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Discriminative stimulus effects of γ-hydroxybutyrate (GHB) in pigeons: role of diazepam–sensitive and –insensitive $GABA_A$ receptors, and of $GABA_B$ receptors.

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Nonstandard abbreviations: GHB, γ-hydroxybutyrate; GABA, γ-aminobutyric acid; 1,4-BDL, 1,4-butanediol; GBL, γ-butyrolactone; CGP 35348, (3-aminopropyl) (diethoxymethyl)phosphinic acid; NCS-382, (2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[a][7]annulen-6-ylidene ethanoic acid; DS, discriminative stimulus

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Abstract

γ-Hydroxybutyrate (GHB) is an emerging drug of abuse with multiple mechanisms of action. This study is part of an effort to examine the role of GHB, $GABA_A$, and $GABA_B$ receptors in the discriminative stimulus (DS) effects of GHB. In pigeons trained to discriminate 100 mg/kg GHB from saline, GHB and its precursors γ-butyrolactone and 1,4-butanediol produced 80-100% GHB-appropriate responding, whereas other compounds (morphine, naltrexone, cocaine, haloperidol) produced at most 34%. Compounds interacting with GABA receptors produced different maximal levels of GHB-appropriate responding: the $GABA_A$ agonist muscimol produced 3%, the $GABA_A$ positive modulators diazepam, pentobarbital, and ethanol, and the $GABA_B$ agonist baclofen, produced levels ranging from 54 to 73%, and the benzodiazepine antagonist flumazenil and inverse agonist Ro 15-4513 both produced 96%. The putative GHB receptor antagonist (2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[a][7]annulen-6-ylidene ethanoic acid (NCS-382) produced 70% GHB-appropriate responding. The $GABA_B$ antagonist (3-aminopropyl)(diethoxymethyl)phosphinic acid (CGP 35348) completely blocked the GHB-like DS effects of NCS-382 and baclofen at a dose of 56 mg/kg. CGP 35348 also blocked the DS effects of GHB, but incompletely and only at a dose of 560 mg/kg. Together, these results are consistent with a role for diazepam-sensitive and diazepam-insensitive $GABA_A$ receptors, and $GABA_B$ receptors, in the DS effects of GHB. Together with previous findings, the present results suggest that diazepam-insensitive $GABA_A$ receptors are more prominently involved in the DS effects of GHB in pigeons than in rats, whereas $GABA_B$ receptors are less prominently involved. Exploring the role of GHB receptors with NCS-382 is hampered by its, $GABA_B$ receptor-mediated, GHB-like agonist activity.

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γ-Hydroxybutyrate (GHB) has therapeutic use, and a recent history of abuse (for a review, see Nicholson and Balster, 2001). GHB was recently approved by the Food and Drug Administration to treat narcolepsy, and is thought to have therapeutic potential to treat alcoholism (e.g., Gallimberti et al., 1989; 1992). GHB, however, is also an emerging drug of abuse, as are some of its metabolic precursors.

GHB occurs naturally in the brain, where it is believed to function as a neurotransmitter (for a review, see Maitre et al., 2000). Its specific binding sites, which can be investigated with the selective radioligand [^{3}H]NCS-382 (Mehta et al., 2001), appear to be G-protein coupled receptors. GHB may also function as a neuromodulator: it affects, for example, the activity of central dopaminergic neurons, and does so in a naloxone-reversible manner (e.g., Feigenbaum and Howard, 1997). Its precise roles in CNS function, however, are not well understood.

GHB may exert its effects not only through specific GHB receptors, but also through other mechanisms. GHB can interact directly with γ-aminobutyric acid $(GABA)_B$ receptors (Lingenhoehl et al., 1999) and indirectly with all GABA receptor subtypes because it can be metabolically converted to GABA (Vayer et al., 1985). A role for $GABA_B$ systems is suggested by the finding that the discriminative stimulus (DS) effects of GHB are mimicked by the $GABA_B$ receptor agonist baclofen and antagonized by the $GABA_B$ receptor antagonist CGP 35348 (e.g., Colombo et al., 1998; Carter et al., 2003). A possible role for $GABA_A$ systems is suggested by overlap in the actions of GHB and positive $GABA_A$ receptor modulators such as diazepam (e.g., Colombo et al., 1998; Carter et al., 2003). Thus, GHB is likely to have multiple mechanisms of action.

Drug discrimination has proved to be useful in identifying mechanisms of action, because it can provide sensitive and selective assays of *in vivo* effects of behaviorally

active drugs (e.g., Colpaert and Balster, 1988). Rats can discriminate GHB from saline (Winter, 1981; Colombo et al., 1995a;1995b;1998; Metcalf et al., 2001; Carter et al., 2003) and its DS effects appear to be selective, because pharmacologically unrelated drugs (e.g., phencyclidine, ketamine) do not occasion GHB-appropriate responding (Winter, 1981; Carter et al., 2003). Also, GHB does not occasion drug-appropriate responding in discriminations using training drugs that are pharmacologically unrelated to GHB (Beardsley et al., 1996; Woolverton et al., 1999). The aforementioned drug discrimination studies in rats suggest that the DS effects of GHB involve multiple mechanisms, with a prominent role for $GABA_B$ receptors, a less important role for $GABA_A$ receptors, and a role for GHB receptors that has as yet not been clearly delineated. The present study, which is part of an effort to explore the generality of these findings across species, was aimed at examining the role of GHB, $GABA_A$, and $GABA_B$ receptors in the DS effects of GHB in pigeons.

Although results of drug discrimination studies in rats and pigeons often appear similar, detailed comparisons have shown some important differences, particularly with compounds that interact with GABA receptors. For example, pentobarbital has been reported to substitute fully for the benzodiazepine midazolam in midazolam-trained rats (e.g., Garcha et al., 1985;Woudenberg and Slangen, 1989), but not in midazolam-trained pigeons (Evans and Johanson, 1989), squirrel monkeys (Spealman, 1985), and baboons (Ator, 2003). These findings suggest (Ator, 2003) that positive modulation of GABA through the barbiturate site is generally not sufficient to produce midazolam-like DS effects in pigeons, squirrel monkeys, and baboons, although it is in rodents. Thus, it is important to examine the DS effects of drugs that interact with GABA receptors, such as GHB, in different species.

Pigeons discriminating 100 mg/kg GHB from saline were used to evaluate the following: 1) possible GHB-like DS effects of metabolic precursors of GHB [i.e., 1,4-butanediol (1,4-BDL) and γ-butyrolactone (GBL)]; 2) the role of dopamine- and opioid systems in the DS effects of GHB; 3) the role of GHB, $GABA_A$, and $GABA_B$ receptors in the DS effects of GHB, by studying whether GHB, the $GABA_B$ agonist baclofen, the $GABA_A$ agonist muscimol, and the positive $GABA_A$ modulators diazepam, pentobarbital, and ethanol produce GHB-like DS effects, and whether these effects can be antagonized by the GHB antagonist NCS-382, the $GABA_B$ antagonist CGP 35348, and the benzodiazepine antagonist flumazenil; and 4) the possibility that full GHB-like DS effects can be produced by adding $GABA_A$ and $GABA_B$ receptor activation, by studying whether combinations of baclofen and diazepam produce more GHB-appropriate responding when given together than when given alone.

Materials and Methods

Animals

Six adult white Carneau pigeons (*Columbia Livia*; Palmetto, Sumter, SC), which were experimentally naïve before use in the present study, were maintained between 80 and 90% of their free-feeding weight, which ranged from 590 to 620 g, by providing mixed grain in the home cage after the daily sessions. The animals were housed individually under a 12/12-h light/dark cycle in stainless steel cages where they had free access to water and grit. 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)

Apparatus

Experiments were conducted in sound attenuating, ventilated chambers (BRS/LVE, Laurel, MD) equipped with two response keys that could be transilluminated by red lights. After completion of each fixed ratio, the key lights were extinguished for 4 s, during which time a white light illuminated the hopper where food (Purina Pigeon Checkers, St. Louis, MO) was available. Chambers were connected by an interface (MED Associates Inc., St. Albans, VT) to a computer that used MED-PC IV software (MED Associates Inc.) to monitor and control inputs and outputs and to record the data.

Procedure

The procedure was similar to that described in detail elsewhere (Brandt and France, 1996). Briefly, before each daily session, subjects received either 100 mg/kg GHB (this training dose was selected because, in preliminary studies, 100 mg/kg was the highest dose that did not affect response rate) or saline (i.m.) and were immediately placed into the chamber; drug and vehicle training sessions occurred with equal frequency. Sessions started with a period of 15 min, during which the lights were off and key pecks had no programmed consequence. Subsequently, the left and the right keys were illuminated red and 20 consecutive responses on the injection appropriate key resulted in 4-s access to food (for half of the pigeons the left key was active after GHB and the right key was active after saline, and for the other half the left key was active after saline and the right key after GHB). Responses on the incorrect key reset the fixed ratio requirement on the correct key. The response period ended after thirty food presentations or 15 min, whichever occurred first. Initially, pigeons had to satisfy the following criteria for at least seven of nine consecutive sessions: ≥ 90% of the total responses on the correct key and fewer than 20 responses on the incorrect key before the first food presentation. Thereafter, tests were conducted when these criteria were met during two consecutive (drug and saline) training sessions.

Test sessions were the same as training sessions (i.e., a 15 min period, followed by a response period that ended after 30 food presentations or 15 min, whichever occurred first), except that food was available after completion of 20 consecutive responses on either key. Drug (or vehicle) treatments were given immediately before the session, and pretreatments were given 10 min before treatments.

To study the duration of action of compounds, shorter test sessions, consisting of a 10 min timeout period followed by a response period that ended after ten food presentations or 5 min, whichever occurred first, were used. Drug treatments were given at different times, ranging from 0 to 240 min, before the session. Each test day, only a single test session (maximum duration: 15 min, i.e., 10 min timeout followed by an at most 5 min long response period) was conducted.

Data analysis

The mean percentage of responses on the drug key ± 1 SEM and the mean rate of responding ± 1 SEM were plotted as a function of dose or time. When an animal responded at a rate less than 20% of its vehicle control rate, discrimination data from that test were not included in the average. Mean percentage of responses on the drug key values were calculated only when they were based on at least half of the animals tested. Doses needed to produce 50% of the maximal response were calculated for each animal by log-linear interpolation, and the log values of these doses were averaged to obtain a mean D_{50} value and its 95% confidence limits (CL). Potency differences were analyzed using Student's t-test for related measures, performed on individual log D50 values. Response rate data were analyzed using one-way repeated measures ANOVA for each drug, which were followed, where appropriate, by comparing the results of each dose with the vehicle control rate by Dunnett's test.

Drugs

The compounds used in this study were dissolved in sterile water or saline, unless otherwise noted, and included the following: (±)-baclofen, 1,4-butanediol (1,4-BDL), γ-

butyrolactone (GBL), γ-hydroxybutyrate (GHB), haloperidol, muscimol, and pentobarbital sodium (Sigma-Aldrich Corp., St. Louis, MO); cocaine hydrochloride, morphine sulfate, and naltrexone hydrochloride (NIDA, Research Technology Branch, Rockville, MD); diazepam (Research Biochemicals International, Natick, MA); flumazenil and Ro 15-4513 (gifts from F. Hoffmann LaRoche Ltd., Basel, Switzerland). NCS-382 (sodium salt) and CGP 35348 (sodium salt) were synthesized as described previously (Maitre et al., 1990; Froestl et al., 1995). Diazepam was dissolved at different concentrations in the same vehicle consisting of 70% emulphor, 20% sterile water, and 10% ethanol (by volume). Flumazenil was dissolved at different concentrations in the same vehicle consisting of 50% saline, 40% propylene glycol, and 10% ethanol (by volume). All compounds were injected i.m. in a volume of 0.1–1.0 ml, except GHB, which was also administered by gavage in a volume of 0.5-5 ml (depending on dose; 10 min before sessions), and ethanol, which was only administered by gavage. The dose of ethanol was manipulated by varying the volume of a 20% (v/v) solution of ethanol in distilled water. Doses are expressed as the form of the drug listed above.

Results

All six animals acquired the 100 mg/kg GHB versus saline discrimination (median sessions to criterion: 31.5; range: 19 - 75). The accuracy of post-criterion discrimination performance during the remainder of the study, which did not differ significantly between drug and saline training sessions, was 78.2 ± 2.8 (mean ± SEM) % responses on the injection-appropriate key.

Under test conditions, GHB dose-dependently increased responding on the GHB-appropriate key (D_{50} = 39 mg/kg, 95% CL 18 - 83) to a maximum of 99 - 100% at 100 and 178 mg/kg (Fig. 1, upper panel). Only a higher dose (i.e., 320 mg/kg) significantly decreased response rate (Fig. 1, lower panel). GHB had similar effects when given by gavage: it produced 100% GHB-appropriate responding (D_{50} = 239, 95% CL 144 - 398) and decreased response rate (at 1000 mg/kg), but was significantly less potent than when administered i.m. (p.o./i.m. potency ratio for GHB-appropriate responding in animals tested both p.o. and i.m. = 5.2, 95% CL 1.9 - 14.5). The GHB precursor, GBL, increased GHB-appropriate responding to a maximum of 95%, with a potency (D_{50} = 24, 95% CL 15 - 38) similar to that of GHB, and decreased response rate (at 100 mg/kg). The GHB precursor, 1,4-BDL, also increased GHB-appropriate responding (to a maximum of 80%), was significantly less potent (D_{50} = 128, 95% CL 60 - 271) than GHB and GBL, and did not significantly affect response rate at any of the doses tested.

GHB produced GHB-appropriate responding in a time-dependent manner: the percentage GHB-appropriate responding observed with the training dose reached a maximum at 15 min and decreased to 0 at 90 min (Fig. 2). GBL produced GHB-like responding with a duration of action similar to that of GHB, but because the rate decreasing effects of 56 mg/kg were statistically significant at 10 min but not later, GBL

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may have a faster onset. The effects of 1,4-BDL on drug-appropriate responding had a slower onset and a longer duration than those of GHB and GBL. Because the effects of 1,4-BDL on GHB-appropriate responding reached a maximum at 60 min after its administration, the dose-response data of 1,4-BDL shown in Fig. 1 were obtained using a 60 min pretreatment.

Morphine produced at most 19.2% GHB-appropriate responding at 1 mg/kg; a higher dose suppressed responding to less than 20% of vehicle control in five of the six animals tested (Fig. 3, left panels). Naltrexone produced at most 17% GHB-appropriate responding when given alone; except at a dose of 1 mg/kg, naltrexone did not significantly affect response rate. Cocaine and haloperidol produced at most 21 and 34% GHB-appropriate responding, respectively, when tested up to and including doses that markedly decreased responding (Fig. 3, right panels).

The $GABA_B$ agonist, baclofen, produced a maximum of 66% GHB-appropriate responding at a dose of 17.8 mg/kg that did not significantly affect the rate of responding (Fig. 4, left panels). At a higher dose of 32 mg/kg, baclofen markedly decreased responding in more than half of the animals tested. The putative GHB receptor antagonist, NCS-382, produced GHB-appropriate responding when given alone: NCS-382 produced 70% GHB-appropriate responding at a dose (i.e., 320 mg/kg) that also significantly decreased response rate. Compounds interacting with $GABA_A$ receptors (Fig. 4, right panel) increased the percentage GHB-appropriate responding to different extents, with maximal effects ranging from 3% (obtained with the $GABA_A$ receptor agonist muscimol) to intermediate (i.e., 54, 68, and 73%, observed with the positive $GABA_A$ modulators, diazepam, ethanol, and pentobarbital, respectively) to levels similar to that obtained with the training dose of GHB (i.e., 96%, observed with the

benzodiazepine antagonist, flumazenil, and with the negative $GABA_A$ modulator, Ro 15-4513). The potency of Ro 15-4513 to produce GHB-appropriate responding responding (D_{50} = 0.069 mg/kg, 95% CL 0.029 – 0.17) was similar to that of flumazenil (D_{50} = 0.047, 95% CL: 0.017 – 0.13). Ro 15-4513, however, had no significant effects on response rate.

When tested alone, the training dose of GHB produced 99% GHB-appropriate responding without affecting response rate (Fig. 5, open circles). These effects of GHB were not attenuated by flumazenil nor by NCS-382, when tested at the highest dose that did not produce marked GHB-appropriate responding when given alone (i.e., 0.01 mg/kg flumazenil, 178 mg/kg NCS-382). Of all the antagonists tested, only CGP 35348 decreased the percentage GHB-appropriate responding produced by GHB, to 23% at a dose of 560 mg/kg.

The percentage GHB-appropriate responding observed with 10 mg/kg baclofen was decreased to 22 – 27 % by diazepam and to 0% by 56 mg/kg CGP 35348 (Fig. 6, left panels). The intermediate responding obtained with 5.6 mg/kg diazepam was not affected by the highest dose of flumazenil (i.e., 0.01 mg/kg) that did not produce marked GHB-appropriate responding when given alone, and was decreased to 27 – 32% by baclofen (Fig. 6, middle panels). The maximal effect of NCS-382 when given alone was decreased by CGP 35348, to 9% at a dose of 56 mg/kg (Fig. 6, right panels). When given alone at 56 and 100 mg/kg, CGP 35348 did not produce more than 10% GHB-appropriate responding (data not shown).

Discussion

The number of sessions to acquire the GHB discrimination in pigeons was similar to those reported with a training dose of 200 mg/kg GHB in rats (Winter, 1981; Carter et al., 2003) suggesting that the discriminability of 100 mg/kg GHB administered i.m. to pigeons is not markedly different from that of 200 mg/kg GHB administered i.p. to rats. The lowest dose that suppressed responding in pigeons was 320 mg/kg, as was the case in rats (Carter et al., 2003). Thus, pigeons can reliably discriminate GHB from vehicle, and their sensitivity to the DS and rate-decreasing effects of GHB does not appear to differ markedly from that of rats.

GBL and 1,4-BDL produced GHB-like DS effects, consistent with the hypothesis that they exert their effects because of metabolism to GHB (e.g., Roth and Giarman, 1968; Roth et al., 1966). The faster onset of GBL than of GHB is compatible with this hypothesis: the bioavailability of GHB as a metabolite of GBL is greater than after administration of GHB because GBL is more rapidly absorbed than GHB (Lettieri and Fung, 1978) and is subsequently converted to GHB. GBL and 1,4-BDL produced more than 80% GHB-appropriate responding in pigeons, but only 1,4-BDL did so in rats (Carter et al., 2003). GBL produced at most 50% GHB-appropriate responding in rats (Winter, 1981; Carter et al., 2003). GBL and 1,4-BDL are metabolized to GHB by different enzymes, which has been suggested to account for the low level of GHB-appropriate responding obtained with GBL in rats (Carter et al., 2003). That 1,4-BDL has GHB-like DS effects in pigeons and in rats is consistent with reports of 1,4-BDL use by human GHB abusers (Mason and Kerns, 2002). Its marked GHB-like effects observed here in pigeons are consistent with GBL being similarly abused (Graeme, 2000).

GHB modulates dopamine function (e.g., Feigenbaum and Howard, 1997); however, neither cocaine nor haloperidol produced more than 35% GHB-appropriate responding, and haloperidol did not attenuate the DS effects of GHB. Also, neither morphine nor naltrexone produced more than 20% GHB-appropriate responding, and naltrexone did not attenuate the DS effects of GHB. These results, which confirm and extend those obtained in GHB-discriminating rats (Winter, 1981; Carter et al., 2003), suggest that the DS effects of GHB do not primarily involve its modulation of dopamine or opioid systems.

Studies in rats (Winter, 1981; Colombo et al., 1995a; 1998; Metcalf et al., 2001; Carter et al., 2003) implicate GABA receptors in the DS effects of GHB. Consistent with this view, the $GABA_B$ agonist baclofen produced intermediate responding (i.e., 66%) in pigeons. In rats, baclofen produced intermediate responding in one study (Winter, 1981), but fully substituted for GHB in two other studies (Colombo et al., 1998; Carter et al., 2003). Baclofen was at least 80-fold more potent than GHB in rats (Carter et al., 2003), but was only 4-fold more potent than GHB in pigeons. In the present study, CGP 35348 completely blocked the GHB-like effects of baclofen at a dose of 56 mg/kg, while incompletely attenuating the DS effects of GHB even at a 10-fold higher dose. In contrast, CGP 35348 blocked the DS effects of GHB in rats at the same dose (i.e., 56 mg/kg) that blocked the GHB-like DS effects of baclofen (Carter et al., 2003). That baclofen was less potent and less efficacious to produce GHB-like DS effects in pigeons than in rats, and that CGP 35348 was less potent and less able to antagonize the DS effects of GHB in pigeons than in rats, suggests a possible species difference in the extent to which $GABA_B$ receptors are involved in these effects. The relative importance of $GABA_B$ mechanisms, however, may also be influenced by the training dose of GHB

(Colombo et al., 1998). Varying the training dose and temporal conditions in both species could help to delineate conditions that determine to what extent $GABA_B$ receptors are involved in the DS effects of GHB.

$GABA_A$ receptors may also play a role in the DS effects of GHB. Ethanol, which positively modulates $GABA_A$ receptors, produced 68% GHB-appropriate responding, in the middle of the range of values previously reported in rats, i.e., 31% (Winter, 1981), 71% (Metcalf et al., 2001), and 91% (Colombo et al., 1995b). The maximal effect of pentobarbital, which positively modulates $GABA_A$ through the barbiturate site, was 73%. In contrast, in a previous study in rats (Carter et al., 2003) pentobarbital did not produce any GHB-appropriate responding. However, the doses of pentobarbital that could be evaluated for possible GHB-like DS effects in the present study in pigeons and in the previous study in rats differed markedly, possibly due to a differential sensitivity to its response rate decreasing effects. In rats, the highest dose tested for GHB-like DS effects was 3.2 mg/kg pentobarbital, because 10 mg/kg eliminated responding in all animals. In pigeons, however, pentobarbital produced GHB-appropriate responding only at 10 and 17.8 mg/kg, without affecting response rate, and eliminated responding at 32 mg/kg. Diazepam produced at most 54% GHB-appropriate responding. In contrast, the GHB precursors GBL and 1,4-BDL did not produce midazolam- or pentobarbital-appropriate responding (McMahon et al., 2003). This apparent asymmetrical generalization resembles that between ethanol and benzodiazepines: i.e., whereas benzodiazepines produce ethanol-appropriate responding in ethanol-trained animals, ethanol does not produce benzodiazepine-appropriate responding in benzodiazepine-trained animals (e.g., De Vry and Slangen, 1986). Together, the maximum percentage GHB-appropriate responding obtained with the $GABA_A$ receptor modulators ethanol (68%), pentobarbital

(73%), and diazepam (54%), and with the $GABA_A$ receptor agonist muscimol (3%), appear to be consistent with a limited role for $GABA_A$ receptors in the DS effects of GHB under these conditions. The finding that diazepam and baclofen did not produce more GHB-appropriate responding when given together than when given alone, suggests that full GHB-like DS effects cannot be produced by adding $GABA_A$ and $GABA_B$ receptor activation. Thus, the DS effects of GHB in pigeons involve, in addition to $GABA_A$ and $GABA_B$ receptors, other (possibly GHB) receptors.

GHB may exert its effects not only through GABA receptors, but also through specific GHB receptors, which can be investigated with [^{3}H]NCS-382 (Mehta et al., 2001). NCS-382 was initially reported to antagonize several effects of GHB (e.g., increased inositol phosphate turnover in hippocampus, increased dopamine release in striatum) as well as GHB-induced seizures (Maitre et al., 1990). Since then, NCS-382 has been reported to antagonize some behavioral effects of GHB [e.g., self-administration in mice (Martellotta et al., 1998), DS effects in rats (Colombo et al., 1995a)]. More recent studies, however, suggest that the antagonism by NCS-382 of the behavioral depressant effects of GHB is very limited (Carai et al., 2001; Cook et al., 2002; Lamb et al., 2003), and that NCS-382 can enhance certain effects of GHB, such as loss of righting (Carai et al., 2001). In a recent study in rats, NCS-382 partially attenuated the DS effects of GHB, but its antagonism was limited by partial GHB-like effects when given alone (Carter et al., 2003). Here, NCS-382 failed to attenuate the DS effects of GHB, and when given alone produced substantial (70%) GHB-appropriate responding. NCS-382-induced GHB-appropriate responding was antagonized by CGP 35348, suggesting that NCS-382 has agonist actions at $GABA_B$ receptors. $GABA_B$ receptors have been reported to mediate the GHB-like effects of NCS-382 on intestinal motility in mice (Carai et al.,

2002). The present results extend this finding to NCS-382-induced GHB-like DS effects and suggest that $GABA_B$ receptors may be involved in the enhancement by NCS-382 of some actions of GHB. Selective GHB receptor antagonists that lack GHB-like effects would greatly facilitate the study of drug actions at specific GHB receptors. Because of its sensitivity to detect GHB-like effects of NCS-382, a GHB discrimination in pigeons may be useful in the search for such antagonists.

Flumazenil, at 5.6 mg/kg, blocked the GHB-like effects of diazepam in rats (Carter et al., 2003). Here, flumazenil did not attenuate the GHB-like effects of diazepam; instead, it substituted completely for GHB when given alone at 0.1 mg/kg. Flumazenil can function as a DS in monkeys (e.g., Gerak and France, 1999), in rats (e.g., De Vry and Slangen, 1985; Woudenberg and Slangen, 1990), and in pigeons (e.g., Wong et al., 1993). In monkeys treated daily with diazepam, flumazenil (0.32 mg/kg) has DS effects that are related to its antagonist actions at diazepam-sensitive $GABA_A$ receptors (Gerak and France, 1999) and that are not mimicked by GBL and 1,4-BDL (McMahon et al., 2003), suggesting that GHB and its precursors lack antagonist actions at diazepam-sensitive $GABA_A$ receptors. In contrast, in monkeys not treated daily with diazepam, the DS effects of flumazenil (1 mg/kg) appear to involve other mechanisms (Gerak and France, 1999). Whereas a flumazenil discrimination in rats has been demonstrated only at doses of 10 mg/kg and higher, pigeons discriminate a 100-fold lower dose of flumazenil. In 0.1 mg/kg flumazenil-trained pigeons, only compounds with high affinity for diazepam-insensitive $GABA_A$ receptors produce flumazenil-appropriate responding (e.g., Ro 15-4513), with potencies that correlate with their affinities at diazepam-insensitive $GABA_A$ receptors, suggesting that the DS effects of flumazenil in this species are mediated by diazepam-insensitive $GABA_A$ receptors (Wong et al., 1993; Acri et al.,

1995; Acri et al., 1997). Despite the opposite functioning of flumazenil and Ro 15-4513 at diazepam-sensitive $GABA_A$ receptors, both compounds produced GHB-appropriate responding, with similar potency. This agrees with the finding that Ro 15-4513 and flumazenil have similar potency to produce flumazenil-like DS effects in pigeons (Wong et al., 1993), and is consistent with a role for diazepam-insensitive $GABA_A$ receptors, for which both have high affinity (Wong et al., 1993), in their GHB-like effects. Thus, a GHB discrimination in pigeons may be especially useful to investigate the role of diazepam-insensitive $GABA_A$ receptors in the effects of GHB.

In summary, the DS effects of GHB in pigeons appear to be similar, but not identical, to those in rats, and involve multiple mechanisms. In pigeons, diazepam-insensitive $GABA_A$ receptors may be more prominently involved in the DS effects of GHB than in rats, whereas $GABA_B$ receptors may be less prominently involved. Varying the training dose of GHB in both species will help to examine the extent to which the DS effects of GHB are species dependent and could help to delineate the conditions that determine the relative importance of diazepam-sensitive and diazepam-insensitive $GABA_A$ receptors, and of $GABA_B$ receptors, in these effects.

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Footnotes:

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Legends for figures:

Figure 1. Effects of GHB, and its precursors, GBL and 1,4-BDL, in pigeons trained to discriminate 100 mg/kg GHB from saline. The percentage of responses on the drug-appropriate key (% DR; upper panel) and the rate of responding (responses/sec; lower panel) are plotted as a function of dose. Symbols represent mean ± SEM; if not shown, SEM values are contained by the symbol. All drugs were tested in six pigeons, except orally-administered GHB, which was tested in five pigeons. Response rate data marked with an asterisk are significantly different from vehicle control.

Figure 2. Time course of the effects of GHB, and its precursors, GBL and 1,4-BDL, in pigeons (n=6) trained to discriminate 100 mg/kg GHB from saline. The percentage of responses on the drug-appropriate key (% DR; upper panel) and the rate of responding (responses/sec; lower panel) are plotted as a function of pretreatment time. Symbols represent mean ± SEM; if not shown, SEM values are contained by the symbol. Response rate data marked with an asterisk are significantly different from vehicle control.

Figure 3. Effects of morphine and naltrexone (left panels) and of cocaine and haloperidol (right panels) in pigeons (n=6) trained to discriminate 100 mg/kg GHB from saline. See Fig. 1 for other details.

Figure 4. Effects of baclofen and NCS-382 (left panels) and of flumazenil, Ro 15-4513, muscimol, diazepam, pentobarbital, and ethanol (right panels) in pigeons trained to discriminate 100 mg/kg GHB from saline. Baclofen, NCS-382, flumazenil, and

diazepam were tested in six pigeons; all other drugs were tested in five pigeons. See Fig. 1 for other details.

Figure 5. Effects of 100 mg/kg GHB alone (open circles; replotted from Fig. 1) and 10 min after pretreatment with flumazenil, haloperidol, naltrexone, NCS-382, or CGP 35348 (solid symbols) in pigeons (n=6) trained to discriminate 100 mg/kg GHB from saline. See Fig. 1 for other details.

Figure 6. Effects of 10 mg/kg baclofen (left panels), 5.6 mg/kg diazepam (middle panels), and 320 mg/kg NCS-382 (right panels), alone (open circles; replotted from Fig. 4) and 10 min after pretreatment with diazepam, CGP 35348, flumazenil, or baclofen (solid symbols) in pigeons trained to discriminate 100 mg/kg GHB from saline. All drugs were tested in six pigeons, except combinations of baclofen and diazepam, which were tested in five pigeons. See Fig. 1 for other details.

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Figure 1

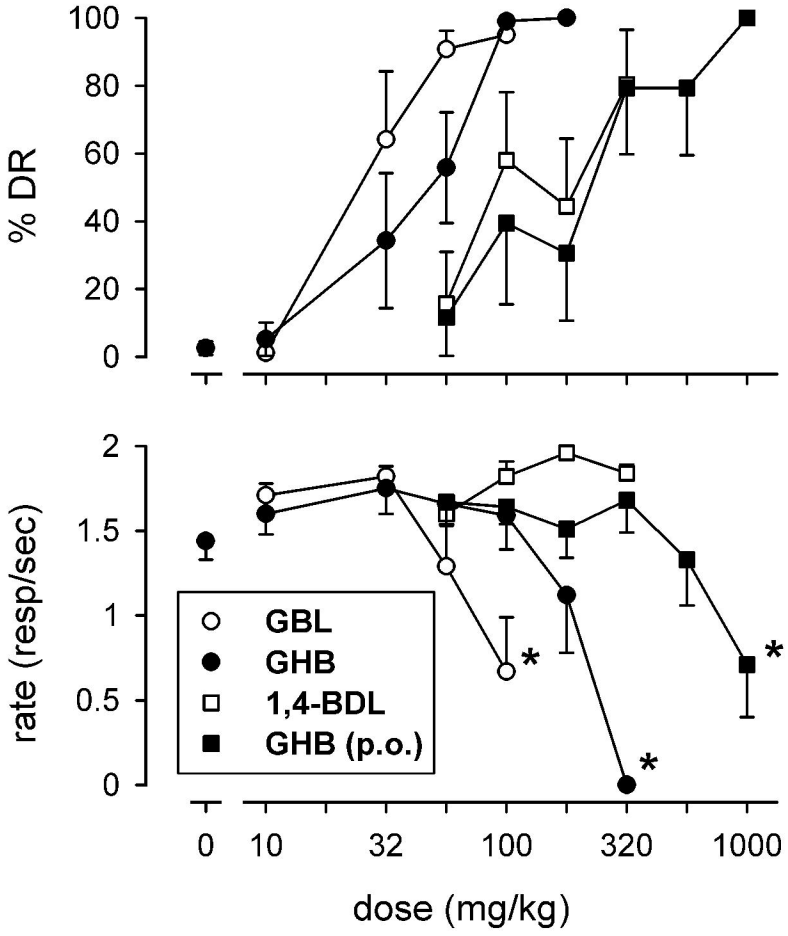

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Figure 2

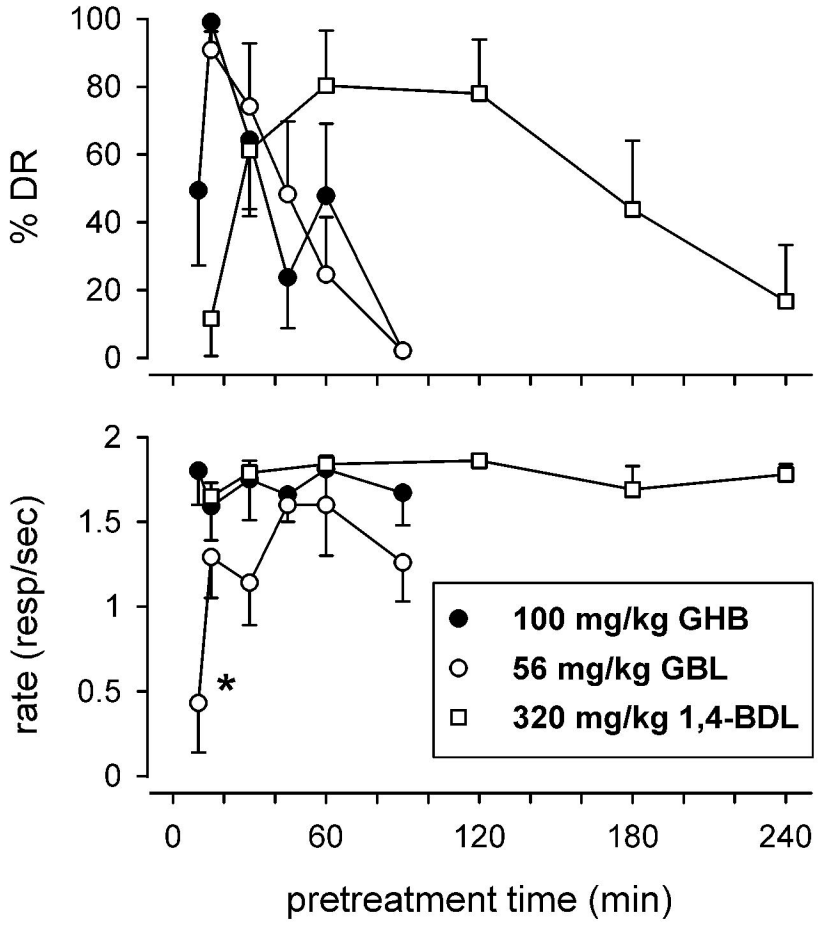

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Figure 3

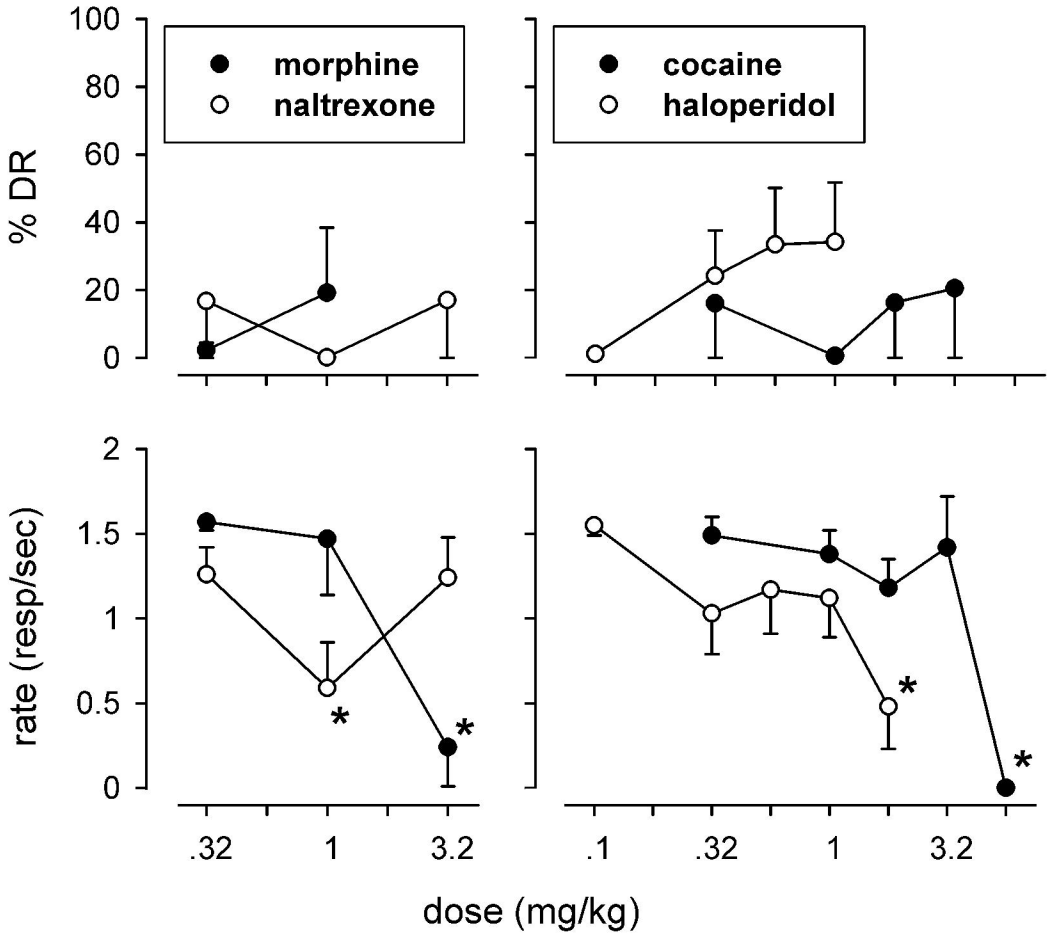

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Figure 4

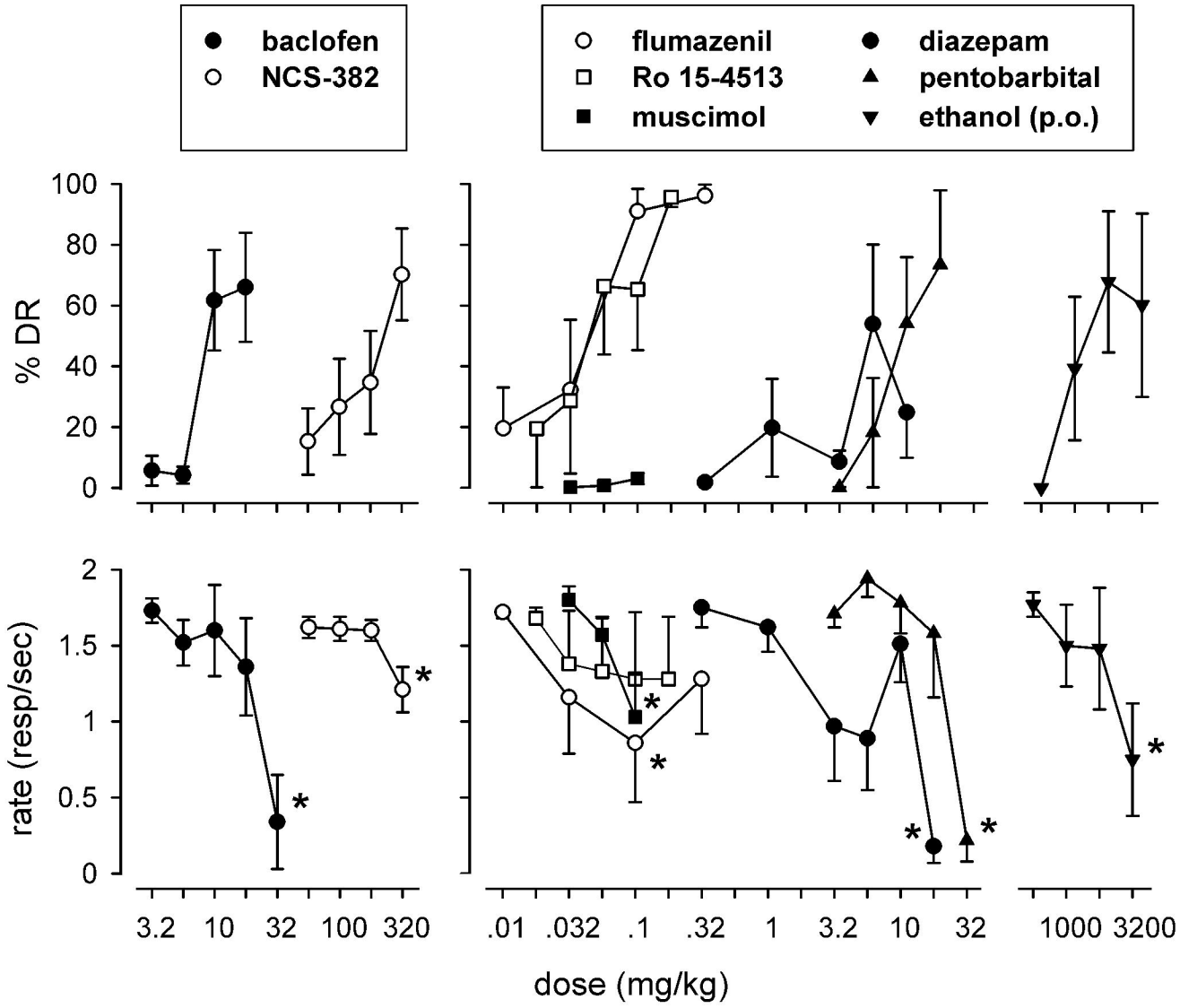

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Figure 5

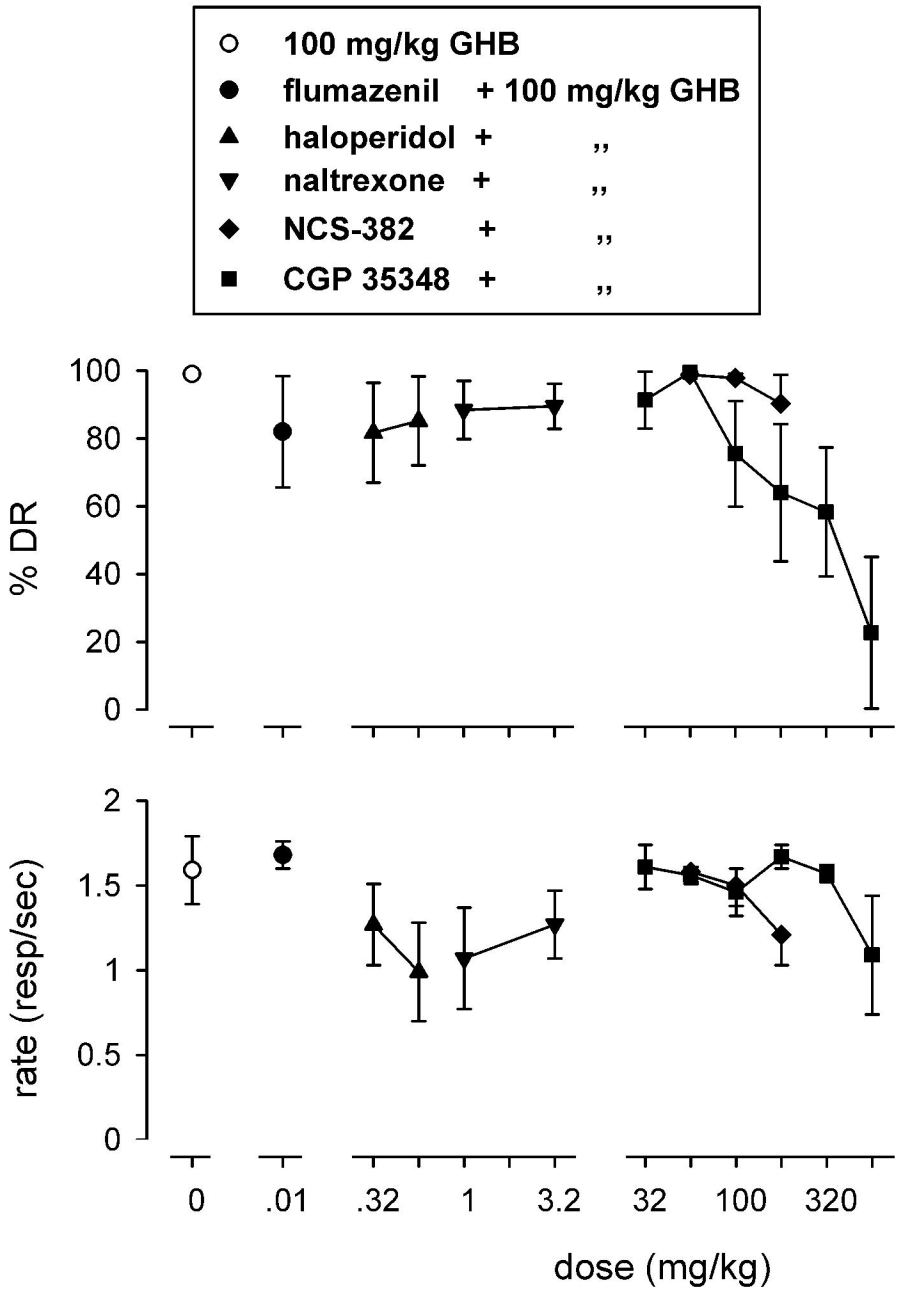

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Figure 6