BASIC INVESTIGATIONS

Gamma-hydroxybutyric Acid Tolerance and Withdrawal in a Rat Model

Theodore C. Bania, MD, MS, Tom Ashar, MD, Gregory Press, MD, Patricia M. Carey, MD

Abstract

Long-term daily use of gamma-hydroxybutyrate (GHB) and related compounds has recently been associated with a withdrawal syndrome. To the best of the authors' knowledge, there are currently no animal models of GHB withdrawal. **Objectives:** The authors studied and described the effect of chronic dosing of GHB (3–6 days) on tolerance and withdrawal in a rat model. **Methods:** Rats were administered GHB every three hours via intraperitoneal catheter. Groups of rats (2 per group) were dosed with GHB for either 3 (24 doses), 4 (32 doses), 5 (40 doses), or 6 (48 doses) days. The GHB dose was 0.25 g/kg for doses 1–8, 0.75 g/kg for doses 9–12, 1 g/kg for doses 13–16, 1.25 g/kg for doses 17–24, 1.5 g/kg for doses 25-32, 1.75 g/kg for doses 33–40, and 2 g/kg for doses 41–48. Following the last dose of GHB, the rats were scored using a 16-point ethanol intoxication–withdrawal scale rating spontaneous behaviors, response to handling, grooming, and neurological signs. Lower scores indicate intoxication, while higher scores indicate withdrawal. Scores were recorded at hours 0, 1, 2, 3, 4, 5, 6, 9, 12, and 24. **Results:** *Tolerance:* Rats dosed with GHB for more days were less intoxicated one hour after their last GHB dose despite receiving higher doses. *Withdrawal:* The scores for all rats dosed with GHB increased at hours 4 ($p = 0.028$), 5 ($p = 0.037$), 6 ($p = 0.007$), and 9 ($p = 0.024$) after the last dose, indicating withdrawal. The scores demonstrated a linear increase dependent upon the number of days of GHB dosing at hours 3 ($p < 0.000$), 4 ($p = 0.004$), 5 ($p = 0.002$), and 12 ($p = 0.039$) as well as prior to the last dose at hour 0 ($p = 0.000$). No rats developed seizures. **Conclusions:** Tolerance and mild withdrawal in rats can be induced by administering intraperitoneal GHB every three hours for 3-6 days. More prolonged dosing and higher doses of GHB may be necessary to induce severe withdrawal. **Key words:** gamma-hydroxybutyrate; GHB; tolerance; withdrawal; drug use. ACADEMIC EMERGENCY MEDICINE 2003; 10:697–704.

Gamma-hydroxybutyric acid (GHB) and its analogues, gamma-butyrolactone and 1-4 butanediol, are used as recreational drugs for their euphoric and sedative effects. They are also used as dietary supplements for their unsubstantiated effect on weight loss and muscle synthesis. Cessation of long-term daily use of GHB may result in a withdrawal syndrome similar to the delirium tremens of ethanol withdrawal.[1–8]

Currently, the treatment of GHB withdrawal is based upon case reports and case series.[1–8] To our knowledge, there are no established animal models in which to evaluate potential therapy. As a first step in the development of an animal model of GHB withdrawal, we studied and described the effect of chronic dosing of GHB (3–6 days) on tolerance and withdrawal in a rat model. We hypothesized that tolerance can be induced in rats with frequent, prolonged dosing of GHB, and withdrawal can be induced following cessation of frequent, prolonged dosing of GHB.

From the Department of Emergency Medicine, St. Luke's–Roosevelt Hospital Center, Columbia University, College of Physicians and Surgeons, New York, NY (TCB, TA, GP, PMC).
Received September 4, 2002; revision received January 27, 2003; accepted January 29, 2003.
Presented at the SAEM annual meeting, St. Louis, MO, May 2001.
Address for correspondence and reprints: Theodore C. Bania, MD, MS, St. Luke's–Roosevelt Hospital Center, Department of Emergency Medicine, 1000 10th Avenue, Room GE-03, New York, NY 10019. Fax: 212-523-8000; e-mail: toxtod@aol.com.

METHODS

Study Design. This was an observational and descriptive laboratory investigation. The Animal Care and Use Committee of the institution approved this protocol, and handling of the animals was in accord with the National Institutes of Health and Food and Drug Administration guidelines.

Animal Subjects and Preparation. Adult male Sprague-Dawley rats weighing between 305 and 362 g were used. The rat model has been used previously to study ethanol withdrawal[9,10] and single doses of GHB.[10,11] The animals were allowed free access to food and water for the duration of the protocol. Alternating 12-hour light and dark cycles were maintained, and the observations were performed in the morning at the beginning of the 12-hour light cycle.

All ten animals were prepared and instrumented in the same manner. The animals were sedated and

anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (8 mg/kg). A 5-Fr pediatric feeding tube was placed into the peritoneum through an abdominal incision. The proximal end of the feeding tube was then subcutaneously tunneled and exteriorized to the back of the neck. The tube was secured in place and the animals were allowed to recover for 48 hours.

Study Protocol. There were five groups, each group consisting of two rats. Four groups received GHB intraperitoneally every three hours for 3, 4, 5, and 6 days, respectively. GHB was dosed for a maximum of six days, because ethanol withdrawal can be induced in rats if ethanol is dosed for six days. The dose of GHB was increased over the first three days. The dose of GHB increased for each additional day of GHB dosing. The 3-day-GHB group received GHB for three days for a total of 24 doses (0.25 g/kg for doses 1–8; 0.75 g/kg for doses 9–12; 1.0 g/kg for doses 13–16; 1.25 g/kg for doses 17–24). The 4-day-GHB group received GHB for three days at the same doses as the 3-day-GHB group, and then 1.5 g/kg on day 4 for doses 25–32. The 5-day-GHB group received the same doses as the 4-day-GHB group for four days, and then 1.75 g/kg on day 5 for doses 33–40. The 6-day-GHB group received the same doses as the 5-day-GHB group for five days, and then 2 g/kg on day 6 for doses 41–48. The control group received 0.3 mL of 0.9% saline intraperitoneal every three hours for three days (24 doses) to make it similar to the 3-day-GHB group.

Measurements. Following the last dose of GHB, the rats were observed and scored at 1, 2, 3, 4, 5, 6, 9, 12, and 24 hours using an ethanol intoxication–withdrawal scale. An ethanol intoxication–withdrawal scale was used because the clinical cases of GHB withdrawal have been observed to closely resemble ethanol withdrawal,[1–8] and no GHB intoxication–withdrawal scale has been developed. The scoring system was originally described by Lal et al.[10] and modified by Colombo and Agabio[9] and uses 16 separate items to evaluate the intensity of both intoxication and withdrawal (Table 1). Most items were graded using a four-point scale (0–3) that reflects the increased frequency of occurrence and degree of severity of each item. For most items, a score of 0 indicates either a sleeping animal or residual intoxication; a score of 1 indicates the normal appearance of a rat that is similar to an undrugged rat; and scores of 2 and 3 indicate increasing severity of withdrawal. The first six items were evaluated for 15 seconds, before any handling, while the rat was in the cage. The next eight items were evaluated upon opening the cage, picking up the rat, and placing it back in the cage. The final two items were evaluated before, during, and after handling. A total score based upon the sum of these items was assigned to each rat on each observation. Scores of 8–9 indicate a neutral state, corresponding to a healthy and undrugged rat. Lower scores indicate intoxication, and higher scores indicate withdrawal. Scores less than 4 indicate severe intoxication, and scores of 18–20 indicate severe withdrawal. The rats were observed for the first six hours for the development of spontaneous seizures.

Data Analysis

Tolerance. For each group, following the last dose of GHB, the scores at hours 1 and 2 were compared with the last dose of GHB in g/kg using a simple linear regression analysis. Only the eight animals that received GHB were included in this evaluation.

Withdrawal. To determine the onset and duration of withdrawal, the average intoxication–withdrawal scores for all GHB dosed rats at hours 1, 2, 3, 4, 5, 6, 9, and 12 were compared with their hour 24 score using the Wilcoxon sign rank test (Figure 1). Hour 24 was taken to represent a "baseline level" for all GHB dosed rats because at this time scores approximated that of the saline placebo group. Only the eight animals that received GHB were analyzed. Alpha was set at 0.05.

To determine whether duration of dosing with GHB affected the intoxication–withdrawal scores, the number of days of GHB dosing was compared with the intoxication-withdrawal scores at hours 3, 4, 5, 6, 12, and 24. Hour 3 is when the next dose of GHB would have been given. A simple linear regression analysis was used, and all ten animals were evaluated in this comparison.

RESULTS

Tolerance. The slope of the regression line was significantly greater than zero at hour 1 (Fig. 1), but not at hour 2 (hour 1 slope = 12.1; 95% CI = 5.5 to 19.7; $p = 0.005$; $Y = -13.4 + 12.1X$; $r^2 = 0.76$). This indicates that within this model (in which longer duration of dosing resulted in increasing doses of GHB), the intoxication–withdrawal score will increase by 12.1 at hour 1 for every additional 1 g/kg of GHB given.

Withdrawal. The rats that received GHB had a decrease in the intoxication–withdrawal score at hour 1 following the last dose of GHB ($p = 0.028$). The intoxication–withdrawal score increased at hours 4 ($p = 0.028$), 5 ($p = 0.037$), 6 ($p = 0.007$), and 9 ($p = 0.024$) when compared with the hour 24 score (Fig. 2).

The slope of the regression line was significantly greater than zero at hours 3, 4, 5, and 12 (hour 3 slope = 1.3 95% CI = 0.9 to 1.6, $p < 0.000$, $r^2 = 0.90$; hour 4 slope = 1.3, 95% CI = 0.5 to 1.9, $p = 0.004$, $r^2 = 0.66$; hour 5 slope = 0.96, 95% CI = 1.7 to 4.5, $p = 0.002$, $r^2 = 0.73$; hour 12 slope = 0.56, 95% CI = 0.04 to 1.07, p =

TABLE 1. Ethanol Intoxication and Withdrawal Scoring Sheet

Spontaneous Behaviors	Observe for 15 seconds.
(1) General	
Immobility	0
Small movements with no walking	1
Occasional walking	2
Continuous or rapid locomotion	3
(2) Treading	Forelimb movement not involved in locomotion, grooming, or feeding. Number of discrete movements of one or both forelimbs.
No treading	0
One to two movements	1
Three to nine movements	2
More than nine movements	3
(3) Shakes	Wet dog type.
Absence of movement	0
One	1
Two to several times	2
Severe or continuous	3
(4) Jerks	Single, usually of head.
Absence of movement	0
One (low incidence compared with normal rats)	1
Two to several times	2
Severe or continuous	3
(5) Twitching	Smaller, twitching movement of isolated body part (flanks, forelimbs, head).
Absence of movement	0
One (low incidence compared with normal rats)	1
Two to several times	2
Severe or continuous	3
(6) Head Tremor	A small amount of phasic movement of the head and vibration of whiskers is normally present.
No movement	0
Normal	1
Increased movement	2
Severe and continuous	3
Response to Handling	The rat's response to opening of the cage and being picked up.
(7) Vocalization	
No sound	0
Single sound, low intensity (squeak or whimper)	1
Loud (screech)	2
Loud, repetitive, continued throughout the time of approach and initial touching	3
(8) Avoidance	Rat's clinging to wire cage top.
Not clinging to floor	0
Brief clinging to floor	1
Moved to back of cage, evasive movement, cling to floor	2
Pressed body firmly against back wall, held extremely forcefully	3
(9) Failure to Groom	
Clean (white, soft, smooth)	0
Rough texture	1
One body part soiled with feces, urine, blood, or food	2
More than one body part soiled with feces, urine, blood, or food	3
Behaviors While Holding the Rat in the Palm of One Hand and Manipulating It Gently with the Other Hand	
(10) Rigidity	Axial musculature palpated.
Flaccid	0
Normal muscle tone	1
Elevated tension	2
Rigidity	3

Continued

TABLE 1. Ethanol Intoxication and Withdrawal Scoring Sheet (*Cont.*)

(11) Tail Tremors	Tail drawn gently between rater's fingers.
Limp	0
Normal tension	1
Tremor and rigidity	2
Very stiff and/or shaking	3
(12) Caudal Tremors	Place palm of hand against the base of the rat's tail.
No tremor	0
Normal body vibrations	1
Tremor	2
Severe shivering or shaking	3
(13) General Tremor	
Limp	0
Normal muscular tension	1
Tremor	2
Constantly shivering or shaking	3
(14) Motor Task	Place the rat on the upper edge of the cage, and observe as the rat descends 6 inches to the cage floor.
Fell	0
Jumped smoothly, touching wall with forepaws	1
Jerky movement, uncoordinated, slowly climbed down cage wall	2
Greater than 5 seconds to climb down cage wall	3
Behaviors, before, during, or after Handling	
(15) Bracing Posture	
Limp	0
Normal waking postures (sitting, crouching, standing)	1
Hind limbs spread in abnormally wide stance	2
Wide stance with lowered body, extension of forelimbs straight ahead	3
(16) Spontaneous Convulsions	
None	0
Forelimb clonus and whole body shakes	1
Tonic/clonic convulsions	2

0.039, $r^2 = 0.43$). The regression line for hour 3 is shown in Figure 3. A similar dose-dependent effect was seen at hour 0 before the last dose of GHB was given (hour 0 slope = 1.13, 95% CI = 0.73 to 1.53, p = 0.000, $r^2 = 0.84$). This indicates that for every additional day GHB was given (in which longer duration of dosing resulted in increasing doses of GHB), the intoxication–withdrawal score will increase by 1.3 at hour 3, 1.3 at hour 4, 0.96 at hour 5, and 1.13 at hour 0.

No rats developed seizures during the first six hours of continuous observation. All rats survived the 24-hour withdrawal period.

DISCUSSION

Gamma-hydroxybutyrate and its analogues are used as recreational drugs as well as dietary supplements. Acute overdose frequently results in patients' presenting to the emergency department with apnea, bradycardia, seizure-like activity, and coma. Deaths have been reported following acute ingestion.[12]

When used as a dietary supplement, GHB or its analogues are taken several times daily over prolonged periods. As a result, a relatively new complication has been described when GHB use is terminated: GHB withdrawal.[1–8] The GHB withdrawal syndrome is being reported with increasing frequency,[1,13] and its incidence is expected to rise as the illicit use of GHB increases.

Currently, there are several published case reports and case series of GHB withdrawal. All are similar with respect to the dosing frequency, duration of use, and withdrawal signs and symptoms. Patients with withdrawal symptoms report using GHB frequently at dosing intervals of every 1–3 hours and over prolonged periods of time (2 months–3 years). Patients also report using increasing doses of GHB and the development of tolerance to the effects of GHB. The signs and symptoms of withdrawal typically develop about 3 hours after taking the last dose but may occur between 20 minutes to 5 hours. The withdrawal symptoms have lasted up to 12 days.[1–8]

With this model, we dosed the animals every three hours for up to six days and used increasing doses of GHB both within and across groups. We were able to demonstrate that tolerance to GHB develops relatively

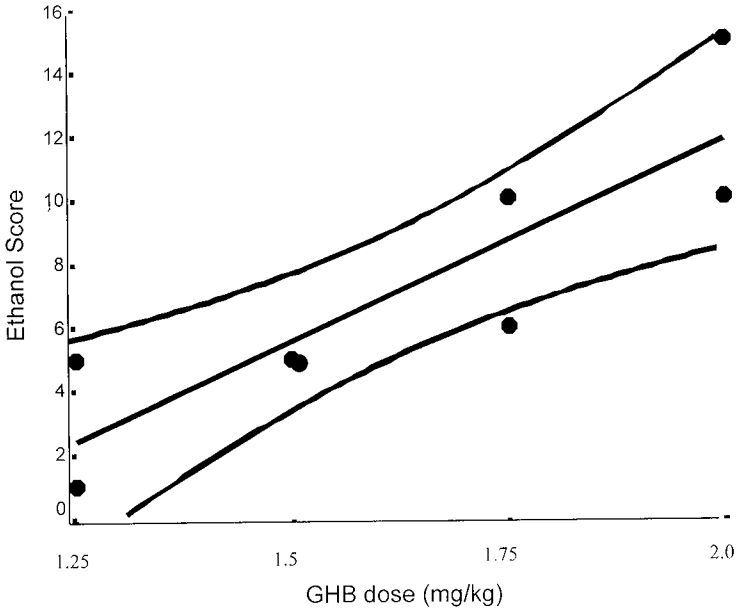

Figure 1. Ethanol intoxication–withdrawal scores one hour after receiving last dose of gamma-hydroxybutyric acid (GHB) are plotted as a function of the dose of GHB administered. Straight line represents the regression line, and curved lines represent the 95% confidence interval. Scores of 8–9 indicate a neutral state, corresponding to a healthy and undrugged rat. Lower scores indicate intoxication. Higher scores indicate withdrawal.

quickly in rats. Increasing the duration of dosing with GHB from three to six days resulted in a decrease in the level of intoxication one hour after the last dose of GHB. This occurred despite the fact that the rats dosed for more days received a larger dose of GHB.

Tolerance is a characteristic of most sedative-hypnotic drugs that result in withdrawal syndromes. Chronic use of sedatives such as ethanol, benzodiazepines, and barbiturates result in the development of tolerance and can produce a withdrawal syndrome.[14] Tolerance can develop due to an increase in the number of receptors for a drug and or a decrease in receptor sensitivity in the central nervous system (CNS). Tolerance can also result from an induction of enzymes causing faster elimination and diminished peak levels of a drug.[14,15] In rats dosed with GHB twice daily for ten days, tolerance to GHB was explained by both the induction of GHB metabolism and a decrease in sensitivity of the CNS for GHB.[16]

This study demonstrated a withdrawal state in rats that started at four hours and lasted until nine hours after the last dose of GHB. The duration of GHB dosing used in this model was far shorter than the duration of use seen in patients with GHB withdrawal. Most animal models of withdrawal from other agents produce withdrawal within a short time frame. Rat models of ethanol withdrawal produce severe ethanol withdrawal after treating rats for only four to six days.[9,10]

In this animal model, an increase in the duration of dosing with GHB from three to six days increased the severity of the withdrawal in a linear manner at hours 3, 4, 5, and 12. The effect of the number of days of dosing with GHB on the intensity of withdrawal was also seen just prior to the last dose of GHB. This is similar to what is seen in clinical withdrawal. Most patients report onset of withdrawal symptoms three hours after their last dose and report some mild signs of withdrawal just prior to their next dose. However, clinical withdrawal in humans has resulted in more severe symptoms lasting for longer periods of time.[1–8]

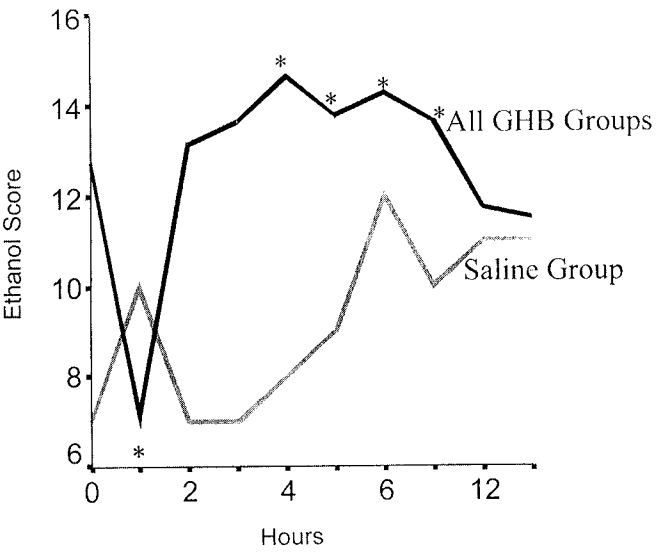

Figure 2. Ethanol intoxication–withdrawal scores for all gamma-hydroxybutyric acid (GHB) groups combined and saline group are plotted as a function of time. Scores of 8–9 indicate a neutral state, corresponding to a healthy and undrugged rat. Lower scores indicate intoxication. Higher scores indicate withdrawal. *$p < 0.05$ compared with 24-hour score.

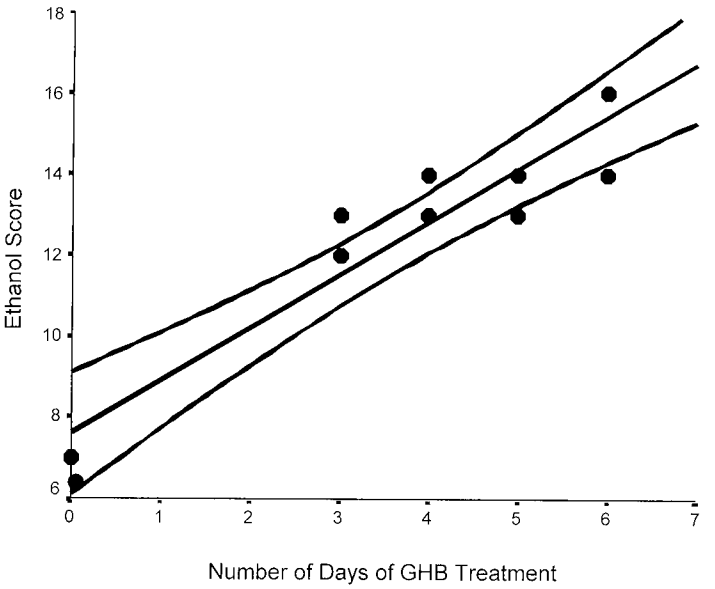

Figure 3. Ethanol intoxication–withdrawal scores three hours after receiving last dose of gamma-hydroxybutyric acid (GHB) are plotted as a function of the number of days GHB was administered. Straight line represents the regression line, and curved lines represent the 95% confidence interval. Scores of 8–9 indicate a neutral state, corresponding to a healthy and undrugged rat. Lower scores indicate intoxication. Higher scores indicate withdrawal.

The reported signs and symptoms of GHB withdrawal are anxiety, restlessness, nightmares, insomnia, tremor, confusion, delirium, hallucinations, tonic-clonic activity, tachycardia, hypertension, hyperthermia, nausea, vomiting, diaphoresis, anorexia, dehydration, and rhabdomyolysis.[1–8] Several of the cases of severe GHB withdrawal reported in the literature resemble delirium tremens of ethanol withdrawal.[1–3] An ethanol withdrawal scale was used in this model because the clinical cases of GHB withdrawal share many similarities with ethanol withdrawal.

Gamma-hydroxybutyrate and its metabolites bind to the GABA-A receptor resulting in an increased chloride flux in the post-synaptic CNS neurons.[15] GHB also act through a number of different receptors and neurotransmitters. GHB binds to its own GHB receptor complex[17,18] and to the GABA-B receptor.[19,20] The GABA-B receptor is located pre-synaptically and inhibits the release of GABA into the synapse. GHB is metabolized by succinic semialdehyde[21,22] to GABA, which then binds to both GABA-A and GABA-B.[23,24] GHB produces a biphasic CNS dopamine response in the substantia nigra, inhibiting release at low doses and promoting release at high doses.[25,26] GHB also mediates the release of an opiate-like substance.[25,26]

The pathophysiology of GHB withdrawal is unknown but it is reasonable to assume that it is similar to other withdrawal syndromes such as ethanol and benzodiazepines. These withdrawal syndromes result from a decrease in receptor sensitivity following chronic exposure to these drugs.

Ethanol and benzodiazepines (binding to different sites on the GABA-A receptor) enhance the binding of gamma-aminobutyric acid (GABA) to the GABA-A receptor, resulting in an increased chloride flux in the post-synaptic CNS neurons.[15] Chronic exposure to ethanol and benzodiazepines results in tolerance to the chronic increase in chloride flux. Chronic exposure decreases GABA-A receptor sensitivity to GABA. Once there is tolerance to chloride flux post-synaptically and there is withdrawal of ethanol or benzodiazepine, GABA is unable to stimulate enough chloride flux to inhibit firing of the post-synaptic neuron. This effect results in post-synaptic activation, which results in the symptoms of CNS stimulation and withdrawal.[15]

Gamma-hydroxybutyric acid has multiple other effects besides an effect on the GABA-A receptors. However, like withdrawal from ethanol and benzodiazepine, GHB withdrawal is probably produced by decreased sensitivity of one of these receptors (GABA-A, GABA-B, dopamine, GHB, opiate) or a combination of receptors to their agonist.

Gamma-hydroxybutyric acid withdrawal has been treated with variable success with benzodiazepines, haloperidol, and/or chlorpromazine.[1–8] A few patients treated with benzodiazepines have continued to suffer life-threatening agitation, delirium, and hallucinations requiring physical restraint and stays in the intensive care unit for 6–10 days.[1–8] One such patient required 2,655 mg of diazepam over 90 hours.[3] Deaths have been reported even with treatment with benzodiazepines.[1] The lack of desired effect of benzodiazepines in some cases implies that the GABA-A receptor may not be the sole receptor mechanism involved in GHB withdrawal, and that either higher doses of benzodiazepines or other medications need to be evaluated. In addition to benzodiazepines, haloperi-

dol, and chlorpromazine, some authors have proposed baclofen and valproic acid as treatment options, but these have not yet been evaluated.[1] The role opioids may play in the treatment of GHB withdrawal has yet to be elucidated, though GHB has been used in opiate withdrawal[27,28] and has been found to release an opiate-like substance.[25,26] There are no clinical trials or animal studies comparing the safety and efficacy of any of these medications in GHB withdrawal. The development of an animal model is needed to evaluate these potential therapies for GHB withdrawal.

To the best of our knowledge, this is the first description of GHB withdrawal in an animal model. Withdrawal displayed in this model shared numerous characteristics with clinical GHB withdrawal in humans; symptoms occurred about three hours after the last dose of GHB (and were actually seen just prior to the last dose), and tolerance was produced. However, signs of severe withdrawal (higher withdrawal scores, seizures, and fatalities) were not seen. A model that develops severe GHB withdrawal would be valuable since mild cases usually do not require treatment.

CLINICAL RELEVANCE

Use of GHB and its analogues is increasing,[29] and the incidence of GHB withdrawal is also expected to rise. This animal model demonstrates that tolerance and withdrawal can develop quickly in rats. This model may be used in the future to study potential therapies and the underlying physiological mechanisms of GHB withdrawal.

LIMITATIONS

Although the study produced significant results, there were only two animals per group, with the potential for a large change in results if even one animal was an outlier.

The dosing used in this model was chosen to approximate that of typical human use reported in patients who develop withdrawal. However, it is unlikely that the pharmacokinetics and or toxicokinetics of GHB are the same in the rat as in humans. The intraperitoneal dosing produced a tighter control of drug delivery, but different GHB absorptions and metabolism, particularly a first-pass effect, may occurr if orogastric delivery is used.

This model resulted in only mild withdrawal with scores ranging from 13.7 to 14.7. Severe ethanol withdrawal scores are usually greater than 20. Incompatibility of the ethanol withdrawal scale with GHB may account for our inability to reproduce severe withdrawal. Another more likely explanation is that the administration of GHB was too limited. Higher doses and or more prolonged dosing may be needed. An estimate of the number of days of dosing needed to generate severe withdrawal scores can be calculated based on the slope of the regression analysis of the withdrawal scores, assuming that the withdrawal scores would continue to be linear at higher doses and longer duration. The withdrawal score increased on average by 1.2 for each additional day of GHB dosing. Since the mean withdrawal score was 14, an additional 6 days of GHB would be needed to produce withdrawal scores greater than 20. In order to create a model of severe GHB withdrawal with seizures and/or fatalities, additional days of therapy and higher doses seem necessary. Once severe GHB withdrawal can be induced, future studies could evaluate potential therapies.

CONCLUSIONS

Gamma-hydroxybutyric acid tolerance and mild withdrawal symptoms can be induced in a rat model of chronic GHB dosing over 3–6 days. Further modifications are needed before this model can be used to evaluate potential therapy.

References

1. Dyer JE, Roth B, Hyme BA. Gamma-hydroxybutyrate withdrawal syndrome. Ann Emerg Med. 2001; 37:147–53.
2. Sharma AN, Nelson L, Hoffman RS. Severe gamma butyrolactone withdrawal [abstract]. J Toxicol Clin Toxicol. 2000; 38:535.
3. Craig K, Gomez H, McManus J, Bania TC. Severe gamma-hydroxybutyrate withdrawal: a case report and literature review. J Emerg Med. 2000; 18:65–70.
4. Galloway G, Frederick S, Stagers F, Gonzalez M, Stalcup S, Smith D. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction. 1997; 9(1):89–96.
5. Addolorato G, Caputo F, Capristo E, Bernardi M, Stefanini G, Gasbarrini G. A case of gamma-hydroxybutyric acid withdrawal syndrome during alcohol addiction treatment: utility of diazepam administration. Clin Neuropharmacol. 1999; 22(1):60–2.
6. Galloway GP, Frederick SL, Staggers F Jr. Physical dependence on sodium oxybate [letter]. Lancet. 1994; 343:57.
7. Hernandez M, McDaniel C, Constanza D, Herandez O. GHB-induced delirium: a case report and review of the literature of gamma hydroxydutyric acid. Am J Drug Alcohol Abuse. 1998; (1):179–83.
8. Miglani J, Kim K, Chahil R. Gamma hydroxybutyrate withdrawal delirium: a case report. Gen Hosp Psychiatry. 2000; 22:213–5.
9. Lal H, Harris CM, Benjamin D. Characterization of a pentylenetetrazol-like interceptive stimulus produced by ethanol withdrawal. Pharmacol Exp Ther. 1988; 247:508–18.
10. Colombo G, Agabio R. Ability of baclofen in reducing alcohol intake and withdrawal severity: I-Preclinical Evidence. Alcohol Clin Exp Res. 2000; 24(1):58–66.
11. Fattore L, Martellotta M, Cossu G, Gratta W. Gamma-hydroxybutyric acid. An evaluation of its rewarding properties in rats and mice. Alcohol. 2000; 20:247–56.
12. Li J, Stokes SA, Woeckner A. A tale of novel intoxication: a review of the effects of gamma-hydroxybutyric acid with

recommendations for management. Ann Emerg Med. 1998; 31:729–36.
13. Porrata T. GHB: The stone cold truth. Available at: www.ashesonthesea.com/ghb. Accessed July 2002.
14. Hamilton R. Substance withdrawal. In: Goldfrank L, Flomenbaum N, Lewin N, Weisman R, Howland M, Hoffman R (eds). Goldfrank's Toxicological Emergencies 5th ed. Stamford CT: Appleton and Lange, 1998, pp 1127–44.
15. Olmeda R, Hoffman R. Psychiatric emergencies. Emerg Med Clin North Am. 2000; 18(2):1–16.
16. Van Sassenbroeck D, De Paepe P, Belpaire F, Buylaert W. Tolerance to gamma-hydroxybutyrate in the rat: pharmacokinetic and pharmacodynamic aspects. Acad Emerg Med. 2002; 9:484–5.
17. Cash CD. What is the role of the gamma-hydroxybutyrate receptor? Med Hypoth. 1996; 47:455–9.
18. Cash CD. Gamma hydroxybutyrate: an overview of the pros and cons for it being a neurotransmitter and/or useful therapeutic agent. Neurosci Behav Rev. 1994; 18:291–304.
19. Ito Y, Ishige K, Zaitsu E, Anzai K, Fukuda H. Gamma-hydroxybutyric acid increases intracelluler Ca^{2+} concentration and nuclear cyclic AMP-responsive elements and activator protein 1 DNA-binding activities through GABA-B receptor in cultured cerebrellar granule cells. J Neurochem. 1995; 65:75–83.
20. Bernasconi R, Lauber J, Marescaux C, et al. Experimental absence seizures: potential role of gamma hydroxybutyric acid and GABA-B receptors. J Neural Transm Suppl. 1992; 35: 155–77.
21. Vayer P, Schmitte M, Bourguignon JJ, Mandel P, Maitre M. Evidence for a role of high Km aldehyde reductase in the degradation of endogenous gamma-hydroxybutyrate from rat brain. FEBS Lett. 1985; 190:55–60.
22. Knapp DJ, Saiers JA, Pohorecky LA. Observations of novel behaviors as indices of ethanol withdrawal-induced anxiety. Alcohol Alcohol Suppl. 1993; 2:489–93.
23. Snead O, Nichols A. Gamma-hydroxybutyric acid binding sites; evidence for coupling to a chloride anion channel. Neuropharmacology. 1987; 26(15):19–23.
24. Hechlor V, Ratompondrine C, Maltra M. Gamma-hydroxybutyrate conversion into GABA induces displacement of GABA-B binding that is blocked by valproate and ethosoximide. J Pharmacol Exp Ther. 1997; 281:753–60.
25. Hechler V, Peter P, Gobaille S, et al. Gamma-hydroxybutyrate ligands possess antidopaminergic and neuroleptic-like activities. J Pharmacol Exp Ther. 1993; 264:1406–14.
26. Hechler V, Gobaille S, Bourguignon JJ, Maitre M. Extracellular events induced by gamma-hydroxybutyrate in striatum: a microdialysis study. J Neurochem. 1991; 56:938–44.
27. Gallimberti L, Schifano F, Forza G, Ferrara M. Clinical efficacy of gamma-hydroxybutric acid treatment of opiate withdrawal. Eur Arch Psychiatry Clin Neurosci. 1994; 244(3):113–4.
28. Gallimberti L, Cibin M, Pagnin P, et al. Gamma-hydroxybutyric acid for treatment of opiate withdrawal syndrome. Neuropsychopharmacology. 1993; 9(1):77–81.
29. Drug Abuse Warning Network. The DAWN report. Available at: http://www.samhsa.gov/oas/dawn.htm. Accessed Jan 11, 2003.