rd OC (1993) Gamma-hydroxybutyric acid induced spike and wave discharges in rats: relation to high-affinity [3H]gamma-hydroxybutyric acid binding sites in the thalamus and cortex. Neuroscience 56:11–21.

Bernasconi R, Mathivet P, Marescaux C (1999) Gamma-hydroxybutyric acid: an endogenous neuromodulator with abuse potential? Trends Pharmacol Sci 20:135–141.

Berton F, Brancucci A, Beghe F, Cammalleri M, Demuro A, Francesconi W, Gessa GL (1999) Gamma-hydroxybutyrate inhibits excitatory post-synaptic potentials in rat hippocampal slices. Eur J Pharmacol 380:109–116.

Deisz R (1999) The GABA(B) receptor antagonist CGP 55845A reduces presynaptic GABA(B) actions in neocortical neurons of the rat *in vitro*. Neuroscience 93:1241–1249.

Emri Z, Antal K, Crunelli V (1996) Gamma-hydroxybutyric acid decreases thalamic sensory excitatory postsynaptic potentials by an action on presynaptic GABAB receptors. Neurosci Lett 216:121–124.

Engberg G, Nissbrandt H (1993) Gamma-hydroxybutyric acid (GHBA) induces pacemaker activity and inhibition of substantia nigra dopamine neurons by activating $GABA_B$-receptors. Naun Schmied Arch Pharmacol 348:491–497.

Erhardt S, Andersson B, Nissbrandt H, Engberg G (1998) Inhibition of firing rate and changes in the firing pattern of nigral dopamine neurons by gamma-hydroxybutyric acid (GHBA) are specifically induced by activation of GABA(B) receptors. Naun Schmied Arch Pharmacol 357:611–619.

Fukuda A, Mody I, Prince DA (1993) Differential ontogenesis of presynaptic and postsynaptic GABAB inhibition in rat somatosensory cortex. J Neurophysiol 70:448–452.

Gallimberti L, Canton G, Gentile N, Ferri M, Cibin M, Ferrara SD, Fadda F, Gessa GL (1989) Gamma-hydroxybutyric acid for treatment of alcohol withdrawal syndrome. Lancet ii:787–789.

Galloway GP, Frederick SL, Staggers FE Jr, Gonzales M, Stalcup A, Smith DE (1997) Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction 92:89–96.

Hoes MJ, Vree TB, Guelen PJ (1980) Gamma-hydroxybutyric acid as hypnotic. Clinical and pharmacokinetic evaluation of gamma-hydroxybutyric acid as hypnotic in man. Encephale 6:93–99.

Hu RQ, Banerjee PK, Snead OC III (2000) Regulation of gamma-aminobutyric acid (GABA) release in cerebral cortex in the gamma-hydroxybutyric acid (GHB) model of absence seizures in rat. Neuropharmacology 39:427–439.

Ingels M, Rangan C, Bellezzo J, Clark RF (2000) Coma and respiratory depression following the ingestion of GHB and its precursors: three cases. J Emerg Med 19:47–50.

Kleinschmidt S, Schellhase C, Martzlufft F (1999) Continuous sedation during spinal anaesthesia: gamma-hydroxybutyrate vs. propofol. Eur J Anaesthesiol 16:23–30.

Korsgaard S (1976) Baclofen (Lioresal) in the treatment of neuroleptic-induced tardive dyskinesia. Acta Psychiat Scand 54:17–24.

Lingenhoehl K, Brom R, Heid J, Beck P, Froestl W, Kaupmann K, Bettler B, Mosbacher J (2000) Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. Neuropharmacology 38:1667–1673.

Liu Z, Vergnes M, Depaulis A, Marescaux C (1992) Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat. Neuroscience 48:87–93.

Lorente P, Lacampagne A, Pouzeratte Y, Richards S, Malitschek B, Kuhn R, Bettler B, Vassort G (2000) Gamma-aminobutyric acid type B receptors are expressed and functional in mammalian cardiomyocytes. Proc Natl Acad Sci USA 97:8664–8669.

Madden T, Johnson SW (1998) Gamma-hydroxybutyrate is a $GABA_B$ receptor agonist that increases a potassium conductance in rat ventral tegmental dopamine neurons. J Pharmacol Exp Ther 287:261–265.

Mamelak M, Scharf MB, Woods M (1986) Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. Sleep 9:285–289.

Mathivet P, Bernasconi R, De Barry J, Marescaux C, Bittiger H (1997) Binding characteristics of gamma-hydroxybutyric acid as a weak but selective $GABA_B$ receptor agonist. Eur J Pharmacol 321:67–75.

Misgeld U, Bijak M, Jaromilek W (1995) A physiological role for $GABA_B$ receptors and the effects of baclofen in the mammalian central nervous system. Prog Neurobiol 46:423–462.

Mody I (1998) Interneurons and the ghost of the sea. Nature Neurosci 1:434–436.

Mott DD, Li Q, Okazaki MM, Turner DA, Lewis DV (1999) GABAB-receptor-mediated currents in interneurons of the dentate-hilus border. J Neurophysiol 82:1438–1450.

Nusser Z, Hajos N, Somogyi P, Mody I (1998) Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. Nature 395:172–177.

Pozza MF, Manuel NA, Steinmann M, Froestl W, Davies CH (1999) Comparison of antagonist potencies at pre- and post-synaptic GABA(B) receptors at inhibitory synapses in the CA1 region of the rat hippocampus. Br J Pharmacol 127:211–219.

Rosen MI, Pearsall HR, Woods SW, Kosten TR (1996) The effect of gamma-hydroxybutyric acid on naloxone-precipitated opiate withdrawal. Neuropsychopharmacology 14:187–193.

Snead OC III (1991) The gamma-hydroxybutyrate model of generalized absence seizures: correlation of regional levels of gamma-hydroxybutyric acid and gamma-butyrolactone with spike and wave discharges. Neuropharmacology 30:161–167.

Takigawa T, Alzheimer C (1999) G protein-activated inwardly rectifying K+ (GIRK) currents in dendrites of rat neocortical pyramidal cells. J Physiol (Lond) 517:385–390.

Thomson AM (2000) Facilitation, augmentation and potentiation at central synapses. Trends Neurosci 23:305–312.

Timby N, Eriksson A, Bostrom K (2000) Gamma-hydroxybutyrate associated deaths. Am J Med 108:518–519.

Xie X, Smart TG (1992a) Gamma-hydroxybutyrate depresses monosynaptic excitatory and inhibitory postsynaptic potentials in rat hippocampal slices. Eur J Pharmacol 223:193–196.

Xie X, Smart TG (1992b) Gamma-hydroxybutyrate hyperpolarizes hippocampal neurones by activating $GABA_B$ receptors. Eur J Pharmacol 212:291–294.