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Gamma-hydroxybutyrate Reduces $GABA_A$-mediated Inhibitory Postsynaptic Potentials in the CA1 Region of Hippocampus

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Gamma-hydroxybutyric acid (GHB) is a psychoactive drug and a putative neurotransmitter, derived from gamma-aminobutyric acid (GABA). At micromolar concentrations GHB binds to specific high and low affinity binding sites present in discrete areas of the brain, while at millimolar concentrations GHB also binds to $GABA_B$ receptors. Previous studies indicated that GHB inhibits both NMDA and AMPA receptor mediated excitatory postsynaptic potentials in hippocampal CA1 pyramidal neurons. This action of GHB occurs in the presence of $GABA_B$ blockade and is antagonized by NCS-382, a specific GHB receptor antagonist, suggesting that it is mediated by GHB receptors. In the present study, we have investigated the effect of GHB on $GABA_A$ mediated inhibitory postsynaptic potentials ($GABA_A$-IPSP) elicited in CA1 hippocampal pyramidal neurons by stimulation of Schaffer collateral-commissural fibers. We observed that GHB inhibited $GABA_A$-IPSPs by about 40% at concentrations of 300–600 μM. GHB inhibition was blocked by NCS-382 (500 μM), which per se failed to modify $GABA_A$-IPSPs. Moreover, GHB failed to modify cell membrane depolarization induced by the brief pressure application of GABA in the presence of tetrodotoxin (TTX), indicating that GHB does not inhibit postsynaptic GABA responses. However, GHB reduced the amplitude of $GABA_A$-IPSPs elicited in pyramidal neurons by paired pulse stimulation and enhanced paired pulse facilitation with respect to control condition, suggesting that GHB reduces GABA release from nerve terminals. Finally, GHB failed to reduce the amplitude of $GABA_A$-IPSPs in the presence of $BaCl_2$, suggesting that the effect of GHB is due to GHB receptor-mediated presynaptic inhibition of Ca^2 + influx.

[Neuropsychopharmacology 27:960-969, 2002]

KEY WORDS: *Gamma-hydroxybutyrate; NCS-382; $GABA_B$ receptors antagonist; Hippocampal slices; Intracellular recordings*

Gamma-hydroxybutyric acid (GHB) is a psychoactive drug and a putative neurotransmitter (Bernasconi et al. 1999; Maitre 1997). Administered peripherally, GHB penetrates freely into the brain and produces dose-related pharmacological effects including euphoria, antidepressant, and anxiolytic effects, sedation, sleep, anesthesia (Agabio and Gessa 2002; Colombo et al. 1998; De Couedic and Voisse 1964; Laborit et al. 1960; Rinaldi et al. 1967; Schmidt-Mutter et al. 1998). GHB has been used clinically as a general anesthetic and as a sleep inducer in the treatment of narcolepsy (Agabio and Gessa 2002; Broughton and Mamelak 1979). GHB is currently marketed in Italy and Austria for the treatment of alco-

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Received January 9, 2002; revised May 17, 2002; accepted May 22, 2002.

Online publication: 5/23/02 at www.acnp.org/citations/Npp052302312/default.htm.

Published by Elsevier Science Inc.
360 Park Avenue South, New York, NY 10010-1710

0893-133X/02/$–see front matter
PII S0893-133X(02)00378-0

holism (Gallimberti et al. 1989, 2000). However, GHB has also gained popularity in the illicit market in the United States, being abused for its euphoriant action, which reportedly resembles that of alcohol and ecstasy (Boyce et al. 2000; Kam and Yoong 1998; Nicholson and Balster 2001). However, GHB is also synthesized and released by specific neurons in the brain and possesses most of the properties required to be classified as a neurotransmitter and/or neuromodulator. In fact, synthesis, release, uptake mechanisms, and specific binding sites for GHB have been identified in the mammalian brain (Benavides et al. 1982a,b; Hechler et al. 1985, 1992; Maitre et al. 1983; Rumigny et al. 1981; Snead and Liu 1984; Vayer et al. 1988). GHB binding sites exhibit high (Kd 30–580 nM) and low (about 20 μM) affinity for GHB (Benavides et al. 1982a) and are sensitive to pertussis toxin, suggesting that these sites could represent G protein coupled receptors (Kemmel et al. 1998; Rotomponirina et al. 1995). GHB binding sites have a discrete brain distribution including the frontal cortex, nucleus accumbens, amygdala, hypothalamus, and, with highest density, the hippocampus (Hechler et al. 1992; Maitre et al. 2000; Snead et al. 1990). The synthetic structural analog of GHB, 6,7,8,9-tetrahydro-5[H]benzocycloheptene-5-ol-4ylidene acid (NCS-382), is the first and the only GHB antagonist currently available. This compound displaces [^{3}H] GHB binding with a low (130–300 nM) and high (5–8 μM) IC_{50} (Maitre et al. 2000).

In vivo, NCS-382 diminishes the sedative effect and the petit mal seizures induced by GHB (Hu et al. 2000; Schmidt et al. 1991; Schmidt-Mutter et al. 1998) and suppresses GHB-intravenous self-administration in mice (Martellotta et al. 1998). Moreover, NCS-382 inhibits GHB-induced increase in Guanosine 3′,5′-cyclic monophosphate (cGMP) levels and inositol phosphate turnover in the hippocampus both in vivo and in vitro (Maitre et al. 1990; Snead 2000). At millimolar concentrations GHB displaces [^{3}H] baclofen from $GABA_B$ (gamma-aminobutyric acid) receptors (Mathivet et al. 1997; Snead 1996). GHB action on $GABA_B$ receptors appears to mediate some of the pharmacological actions of GHB, such as anesthesia in mice and rats (Colombo et al. 2001) and inhibition of intestinal motility in mice (Poggioli et al. 1999). In fact, these effects are not antagonized by NCS-382 but are blocked by $GABA_B$ receptor antagonists.

On the other hand, other effects of GHB such as petit mal seizures and sedation are mimicked by the $GABA_B$ agonist baclofen and are antagonized by either NCS-382 or $GABA_B$ receptor antagonists, suggesting a possible interaction between $GABA_B$ and GHB receptors. Alternatively, it has been suggested that GHB might be converted in vivo into GABA, which in turn could interact with $GABA_B$ receptors (Hechler et al. 1997). Current investigation on the mechanism of action of GHB are aimed at elucidating the role of endogenous GHB in sleep, anxiety, petit mal epilepsy, and alcohol and drug abuse, etc. Previous studies from our laboratory (Berton et al. 1999) have shown that GHB reduces both NMDA and AMPA-mediated excitatory postsynaptic potentials (EPSP) elicited in hippocampal pyramidal neurons by the stimulation of Schaffer collateral/commissural fibers. These effects were seen in hippocampal slices superfused with $GABA_B$ receptor antagonists, ruling out an involvement of $GABA_B$-receptors, but were antagonized by NCS-382, suggesting that they are mediated by GHB receptors.

The present study is aimed at determining whether GHB modifies $GABA_A$-mediated inhibitory postsynaptic potentials (IPSP) evoked in CA1 hippocampal pyramidal neurons by the electrical stimulation of Schaffer collateral-commissural fibers.

METHODS

Slice Preparation

Male Wistar rats (100–150 g) were anesthetized with halothane (3%) and decapitated. Brains were rapidly removed and chilled in ice-cold artificial cerebrospinal fluid (aCSF) gassed with carbogen (95% O_2, 5% CO_2). The aCSF composition (in mM) was: NaCl (130), KCl (3.5), NaH_2PO_4 (1.25), $MgSO_4 7\ H_2O$ (1.5), $CaCl_2\ 2H_20$ (2), $NaHCO_3$ (24), and Glucose (10).

Hippocampal slices of 400 μm thickness were then cut with a vibroslice (Campden Instruments) and incubated at room temperature (23°C) for up to one hour before being placed in the recording chamber. Once in the chamber, and after 15 min of incubation with their upper surface exposed to warmed (33°–34°C) and humidified carbogen, the slices were completely submerged and continuously superfused with aCSF at a constant rate (2–4 ml/min) for the remainder of the experiment.

Electrophysiology

We used sharp glass micropipettes filled with potassium acetate (3 M); tip resistances, 80–120 MΩ to penetrate CA1 pyramidal neurons. We performed current-clamp recordings with an Axoclamp A headstage (Axon Instruments Burlingame, CA). Selected traces were stored for data analysis using a software developed using the Labview package (National Instruments, Austin, Texas). The following criteria were used for the inclusion of cells in the present experiments: stable resting membrane potential of at least −60 mV and no spontaneous firing of action potentials; no sudden drops in the input resistance; and constant amplitude of the spike (> 80 mV) obtained by direct activation of the cell. Postsynaptic inhibitory potentials were evoked by orthodromic stimulation (80 μs stimulus duration, 0.05 Hz frequency) of Schaffer collateral/commissural fibers

with a bipolar tungsten electrode placed in the stratum radiatum. We averaged evoked response from five sweeps and measured the peak amplitude. The testing procedure was the following: inhibitory postsynaptic potentials were recorded for 20 min during superfusion of aCSF containing 10 μM of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 30 μM DL-2-amino-5-phosphonovaleric acid (d-APV), and 1 μM CGP55845A (control); GHB (100, 300, 600, or 1200 μM) was then added to the superfusion solution and the measures were repeated after 5, 10, and 15 min of drug application; the drug was then removed and the measures were repeated (washout). For paired-pulse facilitation (PPF) experiments, paired response were elicited by twin pulse (60 ms apart) in CA1 pyramidal neurons. The PPF is expressed as a ratio of the second to the first $GABA_A$-mediated inhibitory postsynaptic potentials amplitude.

Pharmacological Isolation of $GABA_A$-mediated IPSP

For the pharmacological isolation of synaptic components, we first continuously superfused slices with CNQX (10 μM) and d-APV (30 μM) to block excitatory glutamatergic transmission and then recorded monosynaptic compound IPSPs ($GABA_A$ and $GABA_B$-mediated) in response to local stimulation of Schaffer collateral/commissural fibers. To isolate the $GABA_A$ mediated IPSPs, the $GABA_B$ receptor antagonist, CGP 55845A (1 μM) was added to aCSF. Drugs and receptor channel blockers were added from concentrated stock solutions to the aCSF immediately before its administration to the slice chamber.

We administered GABA by pressure application with a picospritzer II (Parker Instruments, Fairfield, NJ), nol pipette (tip diameter about 2 μm; pressure 5–15 psi; GABA 250 mM) visually positioned close to the recording electrode. The duration of the pressure was decreased and increased several times every 2 min to test the reproducibility and dose-dependency of GABA responses. Trains of hyperpolarizing current pulses (0.2 nA; 100 ms) were injected through the recording electrode at 2–6.6 Hz to measure input resistance (R_{in}) and input conductance (G_{in}), just before and during GABA application. The maximum increase in G_{in} provided a measure of the GABA induced responses (G_{GABA}) in each cell analyzed. G_{GABA} was obtained by subtracting G_{in} before GABA responses from maximal G_{in} during GABA response.

RESULTS

Effects of GHB on $GABA_A$-IPSPs

Monosynaptic IPSPs were recorded from CA1 pyramidal neurons in response to local Schaffer collateral/commissural fiber stimulation by blocking excitatory synaptic transmission with the glutamate receptor antagonists CNQX (10 μM) and d-APV(30 μM) for AMPA and NMDA receptors, respectively. IPSPs were observed in all cells recorded under these conditions (n = 58) and consisted of an early and late component as previously described (Alger and Nicoll 1982; Dutar and Nicoll 1988; Sivilotti and Nistri 1991). Superfusion of CGP 55845A (1 μM), a selective $GABA_B$ receptor antagonist, completely abolished the late component of the IPSPs, suggesting that this component was mediated by $GABA_B$ receptors (Davies et al. 1990). The isolated early IPSPs were found to have a reversal potential of approximately −70 mV, consistent with the reversal potential for Cl^- (Bertrand and Lacaille 2001) and were completely abolished by bicuculline methiodide (30 μM), suggesting mediation of this component by $GABA_A$ receptors (Figure 1, panel A). The effect of GHB on this monosynaptic $GABA_A$-IPSP was then investigated in 39 pyramidal cells.

Bath application of GHB (600 μM) for 15 min reversibly decreased $GABA_A$-mediated IPSP in a concentration-dependent manner, without affecting either the resting membrane potential (r.m.p.) (control = −66.9 ± 0.76 mV; GHB = −68.0 ± 1.09 mV, F = 0.11325 n.s.) or the input resistance (Rin) (control = 36.1 ± 1.1 MΩ; GHB = 34.4 ± 1.69 MΩ, F = 0.011 n.s.) of the cell, as measured by the voltage change in response to a constant current pulse (0.2 nA-200 msec) applied before each stimulus (not shown).

Such a decrease in the amplitude of $GABA_A$-IPSP occurred within 8–10 min after GHB bath application and recovered to control level within 20 min of drug washout at all concentrations. Figure 1, panel A shows a representative cell where 600 μM of the GHB reduced the amplitude of the $GABA_A$-IPSP by 40% of control. Statistical analysis showed that GHB significantly reduced the mean amplitude of this synaptic response from 5.07 ± 0.44 mV to 4.37 ± 0.47 mV ($F_{2,2} = 8.95$, $p < .05$, n = 3), from 4.96 ± 0.29 mV to 3.10 ± 0.48 mV ($F_{2,3} = 8.95$, $p < .001$, n = 4), from 5.52 ± 0.41 mV to 3.24 ± 0.37 mV ($F_{2,11} = 8.95$, $p < .001$, n = 12) and from 5.11 ± 0.55 mV to 3.07 ± 0.51 mV ($F_{2,2} = 8.95$, $p < .001$, n = 3) for GHB 100 μM, 300 μM, 600 μM and 1200 μM, respectively. On average, 100, 300, 600 and 1200 μM of GHB reduced the $GABA_A$-IPSP amplitudes by 13.4% ± 6%, 37.5 ± 4%, 41.3% ± 2.5%, and 39.9% ± 5% of control, respectively (Figure 1, panels B,C).

To determine which receptor type was responsible for the depressant effects of GHB on $GABA_A$-IPSP, we applied GHB (600 μM) in the presence of NCS-382 (500 μM), an antagonist of GHB receptors. As shown in Figure 2, NCS-382 (500 μM) was effective in blocking the depressant effects of GHB on $GABA_A$-IPSPs. NCS-382 (500 μM) had no significant effect on this IPSP when applied alone.

Since it is still a matter of debate whether $GABA_B$ receptors mediate some of the physiological effects of GHB, we compared in the same cell the effect of GHB

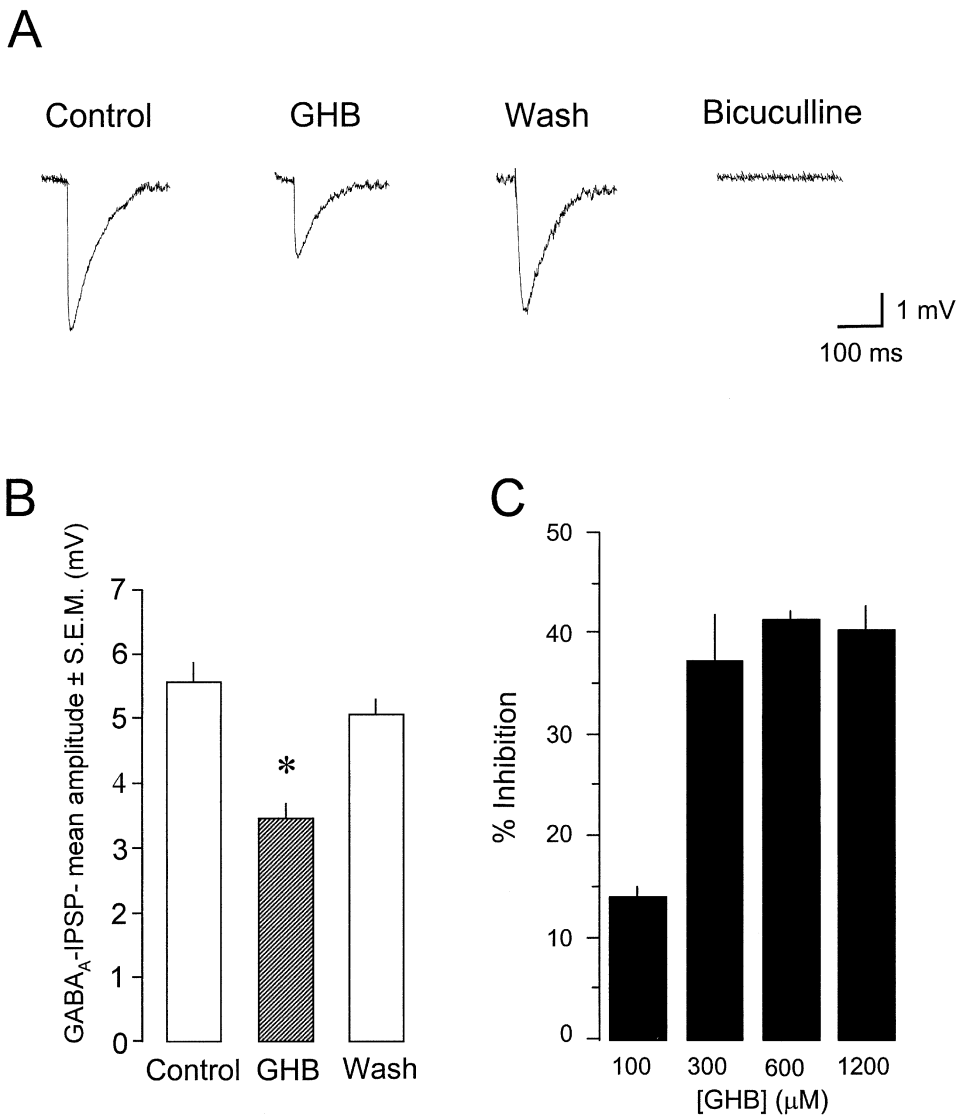

Figure 1. GHB reduces $GABA_A$-IPSPs. **(A)** Recording of isolated $GABA_A$-IPSP from a CA1 neuron in presence of CNQX (10 μM), d-APV (30 μM), and CGP 55845A (1 μM) following stimulation of Schaffer collateral/commisural fibers. GHB (600 μM, 8 min) decreases the $GABA_A$-IPSP size. The response recovered to the control level after washout of GHB (15 min). Bicuculline (30 μM) totally blocked this IPSP. The r.m.p. of the cell was −70 mV. **(B)** Mean peak amplitude of $GABA_A$-IPSPs from 15 cells, showing that GHB (600 μM) significantly (asterisk) attenuated the mean $GABA_A$-IPSP amplitude in a reversible manner. Error bars = S.E.M. **(C)** GHB inhibition of $GABA_A$-IPSPs at different GHB concentrations. Data are percentage inhibition of $GABA_A$-IPSP amplitude (± SEM). Maximal reduction of $GABA_A$-IPSP was seen at GHB concentrations of 300–1200 μM. Therefore a GHB concentration of 600μM was used for the study.

(600 μM) and $GABA_B$ receptor agonist (−)−baclofen (10 μM) on the $GABA_A$-IPSPs.

In slices perfused with the glutamate receptor antagonists CNQX (10 μM) and d-APV (30 μM), the peak amplitude of the compound IPSPs ($GABA_A$ and $GABA_B$-mediated) recorded from CA1 pyramidal neurons in response to local Schaffer collateral/commissural fiber stimulation was dramatically reduced by application of (-)-baclofen (10 μM) from 5.92 ± 0.45 mV to 2.21 ± 0.22 mV ($F_{2,4} = 35.82$, $p < .01$, n = 5) (Figure 3, panels A, B). The peak amplitude of the IPSPs recovered during the washout of (-)-baclofen to 5.81 ± 0.57 mV. After recovery, the CGP 55845A (1 μM), a selective $GABA_B$ receptors antagonist, was applied to block the $GABA_B$-mediated response and to isolate the $GABA_A$-IPSPs (Figure 3, panel B). In the presence of CGP 55845A (1 μM), (-)-baclofen (10 μM) was then unable to reduce the amplitude of the $GABA_A$-IPSPs (from 5.5 ± 0.51 mV to 5.7 ± 0.61 mV). In contrast, after washout of (-)-baclofen in the presence of CGP 55845A, application of GHB (600 μM) significantly reduced the amplitude of the $GABA_A$-IPSPs from 5.7 ± 0.51 mV to 3.8 ± 0.45 mV ($F_{2,4} = 15.55$, $p < .05$) (Figure 3, panel B).

We also sought to determine whether GHB might alter the late IPSPs, likely to be mediated by $GABA_B$ receptors. We isolated $GABA_B$-IPSP applying CNQX (10 μM), d-APV (30 μM), and bicuculline (30 μM) to block AMPA/Kainate, NMDA and $GABA_A$ receptors, respectively. As shown in Figure 4 the mean peak amplitude of isolated $GABA_B$-IPSP was reduced from 4.42 mV ± 0.33 mV to 2.06 mV ± 0.21 mV after 8–10 min GHB (600 μM) superfusion ($F_{2,11} = 15.65$, $p < .001$, n = 12). Washout of GHB with aCSF readily reversed reduction of the $GABA_B$-IPSP amplitude to control level (4.18 mV ± 0.51 mV). The effects of GHB (600 μM) on $GABA_B$-IPSP, were reduced in slices superfused with NCS-382 (500 μM) to block GHB receptors (Figure 4, panel B). In the presence of NCS-382 (500 μM), GHB (600 μM) had only

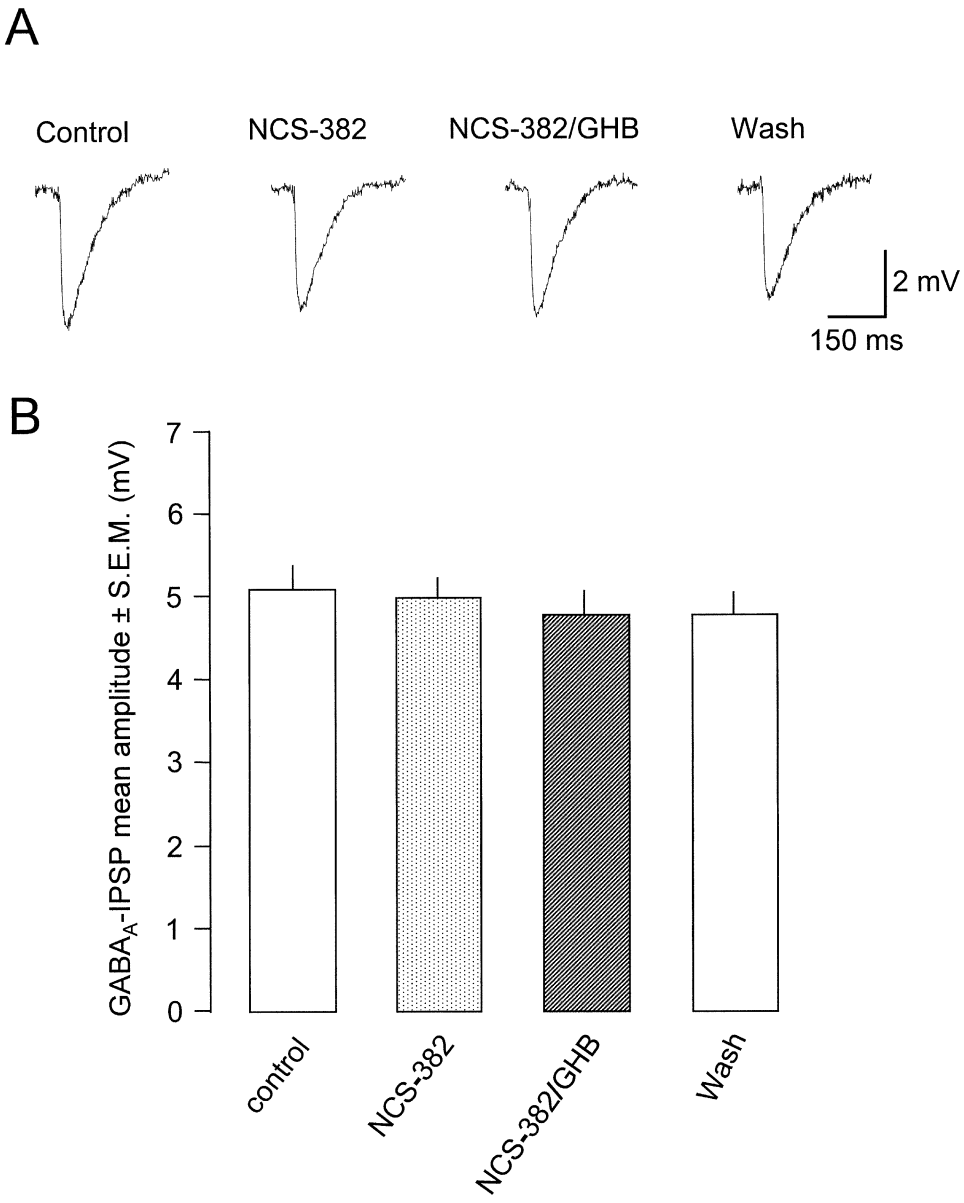

Figure 2. NCS-382 antagonized the inhibition of $GABA_A$-IPSPs by GHB. **(A)** Superfusion of NCS-382 (500 μM) did not alter $GABA_A$-IPSP (representative cell), but blocked the depressant effect of GHB (600 μM). The r.m.p of the cell was −68 mV. **(B)** Mean peak amplitudes from six cells showing that NCS-382 (500 μM) treatment blocked the effect of GHB. Error bars = S.E.M.

a slight depressing effect on the $GABA_B$-IPSP amplitude from 4.25 mV ± 0.48 mV to 3.56 mV ± 0.39 mV.

Site of GHB action on IPSPs

When two stimuli are given in rapid succession, the probability of transmitter release in response to the second stimulation is altered (Zucker 1989). The ratio of the amplitude of the second response to the amplitude of the first inversely correlates with the probability of release, and is therefore usually affected by manipulations that alter release probability (Chieng and Williams 1998; Mennerick and Zorumski 1995)

To determine whether GHB reduces $GABA_A$-IPSPs by a postsynaptic reduction in the sensitivity to synaptically released GABA or through a presynaptic depression of GABA release, we initially examined the effects of GHB on the ratio of the amplitudes of $GABA_A$-IPSPs elicited by paired-pulse stimulation (60 msec, interstimulus interval). The amplitude of both the first and the second IPSPs were reduced by GHB (600 μM) whereas the paired-pulse ratio was significantly enhanced from 1.22 ± 0.08 to 1.79 ± 0.11 ($F_{2,5} = 14.8$, $p < .01$, n = 6) to recover to 1.13 ± 0.10 after 20 min of washout. This result is consistent with a GHB-induced decrease in the probability of GABA release, although it does not rule out contributions of additional postsynaptic mechanisms.

Application of GABA by pressure to cells, kept at resting membrane potential of −75 mV, evoked a dose-dependent depolarization associated with a decrease in R_{in} (data not shown). These GABA responses were blocked by the $GABA_A$ receptor antagonist, bicuculline methio-

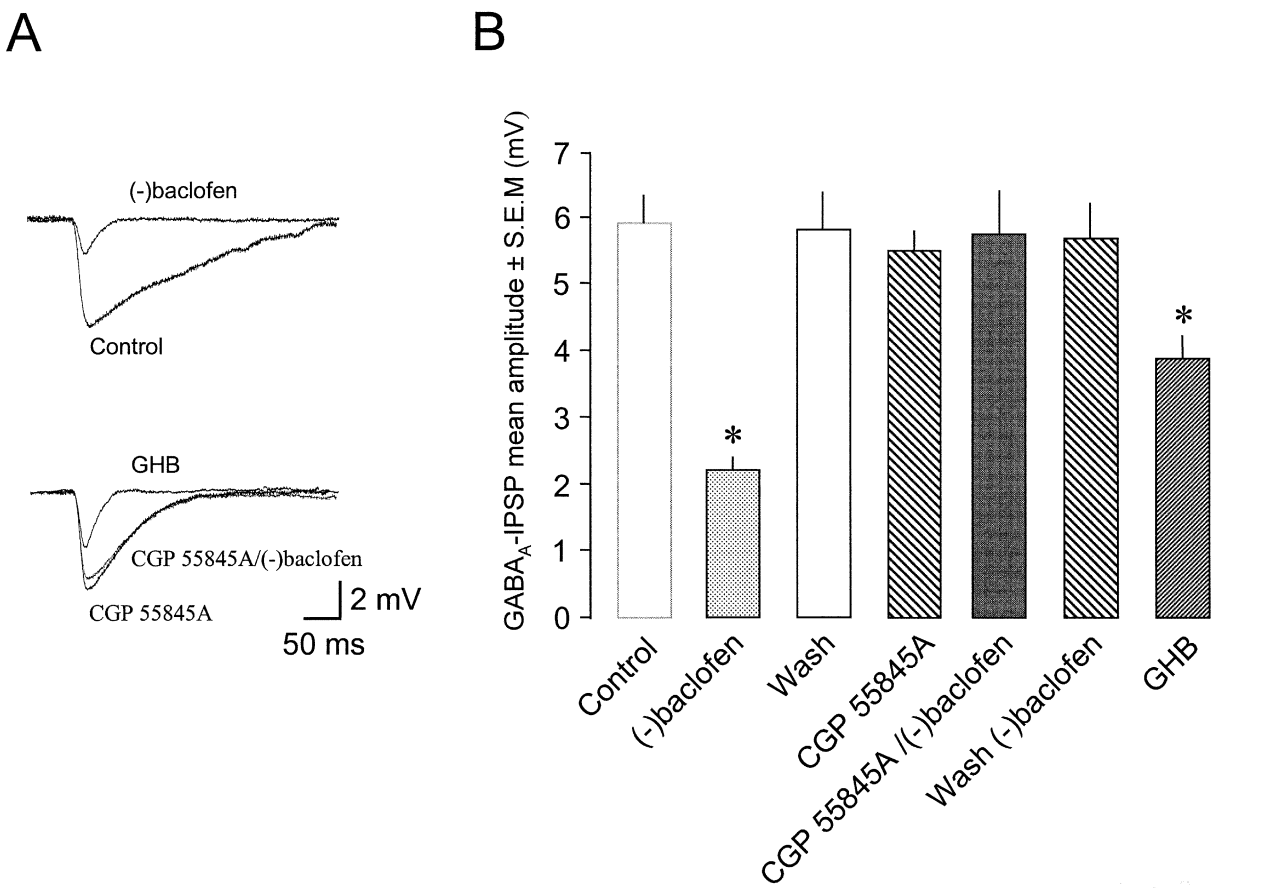

Figure 3. GHB, but not (-)-baclofen, reduced $GABA_A$-IPSP in the presence of $GABA_B$ antagonist. **(A)** Upper traces. (-)-baclofen (10 μM; (-)baclofen trace) dramatically reduced the compound ($GABA_A$ and $GABA_B$-mediated) monosynaptic IPSP (control trace), evoked by electrical stimulation of Schaffer collateral/commissural fibers and recorded in the presence of 10 μM CNQX and 30 μM d-APV to block glutamatergic synaptic potentials. Lower traces. After washout of (-)-baclofen, in the same cell, CGP 55845A (1 μM) superfused for 10 min eliminates the late $GABA_B$-IPSP (CGP 55845A trace) component of the compound IPSP. Superfusion of 10 μM (-)-baclofen (CGP 55845A/(-)-baclofen trace) was unable to reduce the $GABA_A$-IPSP, whereas GHB (600 μM) reduced it (GHB trace). The r.m.p. of the cell was −70 mV. **(B)** Effects of (-)-baclofen and GHB on the mean amplitude of synaptically GABA-mediated responses: Data are mean ± SEM (bars) value of five cells.

dide (30 μM), and were unchanged after addition of 1 μM TTX. These responses therefore were mediated by $GABA_A$ receptors located on the postsynaptic membrane of pyramidal neurons. Superfusion of GHB (600 μM) did not change either the amplitude of depolarization induced by GABA application nor the reduction of membrane conductance (G_{GABA}) observed during GABA induced depolarization. On average G_{GABA} was 47.9 ± 8.8 nS before and 52.6 ± 12.8 nS during GHB application ($F_{1,4} = 0.75$, $p = .437$, n = 5). In the same cells, the benzodiazepine diazepam (100 nM) was effective in increasing GABA-evoked response (Figure 5).

To determine whether the reduction of $GABA_A$-IPSPs induced by GHB was due to an action on presynaptic GABA release, we examined the effects of GHB (600 μM) on evoked $GABA_A$-IPSPs in the presence of $BaCl_2$ (1 mM). Blockade of K^+ channels with Ba^{2+}, broadening the presynaptic action potential waveform, reduces the presynaptic effect of substances controlling Ca^{2+} influx (Nicola and Malenka 1997; Thompson and Gähwiler 1992; Tallent et al. 2001). For monosynaptic $GABA_A$-IPSPs (recorded in CNQX and d-APV), GHB was first applied in aCSF and then in aCSF containing 1 mM $BaCl_2$. In the absence of $BaCl_2$, GHB (600 μM) depressed the $GABA_A$-IPSPs by 41.3 ± 0.2%, whereas in the presence of $BaCl_2$ (1 mM), GHB (600 μM) was unable to reduce the amplitude of the $GABA_A$-IPSPs (from 4.92 ± 0.36 mV to 4.94 ± 0.36 mV, n = 4). These results provide further evidence that GHB reduces inhibitory synaptic transmission by modulating a presynaptic release of neurotransmitters.

DISCUSSION

The present results show that GHB inhibits monosynaptic $GABA_A$-IPSPs in CA1 hippocampal pyramidal

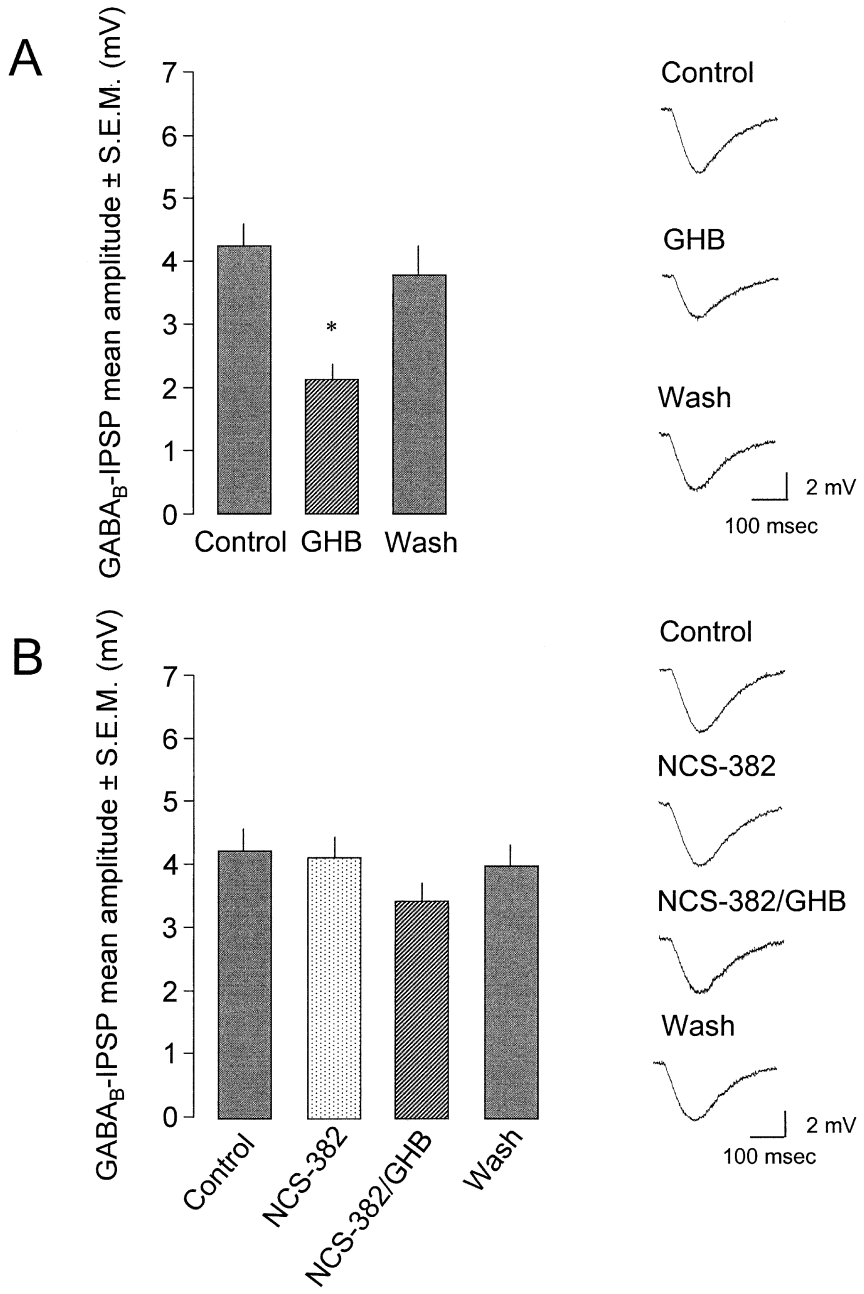

Figure 4. GHB reduced $GABA_B$-IPSPs. **(A)** Mean peak amplitude of $GABA_B$-IPSPs from 12 cells, showing that GHB (600 μM) significantly (asterisk) attenuated the mean $GABA_B$-IPSP amplitude in a reversible manner. Error bars = S.E.M. Traces on right side are isolated $GABA_B$-IPSPs recorded from a CA1 neuron in presence of CNQX (10 μM), d-APV (30 μM), and bicuculline (30 μM) following stimulation of Schaffer collateral/commisural fibers. This synaptic response was reduced by 600 μM GHB application, with recovery in the washout. The r.m.p. of the cell was −65 mV. **(B)** Mean peak amplitude of $GABA_B$-IPSPs from 12 cells, showing that NCS-382 (500 μM) blocked the effect of GHB (600 μM) on $GABA_B$-IPSP amplitude. Error bars = S.E.M. Traces on right side are isolated $GABA_B$-IPSPs records from a CA1 neuron showing that in the presence of GHBr antagonist, NCS-382, GHB was unable to modify the $GABA_B$-IPSPs. The r.m.p. of cell was −69 mV.

neurons evoked by the electrical stimulation of Schaffer collateral-commissural fibers. $GABA_A$-IPSPs were isolated by applying CNQX, d-APV, and CGP 55845A to eliminate NMDA, AMPA and $GABA_B$-mediated synaptic potentials. GHB-induced inhibition of monosynaptic $GABA_A$-IPSPs occurred in the presence of the $GABA_B$ receptor antagonist CGP 55845A at a concentration capable of blocking inhibition of $GABA_A$-IPSPs by the $GABA_B$-receptor antagonist baclofen.

In contrast, the inhibitory effect of GHB on monosynaptic $GABA_A$-IPSPs was suppressed by the GHB receptor antagonist NCS-382, which per se failed to modify $GABA_A$-IPSPs. The results suggest that the effect of GHB is mediated by GHB receptors distinct from $GABA_B$ receptors.

Previous studies have shown that bath application of GHB at the millimolar range hyperpolarizes hippocampal neurons and depresses monosynaptic excitatory and inhibitory postsynaptic potentials in hippocampal slices (Xie and Smart 1992). These effects are inhibited by the $GABA_B$ receptor antagonists GGP 36742 and GGP 33348, suggesting that GHB at high concentrations can activate both pre- and postsynaptic $GABA_B$ receptors (Xie and Smart 1992).

Although GHB concentrations found to be effective in the present study were lower than those needed to activate $GABA_B$ receptors, they were higher than GHB Kds for high and low affinity GHB binding sites. This can be due to the fact that GHB binding is pH depen-

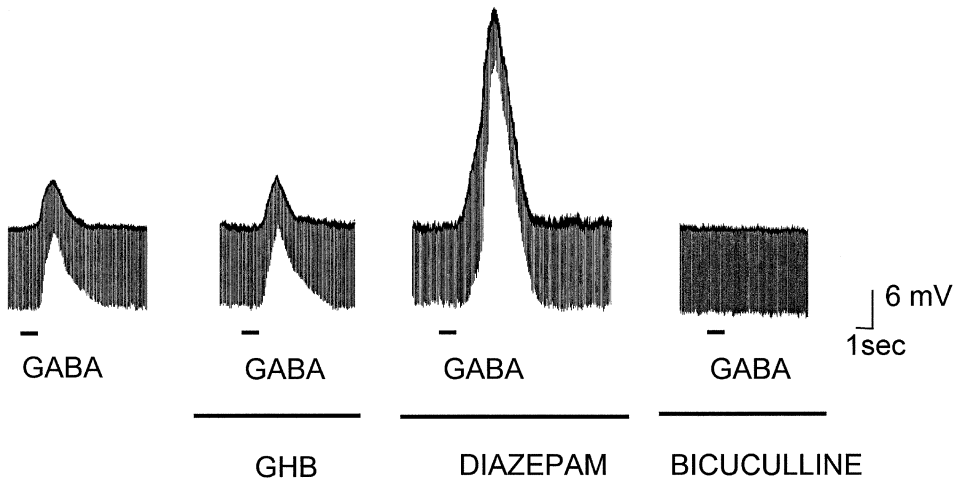

Figure 5. Responses evoked by GABA pressure application were not depressed by GHB. The membrane depolarization and the reduction of membrane conductance (G_{GABA}) induced by brief pressure (0.8 s) application of GABA are not modified during superfusion with GHB (600 μM). Diazepam (100 nM) greatly enhanced the membrane depolarization induced by the same GABA application. Bicuculline (30 μM) totally abolished the response to GABA. The r.m.p. of the cells was −75 mV.

dent, maximum being pH 5.5, and that binding experiments are generally carried out at pH 6.0, and electrophysiological studies are conducted at physiological pH (7.4), where binding of GHB to its receptor is expected be greatly reduced (Maitre et al. 2000). On the other hand, GHB concentrations effective in inhibiting $GABA_A$-IPSPs were within those reached in the rat brain after systemic administration of pharmacologically effective doses of the drug (200–300 mg/kg), which are antagonized by NCS-382 (Maitre 1997).

As to the pre- or postsynaptic site of action of GHB, the finding that GHB failed to modify the membrane depolarization of hippocampal neurons that was produced by pressure application of GABA in the presence of TTX, rules out a site of action at postsynaptic $GABA_A$ receptors. This conclusion is in agreement with binding studies showing that GHB does not alter the function of the $GABA_A$ receptor complex in the rat cerebral cortex (Serra et al. 1991).

On the other hand, our results support the hypothesis that GHB inhibits $GABA_A$-IPSPs by reducing GABA release. Indeed, although GHB reduced the amplitude of $GABA_A$-IPSPs induced by paired pulse stimulation, it increased paired pulse facilitation, which is generally produced by manipulations that reduce transmitter release. These results support the hypothesis that GHB reduces GABA release. Moreover, GHB failed to inhibit $GABA_A$-IPSPs in the presence of $BaCl_2$, which has been shown to reduce the presynaptic effect of substances controlling Ca^{2+} influx (Nicola and Malenka 1997; Thompson and Gähwiler 1992; Tallent et al. 2001). In agreement with this hypothesis, microdialysis studies have shown that GHB reduces GABA release in striatum, thalamus, and cerebral cortex, and that these actions are blocked by NCS-382 (Banerjee and Snead 1995; Gobaille et al. 1999; Hechler et al. 1991; Hu et al. 2000; Maitre et al. 1990). Moreover, previous patch-clamp experiments carried out on NCB-20 neuroblastoma cells, expressing GHB receptors, have shown that GHB inhibited Ca^{2+} conductance and that this action can be antagonized by NCS-382 but not by the $GABA_B$ antagonist CGP 558845 (Kemmel et al. 1998).

It has also recently been shown that GHB inhibits adenylate cyclase activity via presynaptic GHB receptors coupled with a G protein (Snead 2000). Presynaptic adenylate cyclase activation has been shown to open N-type Ca^{2+} channels causing increased influx of Ca^{2+} and neurotransmitter release (Kemp et al. 1994). Thus GHB, by inhibiting adenylate cyclase, could negatively modulate N-type Ca^{2+} channels on gabaergic and glutamatergic nerve endings and reduce transmitter release (Chavez-Noriega and Stevens 1994; Kemp et al. 1994; Dutar and Nicoll 1988).

In conclusion, the present and previous results (Berton et al. 1999) indicate that GHB exerts an inhibitory control on GABA and glutamate release in the hippocampus by acting on presynaptic GHB receptors. These results raise a number of questions, such as whether endogenous GHB has a physiological role in modulating GABAergic and glutamatergic neurotransmission in the hippocampus and in other brain areas where GHB receptors are present, whether GHB and $GABA_B$ receptors are separate entities or whether GHB and some $GABA_B$ receptor subunits might be associated in brain areas where the two are co-expressed and might interact cooperatively or in a negative manner. Hopefully, future cloning of the GHB receptor might provide an answer to these questions.

ACKNOWLEDGMENTS

The authors thank Dr. P.P. Sanna of TSRI for helpful comments on the manuscript. This work was supported by Laboratorio Farmaceutico CT (Sanremo, Italy), The Foundation Cassa di Risparmio di Volterra, and MURST 60% grants (WF).

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