Effects of Sustained Gamma-Hydroxybutyrate Treatments on Spontaneous and Evoked Firing Activity of Locus Coeruleus Norepinephrine Neurons

Steven T. Szabo, Mark S. Gold, Bruce A. Goldberger, and Pierre Blier

Background: Gamma-hydroxybutyrate is currently used to promote nighttime sleep in the treatment of narcolepsy; however, it is also a drug of abuse (“Liquid Ecstasy”) associated with a withdrawal syndrome with anxiety features. Of interest, the activity of locus coeruleus neurons is a reflective index of these above mentioned behavioral states.

Methods: Using in vivo extracellular unitary recordings, sustained administration of gamma-hydroxybutyrate (40 mg/kg/day via minipump implanted subcutaneously) on the spontaneous and sensory-evoked burst firing of locus coeruleus norepinephrine neurons was assessed in rats.

Results: A 2-day and 10-day gamma-hydroxybutyrate administration decreased the spontaneous firing activity of locus coeruleus neurons by 52% and 54%, respectively, when compared with controls. A similar degree of attenuation on evoked burst firing of norepinephrine neurons also occurred in these rats (2-day gamma-hydroxybutyrate: 47% and 10-day gamma-hydroxybutyrate: 58%), when compared with controls. In contrast, rats treated with gamma-hydroxybutyrate for 10 days followed by removal of the minipump for 36 hours resulted in a 33% augmentation in spontaneous locus coeruleus activity as compared with controls. Furthermore, a robust 79% increase in burst firing in response to paw-pinch was exhibited in these rats.

Conclusions: Chronic gamma-hydroxybutyrate treatment inhibits the spontaneous and sensory-evoked burst firing of locus coeruleus norepinephrine neurons, whereas these indices are enhanced during drug withdrawal. The alteration in norepinephrine activity during chronic gamma-hydroxybutyrate administration may contribute to the ability of this agent to induce sleep and regulate narcoleptic episodes. Enhanced norepinephrine activity during withdrawal may be related to symptoms of anxiety on rapid termination of this drug in abusers.

Key Words: Anxiety disorders, withdrawal, addiction, sleep, drug abuse

Gamma-hydroxybutyrate (GHB) is a metabolite of γ-aminobutyric acid (GABA) and is produced naturally in the brain through the semialdehyde reduction pathway (for a review, see Maitre et al 2000; Szabo et al 2003). Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessively inherited defect that leads to the accumulation of GHB (Gibson et al 1998). The clinical presentation of this disease includes significant behavioral disturbances and psychosis (hallucinations, disabling anxiety, aggressive behavior, and sleep disorder), which is accompanied by white-matter hyperintensities of the globus pallidus, thalamus, and brainstem in these patients (Pearl et al 2003). Pharmacologically, GHB binds not only to specific GHB receptors (Benavides et al 1982; Hechler et al 1992; Snead and Liu 1984) but has been largely regarded to mediate its central nervous system (CNS) effects though GABA type B (GABAB) receptors (Bernasconi et al 1999; Emri et al 1996; Erhardt et al 1998; Lingenhöhl et al 1999; Waldmeier 1991; Williams et al 1995), with recent findings indicating a GABA type A (GABA_A) receptor interaction as well (Cammalleri et al 2002; Follesa et al 2003; Lobina et al 1999; Schmidt-Mutter et al 1998).

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correlate with reduced anxiety, opiate-withdrawal attenuation, and sedation leading to sleep or unconsciousness (Szabo and Blier 2001b; Aston-Jones et al 2001).

Given that LC activity is important in regulating many of the aforementioned behavioral states that GHB influences, it was deemed interesting to evaluate the impact of this drug on the firing activity of neurons within this nucleus. The spontaneous firing activity of LC NE neurons was assessed in rats receiving GHB in a sustained fashion for 2 days, 10 days, and 36 hours following washout of the drug in 10-day treated animals. The latter was an attempt to mimic the withdrawal syndrome reported in chronic GHB abusers. Furthermore, as the response to sensory inputs may be altered when administered GHB, it was important to assess the responsiveness of LC neurons to an external stimulus in the above mentioned GHB experiments.

**Methods and Materials**

**Animals**

The experiments were carried out in male Sprague Dawley rats (Charles River, Raleigh, North Carolina), weighing between 300 g and 325 g, which were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water ad libitum). Rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal [IP]) and mounted in a stereotaxic apparatus (David Kopf Instruments Tujunga, California). Supplemental doses (100 mg/kg, IP) were given to prevent any nociceptive reaction to pinching of a hind paw or the tail.

**Short-Term, Long-Term, and Withdrawal Treatments**

Rats were anesthetized with halothane for subcutaneous implantation of osmotic minipumps (ALZA, Palo Alto, California). Two groups of rats were treated with GHB (Sigma Chemicals St. Louis, Missouri) (40 mg/kg/day) for 2 days or 10 days. Groups of rats were treated with the vehicle for GHB (9.9% sodium chloride [NaCl]) in each treatment group to act as respective controls. The rats were tested with the minipumps in place or 36 hours following removal. The effects of other doses as well as different time points of GHB administration and removal on LC activity were not assessed.

**Electrophysiological Experiments**

A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for LC neuron recordings. Extracellular unitary recordings of LC NE neurons were conducted with single-barreled glass micropipettes preloaded with fiberglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1 μm to 3 μm and filled with a 2 mol/L NaCl solution. Their impedance range was between 2 MΩ and 4 MΩ, which provides stable recordings and a signal-to-noise ratio easily allowing action potential discrimination from the baseline. Locus coeruleus NE neurons were recorded with micropipettes lowered at −.7 mm interaural and 1.1 mm to 1.4 mm lateral. Spontaneously active NE neurons of the LC were identified using the following criteria: regular firing rate (1–5 Hz) and positive action potential of long duration (0.8–1.2 milliseconds) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. Norepinephrine neurons were recorded for at least 1 minute to establish basal firing rate. To determine possible changes in the spontaneous firing activity of NE neurons, four to five electrode descents were carried out through this nucleus in control and treated rats.

**Sensory-Evoked Response to an External Stimulus**

The last electrode descent through the LC in each rat was dedicated to assessing sensory-evoked response on NE neuron firing to an external stimulus. Upon a stable firing rate (usually obtained after 1 minute), the contralateral paw was compressed with a surgical forceps that had a rounded end to avoid tissue damage on compression. This compression lasted approximately 1 second with equal pressure being applied to the paw of rats; once the opposite sides of the forceps made contact with each other, the forceps were then released. This is similar to the paradigm detailed by Grant and Weiss (2001). Of interest, it has also been reported that the number of elicited bursts is largely independent of paw-compression intensity (Simson and Weiss 1987). For each neuron tested, the value of burst firing activity assigned to a NE neuron was composed of an average of three paw-pinch trials, each of which was separated by at least 15 seconds.

**Statistical Comparison**

All results were expressed as mean (± SEM) of single neuron values. Statistical comparisons of values obtained in treated and control rats were carried out using Kruskal-Wallis one-way analysis of variance followed by post hoc Dunn method, where multiple comparisons versus control group were performed.

**Results**

**Effects of GHB on the Spontaneous Firing Activity of LC Neurons**

Rats receiving the saline vehicle for 2 days and 10 days, to act as respective controls for the GHB treated animals, did not result in a difference in LC spontaneous firing activity when compared with each other. These data were therefore merged and correspond to a single control group. Sustained 2-day and 10-day GHB treatments attenuated the spontaneous firing activity of LC neurons, whereas rats undergoing a 36-hour washout resulted in augmented NE neuron firing frequency (Figure 1). The 2-day and 10-day GHB treatments produced a significant 52% (range of firing: 2.2–2.6 Hz) and 54% (range of firing: 3.8–2.0 Hz) decrease in mean spontaneous firing rate, respectively. Rats treated with GHB for 10 days followed by a 36-hour washout resulted in a significant 33% increase in the mean spontaneous firing activity of LC neurons (range of firing: 6.0–6.6 Hz) when compared with that of control rats (range of firing: 5.0–4.4 Hz) (Figure 2). Systematic electrode descents into the LC yielded a trend for a decrease and increase in the number of LC neurons discharging spontaneously in rats treated with GHB or following removal of the drug, respectively, but did not reach statistical significance (Table 1).

**Effect of GHB on the Sensory-Evoked Burst Firing of NE Neurons**

Sustained 2-day and 10-day GHB treatments attenuated the sensory-evoked burst firing of LC neurons, whereas rats undergoing a 36-hour washout resulted in an increase in evoked firing rate (Figures 3 and 4). The 2-day and 10-day GHB treatments produced a significant 47% (range of firing: 1–5 Hz) and 58% (range of firing: 1–5 Hz) decrease, respectively, in the number of evoked bursts. Rats treated with GHB for 10 days following a 36-hour washout resulted in a significant 79% increase in the number of elicited bursts of LC activity (range of firing: 5–15 Hz) when compared with that of control rats (range of firing: 3–10 Hz) (Figure 4).
Discussion

A sustained 2-day and 10-day administration of GHB decreased the spontaneous firing activity of LC neurons, whereas GHB discontinuation assessed in the latter treatment group resulted in enhanced activity of this nucleus. The inhibitory action of GHB on LC neuron firing rate is consistent with reports of GHB being able to attenuate brain NE levels (Miguez et al 1988) and the ability of this agent to alter the metabolism of NE (Anden 1974; Gomes et al 1976). The exact mechanism by which GHB attenuates NE activity remains to be elucidated; however, the ability of GHB to activate GABAA and GABAB receptors may explain, at least in part, this effect because these receptors mediate an inhibitory influence on NE neuron firing (Shefner and Osmanovic 1991; Ennis and Aston-Jones 1989). Thus, GHB may attenuate the activity of this nucleus via direct activation of GABA-

Figure 1. Integrated firing rate histograms of LC NE neurons, recorded in single electrode descent in the LC showing their spontaneous firing activity in control (n = 4) (A), 2-day GHB treatment (40 mg/kg/day; n = 4) (B), 10-day GHB treatment (40 mg/kg/day; n = 4) (C), and 10-day GHB treated animals (40 mg/kg/day) where the minipump was removed for 36 hours (n = 4) (D). Each spike corresponds to the number of discharges recorded per a 10-second time lapse. The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded. LC, locus coeruleus; NE, norepinephrine; GHB, gamma-hydroxybutyrate.

Table 1. Locus Coeruleus Norepinephrine Neurons in Controls and Treated Rats

<table>
<thead>
<tr>
<th></th>
<th>Average Number of Noradrenergic Neurons per Descent</th>
<th>Number of Descents</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.7 ± .4</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>GHB (40 mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>3.1 ± .3</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>10 days</td>
<td>3.5 ± .5</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>10 days + 36 hours washout</td>
<td>4.5 ± .6</td>
<td>13</td>
<td>4</td>
</tr>
</tbody>
</table>

*p = .208, among treatment groups using one-way analysis of variance. GHB, gamma-hydroxybutyrate.
receptors on NE neurons or indirectly via alteration of neurons in complex circuitries through which LC activity is regulated (Jodo and Aston-Jones 1997; Szabo and Blier 2001a; Ennis and Aston-Jones 1988; Szabo and Blier 2001c). Studies using GABA receptor antagonists applied iontophoretically in the LC or injected systemically in GHB treated animals while recording the firing activity of NE neurons could help elucidate the mechanism by which GHB alters LC firing.

Endogenous GHB levels and binding sites have previously been reported to be highest in the pontomedullary region of the brain (Doherty et al 1978; Snead and Liu 1984), a region where the LC is located and where the predominant afferent regulation of this nucleus occurs (for a review, see Aston-Jones et al 1991). The distribution of GHB high-affinity binding sites in the rat brain does not match the distribution of GABAA or GABAB binding and appears to be specific for GHB (Maitre et al 2000). Recently, Gould et al (2003) published that newer ligands for the GHB receptor reveal highest binding in cortex and hippocampus and low levels in the LC, while recording the firing activity of NE neurons could help elucidate the mechanism by which GHB alters LC firing.

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A sustained administration of GHB decreased the number of burst firing of NE neurons to a contralateral paw-pinch (Figure 3). The inhibitory effect of GHB on evoked NE neuron burst firing in 2-day GHB treated animals was sustained during a 10-day GHB administration (Figure 4). The mechanism by which NE activity results in a burst-type firing pattern to paw-pinch can be influenced by many factors, including that of the activation of
a variety of receptors (Simson and Weiss 1987). More specifically, Ennis and Aston-Jones (1986) demonstrated that burst firing is due to glutamate, while α2-adrenoceptors and/or calcium (Ca2+) channels are responsible for the inhibition of NE neurons following stimulus activation (Aghajanian et al 1977; Cedarbaum and Aghajanian 1976). Furthermore, α2-adrenoceptors appear to be paramount to the production of burst firing of LC neurons to a paw-pinch independent of altered spontaneous firing rate (Simson and Weiss 1987). It is, however, not known whether an altered activation of α2-adrenoceptors represents a mechanism in which GHB is able to produce its effects on paw-pinch induced LC burst firing or whether a constant increase of GABAergic tone in this nucleus or an action on glutamate, rather than an effect on adrenergic transmission, may underlie this phenomenon. Nonetheless, the ability of GHB to attenuate the perturbation of LC activity to an external stimulus may be important in the regulation of anxiety-like symptoms that occur when chronic opiate or GHB abusers are in withdrawal (Gallimberti et al 1993; Goldrath and Charney 1997). On the other hand, given that the α2-adrenoceptor agonist clonidine is capable of curtailing these effects in the former (Gold 1993; Gold et al 1978), this agent may have some clinical utility in the management of GHB withdrawal.

In conclusion, the attenuation on NE neuron firing rate may be important to the anxiolytic and sedative effects associated with GHB (Miotto et al 2001), as LC activity is linked to these behavioral states (Jones 2003; Szabo and Blier 2001b). In turn, augmented LC activity may be likened to the anxiety symptoms chronic GHB abusers often exhibit when in withdrawal (Miotto et al 2001). This is consistent with agents or stimuli which increase LC activity being capable of triggering anxiety (Szabo and Blier 2001b), whereas anxiolytics which decrease LC activity, such as the benzodiazepines (Grant et al 1980) and barbiturates (Laurent et al 1983), are effective in reducing withdrawal symptoms associated with chronic GHB abuse (Dyer et al 2001; Miotto et al 2001). Furthermore, given that the clinical presentation of GHB withdrawal shares aspects similar with that of withdrawal from opiates, alcohol, and benzodiazepines, these drugs of abuse and potential treatments thereof may pharmacologically converge on common signaling mechanisms (Miotto et al 2001; Nestler et al 1989a, 1989b). For instance, GHB has been reported to substitute for the reinforcing effects of opiates in animal paradigms of drug addiction (Matta et al 1997), as well as being able to curtail the effects of opiate withdrawal in humans (Gallimberti et al 1993). Lastly, the ability of GHB to regulate the activity of NE neurons from internal (decrease in spontaneous firing activity) and external stimuli (paw-pinch evocation of burst firing) may also represent an important mechanism in the ability of this drug to restructure LC activity and regulate sleep-wake patterns in patients with narcolepsy. Further investigation of the mechanism(s) by which GHB alters LC activity and relevance into the production and treatment of the above mentioned disorders is warranted.

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