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Comparing the discriminative stimulus effects of modulators of GABA_A receptors containing α_4 - δ subunits with those of gaboxadol in rats

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Abstract

Rationale—Gaboxadol is a selective agonist at GABA_A receptors that contain α_4 - δ subunits, and it produces anxiolytic and sedative effects. Although adverse effects preclude its clinical use, its mechanism of action suggests that those receptors might provide novel therapeutic targets, particularly for modulators of those GABA_A-receptor subtypes, by retaining therapeutic effects of gaboxadol and not adverse effects.

Objectives—The current study compared discriminative stimulus effects of gaboxadol with those of modulators acting at GABA_A receptors containing α_4 - δ subunits.

Materials—Eight rats discriminated 5.6 mg/kg gaboxadol from vehicle while responding under a fixed-ratio 10 schedule for food. Modulators acting at GABA_A receptors containing α_4 - δ subunits (pregnanolone, ethanol, flumazenil) and receptors that do not contain those subunits (midazolam) were studied alone; pregnanolone and ethanol were also combined with gaboxadol. In addition, gaboxadol was studied in separate groups discriminating 0.32 mg/kg midazolam, 3.2 mg/kg pregnanolone, or 0.75 g/kg ethanol from vehicle.

Results—Gaboxadol produced 80% gaboxadol-lever responding and did not alter rates. No other drug produced, on average, 80% drug-lever responding up to doses that decreased rates, although 1.78 mg/kg midazolam produced 32% gaboxadol-lever responding. Ethanol and pregnanolone did not enhance the effects of gaboxadol. Rats discriminating midazolam, pregnanolone or ethanol from vehicle responded predominantly on the vehicle lever after receiving gaboxadol.

Conclusions—Drugs that modulate GABA_A receptors containing α_4 - δ subunits neither mimicked nor enhanced the discriminative stimulus effects of gaboxadol, indicating that at least some effects of gaboxadol are not shared with modulators of that GABA_A-receptor subtype.

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Keywords

gaboxadol; ethanol; neuroactive steroids; GABA_A receptors; drug discrimination; rats; flumazenil

Gaboxadol is a γ -aminobutyric acid_A (GABA_A) receptor agonist, and like other drugs acting at GABA_A receptors (e.g., benzodiazepines), it has been investigated for its therapeutic potential. Gaboxadol relieves anxiety, decreases sleep onset, and increases sleep duration (Deacon et al. 2007; Hajak et al. 2009; Lankford et al. 2008; Walsh et al. 2008); however, its development as a treatment for anxiety and insomnia was discontinued due to adverse effects and inconsistent therapeutic effectiveness over long treatment periods (Hoehn-Saric 1983; Roth et al. 2010; Walsh et al. 2007). Although gaboxadol does not appear to be a useful therapeutic, interest persists because of its selectivity for GABA_A receptors containing α_4 and δ subunits; these subtypes of GABA_A receptors are located extrasynaptically and regulate neuronal excitability through GABA_A-induced tonic inhibitory currents (Belelli et al. 2005; Brown et al. 2002). Based on the pharmacological effects of gaboxadol and behavioral characterization of rodents lacking δ subunits, these receptors subtypes have been implicated in an array of behavioral functions, suggesting that these receptors might play a role in disorders such as anxiety, insomnia, depression and alcohol abuse (Whissell et al. 2015). Changes in the expression of the δ subunit have also been reported in humans suffering from those pathological conditions (Jin et al. 2012; Klempan et al. 2007; Merali et al. 2004). Thus, gaboxadol continues to be used to investigate these receptors as a potential target for new medications.

Targeting GABA_A receptors containing α_4 - δ subunits to treat anxiety, insomnia, depression, and alcohol abuse will only be helpful if the therapeutic effects of gaboxadol are retained while reducing adverse effects; that objective might be accomplished with modulators of these receptors, which would be expected to produce some but not all of the effects of an agonist like gaboxadol. Unfortunately, modulators that bind exclusively to these particular GABA_A receptor subtypes have not been identified, although there are several drugs that modulate GABA_A receptors containing α_4 - δ subunits in addition to other GABA_A receptor subtypes, including flumazenil and neuroactive steroids like pregnanolone (Brown et al. 2002; Knoflach et al. 1996). In addition, receptors containing α_4 - δ subunits have been shown to be particularly sensitive to ethanol *in vitro* (Hancher et al. 2006; Mitchell et al. 2008; Wallner et al. 2006; Wallner et al. 2003; but see also Borghese et al. 2006), suggesting that smaller doses might be needed *in vivo* to activate these receptors, as compared with doses needed for other GABA_A and non-GABA_A receptors.

In the absence of modulators selective for GABA_A receptors containing α_4 - δ subunits, determining the role of these receptors in the behavioral effects of neuroactive steroids and ethanol is difficult using only pharmacological approaches; however, by using other approaches, such as genetically engineered mice, these receptor subtypes have been shown to contribute to the behavioral effects of these drugs. For example, the effects of neuroactive steroids on locomotion, sleep duration, anxiety-like behavior, and pentylenetetrazol-induced convulsions were diminished in animals lacking δ subunits (Mihalek et al. 1999; Vashchinkina et al. 2014). Similarly, several effects of ethanol were reduced in δ -subunit

knockout mice, including decreased ethanol intake, attenuated withdrawal when ethanol treatment was discontinued, and reduced anticonvulsant effects of ethanol (Mihalek et al. 2001). Not all effects of ethanol or neuroactive steroids are changed when δ subunits are absent; in knockout mice discriminating ethanol, the potencies of ethanol, the benzodiazepine midazolam, the barbiturate pentobarbital, and several neuroactive steroids were unchanged, as compared with wild-type counterparts (Mihalek et al. 2001). Although these studies suggest a role of these receptor subtypes in at least some behavioral effects of neuroactive steroids and ethanol, interpretation of these results is complicated by the possibility that receptor expression is changed during development to compensate for the reduction in δ subunits (Korpi et al. 2002; Peng et al. 2002).

Because of its high pharmacological selectivity (e.g., Lelas et al. 2000), drug discrimination assays might be useful in unraveling *in vivo* differences between drug action at GABA_A receptors containing α_4 - δ subunits and drug action at other receptor subtypes. Previous studies in rats established gaboxadol as a discriminative stimulus and drugs acting at benzodiazepine sites did not produce gaboxadol-lever responding, suggesting that the mechanism of gaboxadol differs from that of benzodiazepines (McDonald et al. 2007; Michelsen et al. 2007). Because benzodiazepines act at modulatory sites on some GABA_A receptors, but not at those containing α_4 - δ subunits (Olsen and Sieghart 2008), one possible explanation is that the discriminative stimulus effects of gaboxadol are mediated by GABA_A receptors containing α_4 - δ subunits, which would not be detected by drugs acting at benzodiazepine sites; however, other drugs that are known to act at this receptor subtype were not studied under those conditions and the role of those receptors remains unknown.

In the current study, gaboxadol was first established as a discriminative stimulus, and then midazolam, pregnanolone, ethanol and flumazenil were studied alone to determine whether any of GABA_A receptor modulators would produce gaboxadol-lever responding. These drugs are modulators of multiple subtypes of GABA_A receptors; however, gaboxadol is selective for one subtype of GABA_A receptors, and pregnanolone, ethanol and flumazenil, but not midazolam, modulate that receptor subtype. Consequently, midazolam will not produce gaboxadol-lever responding, although the other drugs might. Because modulators might not share discriminative stimulus effects with drugs acting directly at the orthosteric binding site (Ator and Griffiths 1986; Grech and Balster 1994; Jones and Balster 1998; McDonald et al. 2007; Michelsen et al. 2007; McMahon and France 2005; Nielsen et al. 1991), their actions at receptors containing α_4 - δ subunits were tested further by administering the test compound immediately before determining a gaboxadol dose-effect curve; to the extent that the modulators are acting at GABA_A receptors containing α_4 - δ subunits, they would be expected to enhance the potency of gaboxadol, shifting its dose-effect curve leftward. The effects of gaboxadol were further compared to those of GABA_A receptor modulators by studying gaboxadol in three separate groups of rats that discriminated midazolam, pregnanolone or ethanol from vehicle.

Materials and methods

Subjects

Male Sprague-Dawley rats were housed individually and maintained under a 12 hr light/dark cycle; experiments were conducted during the light cycle. Water was available ad libitum. Rats received grain-based food pellets (Bio Serv, Inc, Frenchtown, NJ) during experimental sessions and rodent chow (Harlan Teklad, Madison, WI) in the home cage with the amount of chow varying daily to maintain weights between 320 and 330 g. Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee at The University of Texas Health Science Center at San Antonio, and the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council [Department of Health, Education and Welfare, publication No. (NIH) 85-23, revised 2011].

Apparatus

During experimental sessions, each rat was placed in a chamber that contained a houselight, two response levers, two stimulus lights, a pellet trough, a pellet dispenser and a fan for ventilation (MED Associates, Inc., St. Albans, VT). Chambers were located within sound-attenuating cubicles, and white noise was present to mask extraneous noise. Experimental events were controlled and data were collected by a computer which was connected to the chambers by an interface and used Med-PC software (MedAssociates, Inc., St. Albans, VT).

Procedure

One group of 8 rats discriminated 5.6 mg/kg gaboxadol while responding under a fixed-ratio 10 schedule of food presentation. This dose has been established as a discriminative stimulus in other studies (Michelsen et al. 2007), although it produces only modest drug-lever responding in rats discriminating the nonselective GABA_A receptor agonist muscimol (Jones and Balster 1998) or the GABA reuptake inhibitor tiagabine, perhaps suggesting that this dose is selective for a subtype of GABA_A receptor. Initially, saline or 5.6 mg/kg gaboxadol was given immediately before sessions that began with a 15-min timeout and ended with a 15-min response period. During the timeout, chambers were dark and responses had no programmed consequence; during the response period, stimulus lights located above levers were illuminated and 10 consecutive responses on the lever designated correct by the injection given before the session resulted in the delivery of a food pellet. The lever designated correct after administration of gaboxadol was counterbalanced across rats, and responses on the incorrect lever reset the response requirement on the correct lever. Lights were extinguished and response periods ended after 15 min or the delivery of 50 pellets, whichever occurred first. Training under this procedure continued until the following criteria were satisfied for 5 consecutive or for 6 of 7 training sessions: at least 80% of the total responses were emitted on the correct lever and fewer than 10 responses were emitted on the incorrect lever prior to delivery of the first food pellet.

Thereafter, the procedure was changed from single cycles to multiple cycles. Each of the 2-6 cycles in a session began with a 15-min timeout. The response period was shortened to 5 min with a maximum of 10 food pellets available during each cycle of the multiple-cycle

procedure. Injections were given during the first min of each cycle. For some training sessions, 5.6 mg/kg gaboxadol was administered on first cycle, followed by one cycle during which rats received a sham injection; responding on the drug lever resulted in delivery of food during both cycles. For other training sessions, saline or sham was administered for up to 4 cycles before drug was administered and responding on the vehicle lever resulted in delivery of food for cycles that preceded the drug cycle. For still other training sessions, saline or sham was administered for 2-6 cycles. Once the criteria described above were satisfied during all cycles for 5 consecutive or for 6 of 7 sessions, testing began with test sessions conducted every third day as long as the testing criteria were satisfied during the intervening training sessions; otherwise, additional training sessions were conducted until the testing criteria were satisfied for 2 of 3 training sessions. Test sessions were identical to training sessions except that 10 consecutive responses on either lever resulted in the delivery of food during all cycles, regardless of the injection that was administered.

Dose-effect curves were determined using a cumulative dosing procedure. Vehicle was administered during the first cycle with increasing doses of the test compound administered during subsequent cycles. The cumulative dose of the test compound was increased by 0.25 or 0.5 log units per cycle up to a dose that produced 80% responding on the drug lever or decreased rates to <20% of control. In addition to gaboxadol, these studies were conducted with drugs that act at modulatory sites on GABA_A receptors and vary in their actions at GABA_A receptors containing α_4 - δ subunits. One of these drugs, the benzodiazepine midazolam, does not act at these receptor subtypes. Flumazenil acts at benzodiazepine sites on GABA_A receptors, although it also has actions GABA_A receptors containing α_4 - δ subunits, which do not have benzodiazepine binding sites. Ethanol and the neuroactive steroid pregnanolone act at sites other than the benzodiazepine site on GABA_A receptors, including those containing α_4 - δ subunits. In addition, drug combination studies were used to determine whether GABA_A modulators with actions at GABA_A receptors containing α_4 - δ subunits modify the discriminative stimulus effects of gaboxadol. For these studies, an injection of the test compound (ethanol or pregnanolone) replaced the vehicle injection on the first cycle of a test session followed by increasing doses of gaboxadol.

Similarities between gaboxadol and GABA_A receptor modulators were further compared by examining the ability of gaboxadol to produce drug-lever responding in 3 separate groups of rats that discriminated 0.32 mg/kg midazolam (N=5), 3.2 mg/kg pregnanolone (N=5), or 0.75 g/kg ethanol (N=8) from vehicle. These training doses of midazolam and pregnanolone have been used extensively and are pharmacologically equivalent doses (Bai and Gerak 2011; Eppolito et al. 2014). This training dose of ethanol was used because it is smaller than those typically used to study its effects at GABA_A and nonGABAergic receptors (Grant and Colombo 1993), and small doses have been shown to selectively increase currents that are mediated by receptors containing α_4 - δ subunits (Wallner et al. 2003). Training and test sessions were identical to those used in rats discriminating gaboxadol except that cycles were shorter due to the faster onset of these 3 training drugs. During initial training under the single-cycle procedure, a 10-min timeout period was followed by a 20-min cycle. When sessions were changed to multiple cycles, each 15-min cycle began with a 10-min timeout period and ended with a 5-min response period, and the maximum number of cycles was 8. These rats previously satisfied the criteria described above for 5 consecutive or for 6 of 7

training sessions; thereafter, test sessions were separated by at least 3 days and were conducted when the criteria were satisfied during 2 of the 3 training sessions that preceded the test session. Dose-effect curves were determined for gaboxadol, midazolam and pregnanolone in each group with an ethanol dose-effect curve also obtained in rats discriminating ethanol. Under these conditions, gaboxadol was given every other cycle because of its slow onset of action, resulting in a 30-min interval between drug injections; preliminary data in rats responding on a single lever under a fixed-ratio10 schedule of food presentation indicated that the onset of action of the rate-decreasing effects of gaboxadol is >10 min. Vehicle was administered on the first cycle with a sham injection given on the 2nd cycle. Gaboxadol was then administered during the third, fifth and seventh cycle with the cumulative dose increasing in 0.25 log unit increments; sham injections were given during the intervening cycles.

Drugs

Gaboxadol hydrochloride (Sigma-Aldrich Co., St. Louis, MO) was dissolved in sterile 0.9% saline. Pregnanolone (5 β -pregnan-3 α -ol-20-one; National Institute on Drug Abuse Drug Supply Program, Bethesda, MD) was dissolved in 45% (w/v) 2-hydroxypropyl- β -cyclodextrin. Midazolam hydrochloride (Bedford Laboratories, Bedford, OH) and ethanol were purchased as commercially prepared solutions and diluted with sterile 0.9% saline. Flumazenil (Enzo Life Sciences, Farmingdale, NY) was dissolved in a vehicle comprising 10% ethanol, 40% propylene glycol, and 50% sterile water. Drugs were administered i.p. typically in a volume of 1 ml/kg body weight with the exception of ethanol for which a 15% v/v solution was used for all injections. Doses are expressed in the form listed above in mg/kg or g/kg body weight.

Data analyses

The number of training sessions required to satisfy the testing criteria were compared across groups using one-way analysis of variance followed by Tukey's multiple comparisons test. Control response rates were determined for each group by calculating the average rates obtained during 10 training sessions in which rats did not receive drug and they satisfied the testing criteria. Rates were first averaged across cycles within sessions for an individual rat and then averaged across sessions (mean $\pm$ 1 SEM). Group mean response rates were calculated by averaging across rats and were compared between groups using a one-way analysis of variance. The percentage of responses on the drug-appropriate lever and response rates were plotted as a function of dose. Discrimination data for an individual rat were excluded from the figure and from further analyses when response rate was less than 20% of control, and data for the group were not plotted when at least half of the rats were not included because of decreased rates.

Gaboxadol dose-effect curves obtained in the presence or absence of a second drug were compared by simultaneously fitting straight lines to the linear portion of dose-effect curves using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). The linear portion included one data point below 25%, one data point above 75% and all data points in between. Slopes of those lines were compared using an F-ratio test; significant differences in slopes indicated that the test compound altered the gaboxadol dose-effect

curve and that the data were best fit by a model in which slope was allowed to vary. When slopes were not different, a simpler model with a common slope was used. Gaboxadol dose-effect curves obtained in the presence or absence of the second drug were further compared by determining whether the data were best fit by a common intercept. Regression lines were used to calculate doses that produced 50% responding on the drug lever ($ED_{50} \pm 1$ SEM). Significance was set at $p < 0.05$.

Results

Under the single-cycle procedure, the mean (range) number of training sessions needed to establish stimulus control was 34.4 (21-52), 50.2 (14-86), 22.4 (13-31) and 24.1 (18-32) in rats discriminating gaboxadol, pregnanolone, midazolam, and ethanol, respectively. Although there was a significant difference across groups ($F_{3,22}=3.62$, $p=0.03$) and between rats discriminating pregnanolone and those discriminating either midazolam or ethanol, the number of sessions needed in rats discriminating gaboxadol was not different from that of any other group. After the procedure was changed to multiple cycles, the mean (range) number of additional sessions needed to reestablish stimulus control was 26.1 (7-72), 32.6 (21-48), 30.8 (8-52), and 12.6 (5-24) in rats discriminating gaboxadol, pregnanolone, midazolam, and ethanol, respectively, with no significant difference across groups. Although 8 rats discriminating gaboxadol satisfied the initial testing criteria under both the single- and multiple-cycle procedures, stimulus control deteriorated over time in 3 rats and they did not complete the study. Under the multiple-cycle procedure, control response rates (mean $\pm$ 1 SEM) tended to be higher in rats discriminating gaboxadol (0.91 ± 0.08 responses/sec), pregnanolone (0.95 ± 0.09 responses/sec) or ethanol (0.96 ± 0.06 responses/sec), as compared with those in rats discriminating midazolam (0.69 ± 0.07 responses/sec), although rates were not significantly different across groups.

In rats discriminating gaboxadol, the training dose of 5.6 mg/kg produced, on average, 80% drug-lever responding (circles, top panel, Fig 1). In each of the 8 rats, doses of gaboxadol that produced drug-lever responding did not decrease response rates (circles, bottom panel, Fig 1). None of the other drugs produced, on average, 80% gaboxadol-lever responding (top panel, Fig 1) up to doses that markedly decreased rates (bottom panel, Fig 1); however, one rat responded exclusively on the gaboxadol lever after receiving midazolam and pregnanolone while a second subject emitted 90% of the total responses on the gaboxadol lever after receiving midazolam, resulting in an overall average of 32.0% and 14.3% gaboxadol-lever responding at 1.78 mg/kg midazolam and 3.2 mg/kg pregnanolone, respectively. In contrast, those two subjects, along with the rest of the rats tested, responded almost exclusively on the saline lever following administration of flumazenil and ethanol (inverted triangles and diamonds, Fig 1).

Two drugs with actions at GABA_A receptors containing α_4 - δ subunits (ethanol and pregnanolone) were studied in combination with gaboxadol in rats discriminating gaboxadol. When studied alone, the ED_{50} of gaboxadol ($\pm$ SEM) value was 3.1 ± 1 mg/kg. When 0.42 or 0.56 g/kg ethanol was administered on the first cycle, the potency of gaboxadol decreased slightly with ED_{50} values of 5.4 ± 2.7 and 4.6 ± 2.7 mg/kg, respectively (top left panel, Fig 2). Comparison of fits indicated no significant difference in

slope or intercept of gaboxadol dose-effect curves determined in the presence or absence of ethanol. A dose of 0.75 g/kg ethanol decreased response rates to 0.47 responses/sec and rates were decreased further by gaboxadol (bottom left panel, Fig 2). Similarly, pregnanolone did not significantly change the discriminative stimulus effects of gaboxadol (top right panel, Fig 2) as evidenced by ED₅₀ values for gaboxadol that were similar in rats that received saline (ED₅₀= 4.3 ± 1.3 mg/kg), 3.2 mg/kg pregnanolone (ED₅₀=5.4 ± 3.2 mg/kg), and 5.6 mg/kg pregnanolone (ED₅₀=4.2 ± 1.8 mg/kg); there was no significant difference in slopes or intercepts among these 3 gaboxadol dose-effect curves, indicating that they were best fit by a line with a common slope and intercept. Response rates were decreased to 0.51 responses/sec by 10 mg/kg pregnanolone and further decreased by gaboxadol (bottom right panel, Fig 2).

In rats discriminating either midazolam or pregnanolone from vehicle, these two GABA_A receptor modulators occasioned 80% drug-lever responding in both groups at doses that did not significantly change response rates (top left and middle panels, Fig 3). In rats discriminating ethanol, the training drug increased drug-lever responding with 80% of the responses on the drug lever occurring after administration of the dose 1.33 g/kg, although pregnanolone and midazolam (squares, left and middle panels, Fig 3) produced 80% drug-lever responding in only 3 and 4 rats, respectively. When averaged across the 8 rats discriminating ethanol, maximal drug-lever responding was 59% for pregnanolone and 65% for midazolam. Up to doses that markedly decreased responding, gaboxadol did not produce more than 2% drug-lever responding in rats discriminating either pregnanolone or midazolam; 1 of 8 animals discriminating ethanol emitted 80% of responses on the drug-lever after 5.6 mg/kg gaboxadol, resulting on a group average of 17% (right panels, Fig 3).

Discussion

Gaboxadol acts selectively at GABA_A receptors containing α_4 - δ subunits and can produce anxiolytic and sedative effects. Although adverse effects have precluded the clinical use of this particular drug, interest in these GABA_A receptor subtypes as therapeutic targets remains high because of their emerging role in some pathological conditions (Brickley and Mody 2012). Other drugs that have mechanisms of action similar to that of gaboxadol would be expected to produce the same profile of effects and would not be useful therapeutically; however, drugs that modulate the receptor subtypes, rather than act as an orthosteric agonist, would produce only some of the effects of gaboxadol, which might provide a clinical advantage. The current study compared the effects of gaboxadol to those of modulators of GABA_A receptors containing α_4 - δ subunits using drug discrimination.

As shown in previous studies, gaboxadol can be established as a discriminative stimulus (McDonald et al. 2007; Michelsen et al. 2007). The earlier studies compared the discriminative stimulus effects of gaboxadol with those of drugs that have been used clinically for insomnia or shown to have hypnotic effects, including a variety of drugs with actions at benzodiazepine sites on GABA_A receptors; none of those drugs produced gaboxadol-lever responding. While those studies clearly demonstrated differences in the discriminative stimulus effects of gaboxadol and drugs with hypnotic effects, they did not specifically explore the role of GABA_A receptors containing α_4 - δ subunits. Results obtained

with other drugs provide a hint that the discriminative stimulus effects of gaboxadol are selective for these receptor subtypes. For example, tiagabine is an inhibitor of GABA reuptake (GAT-1) and therefore indirectly enhances GABA_A receptor function; in rats discriminating gaboxadol, tiagabine modestly increases drug-lever responding and in rats discriminating tiagabine, gaboxadol produces ~80% drug-lever responding only at doses that markedly disrupt response rates (McDonald et al. 2007; 2008). Similarly, in rats discriminating the nonselective GABA_A receptor agonist muscimol, gaboxadol produces substantial drug-lever responding only at doses that also decrease rates (Jones and Balster 1998). These results are consistent with the hypothesis that the discriminative stimulus effects of gaboxadol are mediated by a subset of GABA_A receptors whereas discriminative stimulus effects tiagabine and muscimol are mediated by multiple GABA_A receptors subtypes, including those containing α_4 - δ subunits.

Given that adverse effects decrease the utility of gaboxadol as a therapeutic and actions at modulatory sites might result in fewer adverse effects, the current study focused on drugs acting at modulatory sites. Because modulators selective for GABA_A receptors containing α_4 - δ subunits are not readily available, ethanol, pregnanolone and flumazenil were examined in rats discriminating gaboxadol; these drugs have been shown to act at modulatory sites on multiple GABA_A receptors subtypes including those containing α_4 - δ subunits (Mitchell et al. 2008; Olsen et al. 2007; Olsen and Sieghart 2009). None of the GABA_A receptor modulators produced gaboxadol-lever responding and neither ethanol nor pregnanolone enhanced the discriminative stimulus effects of gaboxadol. Collectively, these studies have demonstrated that a variety of modulators of GABA_A receptors with actions at receptors containing α_4 - δ subunits do not share discriminative stimulus effects with gaboxadol.

Drug discrimination procedures are often useful in determining mechanism of action *in vivo*, and they have been used widely to examine the actions of drugs at GABA_A receptors. For example, benzodiazepines, neuroactive steroids, and ethanol have been established as discriminative stimuli, and benzodiazepines produce drug-lever responding in subjects discriminating each of those positive GABA_A receptor modulators. Because benzodiazepines do not act at GABA_A receptors containing α_4 - δ subunits, these results indicate that other GABA_A receptor subtypes are an important component of the discriminative stimulus effects of these positive GABA_A receptor modulators (e.g., Bai and Gerak 2011; De Vry and Slangen 1986). Gaboxadol does not produce drug-lever responding in rats (current study) or monkeys (McMahon and France 2005) discriminating midazolam, indicating that the mechanism of action of gaboxadol is different from that of benzodiazepines. One possible explanation for this difference is that the discriminative stimulus effects of gaboxadol are mediated by GABA_A receptors containing α_4 - δ subunits.

Other than gaboxadol, there are few drugs available that act at the GABA binding site of GABA_A receptors and are selective for those receptors located outside of the synapse. There are, however, a number of modulators that act at GABA_A receptors containing α_4 - δ subunits and might be useful in identifying a role of those receptors in the effects of gaboxadol, including ethanol, flumazenil, and neuroactive steroids. For example, GABA_A receptors containing α_4 - δ subunits are reportedly quite sensitive to ethanol with small doses

selectively increasing currents that are mediated by these receptors (Olsen et al. 2007). Flumazenil can enhance GABA-evoked currents mediated by some GABA_A receptors containing δ subunits (Knoflach et al. 1996). Neuroactive steroids increase the effectiveness of GABA to stimulate that receptor subtype (Brown et al. 2002), although they do not enhance the effects of drugs that have greater efficacy than GABA at receptors containing α_4 - δ subunits, such as gaboxadol (Bianchi and Macdonald 2003). These results suggest that modulators will vary in their ability to mimic or enhance the effects of gaboxadol, indicating the importance of using both substitution studies, which determine whether drugs mimic the effects of the training drug, and drug combination studies, which determine whether drugs alter the discriminative stimulus effects of the training drug, to examine the role of GABA_A receptors containing α_4 - δ subunits in the discriminative stimulus effects of gaboxadol.

The use of both substitution and drug combination studies in the current investigation indicates that gaboxadol does not share discriminative stimulus effects with GABA_A receptor modulators with actions at subtypes containing α_4 - δ subunits. One possible explanation for these results is that the discriminative stimulus effects of gaboxadol are not mediated by any GABA_A receptors; however, given the selectivity of gaboxadol for GABA_A receptors in general and subtypes containing α_4 - δ subunits in particular (Belelli et al. 2005; Cope et al. 2005; Drasbek and Jensen 2006; Jia et al. 2005; Meera et al. 2011), this explanation seems improbable. Another possibility is that the role of GABA_A receptors containing α_4 - δ subunits in the behavioral effects of these modulators might be minimal as compared with that of other receptor subtypes, although other results suggest that this possibility is also unlikely. Studies in knockout mice that lack δ subunits of GABA_A receptors have shown decreased behavioral effects of neuroactive steroids and ethanol (e.g., Mihalek et al. 1999; Mihalek et al. 2001; Vashchinkina et al. 2014), and studies in rats suggest that the discriminative stimulus effects of flumazenil are not mediated by benzodiazepine sites on GABA_A receptors (e.g., Wong et al. 1993). A more probable explanation is that the discriminative stimulus effects of GABA_A modulators are distinct from those of drugs acting as orthosteric agonists. Differences between modulators and agonists have also been observed for drugs acting at GABA_A receptors; GABA_A agonists neither mimic nor enhance the discriminative stimulus effects of positive GABA_A receptor modulators (Engel et al. 2001; McMahon and France 2005; Rowlett et al. 1999), and although GABA_A agonists could be established as discriminative stimuli, positive modulators produced no more than 50% responding on the drug lever (Jones and Balster 1998). Whatever the reason, gaboxadol does not share discriminative stimulus effects with GABA_A receptor modulators. Although drug discrimination procedures have been used extensively to determine the mechanism of action of many drugs, including drugs acting at GABA_A receptors, these results indicate that approaches other than drug discrimination are needed to investigate similarities in the behavioral effects of gaboxadol and modulators of GABA_A receptors containing α_4 - δ subunits.

Gaboxadol produces a number of behavioral effects. In addition to its discriminative stimulus effects, which have been studied in several laboratories (current study; McDonald et al. 2007; Michelsen et al. 2007), gaboxadol also has prominent anxiolytic (Hoehn-Saric 1983), and sedative effects (Deacon et al. 2007; Hajak et al. 2009; Lankford et al. 2008; Walsh et al. 2008), suggesting that GABA_A receptors containing α_4 - δ subunits might be

novel therapeutic targets. Interest in developing gaboxadol for clinical use waned due to adverse effects (Hoehn-Saric 1983) or inconsistent effectiveness over long periods of treatment (Roth et al. 2010); however, drugs acting at modulatory sites, rather than at the GABA site, on GABA_A receptors containing α_4 - δ subunits might retain the therapeutic effects of gaboxadol while reducing the adverse effects. Differences in discriminative stimulus effects of modulators and gaboxadol that were observed in the current study suggest that modulators of GABA_A receptors containing α_4 - δ subunits do not share all behavioral effects with gaboxadol; to the extent that they retain effects that can be exploited therapeutically, modulators selective for these receptor subtypes might provide some clinical benefit over gaboxadol.

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Fig.1.

Discriminative stimulus and rate-decreasing effects of gaboxadol and GABA_A modulators in rats discriminating gaboxadol. Data from 8 rats are shown for gaboxadol, midazolam and pregnanolone; data from 6 rats are shown for flumazenil and ethanol. Ordinates: top panel, percentage of total responses emitted on the gaboxadol lever; bottom panel, average response rate. Abscissa: dose in mg/kg

Fig.2.

Discriminative stimulus and rate-decreasing effects of gaboxadol alone and in combination with ethanol (left panels), pregnanolone (right panels). Ordinates: percentage of total responses emitted on the gaboxadol lever. Abscissa: dose in mg/kg

Fig.3.

Discriminative stimulus and rate-decreasing effects of pregnanolone (left panels), midazolam (middle panels), and gaboxadol (right panels) in rats discriminating pregnanolone (triangles), midazolam (circles) or ethanol (squares). Pregnanolone, midazolam and ethanol were administered at the beginning of each 15-min cycle whereas gaboxadol was given at the beginning of every other cycle; thus, the interinjection interval was 15 min for pregnanolone, midazolam and ethanol and 30 min for gaboxadol. Ordinates: percentage of total responses emitted on the drug (i.e., pregnanolone or midazolam) lever. Abscissa: dose in mg/kg