Contribution of GABA<sub>A</sub> Receptor Subtypes to the Anxiolytic-Like, Motor, and Discriminative Stimulus Effects of Benzodiazepines: Studies with the Functionally Selective Ligand SL651498 [6-Fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyridol[3,4-b]indol-1-one]

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ABSTRACT

Benzodiazepines (BZs) are prescribed for a variety of disorders, including those involving anxiety and sleep, but have unwanted side effects that limit their use. Elucidating the GABA<sub>A</sub> receptor mechanisms underlying the behavioral effects of BZs will help develop new drugs having both maximum clinical benefit and minimum adverse side effects. A recently developed compound is SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyridol[3,4-b]indol-1-one], which is a full agonist at GABA<sub>A</sub> receptors containing α<sub>2</sub> and α<sub>5</sub> subunits and a partial agonist at GABA<sub>A</sub> receptors containing α<sub>1</sub> and α<sub>5</sub> subunits. We assessed the ability of SL651498 to engender anxiolytic-like, motor, and subjective effects characteristic of BZ-type drugs in nonhuman primates. Anxiolytic-like activity was assessed with a conflict procedure in rhesus monkeys. Motor effects were evaluated in squirrel monkeys using observational techniques, and the subjective effects of SL651498 were assessed in squirrel monkeys trained to discriminate the nonselective BZ triazolam from saline. SL651498 engendered anxiolytic-like effects similar to conventional BZs. In addition, SL651498 fully induced muscle relaxation, but unlike conventional BZs, engendered minimal ataxia. In drug discrimination studies, SL651498 partially substituted for triazolam. This effect was blocked with the α<sub>1</sub>GABA<sub>A</sub> subtype-preferring antagonist β-CCT (β-carboline-3-carboxylate-t-butyl ester), implicating α<sub>1</sub>GABA<sub>A</sub> receptors in the subjective effects of SL651498. Together, these studies suggest that compounds such as SL651498 that have high intrinsic efficacy at α<sub>5</sub>GABA<sub>A</sub> and/or α<sub>5</sub>GABA<sub>A</sub> receptors may have clinical potential as anxiolytics and muscle relaxants. Moreover, a compound with reduced efficacy at α<sub>1</sub>GABA<sub>A</sub> and/or α<sub>5</sub>GABA<sub>A</sub> receptors may lack some of the motor and subjective effects associated with conventional BZs.

Benzodiazepine (BZ) receptor agonists are commonly prescribed for the treatment of anxiety, sleep, and seizure disorders. Their clinical utility is limited, however, due to undesirable side effects, which include sedation, motor incoordination, memory impairment, abuse, and dependence (Griffiths and Wolf, 1990; Korpi et al., 1997; Belzung et al., 2000; Verster et al., 2002). Research efforts currently are aimed at developing novel BZ agonists that retain therapeutically beneficial properties without the unwanted side effects.

BZ agonists produce their behavioral effects by positive allosteric modulation of GABA binding at the GABA<sub>A</sub> receptor complex. The GABA<sub>A</sub> receptor is a pentameric ionophore formed by the assembly of subunits from at least five different families (Sanger et al., 1994; Lüddens et al., 1995; McKernan and Whiting, 1996). The presence of α, β, and γ subunits are necessary to confer sensitivity to BZ ligands (Pritchett et al., 1989; McKernan and Whiting, 1996; Rudolph et al., 2001), and conventional BZs exert their pharmacological effects via binding nonselective...
tively to GABA\textsubscript{A} receptors containing \(\alpha_1\), \(\alpha_2\), \(\alpha_3\), and \(\alpha_5\) subunits.

Recent research has been aimed at elucidating the receptor mechanisms underlying the specific behavioral effects of BZs, with the goal of developing BZ-type drugs having both maximum clinical benefit and minimum adverse side effects. One such compound that has been developed recently is 6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyridol[3,4-b]indol-1-one (SL651498). SL651498 is a novel pyridoindole derivative that has high affinity for rat native GABA\textsubscript{A} receptors containing \(\alpha_1\) and \(\alpha_5\) subunits and weaker affinity for \(\alpha_6\)GABA\textsubscript{A} receptors, although having intermediate affinity for recombinant rat GABA\textsubscript{A} receptors containing the \(\alpha_5\) subunit (Griebel et al., 2001). However, SL651498 is “functionally selective” in that it acts as a full agonist at \(\alpha_6\)GABA\textsubscript{A} and \(\alpha_5\)GABA\textsubscript{A} receptors and as a partial agonist at \(\alpha_1\)GABA\textsubscript{A} and \(\alpha_6\)GABA\textsubscript{A} receptors, as determined in vitro by modulation of GABA-mediated Cl\textsuperscript{−} flux (Griebel et al., 2001). Behavioral studies in rodents demonstrated that SL651498 elicited anxiolytic-like activity similar to that of diazepam (Griebel et al., 2001, 2003). SL651498 also induced muscle weakness, ataxia, and sedation as measured by motor activity; nevertheless, the doses that engendered these effects were much higher than those producing anxiolytic-like activity. In addition, SL651498 produced neither tolerance to its anticonvulsant effects nor physical dependence after repeated administration (Griebel et al., 2001, 2003). Taken together, these results suggest that the anxiolytic and anticonvulsant activity of SL651498 may make this compound a suitable alternative to currently prescribed drugs for those indications. Furthermore, the observed behavioral effects may be a result of selective actions at specific GABA\textsubscript{A} receptor subtypes.

Our current knowledge of the role of GABA\textsubscript{A} receptor subtypes in the behavioral effects of BZs is predominantly based on studies with transgenic and wild-type rodents. At present, relatively little information is available regarding the roles of particular GABA\textsubscript{A} receptor subtypes in the characteristic effects of BZ-type drugs in either human or nonhuman primates. Therefore, in the present studies, SL651498 was used to investigate the role of specific GABA\textsubscript{A} receptor subtypes in models predictive of the anxiolytic, motor, and subjective effects of BZ-type drugs in nonhuman primates. Anxiolytic-like activity was assessed with a conflict procedure in monkeys in which operant responding maintained by food delivery was suppressed by response-contingent shock presentations. Motor effects characteristic of BZs were evaluated in monkeys using observational techniques based upon naturalistic and drug-induced behavior. Finally, the subjective effects of SL651498 were evaluated in a drug discrimination model, in which monkeys were trained to discriminate the representative nonselective BZ triazolam from saline. Together, these studies aimed to provide insights into the role of GABA\textsubscript{A} receptor subtypes in the therapeutic versus unwanted side effects of BZ-type drugs.

Materials and Methods

Subjects. Four adult rhesus monkeys (Macaca mulatta), two male and two female, were studied in the conflict study. Six adult male squirrel monkeys (Saimiri sciureus) were used for the observational study, and four adult male squirrel monkeys were used in the drug discrimination study. Monkeys used in the conflict and discrimination studies were maintained at 90 to 95% of their free-feeding weight. Monkeys used in the observational study were maintained under free-feeding conditions. All monkeys were housed individually and maintained under a 12-h light/dark cycle in a temperature- and humidity-controlled room. All procedures were conducted with the approval and under the supervision of the Harvard University Institutional Animal Care and Use Committees. Animals in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the “Guide for Care and Use of Laboratory Animals” National Research Council, Department of Health, Education and Welfare Publication No. (NIH 85-23), revised 1996.

Monkeys in the conflict and discrimination studies were prepared with chronic indwelling venous catheters using the general surgical procedures described by Carey and Spealman (1998). Under isoflurane anesthesia and aseptic conditions, one end of a polyvinyl chloride catheter (rhesus monkey = i.d., 0.64 mm; o.d., 1.35 mm; squirrel monkey = i.d., 0.38 mm; o.d., 0.76 mm) was passed to the level of the right atrium by way of a brachial, femoral, or jugular vein. The distal end of the catheter was passed subcutaneously and exited in the mid-scapular region. Rhesus monkey catheters were flushed daily with heparinized saline (150–200 U/ml), whereas squirrel monkey catheters were flushed daily with saline that did not contain heparin. All catheters were sealed with stainless steel obturators when not in use. Monkeys were custom-made nylon mesh jackets (Lomir Biomedical, Toronto, ON, Canada) at all times to protect the catheter.

Conflict Study. Rhesus monkeys were trained to sit in a custom-designed restraint chair (Crist Instruments, Hagerstown, MD) located in a sound-attenuating chamber. A response lever, stimulus lights (Med Associates, Georgia, VT), and a receptacle into which food pellets (1 g of marshmallow-flavored pellets; BioServ, Frenchtown, NJ) were delivered were positioned in front of the monkey. Monkeys were trained on a multiple schedule of food reinforcement consisting of two components: 1) a schedule of food pellet delivery, and 2) a schedule of food pellet delivery plus a schedule of foot shock delivery (0.25-s duration, 1–3 mA depending on the individual monkey). Four components were available in a session, separated by 10-min timeout periods in which responding had no programmed consequences. Responding was maintained in each component under an 18 response, fixed-ratio (FR) schedule of food pellet delivery. Each component consisted of the schedule of food pellet delivery signaled by red stimulus lights, followed immediately by the same schedule of food delivery combined with a 20 response FR schedule of foot shock delivery signaled by green stimulus lights. Each response requirement was followed by a 10-s timeout. Drugs were administered during the 5th min of the 10-min timeout that preceded each component.

Training sessions were conducted 5 days per week until performance (measured as rates of responding, see below) in both “food only” and “food + shock” components was stable (i.e., no upward or downward trends for 3 consecutive days). In addition, if rates of responding in a component during a training session varied by more than 20% of the corresponding response rates in the previous training session, additional training sessions were conducted until responding was again stable. Once training criteria were met, test sessions were conducted once or twice a week, separated by at least 2 days. Dose-response functions were determined for test drugs using a cumulative dosing procedure similar to the one described by Rowlett et al. (2001). Four-point cumulative dose-response functions were determined within a single test session as a result of incremental increases in drug (one-half log units) administered at the 5th min of the 10-min timeout period. Dose-response functions were determined for SL651498 (0.1–3 mg/kg), the classical nonselective BZ agonist chlordiazepoxide (1–30 mg/kg), as well as triazolam (0.001–0.03 mg/kg).

The number of responses in a component, minus responding during pellet delivery and the 10-s timeouts, was divided by the total
ligands were assessed in squirrel monkeys according to the procedures described by Platt et al. (2002). Each monkey was habituated to a ventilated, transparent Plexiglas arena (114 cm × 122 cm × 213 cm). This observation arena was equipped with perches, suspended plastic chains, and a wood chip foraging substrate to allow the monkeys to express a range of species-typical behaviors. A video camera was positioned 1 m in front of the chamber and operated throughout the 30-min session.

Drug testing was conducted once or twice per week with control sessions preceded by saline injections on intervening days. Doses of SL651498 (0.3–10 mg/kg i.m., administered 10 min prior to the start of the session) or chlordiazepoxide (3.0–56 mg/kg i.m., administered 30 min prior to start of the session) were administered on separate test days. Trained observers, unaware of the drug being studied, scored the videotapes by recording the presence or absence of each of eight behaviors (Table 1) at 15-s intervals during three 5-min observation periods across the session (0–5, 12–17, and 24–29 min). For each subject, frequency scores (defined as the total number of 15-s intervals in which a particular behavior was observed; maximum score = 20) for each behavior were averaged across the three observation periods of a session because no reliable differences in scores were identified by separated repeated measures analysis of variance. Scores were then averaged across subjects to obtain group means. To determine statistical reliability of treatment effects on each behavior, the effect of dose was determined for each drug by separate repeated measures analyses of variance. Treatment effects were identified by separated repeated measures analysis of variance.

Scores were then averaged across subjects to obtain group means. To determine statistical reliability of treatment effects on each behavior, the effect of dose was determined for each drug by separate repeated measures analyses of variance. Treatment effects were identified by separated repeated measures analysis of variance.

Drug testing sessions were conducted once or twice per week with training sessions scheduled on intervening days. Test sessions were conducted if 80% or more of the total responses occurred on the lever designated correct for that component for at least four of the five preceding training sessions. Test sessions consisted of i.v. injections of saline, triazolam (0.003–0.1 mg/kg), or SL651498 (0.1–1 mg/kg). Overlapping cumulative dose-response functions were administered on different test sessions until at least one dose of drug produced ≥80% drug-lever responding, decreased response rates to ≤25% of control, or resulted in two or more animals not completing a response requirement. Antagonism studies also were conducted in which selected doses of β-CCT (0.3 and 1.0 mg/kg i.v.) were studied in combination with SL651498. β-CCT and its vehicle control were administered i.v. in the first component similar to previous studies (Lelas et al., 2002).

The percentage of drug-lever responding was computed for individual subjects by dividing the number of responses on the drug lever by the total number of responses on both levers and multiplying by 100. The percentage of drug-lever responses for each dose was then averaged across monkeys and the standard error of the mean computed. A drug was considered to substitute for triazolam if the average maximum percentage of drug-lever responses reached 80%. Average maximum percentages of less than 80% but greater than 20% triazolam-lever responding were considered partial substitution.

Antagonist potencies were obtained by calculating in vivo apparent pKᵦ values using the methods described by Lelas et al. (2002). For each monkey, the dose of agonist that engendered 50% triazolam-lever responding (ED₅₀) was calculated. The ED₅₀ values were used to calculate in vivo apparent pKᵦ values according to the equation described by Negus et al. (1993): pKᵦᵦ = −log [B/DR − 1]. In the equation, “B” is the dose of β-CCT in moles per kilogram and “DR” is the ED₅₀ of agonist combined with antagonist divided by the ED₅₀ of agonist alone. Mean apparent pKᵦ values and 95% confidence intervals were calculated to make comparisons with previous findings (e.g., Lelas et al., 2002; Rowlett et al., 2003).

Drugs. The base forms of triazolam (Research Biochemicals Inc., Natick, MA), β-CCT (Department of Chemistry, University of Wis-
Conflicts of Interest. The authors report no conflicts of interest.

Results

Conflict Study. During the course of the experiments, mean rates of responding during training sessions for nonsuppressed (food only) responding were between 2.0 and 3.0 responses/second and were at or near zero for suppressed (food + shock) responding. During tests with drug vehicle, rates of responding in both components showed a similar pattern (i.e., relatively high rates in the absence of shock, little or no responding when shock was present; Fig. 1, points above “V”). SL651498 produced a dose-dependent increase in suppressed responding at 1.0 and 3.0 mg/kg with no change in nonsuppressed responding (Fig. 1, left panel). Chlordiazepoxide increased rates of suppressed responding at 3.0 and 10 mg/kg (Fig. 1, middle panel), but decreased rates of both nonsuppressed and suppressed responding at the highest dose of 30 mg/kg. Similarly, triazolam increased rates of suppressed responding at 0.003 and 0.01 mg/kg, but decreased rates of suppressed responding at 0.03 mg/kg (Fig. 1, right panel). Rates of nonsuppressed responding also were decreased by 0.03 mg/kg triazolam.

Observation Study. The highest dose of SL651498 (10 mg/kg) engendered mild ataxia (score of 0.4 ± 0.1) [F(4,12) = 7.3, p = 0.003; Bonferroni t test, p < 0.05], unlike chlordiazepoxide which reliably engendered ataxia at three of four doses tested [F(6,30) = 24.7, p < 0.001; Bonferroni t test, p < 0.05] (Fig. 2, top). Similar to chlordiazepoxide, SL651498 produced a dose-dependent decrease in muscle resistance (Fig. 2, bottom). All doses of SL651498 differed from vehicle [F(4,12) = 41.0, p < 0.001; Bonferroni t test, p < 0.05], whereas only the two highest doses of chlordiazepoxide differed reliably from vehicle [F(6,30) = 59.2, p < 0.001; Bonferroni t test, p < 0.05]. Figure 3 shows the effects of SL651498 and chlordiazepoxide on measures of sedative-like behaviors. SL651498 had no effect on locomotion, rest posture, or procumbent posture at any of the doses tested. In contrast, chlordiazepoxide reliably decreased locomotion at the highest dose (56 mg/kg) [F(6,30) = 8.2, p < 0.001; Bonferroni t test, p < 0.05], increased rest posture at an intermediate dose [F(6,30) = 5.0, p = 0.001; Bonferroni t test, p < 0.05], and increased procumbent posture at the two highest doses [F(6,30) = 55.5, p < 0.001; Bonferroni t test, p < 0.05]. No other behaviors were significantly altered by SL651498 (Table 2). In contrast, chlordiazepoxide reliably suppressed environment-directed behavior [F(6,30) = 6.9, p < 0.001; Bonferroni t test, p < 0.05].

Table 3 summarizes the ability of SL651498 to engender typical BZ-like side effects. The maximum score (MS) and minimally effective dose (MD) of SL651498 were compared directly with those of chlordiazepoxide, zolpidem, and triazolam.

Fig. 1. Anticonflict effects of SL651498 compared with chlordiazepoxide in squirrel monkeys. Data are mean ± S.E.M. from N = 6 monkeys. Points above V represent data from vehicle test sessions. Asterisks represent significant differences relative to vehicle (p < 0.05).

Fig. 2. Ataxic and myorelaxant effects of SL651498 compared with chlordiazepoxide in squirrel monkeys. Data are mean ± S.E.M. from N = 6 monkeys. Points above V represent data from vehicle test sessions. Asterisks represent significant differences relative to vehicle (p < 0.05).
that engendered ataxia, in contrast to chlordiazepoxide, zol-
SID651498 occurred at doses at least 33-fold lower than those
procumbent posture. In addition, muscle relaxant effects of
zolpidem, and triazolam by its relative lack of ataxia and
lam. SL651498 was distinguishable from chlordiazepoxide,
chlordiazepoxide, and triazolam were reliably attenuated by
3.0 mg/kg β-CCT pretreatment.

Drug Discrimination Study. In monkeys trained to
discriminate triazolam from saline, triazolam increased
responding on the drug-appropriate lever in a dose-dependent
manner (Fig. 5). As the dose of triazolam increased, rates
of responding decreased, with the 0.1 mg/kg dose reliably
decreasing the rate of responding compared with vehicle (Dun-
nett’s t test, p < 0.05). SL651498 also engendered an increase
in triazolam-appropriate responding; however, the maximum
percentage of drug-lever responding did not reach 80% up to
a dose of 10 mg/kg (Fig. 5). At 10 mg/kg SL651498, two of four
monkeys did not respond, whereas the remaining two mon-
keys responded primarily on the saline lever at rates of
responding that were approximately 25% of vehicle control
levels (Fig. 5, bottom panel). This resulted in an inverted
u-shaped dose-response function for the triazolam-like ef-
fects of SL6514898.

As shown in Fig. 6, the α1GABAA-preferring antagonist
β-CCT inhibited the discriminative stimulus effects of
SL651498 in a dose-dependent manner. Over the dose range
evaluated in this study, the 0.3 mg/kg dose of β-CCT resulted
in antagonism that was fully surmountable (i.e., the max-
imum percentage triazolam-lever responding returned to the
levels observed without β-CCT treatment). It is unknown
whether the effects of the 1.0 mg/kg dose of β-CCT are sur-
mountable because higher concentrations of these drug com-
binations could not be evaluated without compromising cath-
eter patency. Antagonism by the 0.3 mg/kg dose of β-CCT
was analyzed by in vivo apparent pK_B analysis, and an av-
erage apparent pK_B of 6.3 was obtained. The 95% confidence
intervals associated with the apparent pK_B for 0.3 mg/kg
β-CCT and SL651498 overlapped with apparent pK_B values
for antagonism by this dose of β-CCT of the discriminative
stimulus effects of zolpidem in zolpidem-trained monkeys, as
well as β-CCT antagonism of zolpidem and triazolam in tria-
zelam-trained monkeys (Table 4). Neither dose of β-CCT
had any effect on rate of responding when administered alone
(data not shown).

Discussion

The data presented here describe the behavioral effects
engendered by SL651498, a novel BZ agonist that acts as a
full agonist at α1GABAA and α2GABAA receptors and as a
partial agonist at α1GABAA and α3GABAA receptors in non-
human primates. The distribution of the subtypes of the
GABA_A receptors throughout the brain varies such that the
α1GABAA receptor is the most abundant and ubiquitous;
α2GABAA and α3GABAA receptors are located primarily in
limbic areas and in the spinal cord; α1GABAA receptors are
the least abundant, residing mainly in hippocampal pyrami-
dal cells (McKernan and Whiting, 1996). The intrinsic effi-
cacy of SL651498 at the GABA_A receptors and the regional
distribution of those receptors within the central nervous

![Fig. 3. Sedative-like effects of SL651498 compared with chlordiazepoxide in squirrel monkeys. Data are mean ± S.E.M. from N = 6 monkeys. Points above V represent data from vehicle test sessions. Asterisks represent significant differences relative to vehicle (p < 0.05).](Image)

**Table 2**

Behavioral effects of the highest dose of SL651498, in comparison with chlordiazepoxide, in squirrel monkeys (N = 6)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Vehicle</th>
<th>Chlordiazepoxide 10 mg/kg</th>
<th>Chlordiazepoxide 56 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive visual</td>
<td>11.7 ± 2.8</td>
<td>13.3 ± 2.3</td>
<td>8.0 ± 4.4</td>
</tr>
<tr>
<td>Environment-directed</td>
<td>10.4 ± 2.4</td>
<td>6.4 ± 3.9</td>
<td>0.1 ± 0.1*</td>
</tr>
<tr>
<td>Self-directed</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.5</td>
<td>0.5 ± 0.3</td>
</tr>
</tbody>
</table>

* Bonferroni t test, p < 0.05
system together likely play an important role in the behavioral effects produced by SL651498.

Punishment-based conflict procedures are a reliable and clinically relevant method for demonstrating the efficacy of anxiolytic agents (Geller and Seifter, 1962; Kleven and Koek, 1999; Millan and Brocco, 2003). SL651498 increased responding suppressed by contingent shock in a conflict procedure developed for rhesus monkeys. This outcome, moreover, was observed at doses that did not decrease responding in the absence of shock. These results are in agreement with previous studies showing the potent anxiolytic action of SL651498 in the absence of behavioral impairment in various rodent conflict tests (Griebel at al., 2001). In contrast, both chlordiazepoxide and triazolam increased suppressed responding, but decreased rates of nonsuppressed responding at the highest doses tested (cf. Hascoet and Bourin, 1997; Pattij et al., 2000).

Although there are fewer available data attributing the anxiolytic effects of BZ agonists to specific GABAA receptor subtypes in nonhuman primates, studies in rodents have provided valuable insights about the receptor mechanisms underlying these effects. Point mutations in mice rendering the \( \alpha_2 \)GABAA receptors insensitive to diazepam suggested that BZ-induced anxiolytic-like effects are mediated in part by \( \alpha_2 \)GABAA receptors (Rudolph et al., 1999; Löw et al., 2000; McKernan et al., 2000). Additionally, administration of a \( \alpha_2 \)GABAA receptor inverse agonist had anxiogenic effects in rats tested in the elevated plus maze (Collins et al., 2002). Together, these data implicate the \( \alpha_2 \) and/or \( \alpha_3 \)GABAA receptors in anxiolysis. The present results support a similarly critical role for \( \alpha_2 \) and/or \( \alpha_3 \)GABAA receptors in mediating anxiolytic-like effects in primates.

Observational techniques were used to characterize systematically the effects of SL651498 on a range of motor behaviors in squirrel monkeys. Across the range of doses tested, SL651498 decreased muscle resistance, with only a mild ataxic effect. To evaluate the role of \( \alpha_1 \)GABAA receptors in these effects, we examined the ability of the \( \alpha_1 \)GABAA receptor antagonist \( \beta \)-CCT to block the SL651498-induced muscle relaxant and ataxic effects. Neither the relaxation

*Ataxia and procumbent posture data for zolpidem and triazolam derived from Platt et al., 2002.
*Minimum dose that was effective (lowest dose reliably different from vehicle, Dunnett's tests) in milligrams per kilogram, intramuscular.
*Ratio is the MD of a behavioral effect divided by the MD for muscle relaxation.

![Figure 4](https://example.com/fig4.png)  
**Fig. 4.** Myorelaxant and ataxic effects of SL651498, chlordiazepoxide, and triazolam alone and combined with the \( \alpha_1 \)GABAA-preferring antagonist \( \beta \)-CCT in squirrel monkeys (N = 6). Asterisks represent significant differences relative to drug alone (p < 0.05).

![Figure 5](https://example.com/fig5.png)  
**Fig. 5.** Percentage of drug-lever responding (top panel) and rates of responding (bottom panel) engendered by triazolam and SL651498 in squirrel monkeys trained to discriminate triazolam from saline. Data are mean ± S.E.M. from N = 4 monkeys. In parentheses is the number of monkeys responding/total sample size.

### TABLE 3

Summary of observed behavioral effects induced by BZ-type drugs in squirrel monkeys

<table>
<thead>
<tr>
<th></th>
<th>SL651498</th>
<th>Chlordiazepoxide</th>
<th>Zolpidem</th>
<th>Triazolam</th>
</tr>
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<tbody>
<tr>
<td><strong>Muscle relaxation</strong></td>
<td>2.0 ±0.3</td>
<td>1.0</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Ataxia</strong></td>
<td>0.5 ±1.0</td>
<td>10.0 ±3.3</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Procumbent posture</strong></td>
<td>15.0 ±3.0</td>
<td>1.0</td>
<td>1.8</td>
<td>1.0</td>
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<tr>
<th>MS</th>
<th>MD</th>
<th>Ratio</th>
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**Note:**
- Maximum score regardless of dose (maximum possible score: muscle relaxation and ataxia = 2.0; procumbent posture = 20.0).
- Minimum dose that was effective (lowest dose reliably different from vehicle, Dunnett's tests) in milligrams per kilogram, intramuscular.
- Ratio is the MD of a behavioral effect divided by the MD for muscle relaxation.
engendered by SL651498 nor that engendered by chlordiazepoxide or triazolam could be blocked by β-CCT, suggesting that α₂GABA_A receptors may be at the core of the sedative effect caused by the benzodiazepine. In contrast to this, chlordiazepoxide-induced ataxia was not attenuated by SL651498, suggesting that α₂GABA_A receptors are critically involved in motor effects of BZs, and/or procumbent postures and virtually eliminated locomotor activity (Platt et al., 2002). Table 3 shows the lack of sedative-like effects of SL651498 support a role for α₂GABA_A receptors in behavior (Platt et al., 2002).

Recent work in transgenic mice attributes myorelaxation to the α₁ and α₂GABA_A receptors (Crestani et al., 2001), and our results are consistent with these GABA_A receptors mediating muscle relaxation. The muscle relaxant effects of SL651498 reported here were not mediated by α₁GABA_A receptors, as β-CCT did not inhibit this behavior. That β-CCT did attenuate ataxia engendered by SL651498, as well as chlordiazepoxide and triazolam, suggests that ataxia may be mediated by α₂GABA_A receptors, a result consistent with SL651498’s low efficacy at α₁GABA_A receptors. Recent studies have demonstrated that α₂GABA_A receptors are critically involved in motor effects of BZs, suggesting that SL651498’s low efficacy at α₂GABA_A receptors may explain the absence of ataxia/sedation in this study (Rudolph et al., 1999; McKernan et al., 2000; Crestani et al., 2001).

Drug discrimination procedures provide a method for studying receptor mechanisms underlying the subjective effects of drugs. Typically drugs that occasion the same behavioral response as the training drug (i.e., substitute for the training drug) share the same receptor mechanism of action, converge upon the same neurotransmitter system, or both (Lelas et al., 2000). Using this paradigm, the receptor mechanisms mediating the discriminative stimulus effects of SL651498 were assessed in squirrel monkeys trained to discriminate triazolam from saline. The conventional BZ agonist triazolam increased responding on the drug-appropriate lever in a dose-dependent manner, an effect that may be mediated by α₁GABA_A receptors (Lelas et al., 2001, 2002). SL651498 partially reproduced the discriminative stimulus effects of triazolam. To evaluate the role of α₂GABA_A receptors in this effect, we examined the ability of the α₂GABA_A receptor-prefering agonist bretazenil (Puia et al., 1992; Sanne-Srud and Ator, 1995) and the low efficacy BZ agonist bretazenil (Puia et al., 1992; Sanne-Srud and Ator, 1995). The similar substitution profiles of triazolam and the α₁GABA_A receptor-prefering agonist zolpidem (Lelas et al., 2002; Rowlett et al., 2003), dose-dependently prevented SL651498’s partial substitution for triazolam. The apparent pKB value for antagonism of the triazolam-like discriminative stimulus effects of SL651498 was similar to those calculated from previous studies examining β-CCT antagonism of zolpidem- and triazolam-like discriminative effects (Lelas et al., 2002; Rowlett et al., 2003). This analysis suggests that β-CCT blocked the discriminative stimulus effects of SL651498, zolpidem, and triazolam via a similar population of receptors, presumably α₁GABA_A receptors. Consistent with the involvement of α₁GABA_A receptors, SL651498 previously was shown to partially substitute for the α₁GABA_A-prefering BZ agonist zolpidem in rats (Griebel et al., 2001). Altogether, these findings suggest that SL651498 shares some subjective effects with triazolam that may be mediated by α₁GABA_A receptor subtypes.

Drug discrimination studies also provide information about a drug’s intrinsic efficacy, a factor that contributes to its subjective effects. An agonist with low intrinsic efficacy will engender less than full substitution or substitute in a subset of animals trained to discriminate an agonist with high intrinsic efficacy (Aitor and Griffiths, 1999). In the present studies, SL651498 only partially substituted for the high efficacy agonist triazolam (Ducic et al., 1993), suggesting that it has lower intrinsic efficacy than triazolam. Interestingly, the partial substitution of SL651498 for triazolam (64% triazolam-lever responding) is nearly identical to the 66% triazolam-lever responding engendered by chlordiazepoxide in a previous study (Lelas et al., 2001). Thus, it has been suggested that chlordiazepoxide is a partial agonist, a hypothesis that is supported by its similarities to the known low efficacy BZ agonist brextazenil (Puia et al., 1992; Sanne-Srud and Ator, 1995). The similar substitution profiles of SL651498 and chlordiazepoxide in triazolam-trained animals together with the blockade of the partial substitution by the α₁GABA_A receptor antagonist underscore SL651498’s low intrinsic efficacy at α₁GABA_A receptors.

Altogether, these data suggest that the receptor mechanisms underlying the behavioral effects of the BZ agonist SL651498 likely reflect its relative efficacy at the various α

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**Table 4**

In vivo apparent pKB analyses for β-CCT antagonism (0.3 mg/kg) of the triazolam-like discriminative stimulus effects of SL651498, in comparison with zolpidem and triazolam under different training conditions.

<table>
<thead>
<tr>
<th>Test Drug</th>
<th>Training Drug</th>
<th>Dose Ratio</th>
<th>pKB</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL651498</td>
<td>Triazolam</td>
<td>3.5</td>
<td>6.3(6.0–6.5)</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>Zolpidem</td>
<td>4.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Triazolam</td>
<td>Triazolam</td>
<td>2.5</td>
<td>6.1</td>
</tr>
<tr>
<td>Triazolam</td>
<td>Triazolam</td>
<td>3.0</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* Calculated from Rowlett et al. (2003).
* Calculated from Lelas et al. (2002).
* 95% confidence intervals.
subunits of the GABA<sub>A</sub> receptor complex. These studies particularly implicate α<sub>G</sub>GABA<sub>A</sub> receptors in the discriminative stimulus and ataxic effects of SL651498, whereas α<sub>G</sub>GABA<sub>A</sub> and α<sub>G</sub>GABA<sub>A</sub> receptors may mediate the anxiolytic and myorelaxant effects of this drug. These data further suggest that SL651498, a drug with functional selectivity, may have therapeutic potential as a nonsedating anxiolytic and muscle relaxant.

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References


