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Differentiating the discriminative stimulus effects of gamma-hydroxybutyrate and ethanol in a three-choice drug discrimination procedure in rats

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Abstract

Anecdotal reports indicate that GHB produces subjective effects similar to those of ethanol. However, recent investigations comparing the discriminative stimulus effects of GHB to those of ethanol suggest that the subjective effects of these substances may differ considerably. To explore further potential differences between GHB and ethanol, 16 male Sprague–Dawley rats were trained in a three-lever drug discrimination procedure to discriminate ethanol (1.0 g/kg, experiment 1; 1.5 g/kg, experiment 2) and GHB (300 mg/kg) from vehicle. Dose–response functions determined with both training compounds revealed a clear dissociation between the discriminative stimulus effects of these drugs. As expected, the GHB precursors gamma-butyrolactone and 1,4-butanediol produced full substitution for GHB. In addition, the $GABA_B$ receptor agonist baclofen substituted for GHB, whereas the benzodiazepine flunitrazepam and the NMDA receptor antagonist ketamine engendered greater responding on the ethanol-lever. GHB's discriminative stimulus effects were blocked by the $GABA_B$ receptor antagonist CGP-35348 but only partially blocked by the putative GHB receptor antagonist NCS 382. These findings are consistent with previous reports of GHB's discriminative stimulus effects in two-choice drug discrimination procedures and provide additional evidence that these effects are distinct from those of ethanol.

Keywords: Gamma-hydroxybutyrate; Gamma-butyrolactone; 1,4-butanediol; Flunitrazepam; Ketamine; Baclofen; CGP-35348; NCS-382; Drug discrimination; Rats

1. Introduction

Gamma-hydroxybutyrate (GHB) occurs naturally in the mammalian nervous system where it is a putative neurotransmitter with proposed affinity for either a $GABA_B$ metabotropic receptor (Carter et al., 2003) or a specific GHB metabotropic receptor (Snead, 1977). In some European countries GHB has been used in alcohol and opiate detoxification (Gallimberti et al., 1989; Gallimberti et al., 1993) and in 2002 the United States Food and Drug Administration approved GHB (under the trade name Xyrem®) for the treatment of cataplexy in narcoleptic patients (Fuller and Hornfeldt, 2003; Fuller et al., 2005). The abuse of GHB is also a significant health concern and this drug has been characterized as a "date-rape" drug in the popular media (Schwartz et al., 2000).

Human users report that GHB produces feelings of euphoria and sedation that presumably resemble the effects of ethanol and other central nervous system depressants (Couper and Logan, 2001; Miotto et al., 2001; O'Connell et al., 2000). Based on these reports, and assuming that subjective effects of the drugs are similar in humans and non-humans, one would expect to find strong generalization between GHB and ethanol in non-human animals tested in drug discrimination procedures. Several studies have used such procedures to characterize the discriminative stimulus effects of GHB (e.g., Winter, 1981; Colombo et al., 1995a; Colombo et al., 1995b; Colombo et al., 1998; Lobina et al., 1999; Metcalf et al., 2001; Carter et al., 2003; Wu et al., 2003; Koek et al., 2004; Baker et al., 2004; Baker et al., 2005). It is now well accepted that GHB can be readily established as a discriminative stimulus and that the metabolic precursors of GHB, gamma-butyrolactone (GBL) and 1, 4-butanediol (1,4-BDL), produce stimulus generalization in animals trained to discriminate GHB from vehicle (Baker et al.,

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2005). Furthermore, the discriminative cue produced by GHB appears to be mediated by actions at $GABA_B$ receptors (Carter et al., 2003; Carter et al., 2004; Baker et al., 2005).

Results regarding stimulus generalization between GHB and ethanol are somewhat inconsistent. Colombo et al. (1995b) demonstrated cross-generalization between GHB and ethanol, but only within a narrow dose range. More recent investigations using different procedures have demonstrated only partial substitution between GHB and ethanol (Metcalf et al., 2001; Baker et al., 2004; Baker et al., 2005). To date, no one has examined whether animals can learn to discriminate among GHB, ethanol, and vehicle in a three-choice discrimination procedure. Such a procedure may detect differences in drug effects that are not evident in two-choice drug discrimination procedures. For example, research from our laboratory and elsewhere has demonstrated that drugs that show substantial cross-generalization in two-choice (drug versus vehicle) discrimination procedures may be readily discriminated in three-choice (drug 1 versus drug 2 versus vehicle) procedures (Bowen et al., 1997; Bowen and Grant, 1998; Makhay et al., 1998; Baker and Taylor, 1997; Goodwin and Baker, 2000; Goodwin et al., 2003).

The discriminative stimulus effects of ethanol have been examined extensively in two-choice (ethanol-vehicle) procedures. These investigations have consistently found that ethanol's discriminative stimulus effects are mediated by multiple receptor systems. Stimulus generalization to ethanol has been reported with $GABA_A$ positive modulators, including benzodiazepines, barbiturates, and neuroactive steroids (Barry and Krimmer, 1977; York, 1978; Ator et al., 1993; Grant et al., 1996), competitive and non-competitive NMDA receptor antagonists (Grant et al., 1991; Grant and Colombo, 1992; Sanger, 1993; Shelton and Balster, 1994), and 5-HT_1 receptor agonists (Signs and Schechter, 1988; Grant and Colombo, 1993a,b,c; Grant et al., 1997).

In an effort to provide detailed information about the neurochemical mechanisms underlying ethanol's discriminative stimulus properties and the similarity of those properties to those of other drugs, a few studies have implemented three-choice discrimination procedures (Gatto et al., 1995; Bowen et al., 1997; Bowen and Grant, 1998). Two of these investigations reported that ethanol can be discriminated from the non-competitive NMDA receptor antagonist dizocilpine (Gatto et al., 1995; Bowen and Grant, 1998) and one study demonstrated that ethanol can be discriminated from the $GABA_A$ positive modulator pentobarbital (Bowen et al., 1997). When rats were trained to discriminate dizocilpine from ethanol, this essentially eliminated the NMDA receptor component of the ethanol cue, without altering the $GABA_A$ or 5-HT_1 mediated effects. In contrast, when rats were trained to discriminate pentobarbital from ethanol, thereby eliminating the $GABA_A$ component, the NMDA antagonism component of the ethanol cue was also diminished. Moreover, the results of these three-choice discrimination investigations clearly indicate that the pharmacological effects of ethanol involved in establishing discriminative stimulus control may be modified by the discrimination training conditions to the extent that a particular receptor system appears to be no longer involved in the discriminative stimulus effects of the drug (Bowen et al., 1997).

The principle aim of the present investigation was to use a three-choice drug discrimination procedure to determine whether rats could discriminate among GHB, ethanol, and vehicle. Because most prior studies involving two-choice training procedures have reported only partial generalization between GHB and ethanol (Metcalf et al., 2001; Baker et al., 2004; Baker et al., 2005), we assumed that it would be possible to establish this discrimination. It was, and once this discrimination was established we examined whether it was based on qualitative differences between GHB and ethanol by examining stimulus generalization to substances previously shown to substitute for GHB (GBL, 1,4 -BDL, baclofen) or ethanol (flunitrazepam, ketamine) in two-choice drug discrimination investigations.

2. Methods

2.1. Subjects

Sixteen male Sasco Sprague Dawley rats (Charles River, Portage, MI) were individually housed in polycarbonate cages with corn cob bedding in a colony maintained with a 12-h light/dark cycle (lights on 0700 to 1900) and constant temperature (20 °C±2°) and humidity (50%±5%). Animals were experimentally naïve, approximately 60 days old, and weighed approximately 250 g at the beginning of the study. Water was freely available in the home cages, and commercial rodent diet was restricted to maintain body weights at 80–85% of free-feeding levels, accounting for age-related growth. Animals were maintained according to the general principles of animal husbandry outlined by the National Research Council (1996) and the experimental protocol was approved by the Institutional Animal Care and Use Committee of Western Michigan University.

2.2. Apparatus

Experimental sessions were conducted in eight operant testing chambers (MED Associates, Georgia, VT) measuring 30×31×24 cm and housed within sound- and light-attenuating cubicles. The test chambers were equipped with three retractable levers on the front panel, a food pellet delivery mechanism located above the center lever, and a 28-V house light located at the top of the rear panel. Dustless precision food pellets (45 mg, product # F0021, Bioserv®, Frenchtown, NJ) were used as reinforcers. MED-PC (version 4.0 for Windows) instrumentation and software were used to control experimental events and to record data.

2.3. Drugs

Gamma-hydroxybutyrate (National Institute on Drug Abuse, Bethesda, MD) and ethanol (AAPER Alcohol and Chemical Company, Shelbyville, KY) were administered by intragastric (IG) delivery 30 min before training or test sessions. Gamma-butyrolactone, 1,4-butanediol, (±)-baclofen, flunitrazepam, and ketamine-hydrochloride (Sigma Chemical Company, St. Louis, MO) were administered by intraperitoneal (IP) injection 15 min prior to test sessions. NCS-382 (6,7,8,9 Tetrohydro-5-[*H*]