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Discriminative stimulus effects of GHB and $GABA_B$ agonists are differentially attenuated by CGP35348

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Abstract

The aim of this study was to examine the possible heterogeneity of mechanisms that contribute to the discriminative stimulus and rate-decreasing effects of γ-hydroxybutyrate (GHB). Dose effect curves were determined for GHB and two $GABA_B$ receptor agonists (baclofen and SKF97541) alone and together with the selective $GABA_B$ receptor antagonist CGP35348 in rats discriminating GHB. In a second study, GHB and SKF97541 dose effect curves were determined alone and together with baclofen. CGP35348 attenuated the discriminative stimulus and rate-decreasing effects of SKF97541 and baclofen to a greater extent than those of GHB. In the second study, baclofen enhanced the discriminative stimulus and rate-decreasing effects of GHB and SKF97541; however, the GHB dose effect curve was not shifted in a parallel manner. Taken together, these data suggest that multiple mechanisms, possibly including GHB receptors and $GABA_B$ receptor subtypes, are involved in the discriminative stimulus and rate-decreasing effects of GHB.

Keywords: Gamma-hydroxybutyrate; GHB; Baclofen; SKF97541; GABA; Xyrem; Sodium oxybate; Drug discrimination

1. Introduction

Gamma-hydroxybutyrate (GHB) is an endogenous molecule and putative neurotransmitter (Maitre, 1997) that is involved in the regulation of sleep/wake cycles (Mamelak et al., 1977; Van Cauter et al., 1997) and energy metabolism (Boyd et al., 1992; Mamelak, 1997; Ottani et al., 2004). Although its precise mechanism of action is unknown, GHB is used in the US and Europe to treat narcolepsy (Tunnicliff and Raess, 2002; Fuller and Hornfeldt, 2003) and alcoholism (Addolorato et al., 1996; Poldrugo and Addolorato, 1999). GHB [sodium oxybate, marketed as Xyrem® (Schedule III formulation)] increases slow wave (stages III and IV) sleep and the latency to REM sleep, which is thought to be responsible for its therapeutic effect of decreasing daytime episodes of cataplexy in patients with narcolepsy (Mamelak et al., 1986). In addition to its novel therapeutic indications (alcoholism and narcolepsy), GHB is also used recreationally in many countries for its reputed anabolic and euphorigenic effects (Bellis et al., 2003; Caldicott et al., 2004; Couper et al., 2004; Rodgers et al., 2004; Gonzalez and Nutt, 2005). The recreational use of GHB and its alleged involvement in drug-facilitated sexual assaults (ElSohly and Salamone, 1999; Schwartz et al., 2000) led to the placement of GHB into Schedule I of the Controlled Substances Act in 2000 (US Federal Register, 2000).

GHB binds to $GABA_B$ receptors (Mathivet et al., 1997; Lingenhoehl et al., 1999) and specific GHB receptors (Benavides et al., 1982; Snead and Liu, 1984) in brain that are thought to be important for the behavioral effects of GHB. Studies examining the mechanism of action of GHB in vivo (including studies in rats, pigeons and baboons) have shown that $GABA_B$ receptors are important for many of the effects of GHB such as discriminative stimulus effects (e.g., Winter, 1981; Colombo et al., 1998; Carter et al., 2003, 2004a; Koek et

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al., 2004), increased EEG spike wave discharges (Bernasconi et al., 1992; Snead, 1996), loss of righting (Carai et al., 2001), hypothermia (Queva et al., 2003), hypolocomotion (Kaupmann et al., 2003), increased mean arterial pressure, tachycardia and renal sympathetic nerve activity (Hicks et al., 2004), lethality (Carai et al., 2004), catalepsy (Carter et al., 2005b), ataxia (Goodwin et al., 2005), decreased intestinal motility (Carai et al., 2002) and decreased operant responding (Goodwin et al., 2005). Alternatively, a role for GHB receptors in the effects of GHB is less clear, in part, because the study of these receptors has been limited by the lack of selective GHB receptor ligands; however, there is evidence that GHB receptors are involved in some of the behavioral effects of GHB (e.g., Hechler et al., 1993; Carter et al., 2005b).

Despite the similar behavioral profile of baclofen and GHB, baclofen did not increase the latency to REM sleep in rats (Ulloor et al., 2004) or (decerebrate) cats (Takakusaki et al., 2004) and it is not used recreationally. Thus, it is possible that mechanisms of action of GHB, in addition to agonism at $GABA_B$ receptors (e.g., activity at GHB receptors), contribute to the differences between GHB and baclofen. Selective GHB receptor ligands have been shown to produce some GHB-like effects that are not blocked by the selective $GABA_B$ receptor antagonist 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), suggesting that GHB receptors mediate some of the effects of GHB in vivo (Gobaille et al., 2002; Castelli et al., 2003; Carter et al., 2005b). Similarly, there is evidence for $GABA_B$ receptor subtypes (Bonanno and Raiteri, 1992), which might also contribute to some of the differences between GHB and baclofen.

The aim of this study was to examine the possible heterogeneity of mechanisms that contribute to the discriminative stimulus and rate-decreasing effects of GHB by comparing the effects of GHB with those of well-characterized $GABA_B$ receptor agonists. Previous studies have shown similar substitution profiles for different compounds in rats trained to discriminate 200 mg/kg GHB or 3.2 mg/kg baclofen (i.e., the training stimulus was pharmacologically selective and qualitatively similar in each procedure; Carter et al., 2003, 2004a). In the current studies, dose effect curves were determined for GHB and the $GABA_B$ receptor agonists, baclofen and SKF97541, given alone, and together with the $GABA_B$ receptor antagonist CGP35348, in rats discriminating 200 mg/kg GHB from saline. If the effects of these compounds are mediated by the same CGP35348-sensitive $GABA_B$ receptors, then the magnitude of antagonism of these compounds should be the same. In a second study, GHB and SKF97541 were studied together with baclofen to examine whether the effects of GHB and SKF97541 were differentially enhanced by baclofen.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) were housed individually on a 14:10-h light/dark cycle (experiments conducted during the light period) with free access to water in the home cage. Rats ($N=7$) were maintained at 340 to 360 g by providing rodent chow (Rodent sterilizable diet, Harlan Teklad, Madison, WI) in the home cage after daily sessions. The amount of chow that was provided to each animal (5–16 g) was adjusted daily in order to maintain body weights at or near 350 g. The animals in this study had previously been trained to discriminate 200 mg/kg GHB (i.p.) from saline; among the rats trained and used in prior studies (Carter et al., 2003) and this study, a median of 36 (range: 16–72) sessions was required to satisfy testing criteria. All animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and with the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

2.2. Procedures

Experiments were conducted in commercially available chambers (Model #ENV-008CT; MED Associates Inc., St. Albans, VT) located within sound-attenuating, ventilated enclosures (Model #ENV-022M; MED Associates Inc.) that have been described in detail elsewhere (Carter et al., 2003). Data were collected using MED-PC IV software and interface (MED Associates Inc.). Rats were trained to discriminate 200 mg/kg GHB i.p. from saline as described previously (Carter et al., 2003). In the current studies, the daily session was changed from one 30-min cycle (15-min time out period, followed by a 15-min response period) to multiple (between one and six) 20-min cycles, each consisting of a 15-min time out period, followed by a 5-min response period. During the time out period, the chamber was dark and lever presses had no programmed consequence, whereas during the response period the stimulus lights above both levers were illuminated and 10 consecutive responses (fixed ratio 10) on the correct lever resulted in the delivery of a food pellet (45 mg; Research Diets, New Brunswick, NJ). A response on the incorrect lever reset the fixed ratio requirement on the correct lever. The response period ended after 5 min or the delivery of 10 food pellets, whichever occurred first. The minimum and maximum duration of a test session was 20 (one cycle) and 120 (six cycles) min, respectively.

Under training conditions, an injection of saline or 200 mg/kg GHB (i.p.), or a sham injection (pressure applied to the abdomen with a capped needle) was given at the start of each cycle (15 min prior to the response period). Training sessions generally consisted of two to six cycles. On saline training days, animals received an injection of saline prior to the first cycle and a sham injection at the beginning of each subsequent cycle; only responding on the saline-associated lever resulted in food delivery during these cycles. On drug training days, animals received 200 mg/kg GHB in one of the cycles (typically the first or third cycle) followed by a sham injection at the start of a subsequent cycle; only responding on the GHB-associated lever resulted in food delivery during both of these cycles. Sessions