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Contribution of $\alpha 1$ Subunit-Containing γ -Aminobutyric Acid_A (GABA_A) Receptors to Motor-Impairing Effects of Benzodiazepines in Squirrel Monkeys

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Abstract

Rationale—Benzodiazepines (BZs) are effective anxiolytics and hypnotics, but their use is limited by unwanted side effects, such as motor impairment.

Objectives—To assess the contribution of $\alpha 1$ subunit-containing GABA_A receptor subtypes to the motor-impairing effects of BZs, the present study evaluated two observable measures of motor coordination (balance on a pole, resistance to hind-limb flexion) engendered by non-selective and selective BZ-site agonists in squirrel monkeys.

Methods—Multiple doses of non-selective BZs (triazolam, alprazolam, diazepam, chlordiazepoxide) and $\alpha 1$ subunit-preferring agonists (zolpidem and zaleplon) were administered to adult male squirrel monkeys (N=4–6), and experimenters rated the monkey's ability to balance on a horizontal pole ("ataxic-like effects"), as well as the degree of resistance to hind-limb flexion ("myorelaxant-like effects").

Results—Administration of all BZ-type drugs resulted in ataxic-like and myorelaxant-like effects. Pretreatment with the $\alpha 1$ subunit-preferring antagonist β CCCT (β -carboline-3-carboxylate-tbutyl ester) attenuated the ataxic-like effects engendered by both types of drugs. However, β CCCT was largely ineffective at blocking the ability of both BZs and non-BZs to induce myorelaxant-like effects.

Conclusions—These experiments demonstrate dose-dependent motor impairment in squirrel monkeys using quantitative behavioral observation techniques. Altogether, these findings suggest a lack of a prominent role for $\alpha 1$ subunit-containing receptors in the alteration of hind-limb flexion, a putative measure of myorelaxation, induced by BZ-type drugs in monkeys.

Keywords

Ataxia; Myorelaxation; Motor impairment; Benzodiazepine; GABA_A receptor; Monkey

Benzodiazepines (BZs) are among the most widely prescribed drugs worldwide. These drugs produce their pharmacological effects by allosterically modulating the inhibitory action of γ -aminobutyric acid (GABA) at the GABA_A receptor (for review, see Möhler 2006; Whiting 2006). Although the GABA_A receptor is a pentameric structure formed by the assembly of

subunits from at least five different families (α_{1-6} , β_{1-3} , γ_{1-3} , ρ_{1-2} , and δ_1), only the α , β , and γ subunits are necessary to confer BZ sensitivity (McKernan and Whiting 1996; Rudolph et al. 2001). Moreover, the behavioral effects of BZs appear to depend upon the presence of a particular α subunit. In this respect, GABA_A receptors containing $\alpha 1$ subunits ($\alpha 1$ GABA_A receptors) have been proposed to mediate the sedative-like effects of BZs (Rudolph et al. 1999; McKernan et al. 2000). In contrast, the $\alpha 2$ and/or $\alpha 3$ -containing GABA_A receptors ($\alpha 2$ GABA_A, $\alpha 3$ GABA_A receptors) may be the substrates for the anxiolytic and myorelaxant effects of BZs (Rudolph et al. 1999; Löw et al. 2000; McKernan et al. 2000; Collins et al. 2002; Dias et al. 2005), while $\alpha 5$ -containing receptors ($\alpha 5$ GABA_A receptors) may be involved in processes associated with memory (Collinson et al. 2002; Crestani et al. 2002).

In general, the motor-impairing effects of BZs limit their clinical utility as anxiolytics (for review, see Korpi et al. 1997), although some effects, e.g., myorelaxation, can be desirable from a therapeutic perspective. Therefore, understanding the GABA_A receptor mechanisms underlying specific motor-impairing effects such as ataxia and myorelaxation would clearly benefit drug discovery efforts geared toward anxiolytic development. In rodents, BZ-induced ataxia is often measured using the rotarod test (Gallagher et al. 1991; Griebel et al. 1999b), while the myorelaxant effects of BZs are typically assessed using a test of grip strength (Griebel et al. 1999a; Crestani et al. 2001; Bach-Rojecky and Samarzija 2005). In non-human primates, observational procedures have been used to evaluate the effects of BZs on these measures. For example, observers were trained to interpret slow and uncertain movements as ataxia in baboons, while lip droop was interpreted as myorelaxation (Weerts et al. 1998; Ator et al. 2000).

Platt et al. (2002) first described a quantitative procedure to assess motor effects in squirrel monkeys in which trained handlers evaluated the ability of monkeys to maintain normal balance on a transport pole following administration of BZ agonists. Subsequent studies employing this procedure have suggested that $\alpha 1$ GABA_A receptors may play a critical role in mediating this putative measure of ataxia, a term defined medically as a lack of muscle coordination when performing voluntary movements (Licata et al. 2005; Rowlett et al. 2005b). Preliminary descriptions of methods for evaluating myorelaxation have been described in later studies from our laboratory (Licata et al. 2005; Rowlett et al. 2005b). This procedure involves evaluation of the degree to which monkeys resist the extension of a hind limb. Results from studies using this procedure have suggested that in contrast to the effects of BZs on balancing on a horizontal pole, the ability of BZ-type drugs to decrease resistance to hind-limb flexion may involve $\alpha 2$ and/or $\alpha 3$ GABA_A subtypes (Licata et al. 2005; Rowlett et al. 2005b).

The present study evaluated hands-on measures of motor behavior in squirrel monkeys, with the overall aim of examining the contribution of specific GABA_A receptor subtypes to the ataxic vs. myorelaxant effects of BZs and BZ-site agonists. For these experiments, we chose representative 1,4-benzodiazepines and triazolobenzodiazepines with high intrinsic efficacy and no selectivity for GABA_A receptor subtypes (triazolam, alprazolam, diazepam, and chlordiazepoxide), as well as two $\alpha 1$ GABA_A receptor-selective agonists with high intrinsic efficacy (zolpidem and zaleplon). To delineate further the extent to which $\alpha 1$ GABA_A receptors contribute to BZ-induced ataxia and myorelaxation, studies were conducted with the $\alpha 1$ GABA_A-preferring antagonist β -carboline-3-carboxylate-t-butyl ester (β CCCT; Cox et al. 1995; for review see Rowlett et al. 2005a). In squirrel monkeys, β CCCT has been shown to block motor behaviors involving $\alpha 1$ GABA_A receptors. For example, β CCCT antagonized both zolpidem-induced decreases in operant responding, as well as zolpidem- and triazolam-induced increases in ataxia (Paronis et al. 2001; Platt et al. 2002). While other studies conducted by our group have found that β CCCT did not attenuate the ability of BZ-site compounds to decrease hind-limb flexion at a dose that did reduce ataxic-like effects (Licata et al. 2005; Rowlett et al. 2005b), those experiments were conducted with single doses of a relatively limited range of

BZ agonists. Thus, another goal of the present study was to compare β CCT's ability to shift full dose-response functions of conventional and receptor-preferring BZ-site ligands.

Methods

Subjects

Adult male squirrel monkeys (*Saimiri sciureus*, N= 4–6), weighing 750–1100g, were studied in daily experimental sessions (Monday through Friday). Between experimental sessions all monkeys were housed individually and maintained under a 12 hr light/dark cycle in a temperature- and humidity-controlled room where they had unrestricted access to food (Teklad Monkey Diet supplemented with fresh fruit) and water. 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.

Apparatus

Studies were conducted in a ventilated, transparent Plexiglas arena (114 cm $\times$ 122 cm $\times$ 213 cm) located in an isolated room. This 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. Monkeys were transferred to and from the observation arena using a pole and leash system. The stainless steel chain leash was attached to a collar around the neck of the monkey, and during transfer the leash was passed through a ring at the end of a 1 cm diameter stainless steel transport pole. This technique restricted the monkey's movement to the region of the transport pole farthest away from the handler and required the monkey to balance atop the transport pole.

Procedure

Monkeys were habituated to the observation arena and the experimental procedures described below for a period of approximately one month. Following habituation, 30-min behavioral assessment sessions were conducted daily. During the 6th, 18th, and 30th minute of each 30-min session, the monkeys were removed briefly from the observation arena by a trained handler following the technique described above for transferring the monkeys, and evaluated for ataxic-like effects and myorelaxant-like effects. Ataxic-like effects were evaluated according to the criteria described by Platt et al. (2002), and were defined as the inability to balance on the transport pole held in the horizontal plane. A score of 0 indicated that the monkey was able to balance normally on the pole, a score of (+1) indicated that the monkey was able to hold onto the pole, but unable to maintain balance (e.g. suspended by limbs below the pole), and a score of (+2) indicated that the monkey could neither balance on nor hold onto the pole. Myorelaxant-like effects were defined as decreased resistance to extension of a hind limb. A score of 0 indicated normal resistance to extension, a score of (–1) indicated a decreased resistance to extension, and a score of (–2) indicated no resistance to extension (i.e., the monkey was flaccid and completely relaxed). Handlers were trained for at least 1 month prior to data collection, and were unaware of the drug being tested (i.e., “blinded” to the drug condition). Inter-rater reliability scores were determined prior to the study to be ≥ 0.90 .

Drug testing occurred once or twice per week, with control sessions preceded by saline injections on intervening days. Doses of diazepam (0.3–10 mg/kg; 30 min pretreatment), chlordiazepoxide (3.0–56 mg/kg; 30 min pretreatment), triazolam (0.01–0.3 mg/kg; 30 min pretreatment), alprazolam (0.03–1.0 mg/kg; 30 min pretreatment), zolpidem (1.0–17.8 mg/kg; 10 min pretreatment), or zaleplon (1.0–17.8 mg/kg; 10 min pretreatment) were evaluated in

separate test sessions. Pretreatment intervals were chosen on the basis of preliminary studies which determined the approximate time to peak effect, or were obtained from published reports of these drugs administered i.m. to squirrel monkeys (cf. Platt et al., 2002; Licata et al., 2005). In a subset of four monkeys, antagonism studies were conducted in which selected doses of β CCT (3.0 mg/kg; 10 min pretreatment) were studied in combination with a range of doses of the agonists. All drugs, as well as saline controls, were administered via intramuscular injections in a calf or thigh muscle.

Analysis of drug effects

For each subject, the median scores for each measure were obtained across the three evaluation time points of a session, and median scores were obtained across subjects for each treatment. To determine statistical reliability of treatment effects, the effect of dose was determined for each drug by separate Friedman repeated measures analysis of variance (ANOVA) on ranks. Treatment effects were assessed further using Dunn's Q statistic, in which the effects of different doses of each drug were compared to data from saline control sessions. Data are presented graphically as medians with variability presented as the interquartile range.

The potency of compounds to engender ataxia and muscle relaxation was estimated by calculating the ED_{50} for each drug. ED_{50} values correspond to the doses that produced 50% of the maximum effect observed for a particular drug or drug combination. Linear regression analysis was used in cases where the portion of the log dose-response function was defined by three or more data points or by linear interpolation in cases where the log dose-response function was defined best by two points.

Drugs

Triazolam, alprazolam, diazepam (Sigma/RBI; St. Louis, MO), zolpidem (Merck Sharp Dohme; Harlow, UK; gift from G. Dawson), β CCT (Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI), and zaleplon (Wyeth-Ayerst; Princeton, NJ) were prepared in a vehicle of 50% propylene glycol (Fisher Scientific; Suwanee, GA), 40% saline, and 10% ethanol. Chlordiazepoxide (Research Biochemicals; Natick, MA) was dissolved in 0.9% saline. All doses are expressed in the base form of the compound. Injection volumes were 0.1–0.4 ml/kg body weight. All drugs were prepared the day of a test session.

Results

Ataxic-Like and Myorelaxant-Like Effects of Non-selective BZs

Following saline administration, no animal exhibited ataxic-like or myorelaxant-like effects (i.e., ataxic-like effects score=0, resistance score=0). Dose-related increases in ataxic-like effects were observed following administration of the non-selective BZs triazolam, alprazolam, diazepam, and chlordiazepoxide (Fig. 1, top panels). Triazolam engendered increases in ataxic-like effects such that doses of 0.1 or 0.3 mg/kg produced ataxic-like effects scores that were reliably greater than those obtained following saline administration [$\chi^2(4)=19.30$, $p<0.05$, Dunn's Q statistic, $p<0.05$]. Similarly, the highest dose of alprazolam (1.0 mg/kg) [$\chi^2(4)=15.51$, $p<0.05$, Dunn's Q statistic, $p<0.05$], diazepam (10.0 mg/kg) [$\chi^2(4)=14.51$, $p<0.05$, Dunn's Q statistic, $p<0.05$], and the 30 mg/kg dose of chlordiazepoxide [$\chi^2(4)=12.97$, $p<0.05$, Dunn's Q statistic, $p<0.05$] engendered reliable increases in the scores for ataxic-like effects compared to those obtained following saline.

Dose-related decreases in hind limb resistance also were observed following administration of triazolam [$\chi^2(4)=19.50$, $p<0.05$], alprazolam [$\chi^2(4)=15.78$, $p<0.05$], diazepam [$\chi^2(4)=13.13$, $p<0.05$], and chlordiazepoxide [$\chi^2(4)=15.13$, $p<0.05$] (Fig. 1, bottom panels). The highest

doses of alprazolam, diazepam, and chlordiazepoxide induced reliable decreases in resistance scores, with the two highest doses reliable for triazolam (Dunn's Q statistic, $p < 0.05$).

Ataxic-Like and Myorelaxant-Like Effects of the $\alpha 1\text{GABA}_A$ -Preferring Agonists

Similar to the non-selective BZs, the $\alpha 1\text{GABA}_A$ -preferring BZ-site agonists zolpidem and zaleplon engendered dose-dependent increases in ataxic-like effects above saline levels (Fig. 2, top panels). Zolpidem engendered a significant increase in the ataxic-like effects score at doses of 10 and 17.8 mg/kg [$\chi^2(4) = 13.11$, $p < 0.05$, Dunn's Q statistic, $p < 0.05$]. The effects of zaleplon were similar such that doses of 10 and 17.8 mg/kg engendered a significant ataxic-like effects [$\chi^2(4) = 15.64$, $p < 0.05$, Dunn's Q statistic, $p < 0.05$].

Zolpidem and zaleplon also engendered dose-related decreases in the hind limb resistance scores (Fig. 2, bottom panels). Unlike the scores for ataxic-like effects, decreases in resistance (i.e. increases in myorelaxant-like effects) only were induced by the highest dose of both zolpidem [$\chi^2(4) = 14.75$, $p < 0.05$, Dunn's Q statistic, $p < 0.05$] and zaleplon [$\chi^2(4) = 14.86$, $p < 0.05$, Dunn's Q statistic, $p < 0.05$].

Effects of the $\alpha 1\text{GABA}_A$ -Preferring Antagonist βCCT

Previous results have shown that βCCT (3.0 mg/kg, i.m.) does not affect motor behavior when administered alone, and it antagonizes some of the behavioral effects of zolpidem and non-selective BZs (Platt et al. 2002). When administered as a pretreatment, βCCT attenuated the ataxic-like effects of low-to-high doses of both the non-selective BZs and the $\alpha 1\text{GABA}_A$ -preferring agonists (Figs. 3 and 4, top panels). With the exception of chlordiazepoxide, this attenuation of ataxic-like effects by βCCT was surmountable (higher doses of chlordiazepoxide were not tested due to solubility limitations leading to relatively high injection volumes). Overall, βCCT pretreatment resulted in rightward shifts in the dose-response functions for ataxic-like effects for all compounds except chlordiazepoxide, and this antagonism is evident in the 2- to 4-fold greater ED_{50} values computed for the combination of each agonist plus βCCT compared to agonist alone (Table 1).

βCCT also attenuated myorelaxant-like effects induced by the non-selective BZs but only at the lowest dose of agonist (or "middle" dose for chlordiazepoxide, 30 mg/kg); and for the $\alpha 1\text{GABA}_A$ -selective agonists no antagonism was apparent (Figs. 3 and 4, bottom panels). In fact, the myorelaxant-like effects of one dose of zolpidem (3.0 mg/kg) were enhanced by administration of βCCT (Bonferroni t -test, $p < 0.05$). For both the non-selective BZs and the $\alpha 1\text{GABA}_A$ -preferring agonists, βCCT did not significantly alter the ED_{50} values (Table 1).

Discussion

These studies further established observational methods for evaluating potential measures of ataxia and myorelaxation induced by BZ-type drugs in a non-human primate species (squirrel monkey). We also investigated the potential role of $\alpha 1\text{GABA}_A$ receptor mechanisms underlying these effects. Although previous reports from our laboratory have provided an analysis of the ability of BZ-type compounds to engender motor effects (Platt et al. 2002; Licata et al. 2005; Rowlett et al. 2005b), the present studies were designed to compare more fully the ability of both conventional BZs and $\alpha 1\text{GABA}_A$ receptor-preferring agonists to disrupt balance on a pole (ataxic-like effects) and decrease resistance to hind-limb flexion (myorelaxant-like effects). The primary advantage of these techniques, in addition to the use of quantitative observation methods to improve reliability and reproducibility across laboratories, is the ease of use and non-invasive nature of the methodology (i.e., no implants are required, as with electromyography). However, it should be noted that as with all indirect measures of motor coordination and function, validation by direct measures is required before

firm conclusions can be made regarding the actual phenomena of interest (e.g., myorelaxation is most precisely measured via electromyography), hence the use of the phrases “ataxic-like” and “myorelaxant-like” in this report. Finally, our data provide support for the hypothesis that $\alpha 1$ subunit-containing GABA_A receptors likely do not play a key role in the myorelaxant-like effects of BZ-type drugs in monkeys.

Of the compounds tested, nearly all induced maximum median scores for both ataxic-like and myorelaxant-like effects. The primary exception to this pattern of effects was chlordiazepoxide, which consistently engendered lower-than-maximal median scores for ataxic-like effects. These observations are consistent with reports of chlordiazepoxide’s relatively distinctive profile in behavioral studies. For example, drug discrimination studies have suggested that chlordiazepoxide may interact more selectively with $\alpha 2$, $\alpha 3$, and $\alpha 5$ GABA_A receptors than $\alpha 1$ GABA_A receptors (Sanger and Benavides 1993; Lelas et al. 2001a). However, the complete abolition of chlordiazepoxide’s ataxic effects by pretreatment with β CCT in the present study suggests that chlordiazepoxide’s effects may be influenced by $\alpha 1$ GABA_A receptors as well, consistent with its profile as a non-selective agonist. Alternatively, the variable behavioral effects induced by chlordiazepoxide could result from its complex metabolic pathway since it is transformed into four active metabolites, none of which have been characterized in the present behavioral methods or with respect to their interactions with the GABA_A receptor (Greenblatt et al. 1978). However, diazepam shares many of these same metabolites and did not differ appreciably from the triazolobenzodiazepines with respect to ataxic-like and myorelaxant-like profiles.

Our findings are consistent with a growing body of literature suggesting that BZ-induced ataxia may be an $\alpha 1$ GABA_A receptor-mediated phenomenon. In support of this idea, the experiments in which monkeys were pretreated with the $\alpha 1$ GABA_A-preferring antagonist β CCT showed a clear attenuation of the ataxic-like effects produced by both the $\alpha 1$ GABA_A-preferring and non-selective BZ agonists. Rightward shifts were observed for those dose-response functions. These findings are consistent with an earlier study from our laboratory (Platt et al. 2002), and more recently, we have shown that L-838,417, a compound lacking efficacy at $\alpha 1$ GABA_A receptors, failed to engender ataxia (Rowlett et al. 2005b), and SL651498 (6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyridol[3,4-b]indol-1-one), a compound with only partial agonist efficacy at $\alpha 1$ receptors, demonstrated a relatively weak ataxic effect at the highest dose tested (Licata et al. 2005). Nonetheless, it is important to note that our studies cannot rule out a role for other receptor subtypes in mediating ataxic-like effects induced by BZ-type drugs—this type of information awaits the availability of selective antagonists for each of the subtypes.

Our findings with monkeys are very similar to data obtained in studies using rodent models. For example, reports with several compounds that have weak or no activity at $\alpha 1$ GABA_A receptors compared with $\alpha 2$ GABA_A, $\alpha 3$ GABA_A, and $\alpha 5$ GABA_A receptors have shown a reduced propensity to engender ataxia evaluated by the rotarod test and measures of spontaneous locomotor activity (e.g., Savic et al. 2007; but see also Popik et al. 2006). Finally, studies performed in genetically altered mice in which the $\alpha 1$ subunit is rendered insensitive to the effects of BZs have shown that diazepam engendered less ataxia in these mice compared to their wild-type counterparts (McKernan et al. 2000; Rudolph et al. 2001). Together, these findings provide support for the idea that stimulation of $\alpha 1$ GABA_A receptors plays a role in BZ-induced ataxia, and extend these observations to a monkey species.

In addition to BZ-induced ataxic-like effects, all six agonists engendered robust myorelaxant-like effects. Unlike ataxic-like effects, studies using functionally selective compounds in monkeys (Licata et al. 2005; Rowlett et al. 2005b) or genetically altered mice (Crestani et al. 2001; 2002) have suggested a more prominent role for $\alpha 2$ and/or $\alpha 3$ GABA_A receptors in the

myorelaxant effects of BZs and BZ-type compounds. At first glance, this hypothesis does not appear to be supported by the data presented here, since both zolpidem and zaleplon induced significant myorelaxant-like effects. Despite their binding preference for $\alpha 1$ GABA_A receptors, however, zolpidem and zaleplon bind to and have intrinsic efficacy at $\alpha 2$ GABA_A and $\alpha 3$ GABA_A receptors (see Sanna et al. 2002). Potential mediation of zolpidem- and zaleplon-induced myorelaxation by these subtypes of the GABA_A receptor may be implied in our studies by the need for somewhat higher (and presumably less selective) doses of both drugs to induce myorelaxant-like effects.

Although β CCCT attenuated the myorelaxant-like effects engendered by triazolam, alprazolam, diazepam, and chlordiazepoxide, this antagonism occurred only at relatively low doses of the agonists and did not result in an overall change in potency (measured via ED₅₀ values). Consistent with this finding, we previously demonstrated that β CCCT was generally ineffective in blocking myorelaxation, but not ataxia, induced by single doses of alprazolam, triazolam, chlordiazepoxide, and SL651498 (Licata et al. 2005; Rowlett et al. 2005a). Most strikingly, β CCCT clearly did not antagonize the myorelaxant-like effect engendered by zolpidem and zaleplon, raising the possibility that myorelaxant effects induced by these agonists primarily involve the $\alpha 2$ GABA_A and/or $\alpha 3$ GABA_A receptor subtypes. Overall, this hypothesis is supported by previous results in which myorelaxant-like effects in monkeys were engendered by compounds functionally-selective for $\alpha 2$ GABA_A and/or $\alpha 3$ GABA_A receptor subtypes (Rowlett et al. 2005b; Licata et al. 2005).

In summary, these findings suggest that BZ agonist-induced ataxic-like and myorelaxant-like effects in squirrel monkeys can be dissociated by antagonism studies with β CCCT, implying that these two observable effects may require activation of different GABA_A receptor subtypes. These studies particularly raise the possibility that $\alpha 1$ GABA_A receptors contribute to a lesser extent to the myorelaxant effects of these drugs. These studies further suggest that compounds with limited efficacy and/or potency at $\alpha 1$ GABA_A receptors may be better poised to have fewer motor side effects than either non-selective or $\alpha 1$ GABA_A-selective compounds.

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Figure 1.

Ataxic-like and myorelaxant-like effects of the non-selective benzodiazepines triazolam, alprazolam, diazepam, and chlordiazepoxide. Data are median $\pm$ interquartile range from N=4–5 monkeys. Asterisks represent significant differences relative to saline control sessions ($p < 0.05$).

Figure 2.

Ataxic-like and myorelaxant-like effects of the $\alpha 1$ GABA_A receptor-preferring agonists zolpidem and zaleplon. Data are median $\pm$ interquartile range from N=4 monkeys. Asterisks represent significant differences relative to saline control sessions ($p < 0.05$).

Figure 3.

Ataxic-like and myorelaxant-like effects of the non-selective benzodiazepines triazolam, alprazolam, diazepam, and chlordiazepoxide, in the presence and absence of the α^1 GABA_A receptor-preferring antagonist β CCT (3.0 mg/kg). Data are median $\pm$ interquartile range from N=4 monkeys. Asterisks represent significant differences relative to agonist alone ($p < 0.05$). Horizontal dashed lines represent median values for vehicle and 3.0 mg/kg β CCT alone. β CCT had no effects when tested alone under any condition (all scores = 0).

Figure 4.

Ataxic-like and myorelaxant-like effects of the $\alpha 1$ GABA_A receptor-preferring agonists zolpidem and zaleplon in the presence and absence of the $\alpha 1$ GABA_A receptor-preferring antagonist β CCT (3.0 mg/kg). Data are median $\pm$ interquartile range from N=4 monkeys. Asterisks represent significant differences relative to agonist alone ($p < 0.05$). Horizontal dashed lines represent median values for vehicle and 3.0 mg/kg β CCT alone. β CCT had no effects when tested alone under any condition (all scores = 0).

Table 1
Potencies and antagonism of the ataxic and myorelaxant effects of benzodiazepine-type drugs by β CCT in squirrel monkeys

Drug	ED ₅₀ mean mg/kg ± SEM)		Dose Ratio ^a (mean ± SEM)	P<0.05? ^b
	Alone	+ βCCT (3.0 mg/kg)		
<i>Ataxia</i>				
lam	0.07 ± 0.01	0.3 ± 0.05	4.0 ± 0.50	Yes
Alprazolam	0.3 ± 0.03	0.8 ± 0.08	2.7 ± 0.27	Yes
Diazepam	2.6 ± 0.8	8.8 ± 0.5	3.6 ± 0.73	Yes
Chlordiazepoxide	11± 7.1	>56	> 3.0	
Zolpidem	3.0 ± 0.9	16 ± 1.2	6.0 ± 1.1	Yes
Zaleplon	3.3 ± 0.3	15 ± 1.6	4.5 ± 0.8	Yes
<i>Muscle Resistance</i>				
Triazolam	0.09 ± 0.01	0.1 ± 0.01	1.1 ± 0.1	No
Alprazolam	0.2 ± 0.02	0.3 ± 0.00	1.5 ± 0.3	No
Diazepam	1.7 ± 0.2	4.4 ± 1.7	2.3 ± 0.8	No
Chlordiazepoxide	27 ± 2.8	41 ± 0.00	1.5 ± 0.2	No
Zolpidem	8.8 ± 0.8	7.6 ± 0.9	0.9 ± 0.2	No
Zaleplon	9.0 ± 0.7	9.2 ± 0.8	1.0 ± 0.4	No

ED₅₀ is the dose of drug resulting in 50% of the maximum effect.

^aDose ratio = agonist + β CCT ED₅₀ divided by agonist alone ED₅₀.

^bDifference between agonist and agonist + β CCT, Student's t-tests.