JPET Fast Forward. Published on June 29, 2009 as DOI: 10.1124/jpet.109.151845

Title Page

Behavioral effects of GHB, its precursor GBL, and $GABA_B$ receptor agonists: time course, and differential antagonism by the $GABA_B$ receptor antagonist CGP35348.

Wouter Koek, Susan L. Mercer, Andrew Coop, and Charles P. France

Departments of Psychiatry and Pharmacology (W.K., C.P.F.), The University of Texas Health Science Center at San Antonio, San Antonio, TX, Department of Pharmaceutical Sciences (S.L.M.), Lipscomb University College of Pharmacy, Nashville, TN, and Department of Pharmaceutical Sciences (A.C.), University of Maryland, Baltimore, MD

JPET Fast Forward. Published on June 29, 2009 as DOI: 10.1124/jpet.109.151845
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title Page

Running title: GHB, GBL, and $GABA_B$ receptor agonists

Corresponding Author: Wouter Koek, Ph.D.

Departments of Psychiatry and Pharmacology

University of Texas Health Science Center at San Antonio

7703 Floyd Curl Drive, Mail Code 7792

San Antonio, Texas 78229-3900

Tel: (210) 567 5478

Fax: (210) 567 5381

E-mail: koek@uthscsa.edu

Text pages: 32

Tables: 1

Figures: 5

References: 40

Abstract: 246 words

Introduction: 717 words

Discussion: 1016 words

Abbreviations: GHB, γ-hydroxybutyrate; GBL, γ-butyrolactone; SKF97541, 3-aminopropyl(methyl)phosphinic acid hydrochloride; CGP35348, 3-aminopropyl(diethoxymethyl)phosphinic acid; NMDA, N-methyl-d-aspartate; PCP, phencyclidine

Recommended Section Assignment: Behavioral Pharmacology

Abstract

γ-Hydroxybutyrate (GHB) is used therapeutically and recreationally. The mechanism by which GHB produces its therapeutic and recreational effects is not entirely clear, although $GABA_B$ receptors appear to play an important role. This role could be complex, because there are indications that different $GABA_B$ receptor mechanisms mediate the effects of GHB and the prototypical $GABA_B$ receptor agonist baclofen. To further explore possible differences in underlying $GABA_B$ receptor mechanisms, the present study examined the effects of GHB and baclofen on operant responding, and their antagonism by the $GABA_B$ receptor antagonist CGP35348. Pigeons were trained to peck a key for access to food during response periods that started at different times after the beginning of the session. In these pigeons, GHB, its precursor GBL, and the $GABA_B$ receptor agonists baclofen and SKF97541 decreased the rate of responding in a dose- and time-dependent manner. CGP35348 shifted the dose-response curve of each agonist to the right, but the magnitude of the shift differed among the agonists. Schild analysis yielded a pA_2 value of CGP35348 to antagonize GHB and GBL (i.e., 3.9 [3.7-4.2]) that was different (P=0.0011) from that to antagonize baclofen and SKF97541 (i.e., 4.5 [4.4-4.7]. This finding is further evidence that the $GABA_B$ receptor mechanisms mediating the effects of GHB and prototypical $GABA_B$ receptor agonists are not identical. A better understanding of the similarities and differences between these mechanisms, and their involvement in the therapeutic effects of GHB and baclofen, could lead to more effective medications with fewer adverse effects.

Introduction

Gamma-hydroxybutyric acid (GHB) is an endogenous molecule, a marketed therapeutic drug, and a drug of abuse. GHB is a putative neuromodulator (Maitre, 1997) involved in the regulation of sleep and used clinically to treat narcolepsy (Fuller and Hornfeldt, 2003) and alcoholism (Poldrugo and Addolorato, 1999). GHB is also used recreationally (Gonzalez and Nutt, 2005). The precise mechanism by which GHB exerts its various effects is unknown.

GHB binds to specific sites in brain (Benavides et al., 1982) and to $GABA_B$ receptors (Mathivet et al., 1997). At present, there is little evidence that specific GHB binding sites mediate the in vivo effects of GHB (Wong et al., 2004). Instead, many studies suggest that $GABA_B$ receptors are particularly important for various behavioral effects of GHB, including hypolocomotion (Kaupmann et al., 2003), catalepsy (Carter et al., 2005), ataxia (Goodwin et al., 2005), loss of righting (Carai et al., 2001), decreased operant responding (Goodwin et al., 2005), and discriminative stimulus effects (Carter et al., 2003, 2009; Colombo et al., 1998; Koek et al., 2004, 2006; Winter, 1981). All of these effects of GHB are also produced by the prototypical $GABA_B$ agonist baclofen (Carter et al., 2003, 2004, 2005), consistent with the involvement of $GABA_B$ receptors in the effects of GHB.

Although $GABA_B$ receptors likely mediate behavioral effects that GHB has in common with baclofen, there is growing evidence that the underlying $GABA_B$ receptor mechanisms are not identical. One line of evidence is from studies that examined the interactions of GHB and baclofen with antagonists at the N-methyl-d-aspartate (NMDA) subtype of glutamate receptors. The NMDA antagonist dizocilpine (MK-801) enhances GHB-induced catalepsy in rodents (Sevak et al., 2004, 2005; Koek and France, 2008). The cataleptic effects of GHB are enhanced not only by MK-801, but also by other drugs with

NMDA antagonist activity, such as phencyclidine (PCP) and ketamine. However, these NMDA antagonists do not affect the cataleptic effects of baclofen (Koek and France, 2008). Similar interactions have been observed in drug discrimination studies: PCP enhances the discriminative stimulus effects of GHB but not of baclofen (Koek et al., 2007a). Differential enhancement of the effects of GHB and baclofen by NMDA antagonists suggest that the $GABA_B$ receptor mechanisms involved in the effects of GHB and baclofen may not be identical.

A second line of evidence that the $GABA_B$ receptor mechanisms underlying the effects of GHB and baclofen may be different is from antagonism studies. The $GABA_B$ receptor antagonist CGP35348 antagonizes the discriminative stimulus effects of GHB and baclofen, consistent with the involvement of $GABA_B$ receptors, but is less potent to antagonize these effects of GHB than those of baclofen (Carter et al., 2006). Recently, we reported that CGP35348 was also less potent to antagonize the cataleptic effects of GHB than those of baclofen (Koek et al., 2007b). Together, these findings suggest a possible role for $GABA_B$ receptor subtypes or different interactions with the same $GABA_B$ receptor in the behavioral effects of GHB and baclofen.

A detailed characterization of antagonist actions requires complete dose-response curves of the agonist in the presence of several doses of the antagonist. Such data are often analyzed by Schild regression, which yields information about the nature of the antagonism and the potency of the antagonist. This method compares the pattern of antagonism to that predicted by the simple competitive model (i.e., the agonist and antagonist compete for the same recognition sites on the receptor). If the Schild regression has a slope of unity, this is consistent with simple competitive antagonism; deviations from unity can signify non-competitive antagonism, non-equilibrium steady states, or receptor-population heterogeneity

(Kenakin, 1997). A limitation of the studies examining antagonism of GHB and baclofen that we have conducted to date is that their results could not be analyzed by Schild regression. The present study, aimed at remedying this limitation, is an effort to characterize in detail the antagonism by CGP35348 of behavioral effects of GHB, its precursor GBL, and the $GABA_B$ receptor agonists baclofen and SKF97541. The behavioral measure used was decreased operant responding, assessed in a procedure that provided information about the time course of agonist and antagonist effects. The results confirm preliminary findings of differential antagonism by CGP35348 of GHB and baclofen, and suggest that the underlying $GABA_B$ receptor mechanisms are different, which may have implications for their different profiles of preclinical and clinical activities.

Methods

Animals

Ten adult white Carneau pigeons (*Columbia Livia*; Palmetto, Sumter, SC) were individually housed under a 12/12-h light/dark cycle. They had free access to water and were maintained between 80 and 90% of their free-feeding weight by food (Purina Pigeon Checkers) received during experimental sessions and supplemental post-session feedings (Purina Pigeon Checkers or mixed grain). All subjects had drug discrimination histories (Koek et al., 2006) and had not received any drug for at least one month before the start of the current study. 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 a response key that could be illuminated by a red light. After completion of each fixed ratio, the key light was 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 by Schlinger and Poling (1988). Briefly, pigeons trained to peck the response key for access to food were exposed to 12-h overnight sessions that were conducted four times per week, Monday through Thursday. During each session, the key was illuminated during ten response periods, each starting at a different time after the beginning of the session (i.e., 0, 15, 30, 60 min, 2, 4, 6, 8, 10, 12 h). When the key was illuminated, 20 responses resulted in 4-s access to food (i.e., fixed ratio 20). A response period ended after 5 food presentations or 5 min, whichever occurred first. Between response periods the key light was off, and responses had no programmed consequence. Response periods began with a brief (0.25 s) operation and illumination of the hopper (i.e., a brief auditory and visual stimulus).

Once responding stabilized under the fixed ratio schedule during each of the 10 response periods (i.e., no visible trend was evident for at least five consecutive sessions), subjects received an i.m. injection of physiological saline before each session. Monday and Wednesday sessions were always preceded by a saline injection. If responding during a saline session did not differ by more than 20% from responding during the previous saline session, an antagonist and/or agonist was given before the next session (i.e., on Tuesday or Thursday). Otherwise, a saline session was conducted. Agonists were given immediately before the session, and the antagonist was given 10 min before an agonist.

Data Analysis

Response rates were calculated for each response period by dividing the number of responses by the duration (in s) of the period. For each animal, response rates during the periods that started at different times after drug administration were expressed as a

percentage of the corresponding control values obtained during the previous vehicle session. The response rates during drug test sessions, expressed as percentage control, were averaged across animals and mean values +/- S.E.M. were plotted as a function of dose and time after drug administration.

To calculate doses needed to produce 50% of the maximal response (ED_{50}) and their 95% confidence limits, the linear portion of dose-response curves was analyzed by log-linear regression (Tallarida 2000) of data from individual subjects using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA; www.graphpad.com), using the following equation: effect = slope x log(dose) + intercept. The linear portion comprised the data points at doses immediately below and above 50% and included not more than one dose with an effect larger than 80% and not more than one dose with an effect smaller than 20%. For each dose-response curve fitted to more than two doses, the replicates test (also called lack of fit test; Draper and Smith, 1998) implemented in GraphPad Prism was used to examine whether the log dose-response data used in the log-linear regression deviated from linearity. F ratio tests in GraphPad Prism were used to compare dose-response curves with respect to their slopes and intercepts. For example, a non-significant F ratio for slopes and a significant F ratio for intercepts shows dose-response curves to be parallel but occupying different positions on the dose axis.

To examine the onset and duration of agonist effects, ED_{50} values for each agonist were plotted as a function of time after drug administration. Differences among ED_{50} values were analyzed using the F ratio test implemented in GraphPad Prism and using the common slope calculated by GraphPad Prism to constrain the fit of the parallel line assay (Tallarida 2000), as detailed elsewhere (Koek et al., 2006). Dose-response data obtained at the time that the ED_{50} values were lowest (i.e., when the agonist appeared to reach

peak effect) were used to examine antagonist effects. For each agonist, differences among its dose-response curves to decrease response rate in the presence of different doses of antagonist were analyzed by simultaneously fitting straight lines to the linear portion of the dose-response curves. Differences among the slopes and intercepts of the curves were analyzed with the F ratio test, and ED_{50} values and potency ratios were calculated by parallel line analysis (Tallarida 2000). For each agonist, potency ratios were used to calculate an apparent pA_2 value for the antagonist according to the methods described by Arunlakshana and Schild (1959). The Schild plots were analyzed with the F ratio test to examine if the slopes of the Schild regressions deviated significantly from -1, and then to examine differences among pA_2 values by comparing the following models of increasing complexity (i.e., increasing number of parameters): 1) same pA_2 value for all agonists; 2) same pA_2 value for GHB and SKF97541, same pA_2 value for GHB and GBL; and 3) individual pA_2 value for each agonist. Thus, Schild plots were used to examine if the effects of the antagonist differed for different agonists. In addition, Schild analyses of data obtained at various times after the administration of the antagonist were used to examine the duration of antagonist activity.

Drugs

Gamma-hydroxybutyrate sodium (GHB), gamma-butyrolactone (GBL), and (±)-baclofen were purchased from Sigma-Aldrich Corp. (St. Louis, MO). CGP35348 (sodium salt) and SKF97541 hydrochloride were synthesized at the University of Maryland. All compounds were dissolved in physiological saline (0.9% NaCl), except GHB, which was dissolved in sterile water. All compounds were injected i.m. in a volume of 0.1–1.0 ml. Doses are expressed as the form of the drug listed above.

Results

Under control conditions, key peck responses occurred at a rate that varied not more than 20% within pigeons, and that varied from 0.83 to 1.94 responses/second between pigeons. Baclofen decreased the rate of responding in a dose- and time-dependent manner (Fig. 1). Responding was decreased to less than 50% of control between 30 min and 4 h after the injection of baclofen (Fig. 1, closed symbols), but not at shorter or longer intervals (Fig. 1, open symbols). The dose-response data obtained at 30 min, 1, 2, and 4 h after 5.6, 10, and 17.8 mg/kg baclofen were analyzed by log-linear regression. The four dose-response curves, which did not deviate from linearity (replicates test: $P>0.20$), had a common slope ($F[3,84]=0.31$, $P=0.82$), but not a common ED_{50} ($F[3,87]=2.73$, $P=0.049$). The ED_{50} of baclofen to decrease responding was lowest at 60 min after injection (i.e., 8.6 [95% confidence limits: 7.1-10] mg/kg; Fig. 2, Table 1), was not different at 30 and 120 min ($F[1,43]\leq1.69$, $P\geq0.20$), but was higher at 240 min ($F[1,43]=8.28$, $P=0.0062$). Based on these results, baclofen appeared to reach peak effect 30-120 min after injection. The other drugs reached peak effect at 30-120 (SKF97541) or at 15-30 min (GHB, GBL). Thus, at 30 min after injection, all drugs were maximally active.

When injected alone at a dose of 32 mg/kg, the $GABA_B$ receptor antagonist CGP35348 did not alter the rate of responding (data not shown). The response rate, expressed as a percentage of saline control, did not significantly change during the session and varied between 98 and 109% (S.E.M. 2.5-6.3). When injected 10 min before each agonist, CGP35348 dose-dependently shifted the dose-response curve of each agonist to the right (Fig. 3, upper and middle panels; Table 1). However, the extent and nature of these shifts appeared to differ among some of the agonists. To examine these

apparent shifts, the linear portion of each dose-response curve (see Materials and Methods) was analyzed by log-linear regression. None of the dose-response data used in the regression analyses deviated from linearity (replicates test: P values ranged from a minimum of 0.13 obtained for $F[1,9]=2.73$ to a maximum of 0.98 for $F[2,14]=0.02$). CGP35348 shifted the dose-response curves of baclofen and SKF97541 to the right (common intercept: $F[3,49]\geq 7.80$, $P\leq 0.0002$), in a parallel manner (common slope: $F[3,46]\leq 1.29$. $P\geq=0.29$), and to an apparently similar extent. At the same doses, CGP35348 shifted the dose-response curves of GHB (common intercept: $F[3,41]=7.53$, $P=0.0004$) and GBL (common intercept: $F[3,47]=3.37$, $P=0.026$), but apparently less extensively, and in the case of GHB, in a non-parallel manner (GHB, common slope: $F[3,41]=6.34$, $P=0.0012$; GBL, common slope: $F[3,44]=2.02$, $P=0.12$). The antagonist effects of CGP35348 were quantified by means of Schild regression plots (Fig., 3, lower panel). These plots, with a common slope ($F[3,4]=0.47$, $P=0.72$) not different from -1 ($F[1,10]=2.04$, $P=0.18$) , yielded the following pA_2 values (Table 1): 4.46 (95% CL: 4.10-4.82) for baclofen, 4.63 (4.17-5.09) for SKF97541, 3.97 (3.47-4.47) for GHB, and 3.91 (2.98-4.85) for GBL. The confidence interval of the estimated pA_2 value was wider for GBL (i.e., 1.87) than for baclofen, SKF97541, and GHB (i.e., 0.72, 0.92, and 1, respectively), indicating that the Schild regression fitted the data obtained with GBL less well than those obtained with the other drugs. To examine similarities and differences among the pA_2 values obtained for CGP35348 with each of the four agonists, the following models of increasing complexity (i.e., larger number of parameters) were compared by means of F tests: model 1) a common pA_2 value for all agonists; model 2) a common pA_2 value for baclofen and SKF97541 and a common pA_2 value for GHB and GBL; and model 3) an individual pA_2 value for each agonist. Model 2 fitted the data

better than model 1 (F[1,10]=20.74, P=0.0011), which indicates that the data could not be adequately fitted with a single pA_2 value. However, adding more parameters, by assuming the pA2 values to differ for each agonist, did not further increase the fit (comparison of model 3 with model 2: F[2,8]=0.42, P=0.67). Thus, model 2 appeared to be the simplest model that could be fitted to the Schild regression data obtained with all four drugs, and consisted of one plot for baclofen and SKF97541 and one for GHB and GBL. Based on these plots, the pA_2 value of CGP35348 was 4.54 [4.36-4.73] to antagonize baclofen and SKF97541, and was 3.94 [3.66-4.23] to antagonize GHB and GBL. Thus, CGP35348 appeared to be 4-fold less potent to antagonize the response rate decreasing effects of GHB and GBL than to antagonize those of baclofen and SKF97541.

SKF97541 dose-dependently decreased response rate not only at 30 min, but also at shorter and longer injection-test intervals (Fig. 4, all panels except lower right). None of the dose-response data used in the regression analyses deviated from linearity (replicates test: P values ranged from a minimum of 0.19 obtained for F[2,15]=1.88 to a maximum of 0.98 for F[2,14]=0.02). CGP35348, injected 10 min before SKF97541, dose-dependently shifted the dose-response curves of SKF97541 at each interval to the right (common intercept: 15 min, F[3,42]=3.53, P=0.023; 30 min, F[3,49]=7.80, P=0.0002; 60 min, F[3,50]=13.13, P<0.0001; 120 min, F[3,46]=12.38, P<0.0001; 240 min, F[4.29, P=0.009), in a parallel manner (common slope: 15 min, F[3,39]=0.44, P=0.73; 30 min, F[3,46]=0.50, P=0.69; 60 min, F[3,47]=0.99, P=0.41; 120 min, F[3,43]=2.45, P=0.077; 240 min, F[3,47]=1.84, P=0.15). However, the pA_2 values for CGP35348 obtained at these intervals were not the same (Fig. 4, lower right panel; Table 1). The Schild regression plots, with a common slope (F[4,5]=3.55, P=0.10) not different from -1 (F[1,13]=2.48, P=0.14), yielded a pA_2 value at 240 min after the injection of

SKF9754 (i.e., 4.18 [3.93-4.43]) that differed from the values obtained at shorter intervals (Table 1). To examine similarities and differences among the pA_2 values obtained at each of the intervals, the following models of increasing complexity (i.e., larger number of parameters) were compared by means of F tests: model 1) a common pA_2 value for all intervals; model 2) a common pA_2 value for the 15-120 min intervals, and a pA_2 value for the 240 min interval; and model 3) an individual pA_2 value for each interval. Model 2 fitted the data better than model 1 ($F[1,13]=14.87$, $P=0.002$), which indicates that the data could not be adequately fitted with a single pA_2 value. However, adding more parameters, by assuming the pA2 values to differ for each interval, did not further increase the fit (comparison of model 3 with model 2: $F[3,10]=0.62$, $P=0.62$). Thus, model 2 appeared to be the simplest model that could be fitted to the Schild regression data obtained at all five intervals, and consisted of one plot for the 15-120 min intervals and one the 240 min interval. Based on these plots, the pA_2 value of CGP35348 was 4.53 [4.44-4.63] to antagonize SKF97541 at 15-120 min after its injection, and was 4.18 [3.93-4.43] at 240 min. Thus, the potency of CGP35348 to antagonize SKF97541 was not different from 15 to 120 min after the injection of SKF97541 (i.e., 25 to 130 min after CGP35348); however, 250 min after the injection of CGP35348, its potency decreased about two-fold. A similar trend was apparent when CGP35348 was used to antagonize the effects of baclofen at various time intervals (Fig. 5), but this failed to reach statistical significance ($F[1,10]=1.46$, $P=0.25$). The pA_2 values obtained with GHB and GBL did not differ across intervals (GHB: $F[2,5]=0.40$, $P=0.69$; GBL: $F[1,4]=0.63$, $P=0.47$) but were lower than those obtained with baclofen and SKF97541 at the same interval (30 min: GHB vs baclofen, $F[1,4]=11.40$, $P=0.028$; GHB vs SKF97541, $F[1,4]=17.39$, $P=0.014$; GBL vs baclofen, $F[1,4]=5.51$, $P=0.079$; GBL vs SKF97541, $F[1,4]=8.85$,

P=0.041; 60 min: GHB vs baclofen, F[1,3]=7.63, P=0.07; GHB vs SKF97541, F[1,3]=12.80, P=0.037; GBL vs baclofen, F[1,4]=20.42, P=0.011; GBL vs SKF97541, F[1,4]=29.60, P=0.0055). Thus, CGP35348 appeared to be most potent between 25 and 130 min after its administration, and 4-fold more potent to antagonize baclofen and SKF97541 than GHB and GBL (Fig. 5).

Discussion

$GABA_B$ receptors appear to play an important role in the effects of GHB; however, the effects of GHB, although similar, are not identical to those of the prototypical $GABA_B$ receptor agonist baclofen (e.g., Carter et al., 2009). The main finding of the present study is that the $GABA_B$ receptor antagonist CGP35348 was significantly more potent to antagonize behavioral effects of the $GABA_B$ receptor agonists baclofen and SKF97541 than those of GHB and its precursor GBL. Schild plots with slope values not significantly different from -1, consistent with simple competitive antagonism, yielded a pA_2 value of CGP35348 to antagonize baclofen and SKF97541 (i.e., 4.5 [4.4-4.7]) that was similar to in vitro pA_2 values reported previously for CGP35348 to antagonize baclofen (i.e., 4.3-4.7; [Kerr et al., 1993; Olianas and Onali, 1999]), but that was about 4-fold higher than its pA_2 value to antagonize GHB and GBL (i.e., 3.9 [3.7-4.2]). These findings are consistent with previous observations of differential antagonism by CGP35348 of the discriminative stimulus effects of baclofen and GHB in rats (Carter et al., 2006) and of the cataleptic effects of baclofen and GHB in mice (Koek et al., 2007b), and extend them to effects on operant responding in pigeons. Together, these data are further evidence that $GABA_B$ receptors mediate many behavioral effects of GHB; however, they also suggest that the underlying $GABA_B$ mechanisms differ from those mediating the effects of prototypical $GABA_B$ receptor agonists such as baclofen.

Additional evidence that the $GABA_B$ receptor mechanisms underlying the effects of GHB and baclofen are not identical has recently been obtained in interaction studies with NMDA antagonists. The NMDA antagonist PCP and GHB enhance each other's discriminative stimulus effects, but PCP and baclofen do not, suggesting that the

mechanisms underlying these effects of GHB and baclofen are differentially modulated by the glutamatergic system with which PCP interacts (Koek et al., 2007a). The recent finding that PCP and other antagonists at the NMDA subtype of glutamate receptors enhance the cataleptic effects of GHB but not those of baclofen (Koek and France, 2008) provides further evidence that the $GABA_B$ receptor systems mediating the effects of GHB and baclofen are differentially modulated by glutamate.

Recent electrophysiological studies offer further evidence of differing effects of GHB and baclofen. At concentrations described as clinically relevant, GHB disinhibits and baclofen inhibits ventral tegmental dopamine neurons (Cruz et al., 2004). This suggests that GHB is more likely to activate the dopamine system implicated in addiction, whereas baclofen, which may be useful to reduce relapse to cocaine-taking (Weerts et al., 2007), may have more pronounced anti-craving effects (Cruz et al., 2004). GHB and baclofen differ also in their effects on neurotransmission at glutamate receptors (Li et al., 2007). GHB and baclofen both inhibited currents elicited by NMDA and AMPA, and their effects could be reversed by the $GABA_B$ receptor antagonist CGP62349. However, GHB was more potent to inhibit NMDA elicited currents, whereas baclofen was more potent to inhibit AMPA elicited currents (Li et al., 2007). These latter findings, together with previous in vivo findings (Koek et al., 2007a; Koek and France, 2008), suggest a more prominent role for NMDA receptors in the $GABA_B$ receptor-mediated effects of GHB. Thus, there is emerging evidence that the effects of GHB and baclofen, although in many respects similar, may be mediated by different $GABA_B$ systems.

There is evidence for functional $GABA_B$ receptor subtypes (Bonanno and Raiteri, 1992; Fassio et al., 1994; Lanza et al., 1993; Seabrook et al., 1990; Yamada et al., 1999).

Conceivably, differential activity of GHB and baclofen at $GABA_B$ auroreceptors and heteroreceptors could account for the differential enhancement of their effects by NMDA antagonists, with effects of GHB mediated by glutamatergic $GABA_B$ heteroreceptors and effects of baclofen by $GABA_B$ autoreceptors. Differential activity of GHB and baclofen at these receptors could also account for the differential ability of CGP35348 to antagonize their effects. Alternatively, GHB and baclofen may interact differently with the same $GABA_B$ receptor (e.g., GHB may induce conformational changes in the $GABA_B$ receptor that differ from those of induced by baclofen). Further studies examining the different $GABA_B$ mechanisms underlying effects of GHB and baclofen may help to explain why GHB is effective to treat narcolepsy and is abused, whereas there is no evidence that baclofen is effective in any sleep disorder or that it is abused.

The procedure used in the present study yielded not only quantitative measures of antagonist potency, but also information about the time course of agonist and antagonist effects. GHB and GBL had a more rapid onset and a shorter duration of action than baclofen and SKF97541 to decrease operant response rate in pigeons, in agreement with and extending previous observations in rats (Carter et al., 2004). Because baclofen and SKF97541 had long-lasting effects, pA_2 values for CGP35348 to antagonize these effects could be calculated for injection-test intervals ranging from 15 to 240 min. Changes of pA_2 values over time have been used to provide a description of the duration of action of antagonists (Gerak and France, 2007). From these pA_2 values obtained at different injection-test intervals, it appears that the antagonist potency of CGP35348 was maximal and remained unchanged from 25 to 130 min after its injection. At 240 min, the antagonist potency of CGP35348 decreased, perhaps resulting from it being eliminated. A duration of antagonist action of at least 120 min makes CGP35348 suitable for use as a

pretreatment in cumulative dosing procedures. Cumulative dosing is generally more rapid and economical than single dosing to obtain full dose-response curves. Thus, future studies with CGP35348 in pigeons will use cumulative dosing of agonists.

In summary, the $GABA_B$ receptor antagonist CGP35348 was significantly more potent to antagonize behavioral effects of the $GABA_B$ receptor agonists baclofen and SKF97541 than those of GHB and its precursor GBL. This is further evidence that the effects of GHB and prototypical $GABA_B$ agonists are mediated by $GABA_B$ receptor mechanisms that are not identical. A better understanding of these mechanisms may help to explain why GHB is effective for treating narcolepsy and is abused, whereas baclofen is not, and could lead to more effective medications with fewer adverse effects.

Acknowledgments

We thank Debbie Rodriguez, Adela Garza, Jason Persyn, and Christopher Limas for technical assistance.

References

Arunlakshana O, Schild HO (1959) Some quantitative uses of drug antagonists. *Br J Pharmacol Chemother* **14**:48-58.

Benavides J, Rumigny JF, Bourguignon JJ, Cash C, Wermuth CG, Mandel P, Vincendon G, Maitre M (1982) High affinity binding sites for gamma-hydroxybutyric acid in rat brain. *Life Sci* **30**:953-961.

Bonanno G and Raiteri M (1992) Functional evidence for multiple gamma-aminobutyric acidB receptor subtypes in the rat cerebral cortex. *J Pharmacol Exp Ther* **262**:114-118.

Carai MA, Colombo G, Brunetti G, Melis S, Serra S, Vacca G, Mastinu S, Pistuddi AM, Solinas C, Cignarella G, Minardi G, Gessa GL (2001) Role of GABA(B) receptors in the sedative/hypnotic effect of gamma-hydroxybutyric acid. *Eur J Pharmacol* **428**:315-321.

Carter LP, Chen W, Coop A, Koek W, France CP (2006) Discriminative stimulus effects of GHB and GABA(B) agonists are differentially attenuated by CGP35348. *Eur J Pharmacol* **538**:85-93.

Carter LP, Flores LR, Wu H, Chen W, Unzeitig AW, Coop A, France CP (2003) The role of GABAB receptors in the discriminative stimulus effects of gamma-hydroxybutyrate in rats: Time course and antagonism studies. *J Pharmacol Exp Ther* **305**:668-674.

Carter LP, Koek W, France CP (2009) Behavioral analyses of GHB: Receptor mechanisms. *Pharmacol Ther* **121**:100-114.

Carter LP, Wu H, Chen W, Cruz CM, Lamb RJ, Koek W, Coop A, France CP (2004) Effects of gamma-hydroxybutyrate (GHB) on schedule-controlled responding in rats: Role of GHB and GABAB receptors. *J Pharmacol Exp Ther* **308**:182-188.

Carter LP, Wu H, Chen W, Matthews MM, Mehta AK, Hernandez RJ, Thomson JA, Ticku MK, Coop A, Koek W, France CP (2005) Novel gamma-hydroxybutyric acid (GHB) analogs share some, but not all, of the behavioral effects of GHB and GABAB receptor agonists. *J Pharmacol Exp Ther* **313**:1314-1323.

Colombo G, Agabio R, Lobina C, Reali R, Gessa GL (1998) Involvement of GABA(A) and GABA(B) receptors in the mediation of discriminative stimulus effects of gamma-hydroxybutyric acid. *Physiol Behav* **64**:293-302.

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C (2004) Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nat Neurosci* **7**:153-159.

Draper N and Smith H (1998) *Applied regression analysis,* Wiley Interscience, 3rd edition, pp 47-56.

Fassio A, Bonanno G, Cavazzani P, Raiteri M (1994) Characterization of the GABA autoreceptor in human neocortex as a pharmacological subtype of the GABAB receptor. *Eur J Pharmacol* **263**:311-314.

Fuller DE and Hornfeldt CS (2003) From club drug to orphan drug: Sodium oxybate (xyrem) for the treatment of cataplexy. *Pharmacotherapy* **23**:1205-1209.

Gerak LR and France CP (2007) Time-dependent decreases in apparent pA2 values for naltrexone studied in combination with morphine in rhesus monkeys. *Psychopharmacology (Berl)* **193**:315-321.

Gonzalez A and Nutt DJ (2005) Gamma hydroxy butyrate abuse and dependency. *J Psychopharmacol* **19**:195-204.

Goodwin AK, Froestl W, Weerts EM (2005) Involvement of gamma-hydroxybutyrate (GHB) and GABA-B receptors in the acute behavioral effects of GHB in baboons. *Psychopharmacology (Berl)* **180**:342-351.

Kaupmann K, Cryan JF, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Brauner-Osborne H, Waldmeier P, Bettler B (2003) Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. *Eur J Neurosci* **18**:2722-2730.

Kenakin T (1997) *Pharmacologic analysis of drug-receptor interaction,* Lippincott-Raven, 3rd edition, pp 331-373, Philadelphia, PA.

Kerr DI, Ong J, Doolette DJ, Abbenante J, Prager RH (1993) 3-amino-2-(4-chlorophenyl)-nitropropane is a new GABAB receptor agonist, more active peripherally. *Eur J Pharmacol* **236**:239-245.

Koek W, Chen W, Mercer SL, Coop A, France CP (2006) Discriminative stimulus effects of gamma-hydroxybutyrate: Role of training dose. *J Pharmacol Exp Ther* **317**:409-417.

Koek W, Flores LR, Carter LP, Lamb RJ, Chen W, Wu H, Coop A, France CP (2004) Discriminative stimulus effects of gamma-hydroxybutyrate in pigeons: Role of diazepam-sensitive and -insensitive GABA(A) and GABA(B) receptors. *J Pharmacol Exp Ther* **308**:904-911.

Koek W and France CP (2008) Cataleptic effects of gamma-hydroxybutyrate (GHB) and baclofen in mice: Mediation by GABA(B) receptors, but differential enhancement by N-methyl-d-aspartate (NMDA) receptor antagonists. *Psychopharmacology (Berl)* **199**:191-198.

Koek W, Khanal M, France CP (2007a) Synergistic interactions between 'club drugs': Gamma-hydroxybutyrate and phencyclidine enhance each other's discriminative stimulus effects. *Behav Pharmacol* **18**:807-810.

Koek W, Mercer SL, Coop A (2007b) Cataleptic effects of gamma-hydroxybutyrate (GHB), its precursor gamma-butyrolactone (GBL), and GABAB receptor agonists in mice: Differential antagonism by the GABAB receptor antagonist CGP35348. *Psychopharmacology (Berl)* **192**:407-414.

Lanza M, Fassio A, Gemignani A, Bonanno G, Raiteri M (1993) CGP 52432: A novel potent and selective GABAB autoreceptor antagonist in rat cerebral cortex. *Eur J Pharmacol* **237**:191-195.

Li Q, Kuhn CM, Wilson WA, Lewis DV (2007) Effects of gamma hydroxybutyric acid on inhibition and excitation in rat neocortex. *Neuroscience* **150**:82-92.

Maitre M (1997) The gamma-hydroxybutyrate signalling system in brain: Organization and functional implications. *Prog Neurobiol* **51**:337-361.

Mathivet P, Bernasconi R, De Barry J, Marescaux C, Bittiger H (1997) Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABAB receptor agonist. *Eur J Pharmacol* **321**:67-75.

Olianas MC and Onali P (1999) GABA(B) receptor-mediated stimulation of adenylyl cyclase activity in membranes of rat olfactory bulb. *Br J Pharmacol* **126**:657-664.

Poldrugo F and Addolorato G (1999) The role of gamma-hydroxybutyric acid in the treatment of alcoholism: From animal to clinical studies. *Alcohol Alcohol* **34**:15-24.

Schlinger H, Poling A (1988) Evaluation of a procedure to measure the time course of a drug's behavioral action. *J Pharmacol Methods* **20**:169-174

Seabrook GR, Howson W, Lacey MG (1990) Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic GABAB receptors on neurones in rat brain slices. *Br J Pharmacol* **101**:949-957.

Sevak RJ, France CP, Koek W (2004) Neuroleptic-like effects of gamma-hydroxybutyrate: Interactions with haloperidol and dizocilpine. *Eur J Pharmacol* **483**:289-293.

Sevak RJ, Koek W, France CP (2005) Streptozotocin-induced diabetes differentially modifies haloperidol- and gamma-hydroxybutyric acid (GHB)-induced catalepsy. *Eur J Pharmacol* **517**:64-67.

Tallarida RJ (2000) *Drug synergism and dose-effect data analysis*, Chapman & Hall/CRC, pp 44-50, Boca Raton, FL.

Weerts EM, Froestl W, Kaminski BJ, Griffiths RR (2007) Attenuation of cocaine-seeking by GABA B receptor agonists baclofen and CGP44532 but not the GABA reuptake inhibitor tiagabine in baboons. *Drug Alcohol Depend* **89**:206-213.

Winter JC (1981) The stimulus properties of gamma-hydroxybutyrate. *Psychopharmacology (Berl)* **73**:372-375.

Wong CG, Gibson KM, Snead OC,3rd (2004) From the street to the brain: Neurobiology of the recreational drug gamma-hydroxybutyric acid. *Trends Pharmacol Sci* **25**:29-34.

Yamada K, Yu B, Gallagher JP (1999) Different subtypes of GABAB receptors are present at pre- and postsynaptic sites within the rat dorsolateral septal nucleus. *J Neurophysiol* **81**:2875-2883.

Footnotes

This work was supported by the National Institutes of Health National Institute on Drug Abuse [Grants DA15692, DA17918, DA19634].

Address correspondence to: Wouter Koek, Ph.D., Departments of Psychiatry and Pharmacology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, Mail Code 7792, San Antonio, Texas 78229-3900. E-mail: koek@uthscsa.edu

Legends for Figures

Figure 1. Effects of baclofen, administered i.m. immediately before the session, on fixed ratio 20 responding in a one-key food-reinforced procedure in pigeons (n=5-9). Each 12 h session consisted of ten 5-min response periods starting at different times since the beginning of the session. For each of these response periods, rate of key peck responding is plotted as a function of dose. Symbols represent mean ± S.E.M.; if not shown, S.E.M. values are contained by the symbol.

Figure 2. Effects of baclofen, SKF97541, GHB, and GBL, administered i.m. immediately before the session, on fixed ratio 20 responding in a one-key food-reinforced procedure in pigeons (n=4-9). The potency to decrease the rate of key peck responding is plotted as a function of time since the beginning of the session. Circles represent ED_{50} and 95% confidence limits. For each drug, open circles indicate ED_{50} values that differed significantly from the lowest observed value, and closed circles indicate ED_{50} values that did not. Squares represent interval estimates of ED_{50} values.

Figure 3. Attenuation by CGP35348 of response rate-decreasing effects of baclofen, SKF97541, GHB, and GBL in pigeons (N=5-6) (upper and middle panels). CGP35348 or saline was injected 10 min before each agonist, and 30 min after the agonist was injected, key peck responding was measured. For each agonist, the rate of key peck responding, expressed as a percentage of saline control, is plotted as a function of dose after pretreatment with saline (black circles) or different doses of CGP35348 (open symbols: data comprising the linear portion of the dose-response curves, and fitted with log-linear

regression lines; grey-filled symbols: data obtained with the same doses of CGP35348 as the corresponding open symbols, but not part of the linear portion of the dose-response curves, and not used in the regression calculations [see Data Analysis]). Symbols represent mean ± S.E.M.; if not shown, S.E.M. values are contained by the symbol. Lower panel: Schild regression plots for antagonism by CGP35348 of the response rate-decreasing effects of baclofen, SKF97541, GHB, and GBL. Dose-ratios are the ED50 values of SKF97541 in the presence of CGP35348 (3.2-32 mg/kg) divided by the ED50 value after pretreatment with saline. ED_{50} values were calculated from the regression lines shown in the upper and middle panels. Data obtained with each agonist could be fitted with a regression line with a slope of -1. Calculated from these regression lines, the pA_2 value of CGP35348 ranged from 3.91 (95% confidence limits: 2.98-4.85) for GBL to 4.63 (4.17-5.09) for SKF97541.

Figure 4. Time-dependent attenuation by CGP35348 of the response rate-decreasing effects of SKF97541 in pigeons (N=5-6) (left panels, and upper and middle right panels). CGP35348 or saline was injected 10 min before SKF97541, and key peck responding was measured at different times after the agonist was injected (ranging from 15 to 240 min). For each time interval, the rate of key peck responding, expressed as a percentage of saline control, is plotted as a function of dose after pretreatment with saline (closed circles) or different doses of CGP35348 (open symbols: data comprising the linear portion of the dose-response curves, and fitted with log-linear regression lines; grey-filled symbols: data obtained with the same doses of CGP35348 as the corresponding open symbols, but not part of the linear portion of the dose-response curves, and not used in the regression calculations [see Data Analysis]). Symbols represent mean ± S.E.M.; if not

shown, S.E.M. values are contained by the symbol. Lower right panel: Schild regression plots for the ability of CGP35348 to antagonize, at different times after its administration (in min), the response rate-decreasing effects of SKF97541. Dose-ratios are the ED_{50} values of each agonist in the presence of CGP35348 (3.2-32 mg/kg) divided by the ED_{50} value of the agonist after pretreatment with saline. Data obtained at each time interval could be fitted with a regression line with a slope of -1. Calculated from these regression lines, the pA_2 value of CGP35348 to antagonize SKF97541 at the different intervals ranged from 4.18 (95% confidence limits: 3.92-4.43) at 240 min after SKF97541 to 4.63 (4.17-5.09) at 30 min after SKF97541 (i.e., 250 and 40 min after CGP35348, respectively).

Figure 5. Antagonism by CGP35348 of the rate-deceasing effects of different agonists at different times after their administration. PA_2 values are plotted for each agonist at the times it had rate decreasing effects. CGP35348 was about four-fold less potent to antagonize the effects of GHB and GBL than to antagonize the effects of baclofen and SKF97541. CGP35348 was about 2-fold less potent to antagonize SKF97541 at 240 min after its administration than at shorter intervals. **a**: $P<0.05$ compared with baclofen and SKF; **b**: $P<0.05$ and <0.10 compared with SKF97541 and baclofen, respectively; **c**: $P<0.05$ compared with SKF97541 at all shorter intervals.

TABLE 1. ED_{50} values (in mg/kg) for effects of agonists on food-reinforced responding in pigeons at different injection-test intervals and pA_2 values of CGP35348 [in -log (mol/kg)] to antagonize these effects.

		CGP35348 (mg/kg)				
agonist	time (min)	0	3.2	10	32	pA2
baclofen	30	9.4 (7.4-12)	14 (10-nd)	22 (16-30)	33 (24-47)	4.46 (4.10-4.82)
	60	8.6 (7.1-10)	15 (12-nd)	18 (14-22)	31 (25-40)	4.49 (3.93-5.05)
	120	9.1 (7.9-10)	14 (12-17)	19 (16-22)	34 (28-40)	4.46 (4.07-4.85)
	240	12 (9.6-15)	17 (13-nd)	22 (17-28)	36 (27-48)	4.34 (3.95-4.73)
SKF97541	15	0.077 (0.047-0.14)	0.12 (0.068-nd)	0.19 (nd)	0.42 (0.20-nd)	4.46 (4.11-4.81)

	30	0.046 (0.034-0.063)	0.079 (0.057-0.11)	0.15 (nd)	0.20 (0.14-0.30)	4.63 (4.17-5.09)
	60	0.046 (0.036-0.059)	0.078 (0.061-0.098)	0.11 (0.085-0.15)	0.18 (0.14-0.24)	4.54 (4.09-4.98)
	120	0.051 (0.041-0.064)	0.079 (0.064-0.098)	0.11 (0.085-0.14)	0.22 (nd-30)	4.52 (4.21-4.82)
	240	0.091 (0.067-0.13)	0.10 (0.074-0.16)	0.15 (0.10-nd)	0.30 (0.18-nd)	4.18 (3.92-4.43)
GHB	15	150 (120-190)	200 (160-250)	250 (190-nd)	250 (190-nd)	4.14 (3.26-5.02)
	30	150 (130-180)	170 (140-200)	240 (210-270)	260 (220-nd)	3.97 (3.47-4.47)
	60	160 (130-200)	180 (150-210)	280 (230-nd)	340 (280-400)	3.96 (2.38-5.54)
GBL	30	79 (67-93)	100 (82-130)	94 (76-120)	130 (nd-170)	3.91 (2.98-4.85)
	60	100 (81-130)	110 (86-140)	140 (130-150)	150 (120-nd)	3.72 (3.24-4.19)

Numbers between parentheses: 95% confidence limits; nd, not determined, because the confidence limit could not be calculated.

Figure 1

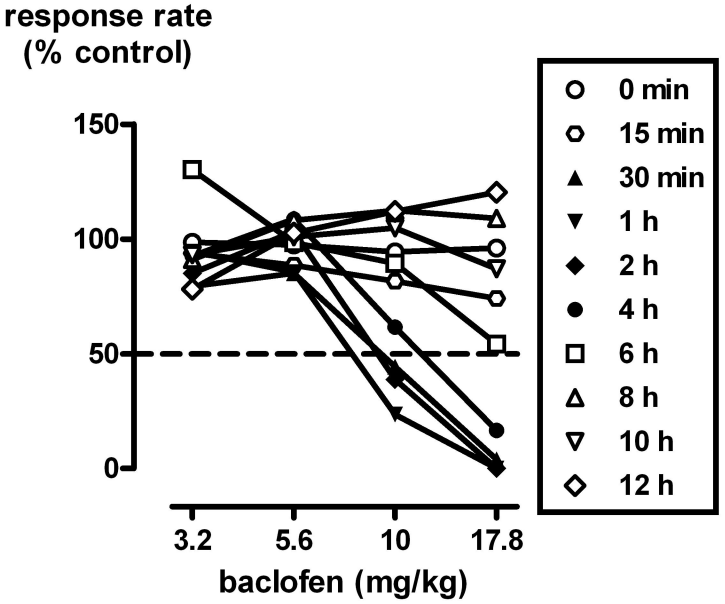

response rate
(% control)
150
100
50
0
3.2
5.6
10
17.8
baclofen (mg/kg)
0 min
15 min
30 min
1 h
2 h
4 h
6 h
8 h
10 h
12 h

Figure 2

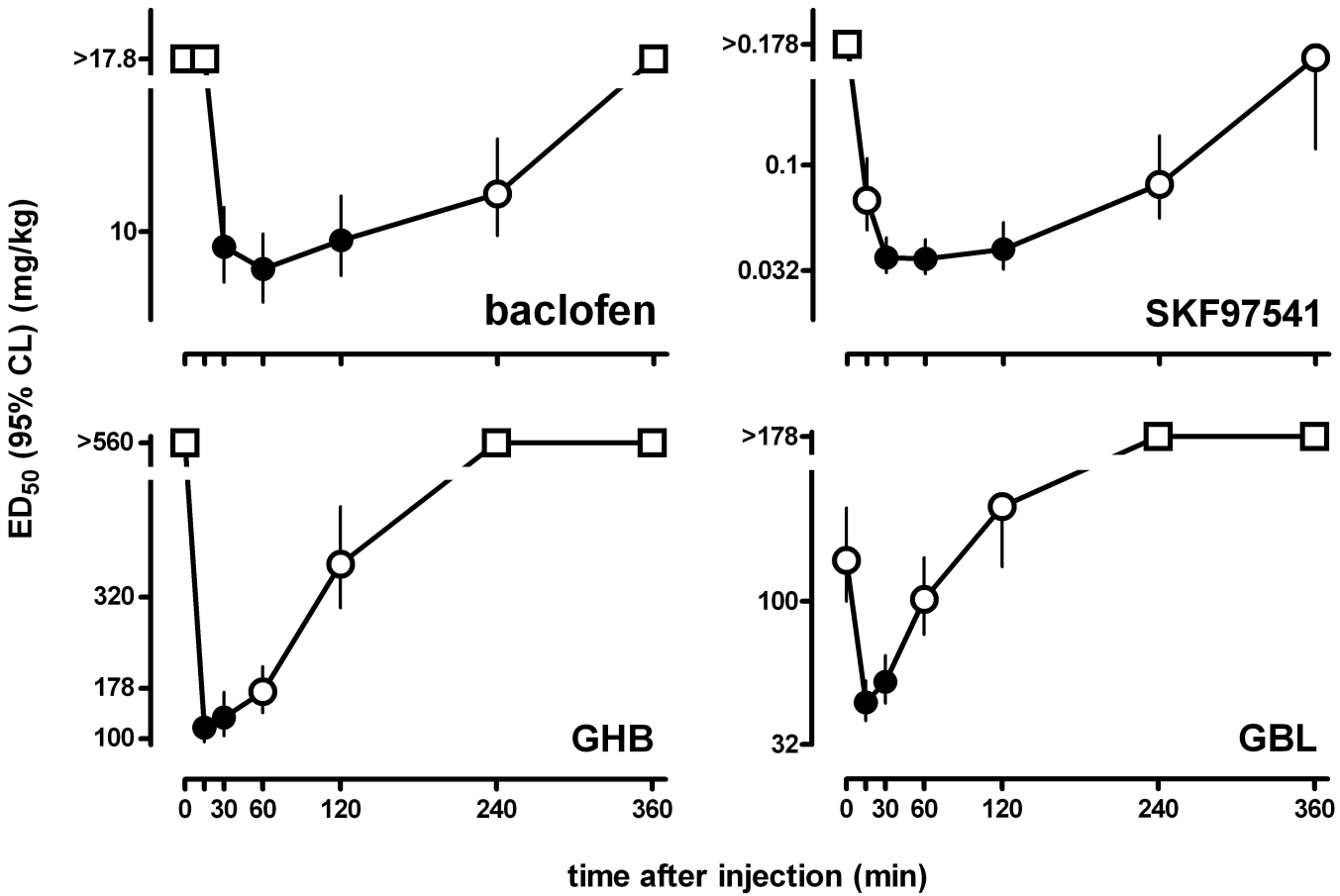

>17.8
10
baclofen
>0.178
0.1
0.032
SKF97541
>560
320
178
100
GHB
>178
100
32
GBL
0 30 60 120 240 360
0 30 60 120 240 360
ED50 (95% CL) (mg/kg)
time after injection (min)

Figure 3

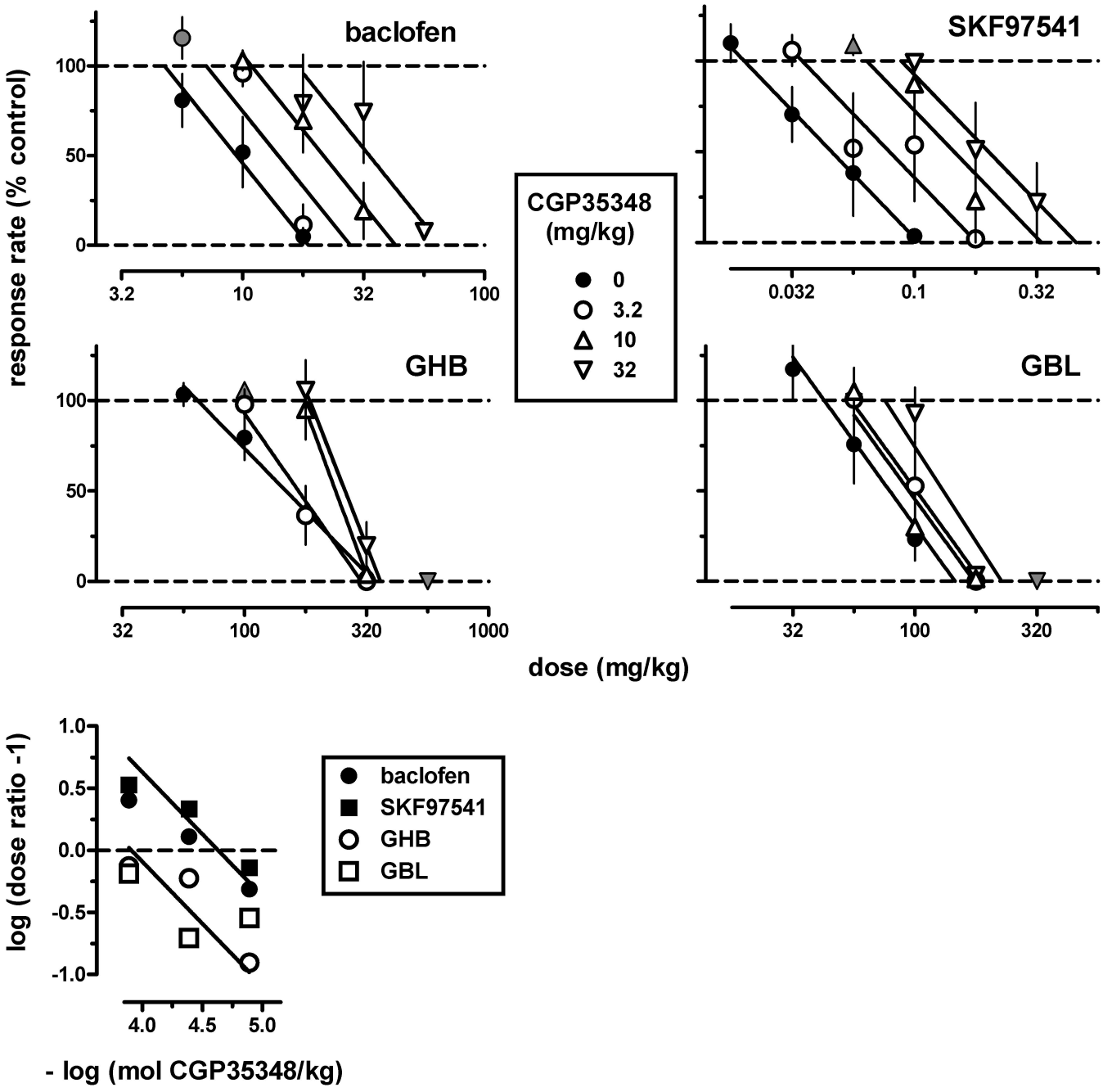

baclofen
SKF97541
GHB
GBL
response rate (% control)
100
50
0
3.2
10
32
100
0.032
0.1
0.32
320
1000
CGP35348
(mg/kg)
0
3.2
10
32
dose (mg/kg)
log (dose ratio -1)
1.0
0.5
0.0
-0.5
-1.0
4.0
4.5
5.0
- log (mol CGP35348/kg)

Figure 4

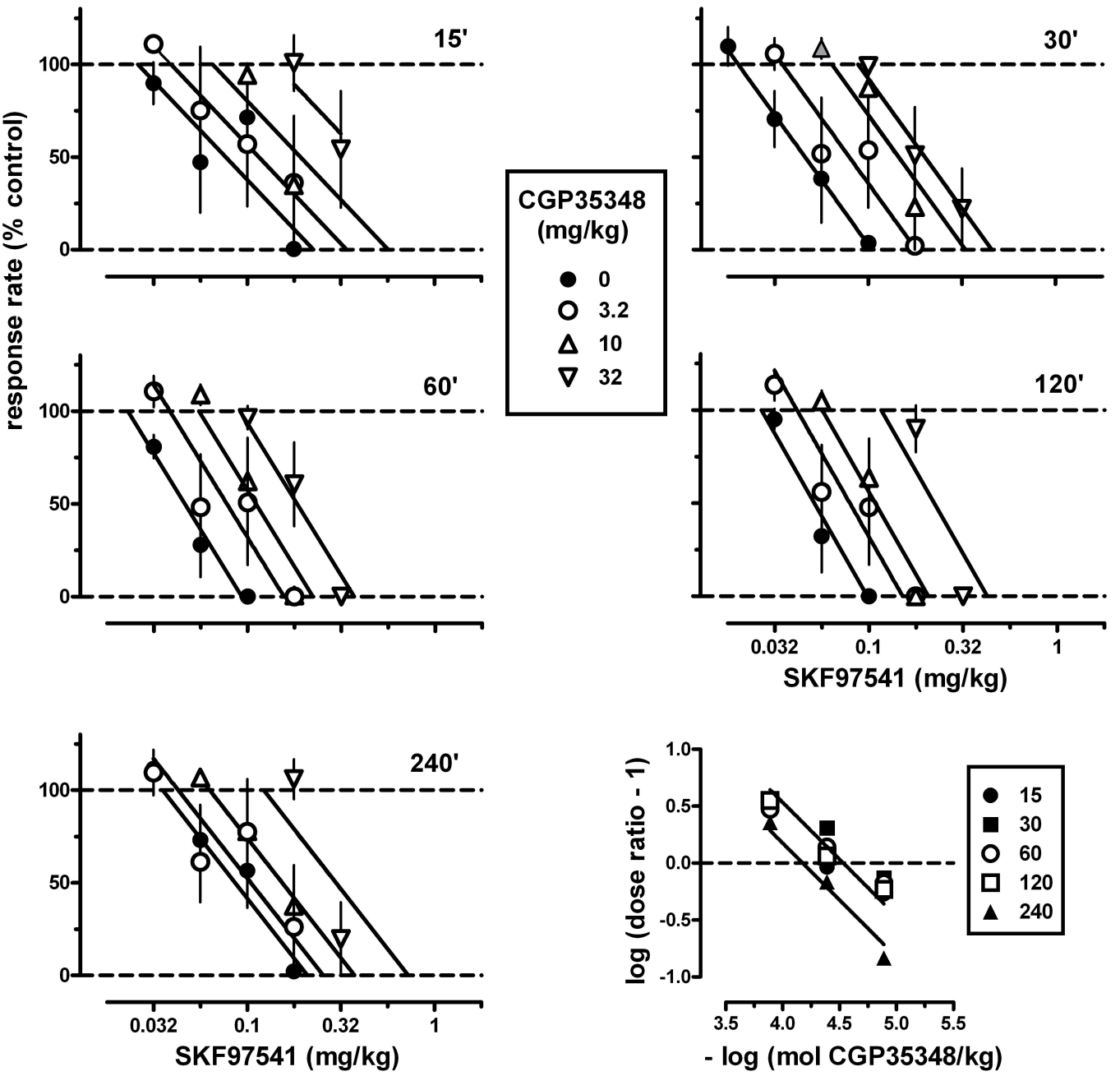
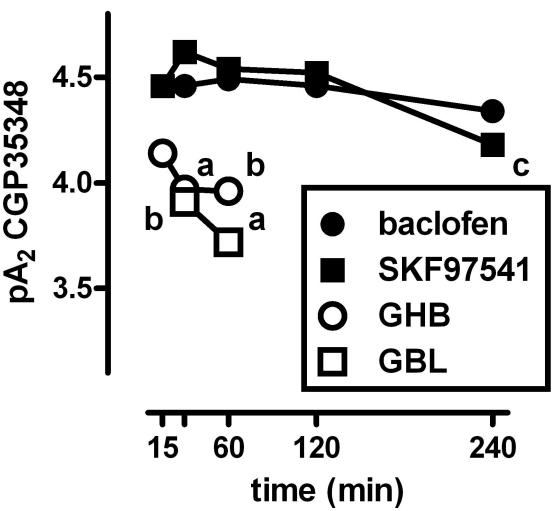

15'
30'
60'
120'
240'
response rate (% control)
100
50
0
CGP35348
(mg/kg)
0
3.2
10
32
0.032
0.1
0.32
1
SKF97541 (mg/kg)
log (dose ratio - 1)
1.0
0.5
0.0
-0.5
-1.0
15
30
60
120
240
3.5
4.0
4.5
5.0
5.5
- log (mol CGP35348/kg)

Figure 5


pA2 CGP35348
4.5
4.0
3.5
a
b
b
a
c
baclofen
SKF97541
GHB
GBL
15
60
120
240
time (min)