

Presynaptic Modulation of Spontaneous Inhibitory Postsynaptic Currents by Gamma-hydroxybutyrate in the Substantia Nigra Pars Compacta

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The regulation of GABA release from the inhibitory input to dopamine cells in the substantia nigra pars compacta (SNc) plays a key role in different reward-related behaviors. Gamma-hydroxybutyrate (GHB) has therapeutical properties in various psychiatric disorders, especially in alcohol abuse. GHB is also used as a drug of abuse, which induces sedation and euphoria. Using whole-cell patch-clamp recordings, we studied the effects of GHB on GABA release in the SNc by recording spontaneous inhibitory postsynaptic currents (sIPSCs) in brain slices of 21- to 25-day-old rats. We found that GHB depressed the frequency and amplitude of sIPSCs, while the frequency and the amplitude of miniature inhibitory postsynaptic currents (mIPSCs), recorded in the presence of TTX, were not affected. However, in the presence of high extracellular potassium (15 mM), which increases the contribution of voltage-dependent calcium channels, GHB induced a reduction in the frequency of the mIPSCs without any effect on their amplitude. All of these effects were GABA_B-independent and they were blocked by the GHB receptor antagonist NCS-382. The present results indicate that GHB inhibits spontaneous inhibitory synaptic transmission recorded from dopaminergic neurons in the SNc likely by reducing voltage-dependent calcium influx involved in presynaptic GABA release.

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INTRODUCTION

Gamma-hydroxybutyrate (GHB) is a naturally occurring compound of the mammalian brain (Bessman and Fishbein, 1963; Roth and Giarman, 1969). Although the physiological function of GHB has not been clearly defined, the existence of binding sites as well as mechanisms for synthesis, release, and uptake in the brain indicate that GHB acts as neuromodulator on specific neuronal populations (Maitre, 1997). High-affinity binding site studies revealed a G protein-coupled GHB receptor (Snead, 2000) present mainly in the cortex, hippocampus, dopaminergic areas of the midbrain such as the substantia nigra, and the striatum (Benavides *et al*, 1982; Hechler *et al*, 1987, 1992; Castelli *et al*, 2000). GHB reportedly possesses several neurophar-

macological properties such as reduction of voluntary alcohol intake in rats (Fadda *et al*, 1988) and humans (Addolorato *et al*, 1998), treatment of several psychiatric (Poldrugo and Addolorato, 1999) and sleep disorders (Mamelak *et al*, 1986), and efficacy as an anesthetic agent (Kleinschmidt *et al*, 1999). Recently, GHB has become a popular drug of abuse due to its ability to induce euphoria, hallucinations, sedation, and relaxation (Galloway *et al*, 1997; Bernasconi *et al*, 1999). GHB, like many other drugs of abuse, induces a modulation of dopamine release in the nigrostriatal and mesocorticolimbic system (Hechler *et al*, 1991; Hedou *et al*, 2000). Furthermore, electrophysiological studies revealed that GHB modulates the spontaneous firing rate and suppresses burst activity of nigral neurons (Diana *et al*, 1991; Nissbrandt *et al*, 1994; Madden and Johnson, 1998).

The substantia nigra pars compacta (SNc) is located in the midbrain and contains the cell bodies of dopaminergic and GABAergic neurons. Dopaminergic neurons give rise to diffuse mesolimbic and nigrostriatal projections, which play an important role in the actions of many drugs of abuse. Dopaminergic projections are involved in addiction by controlling reward effects of psychoactive substances and

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by generating compulsive drug-seeking behaviors (Hyman and Malenka, 2001; Maharajan *et al*, 2001; Fasano and Brambilla, 2002). The great majority of synapses on the dopaminergic neurons of the SNc are GABAergic (Bolam and Smith, 1990). These inhibitory inputs arise from local interneurons as well as from other areas such as globus pallidus, striatum, and substantia nigra pars reticulata (Somogyi *et al*, 1981; Smith and Bolam, 1990; Smith *et al*, 1996; Chen and Rice, 2002). GABA exerts its action on dopaminergic neurons of the SNc through GABA_A and GABA_B receptors (Bunney *et al*, 1991; Hausser and Yung, 1994; Paladini *et al*, 1999).

GHB has been shown to reduce GABA-mediated synaptic transmission in different brain systems (Cammalleri *et al*, 2002; Xie and Smart, 1992b; Hu *et al*, 2000). However, regulation by GHB of GABA release in the SNc has not been investigated and could be important in shaping the pattern of activity of dopamine neurons. The goal of the present study was to determine the acute actions of GHB on spontaneous GABA-mediated synaptic transmission in the SNc as revealed by whole-cell patch-clamp recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) from dopaminergic neurons.

METHODS

Slice Preparation

Experiments were performed on horizontal slices of the rat ventral midbrain containing the substantia nigra pars compacta, obtained as described previously (Mercuri *et al*, 1995). Briefly, male Wistar rats (P21–P25, 60–80 g) were anesthetized with intraperitoneal ketamine injection and decapitated. The brain was rapidly removed from the skull and horizontal slices (250 μ m thick) were cut in 8–12°C artificial cerebrospinal fluid solution (ACSF) using a vibratome. After 1 h incubation in 34°C ACSF, containing (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 1.2 NaH₂PO₄, 2.4 CaCl₂, 10 glucose, 19 NaHCO₃ and gassed with 95% O₂–5% CO₂, a single slice was transferred to a recording chamber. Here the slice was completely submerged in continuously flowing (2.5 ml/min) ACSF (33–34°C, pH 7.4) and immobilized with a platinum grid. The chamber was mounted on the stage of an upright microscope Axioskop (Carl Zeiss, Oberkochen, Germany) equipped for infrared video microscopy (Hamamatsu, Hamamatsu City, Japan).

Whole-cell Recordings

sIPSCs were recorded in the whole-cell configuration of the patch-clamp technique (voltage-clamp mode) with an Axopatch 200B amplifier (Axon Instruments; Foster City, CA), at a holding potential of –60 mV. Patch electrodes, pulled with a PP-83 Narishige puller (Tokyo, Japan) using 1.5 mm borosilicate glass, had a resistance of 3–6 M Ω when filled with an intracellular solution containing (in mM) 130 KCl, 2 MgCl₂, 1 CaCl₂, 1 NaCl, 11 EGTA, 10 HEPES, 2 Mg-ATP, 0.3 Na₃-GTP; pH was adjusted to 7.3 with KOH. Individual neurons within the SNc were visualized by infrared video imaging and approached by applying positive pressure in the electrode. Dopaminergic neurons were recognized on the basis of the presence of a large

hyperpolarization-induced inward current, which is typical in these cells (Grace and Bunney, 1983; Mercuri *et al*, 1995). Series resistance and whole-cell capacitance were monitored continuously during the experiment and recordings were discarded if series resistance changed by more than 20% from control conditions.

Drugs

Drugs were dissolved in ACSF and applied in the bath by changing the superfusion solution to one that differed only in its content of drug(s). The ratio of flow rate to bath volume ensured complete exchange within 1 min. Drugs used were GHB, 3-*N*[1-(*s*)-(3,4-dichlorophenyl) ethyl] amino-2(*s*)-hydroxypropyl-*p*-benzyl-phosphinic acid (CGP 55845A) and 6,7,8,9-tetrahydro-5(-)[*H*]-benzocycloheptene-5-ol-4-ylidene acetic acid (NCS-382) from Laboratorio Farmaceutico CT (SanRemo, Italy), tetrodotoxin (TTX) from Alomone Labs, and bicuculline from Sigma.

Data Analysis

Recordings were digitized online with the p-Clamp 7 software (Axon Instruments) using a Digidata 1200 acquisition board (Axon Instruments). Spontaneous events were analyzed with the MiniAnalysis software (Synaptosoft Inc., Decatur, GA). The data were presented as mean \pm standard error of the mean (SEM). Statistical differences were determined on raw data by repeated measures analysis of variance (ANOVA) and Kolmogorov–Smirnov test at a significance level of 0.05.

RESULTS

We recorded from 52 dopaminergic neurons within the SNc. Dopaminergic cells were identified by their prominent hyperpolarization-activated inward current (Grace and Bunney, 1983; Mercuri *et al*, 1995). Neurons had a mean input resistance of 110 ± 15 M Ω , which was not statistically affected by GHB (106 ± 19 M Ω , $p = 0.48$).

Effects of GHB on sIPSCs

Spontaneous synaptic currents were recorded from SNc dopaminergic neurons in the presence of the GABA_B antagonist CGP 55845A (1 μ M) to prevent the effects of GHB on the GABA_B receptor (Xie and Smart, 1992a, b; King *et al*, 1997). This spontaneous activity appeared as rapidly rising inward currents at a frequency of 8.91 ± 0.75 Hz and an amplitude of 28.1 ± 2.4 pA ($n = 8$). In this slice preparation, most synaptic drive to SNc dopaminergic neurons is GABAergic, since extrinsic glutamatergic afferents are lost, while the intrinsic GABAergic drive is preserved. Indeed, the spontaneous synaptic currents could be blocked by bicuculline (30 μ M; Figure 1a), indicating that they were spontaneous inhibitory synaptic currents (sIPSCs) mediated by GABA_A receptors.

Bath application of GHB (600 μ M) reduced sIPSCs frequency to 5.46 ± 0.58 Hz ($-38.7 \pm 6.5\%$; $F = 25.5$, $p < 0.01$) and sIPSCs amplitude to 21.4 ± 2.2 pA ($-23.8 \pm 7.8\%$; $F = 28.1$, $p < 0.01$; $n = 8$; Figure 1b–e). After 10 min GHB washout, sIPSCs frequency recovered to

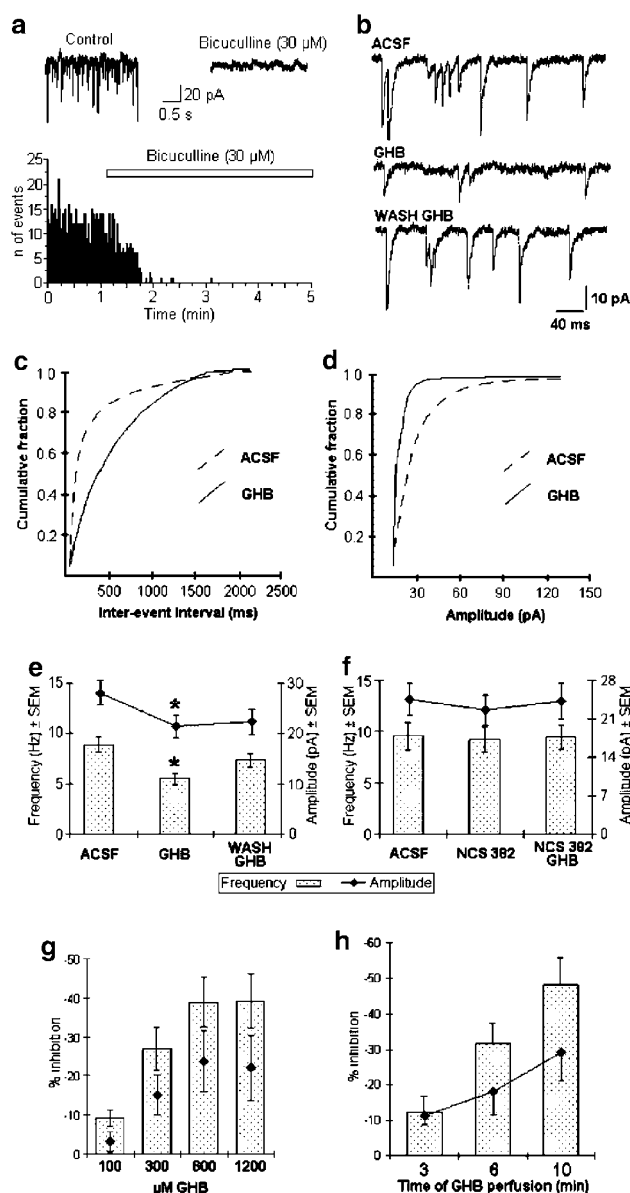


Figure 1 Effects of GHB on sIPSCs. (a) Bicuculline blocked sIPSCs, indicating that they are GABA_A-dependent. Top: recordings of sIPSC in control (ACSF) and during bicuculline (30 μM) perfusion in a representative neuron. Bottom: histogram showing the number of events recorded from the same neuron in control (ACSF) and during bicuculline perfusion. (b) Recordings of sIPSC in control (ACSF), during GHB (600 μM) perfusion, and upon washout of GHB in a representative neuron. (c) Cumulative interevent interval distribution of a representative cell, revealing a significant increase in the interevent interval (ie decreased frequency) during GHB application. (d) Cumulative amplitude distribution obtained from the same cell reveals a significant decrease in sIPSC amplitude during GHB application. (e) Summary of the mean effect of GHB on the frequency and amplitude of the sIPSCs. Significant reductions (*) were observed in both frequency and amplitude ($n = 8$). (f) NCS 382, an antagonist of GHB receptors, blocks the effects of GHB on sIPSCs. Application of NCS 382 (500 μM) alone showed no effects but blocked the effects of GHB (600 μM) on both frequency and amplitude ($n = 4$). (g) GHB reduction of sIPSCs frequency and amplitude at concentrations of 100 μM ($n = 6$), 300 μM ($n = 7$), 600 μM ($n = 8$), and 1200 μM ($n = 8$). Data are percentage inhibition of frequency and amplitude (\pm SEM). Maximal reduction was seen at GHB concentrations of 600–1200 μM. Therefore, a GHB concentration of 600 μM was used for the study. (h) Time course of the effect of GHB (600 μM). Chart shows percentage inhibition of frequency and amplitude (\pm SEM) observed 3, 6, and 10 min after GHB application.

7.36 ± 0.66 Hz and sIPSCs amplitude to 22.3 ± 2.6 pA. To investigate the dose–response relationship, we further tested the effects of GHB at 100, 300, and 1200 μM. GHB (100 μM) reduced mean sIPSC frequency from 4.44 ± 1.55 to 4.03 ± 1.89 Hz (−9.2 ± 2.2%; $F = 0.564$, $p = 0.486$) and mean sIPSC amplitude from 21.26 ± 5.65 to 20.59 ± 6.56 pA (−3.2 ± 2.7%; $F = 0.609$, $p = 0.470$; $n = 6$). GHB (300 μM) exerted a significant reduction of sIPSC mean frequency from 5.67 ± 1.68 to 4.14 ± 1.33 Hz (−26.9 ± 5.5%; $F = 10.34$, $p = 0.018$) and mean sIPSC amplitude from 22.11 ± 4.75 to 18.76 ± 6.56 pA (−15.1 ± 5.1%; $F = 9.78$, $p = 0.020$; $n = 7$). Finally, GHB (1200 μM) exerted a significant reduction of sIPSC mean frequency from 5.55 ± 1.59 to 3.38 ± 1.21 Hz (−39.0 ± 7.1%; $F = 12.53$, $p = 0.009$) and mean sIPSC amplitude from 22.32 ± 5.12 to 17.38 ± 4.83 pA (−22.1 ± 8.3%; $F = 6.29$, $p = 0.041$; $n = 8$; Figure 1g). Cumulative interevent interval and amplitude distributions were significantly different between control and GHB ($p < 0.01$, KS test) in six out of eight cells tested. Analysis of the time course (Figure 1h) of the effect of GHB (600 μM) showed that in the first 3 min of drug application, the mean reduction of the IPSCs was −12.2 ± 4.5% (frequency) and −11.2 ± 2.4% (amplitude). Within 6 min from GHB application, the reduction of IPSCs reached −31.8 ± 5.7% (frequency) and −18.1 ± 6.6% (amplitude), while at 10 min the reduction of the IPSCs reached a steady-state level of −38.7 ± 6.5% (frequency) and −23.8 ± 7.8% (amplitude). To characterize the receptors involved in GHB-induced effects, we repeated the experiments in the presence of the GHB antagonist NCS-382 (500 μM). As shown in Figure 1f, NCS-382 (500 μM) applied alone for 15 min had no significant effect on the frequency (from 9.55 ± 1.33 to 9.25 ± 1.22 Hz) and amplitude (from 24.55 ± 2.98 to 22.62 ± 2.84 pA, $n = 4$) of the sIPSCs. However, after 15 min of application of NCS-382 (500 μM), this drug was able to block the effect of GHB (600 μM, applied for 10 min) on the frequency (+3.14 ± 12.77%, $p > 0.05$) and amplitude (+7.86 ± 11.56%, $p > 0.05$) of the sIPSCs.

These results indicate a depressive effect of GHB on sIPSCs in the SNc. Since both frequency and amplitude of sIPSCs were reduced by GHB, these observations suggest a reduced probability of GABA release or a reduced postsynaptic responsiveness to GABA. In order to discriminate between these two possibilities, we investigated the effects of GHB on action-potential-independent, miniature IPSCs (mIPSCs) recorded in the presence of the voltage-dependent Na⁺ channel blocker TTX.

Effects of GHB on Miniature IPSCs

GABA_A-induced mIPSCs were recorded from dopaminergic neurons of the SNc in the presence of TTX (1 μM) and the GABA_B antagonist CGP 55845A (1 μM). TTX alone reduced the sIPSCs frequency from 9.76 ± 0.96 to 1.34 ± 0.16 Hz and the sIPSCs amplitude from 25.61 ± 2.4 to 23.3 ± 2.2 pA ($n = 7$). The frequency of mIPSCs during GHB (600 μM) perfusion was 1.23 ± 0.14 Hz, and was not significantly different from controls ($F = 0.1$, $p > 0.05$). Similarly, mIPSCs amplitude with GHB was 22.9 ± 1.9 pA, and the difference from controls was not significant ($F = 0.3$, $p > 0.05$) (Figure 2). Interevent interval and amplitude distributions

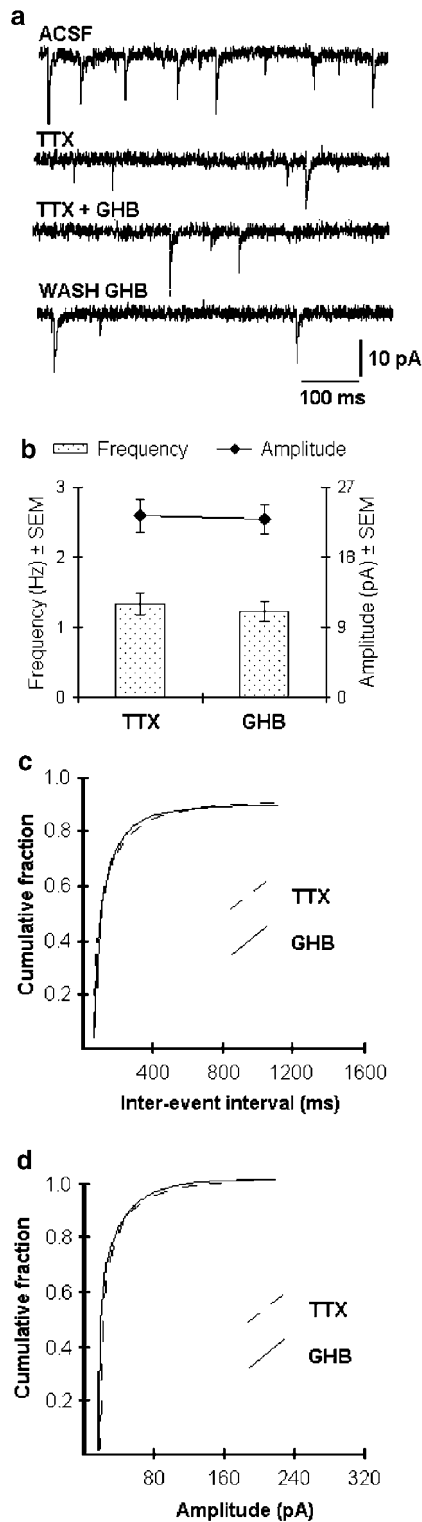


Figure 2 Effects of GHB on mIPSCs. (a) Traces representing recordings of mIPSCs in ACSF, in the presence of TTX (1 μ M), during GHB (600 μ M) perfusion, and during GHB washout in one representative neuron. (b) Mean effect of GHB (600 μ M) on the frequency and amplitude of the mIPSCs. No effects were observed in both frequency and amplitude ($n=7$). (c) Cumulative interevent interval distribution of a representative cell, revealing no changes in mIPSC frequency during application of GHB (600 μ M). (d) Cumulative amplitude distribution obtained from the same cell revealing no changes in mIPSCs amplitude during application of GHB (600 μ M).

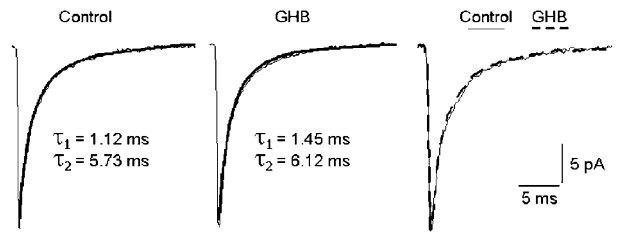


Figure 3 Averages of 200 mIPSCs recorded (left) in control (TTX, 1 μ M) and (middle) during GHB (600 μ M) application (representative cell). Traces are fit with a double exponential function. Statistical analysis of τ_1 and τ_2 showed that there were no significant differences in the mIPSCs kinetics between TTX (control) and GHB. Right: control and GHB mean mIPSCs are superimposed.

were not significantly different in the controls and in the GHB ($p > 0.05$, $n=7$, KS test).

We also analyzed the kinetics of these miniature events ($n=7$), by fitting the decay phase of the averaged mIPSCs with a double exponential function, in control and in GHB for each cell. GHB showed no effects on τ_1 (from 1.21 ± 0.56 to 1.57 ± 0.84 ms, $p=0.61$, $n=7$, ANOVA test) nor on τ_2 (from 5.71 ± 2.83 to 5.98 ± 1.92 ms, $p=0.63$, $n=7$, ANOVA test; Figure 3).

These experiments on mIPSCs demonstrate that, when voltage-dependent Na^+ channels are blocked, the depressive effect of GHB on frequency and amplitude of sIPSCs is suppressed. Thus, GHB does not reduce postsynaptic responsiveness to GABA, and GHB may have an effect at a presynaptic site. The observation that mIPSCs frequency was unaffected by GHB also suggests that the depressing action of this compound on sIPSCs may be ascribed either to a direct action on voltage-dependent Na^+ channels or to a reduction of voltage-dependent calcium conductances activated following the Na^+ channel-induced depolarization. To discern between these possibilities, we examined the actions of GHB on mIPSCs recorded in the presence of high extracellular potassium (15 mM $[\text{K}^+]_0$) in order to promote the release of GABA through an artificial depolarization of the presynaptic terminal.

Effects of GHB on mIPSCs during Depolarization with KCl

In the presence of TTX (1 μ M), the elevation of extracellular K^+ from 3 to 15 mM produced a significant increase in both mIPSCs frequency (from 0.91 ± 0.12 to 1.35 ± 0.19 Hz; $n=9$; $p < 0.05$) and amplitude (from 13.8 ± 0.33 to 18.5 ± 0.35 pA; $n=9$, $p < 0.05$). To confirm that the increased frequency and amplitude of mIPSCs observed in high $[\text{K}^+]_0$ was due to voltage-dependent calcium channel activation, we applied CdCl_2 (200 μ M) in three neurons. In these cells, CdCl_2 reduced the frequency to 0.88 ± 0.51 Hz and amplitude to 12.9 ± 1.1 pA, values not statistically different from those recorded in the presence of TTX alone ($p > 0.05$ for frequency and amplitude).

Under conditions of elevated $[\text{K}^+]_0$, perfusion of GHB (600 μ M) significantly reduced the frequency of mIPSCs to 0.84 ± 0.20 Hz ($-37.8 \pm 5.2\%$; $p < 0.05$) while their amplitude was not affected (16.8 ± 2.8 pA; $p > 0.05$). Upon GHB washout, mIPSCs frequency recovered to control levels

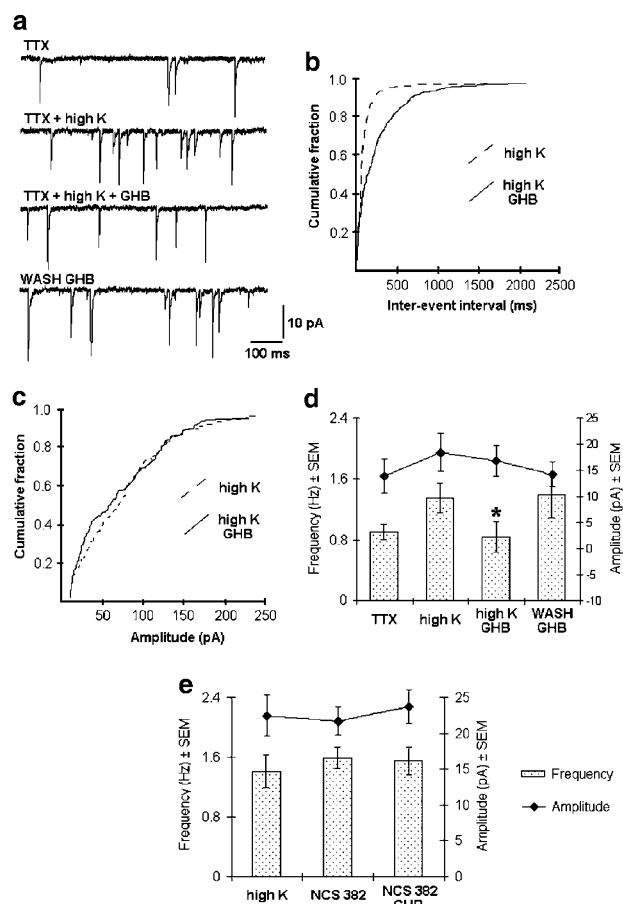


Figure 4 Effects of GHB on mIPSCs recorded in elevated $[K^+]_o$. (a) Recordings of mIPSCs in TTX ($1 \mu\text{M}$), TTX ($1 \mu\text{M}$), and elevated $[K^+]_o$ (15 mM), during GHB ($600 \mu\text{M}$) application and upon washout of GHB. (b) Cumulative interevent interval distribution of a representative cell, revealing a significant reduction of the mIPSCs frequency during GHB application. (c) Cumulative amplitude distribution obtained from the same cell revealing no changes in mIPSC amplitude during GHB ($600 \mu\text{M}$) application. (d) Summary of the mean effect of GHB on the frequency and amplitude of the mIPSCs recorded in elevated $[K^+]_o$. GHB reduced the frequency (*) but not the amplitude of the mIPSCs ($n = 9$). (e) NCS 382 blocked the effect of GHB on the frequency of the mIPSCs recorded during elevated $[K^+]_o$ (15 mM). NCS 382 showed no effects *per se* on the frequency and amplitude of the mIPSCs ($n = 3$).

($1.39 \pm 0.29 \text{ Hz}$, $p > 0.05$). Cumulative interevent interval distributions in control and GHB were significantly different ($p > 0.05$, KS test) (Figure 4a–d).

The specificity of the effect of GHB in high $[K^+]_o$ was tested by repeating the same experiments in the presence of the GHB receptor antagonist NCS-382. As shown in Figure 4e, NCS-382 ($500 \mu\text{M}$) applied alone for 10 min had no significant effect on the frequency (from 1.41 ± 0.22 to $1.59 \pm 0.14 \text{ Hz}$, $p > 0.05$) and amplitude (from 22.45 ± 2.88 to $21.72 \pm 1.99 \text{ pA}$, $n = 3$) of the mIPSCs. However, NCS-382 ($500 \mu\text{M}$) prevented the effect of GHB ($600 \mu\text{M}$) on the frequency ($-1.95 \pm 2.71\%$, $p > 0.05$) of the mIPSCs.

These results show that, although there is no direct evidence of an action of GHB on voltage-dependent calcium channels, the depression effect of GHB on the frequency of mIPSCs is most likely due to its action on presynaptic voltage-dependent calcium influx activated following action potential invasion of the presynaptic terminal.

DISCUSSION

The present study demonstrates that GHB reversibly reduces both frequency and amplitude of sIPSCs in dopaminergic neurons of the SNc, following a reduction of GABA release, most likely due to inhibition of calcium influx in the presynaptic terminal.

The concentrations of GHB used in this study (100 – $1200 \mu\text{M}$) are in the range of those measured in the rat brain after systemic administration of pharmacologically effective doses (200 – 400 mg/kg) that reduced voluntary alcohol intake (Fadda *et al*, 1988). Moreover, the dose–response relationship (Figure 1g) and previous reports showed maximal and specific action of GHB at the concentration of $600 \mu\text{M}$ (Berton *et al*, 1999; Cammalleri *et al*, 2002). It should be noted that previous studies (Madden and Johnson, 1998; Erhardt *et al*, 1998; Shepard and Connelly, 1999) reported an inhibitory effect of GHB on nigral dopamine neurons. However, higher doses of GHB were used by these authors, and no GABA_B receptor antagonist was present in the perfusing medium. Conversely, the continuous presence of CGP 55845A in our work prevented GABA_B receptor activation by GHB and we never observed changes in the holding current or in cell input resistance such as those reported by other authors (see also Olpe and Koella, 1979; Kozhechkin, 1980; Xie and Smart, 1992a).

The action of GHB can be at least in part ascribed to specific depressing mechanisms, since the reduction of sIPSCs frequency and amplitude by GHB was prevented by NCS-382, a GHB receptor antagonist (Maitre *et al*, 1990). This result suggests that the depressant effect of GHB shown in this study, which leaves out GHB effects on the GABA_B receptor, is mediated by the activation of the GHB receptor. In this regard, it should be mentioned that the selectivity and specificity of NCS-382 for the GHB receptor is still a matter of debate, as some laboratories have reported divergent results regarding this issue (Metcalf *et al*, 2001; Carter *et al*, 2003; Gould *et al*, 2003; Lamb *et al*, 2003).

A GHB-induced depression of the spontaneous inhibitory synaptic transmission has been reported previously in neocortical neurons (Jensen and Mody, 2001). This depression was obtained at higher concentrations (10 mM) than those of the present study and it was reversibly blocked by a selective GABA_B antagonist. Thus, GHB can also activate presynaptic GABA_B receptors to reduce inhibitory and excitatory transmitter release as described in other reports (Xie and Smart, 1992a, b; King *et al*, 1997). In the presence of TTX ($1 \mu\text{M}$), no reduction in mIPSCs amplitude and frequency was observed indicating that the depressing effect of GHB on sIPSCs was not due to a reduced postsynaptic sensitivity to GABA but to a presynaptic depression of GABA release. Our observation that GHB did not alter the kinetic properties of mIPSCs contradicts its postsynaptic mechanism of action. Moreover, the observation that K^+ -evoked release of GABA was attenuated by GHB also implies a presynaptic mechanism of action (Banerjee and Snead, 1995). Additionally, it has been previously shown that GHB receptors are found mostly on nerve terminals (Maitre *et al*, 1983). A presynaptic mechanism of action of GHB has also been proposed in the hippocampus in experiments on paired pulse facilitation (Berton *et al*, 1999) or following exogenous application of GABA (Cammalleri *et al*, 2002).

A presynaptic mechanism underlying the effect of GHB does not conflict with the observed reduction of sIPSCs amplitude. A conceivable explanation for this result is the presence of multiple release sites on the GABAergic terminals that simultaneously release GABA when invaded by an action potential. Thus, a presynaptic depression of action-potential-driven events would result in the suppression of higher amplitude multiquantal events. In agreement with this hypothesis, perfusion with TTX also resulted in reduction of both frequency and amplitude of spontaneous synaptic events.

The lack of effect in the presence of TTX indicates that GHB only depresses synaptic GABAergic events triggered by action potentials. Hence GHB may affect the action potential itself by inhibiting its generation or its ability to invade the terminal. However, GHB may also reduce the calcium influx, following spike-driven activation of voltage-gated calcium channels. In order to discriminate between these possibilities, we bypassed the possible effects on action potentials by performing experiments in the presence of TTX and then depolarizing the terminals with high extracellular K^+ (15 mM). In this condition, we were able to promote a cadmium-sensitive increase in mIPSC frequency, indicating an increased GABAergic neurotransmission due to calcium channel activation independent of spike generation. In these conditions, the ability of GHB to reduce sIPSC frequency was restored. This result gives a strong indication that voltage-dependent calcium entry could be a specific target of GHB action, whereby spontaneous synaptic GABAergic release could be inhibited by reducing the spike-dependent calcium influx into the terminal.

The inhibitory effect of GHB on GABAergic neurotransmission reported here is expected to have substantial influences on the activity of the dopaminergic neurons of the ventral midbrain, and consequently on a wide range of behaviors related to drug abuse.

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