Different GABA_A receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates

James K. Rowlett*,1, Donna M. Platt*, Snjezana Lelas*, John R. Atack‡, and Gerard R. Dawson‡

*Harvard Medical School, New England Primate Research Center, One Pine Hill Road, Box 9102, Southborough, MA 01772; and ‡Neuroscience Research Centre, Merck, Sharp, and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, United Kingdom

Edited by Eugene Roberts, Beckman Research Institute of the City of Hope, Duarte, CA, and approved December 6, 2004 (received for review August 17, 2004)

Benzodiazepines exert their effects by binding to multiple subtypes of the GABA_A receptor, the predominant subtypes in the brain being those that contain α1-, α2-, α3- and α5-subunits. To understand the potentially different roles of these subtypes in the therapeutic and side effects of benzodiazepines, we evaluated GABA_A receptor subtype-preferring compounds in nonhuman primate models predictive of anxiolytic, sedative, motor, subjective, and reinforcing effects of benzodiazepine-type drugs. These compounds included zolpidem, which shows preferential binding to GABA_A receptors containing α1-subunits (α1GABA_A receptors); L-838,417, which shows functional selectivity for α2GABA_A, α3GABA_A, and α5GABA_A receptors; and nonselective conventional benzodiazepines. The results provide evidence in nonhuman primates that α1GABA_A receptors do not play a key role in the anxiolytic and muscle-relaxant properties of benzodiazepine-type drugs; instead, these effects involve α2GABA_A, α3GABA_A, and/or α5GABA_A subtypes. Our results also suggest that the α1GABA_A receptor subtype might be critically involved in the subjective, sedative, and motor effects of benzodiazepine-type drugs. In contrast, stimulation of α1GABA_A receptors is sufficient, but not necessary, for mediation of the abuse potential of these drugs.

Receptors for the neurotransmitter GABA, in particular the type A subtype (GABA_A receptor), have received considerable attention as the site of action for drugs acting as anxiolytics, sedatives, anticonvulsants, and muscle relaxants. These clinically beneficial effects are exhibited by the benzodiazepines, which act by allosterically binding to GABA_A receptors and enhancing the ability of GABA to increase chloride conductance. The therapeutic use of benzodiazepines is constrained, however, by other characteristic effects of these drugs, such as daytime drowsiness and impairment of motor coordination. Benzodiazepines additionally have subjective and reinforcing effects that might contribute to their widespread abuse (1). Recent studies have revealed the existence of multiple subtypes of the GABA_A receptor (2, 3), and research with transgenic mice has postulated that the diverse behavioral effects of benzodiazepine-like drugs may reflect action at different subtypes of GABA_A receptors (3–5). Although provocative, the extent to which these findings in transgenic mice are applicable to other species, especially primates, is not known. Moreover, virtually no information is available regarding the role of specific GABA_A receptor subtypes in the addictive properties of benzodiazepines in any species.

The GABA_A receptors in the central nervous system are pentamers constituted from structurally distinct proteins, with each protein family consisting of different subunits (for review, see ref. 3). The majority of GABA_A receptors consist of α-, β-, and γ-subunit families, and benzodiazepine action appears to be determined by the presence of particular α-subunits. Benzodiazepine-like drugs bind predominantly to a site on the native GABA_A receptor that occurs at the interface of the γ2-subunit with either α1-, α2-, α3-, or α5-subunits, whereas these drugs are inactive at α2-subunit- and α6-subunit-containing receptors. More than 90% of the GABA_A receptors in the brain contain α1-, α2-, and α3-subunits (6), and GABA_A receptors containing the α1-subunit (α1GABA_A receptors) recently have been implicated in the sedative effects of benzodiazepines, whereas GABA_A receptors containing α2- and α3-subunits (α2GABA_A and α3GABA_A receptors) have been implicated in the anxiolytic effects of benzodiazepines (4, 5). Receptors containing α5-subunits, in contrast, are a relatively minor population that might play a role in memory processes, but not anxiolysis or motor effects (7, 8).

Efforts to delineate the contribution of GABA_A receptor subtypes in mediating the multiple effects of benzodiazepines have been hampered by the absence of compounds with substantial selectivity for the individual receptor subtypes. Recently, McKernan and colleagues (4) described the compound L-838,417 that, unlike previous benzodiazepine compounds, exhibits “functional selectivity” rather than binding selectivity for the α2GABA_A, α3GABA_A, and α5GABA_A subtypes. That is, L-838,417 does not bind differentially to GABA_A receptor subtypes; instead it is antagonist at GABA_A receptors containing α5-, α3-, and α2-subunits, but an agonist at α1GABA_A receptors. Because L-838,417 exhibits no appreciable efficacy at α1GABA_A receptors, the extent to which this compound lacks an effect characteristic of conventional benzodiazepines can be used to determine the role for the α1GABA_A subtype in benzodiazepine agonist action. Using this approach, in the present study, we compared the ability of L-838,417 with those of the α1GABA_A-prefering agonist zolpidem, as well as nonselective benzodiazepines to engender characteristic anxiolytic, motor, and sedative effects in monkeys. We also compared the effects of L-838,417 with reference drugs in primate models of the subjective and reinforcing effects of benzodiazepines to determine whether the unique subjective properties and the abuse potential associated with benzodiazepine-type drugs involve different GABA_A receptor subtypes.

Methods

Animals. Subjects were adult rhesus monkeys (Macaca mulatta) for the conflict and self-administration procedures and adult squirrel monkeys (Saimiri sciureus) for the observation and drug discrimination studies. Monkeys in the conflict and discrimination studies were maintained at 85–95% of their free-feeding weights, whereas the other monkeys were not food-restricted. Monkeys were individually housed and maintained on a 12-h
Catheters were implanted into a major vein (jugular, brachial, or femoral) according to the procedures described by Carey and Spealman (9). 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).

**Conflict Procedure.** Four rhesus monkeys (two males and two females) were trained under a multiple schedule of food reinforcement consisting of two components: (i) a schedule of food delivery and (ii) a schedule of food delivery plus a schedule of foot shock delivery. At the beginning of a session, monkeys were seated in restraint chairs (Crist Instruments, Hagerstown, MD) and placed in an experimental chamber (Med Associates, Georgia, VT). 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 a 18-response, fixed-ratio schedule of food pellet delivery (1 g, Bioserve, Frenchtown, NJ). Each component consisted of the schedule of food delivery signaled by red stimulus lights, followed immediately by the same schedule of food delivery combined with a 20-response, fixed-ratio schedule of foot shock delivery (1.5–3.0 mA, 0.25-s duration), signaled by green stimulus lights. Each response requirement was followed by a 10-s timeout. On training days, monkeys received i.v. injections of saline (2 ml) in the fifth minute of each 10-min timeout. On test days (Tuesdays and Fridays), i.v. injections of vehicle or drug were administered in the fifth minute. Data were expressed as the mean responses per s (±SEM) for each dose of test compound.

**Observation Procedure.** Four male squirrel monkeys were initially habituated to an observation arena (described in ref. 10) for ~1 month. After habitation, 30-min observational sessions were conducted daily, during which the animal's behavior was videotaped continuously. Drug test sessions were conducted once or twice per week, with saline control sessions on intervening days. All drugs, as well as saline controls, were administered i.m. in a calf or thigh muscle. During the 6th, 18th, and 30th min of each 30-min session, the monkeys were removed briefly from the observation arena by a trained handler and evaluated for ataxia, defined as the inability to balance on a stainless-steel transport pole (length, 56.0 cm; diameter, 1.0 cm) held in the horizontal plane. During each ataxia assessment, a score of 0 indicated that the monkey was able to balance normally on the transport pole, a score of 1 indicated inability to balance (e.g., hang suspended by limbs below pole), and a score of 2 indicated that the monkey could neither balance nor support its weight on the pole.

A different technique was included during the hands-on observation arena by a trained handler and evaluated for ataxia, defined as the inability to balance on a stainless-steel transport pole (length, 56.0 cm; diameter, 1.0 cm) held in the horizontal plane. During each ataxia assessment, a score of 0 indicated that the monkey was able to balance normally on the transport pole, a score of 1 indicated inability to balance (e.g., hang suspended by limbs below pole), and a score of 2 indicated that the monkey could neither balance nor support its weight on the pole.

Scoring of videotapes was conducted by observers trained to use the behavioral scoring system described in ref. 10. The observer was not informed about the drugs under investigation. Four observers performed the videotape scoring for the duration of this study. To assure reliability across observers, all individuals underwent at least 20 h of training until they reached an interobserver reliability criterion of ≥90% based on percent agreement scores. The monkeys were scored for locomotor activity, defined as any two or more directed steps in the horizontal and/or vertical plane, and the appearance of sedation, defined as procumbent posture (i.e., loose-limbed, sprawled, unable to maintain an upright position). These behaviors were scored by recording their presence or absence in 15-s intervals during three 5-min observation periods across the session (0–5, 12–17, and 24–29 min). Frequency scores were calculated from these data as the proportion of 15-s intervals in which a particular behavior occurred, and the maximum possible score was 20.

**Drug Discrimination.** Monkeys were previously trained to discriminate 0.03 mg/kg triazolam i.v. from saline (11). Briefly, five male squirrel monkeys were placed in restraint chairs (Med Associates), and each monkey was trained to respond on both levers under a fixed-ratio 10-response schedule of food reinforcement. Training sessions consisted of one to four 15-min cycles. A cycle consisted of a 10-min pretreatment period and a 5-min response period. During the pretreatment period, the chamber was dark and responses had no programmed consequence. An injection of either saline or drug (triazolam, 0.03 mg/kg, i.v.) was administered during the fifth minute of the pretreatment period. During the response period, stimulus lights were illuminated, and 10 consecutive responses on the lever designated correct by the injection administered during the pretreatment period of the cycle resulted in food delivery. A 10-s timeout occurred after food delivery or if the response requirement was not met within 60 s. Responses on the incorrect lever did not result in food delivery and reset the response requirement on the correct lever. Response periods ended after 5 min or the delivery of 10 pellets, whichever occurred first. The number of cycles per session and order of saline and drug cycles varied randomly with the constraint that if a drug cycle was scheduled it was always the last cycle of the session.

Drug test sessions were conducted once or twice per week with training sessions scheduled on intervening days. Test sessions were conducted if 80% or more of total responses occurred on the correct lever for at least four of five training sessions. Test sessions were conducted if 80% or more of total responses occurred on the correct lever during the fifth minute of each cycle. Testing with a particular drug continued up to the doses that engendered 80% or more of responses on the drug-appropriate lever or that decreased response rates to 20% or less of control response rates. Percent drug-lever responding was computed for individual subjects in each cycle of a test session by dividing the number of responses on the drug lever by the total number of responses on both levers and multiplying by 100. Discrimination data for an individual subject were excluded from the analyses if response rates were 20% or less of response rates during vehicle tests. Full substitution was defined as 80% or more drug-lever responding, partial substitution as 20–80% drug-lever responding, and no substitution as <20% or less drug-lever responding.

**Self-Administration Procedure.** Five rhesus monkeys (three male, two female) were trained to self-administer the short-acting barbiturate methohexital under a progressive-ratio schedule of i.v. drug injection as described (12). Monkeys were housed individually in stainless-steel primate cages (Harford Metal Products, Aberdeen, MD) that served as the experimental chambers. A removable panel was placed on the front of each cage and contained four stimulus lights (two red and two white; 3 cm, 1.1 W; Med Associates) and a response lever (Med
Associates). Each monkey was fitted with a nylon-mesh jacket (Lomir Biomedical, Malone, NY) that was connected to a 1-in stainless-steel flexible tether (Lomir Biomedical). The monkey’s catheter was routed through the tether and attached to a fluid swivel (Lomir Biomedical) on top of the cage. The swivel was attached to an injection pump (Med Associates) located on top of the cage, which could infuse drug solutions at a rate of 0.2 ml/s. The stimulus lights, response levers, and infusion pump were connected to interfaces (Med Associates) and PC-compatible computers located in an adjacent room.

At the beginning of a daily session, the white stimulus lights above the lever were illuminated to signal the start of a trial. Upon completion of the response requirement, the white lights were extinguished and the red stimulus lights were illuminated for 1 s, coinciding with a 1-s infusion. Each trial ended with either an injection or the expiration of a 30-min limited hold. Trials were separated by a 30-min timeout period, during which all lights were extinguished and responding had no programmed consequences.

Experimental sessions consisted of five components made up of four trials each, for a maximum possible of 20 trials per session. The response requirement remained constant for each of the four trials within a component and doubled during each successive component. The session ended when a monkey self-administered a maximum of 20 injections or when the response requirement was not completed for two consecutive trials. The number of trials per response requirement was chosen so that the maximum number of injections could be completed within a day (maximum session time was ~10 h).

Monkeys were trained to self-administer 0.3 mg/kg per injection of methohexital under a progressive-ratio schedule beginning with a response requirement of 40 responses per injection. Thus, the sequence of possible response requirements was 40, 80, 160, 320, and 640 responses per injection. Once performance was stable under these conditions (no increasing or decreasing trend in the number of injections per session for three consecutive sessions), saline was substituted for methohexital until responding declined to low levels and was stable. Methohexital was again made available for at least three sessions, and doses of benzodiazepine agonists were available in each monkey for the same number of sessions required for responding to decline under conditions of saline availability. The number of injections per session and the break points (BPs) were determined for individual monkeys under each test condition. BP, defined as the session and the break points (BPs) were determined for individual monkeys under each test condition. BP, defined as the

**Results**

**Anxiolytic-Like Effects.** The reference benzodiazepine diazepam induced anticonflict effects, characterized as a reliable increase in food-maintained behavior that was suppressed by electric shock presentations (Fig. 1, ○; Dunnett’s tests, P < 0.05). Similarly, the functionally selective α2,3,5GABA_A agonist L-838,417 induced anticonflict behavior to the same degree as diazepam (Fig. 2, □). In contrast, the preferential α1GABA_A agonist zolpidem lacked anticonflict effects (Fig. 2, Dunnett’s tests, P > 0.05), implying that preferential action at α2,3,5GABA_A, α1GABA_A, and/or α4GABA_A receptors mediates the anxiolytic-like effects of benzodiazepine-type drugs.

Conventional benzodiazepines characteristically have anticonflict effects at doses lower than those that disrupt nonconflict behavior, a pattern of effects that was observed in the present study with diazepam (Dunnett’s tests, P < 0.05; data not shown, see Fig. 7, which is published as supporting information on the

**Fig. 1.** Effects of zolpidem (○, α1GABA_A-preferring agonist), L-838,417 (□, agonist with functional selectivity for α2GABA_A, α3GABA_A, and α5GABA_A receptors), and diazepam (●, nonselective agonist) on behavior maintained by food presentation that was suppressed by presentation of mild electric shock (n = 4 rhesus monkeys). V, vehicle (50% propylene glycol, 50% saline). * P < 0.05 vs. vehicle, Dunnett’s tests.

**Fig. 2.** Ability of zolpidem (○), L-838,417 (□), and diazepam (●) to engender muscle relaxation (a) and ataxia (b) in squirrel monkeys (n = 4). Data are the mean cumulative score (±SEM). * P < 0.05 vs. vehicle, Dunnett’s tests.
PNAS web site). Zolpidem also disrupted nonconflict behavior at the two highest doses tested (Dunnett’s tests, P < 0.05; data not shown; see Fig. 7). However, over the dose range tested L-838,417 did not reliably disrupt nonconflict behavior.

**Motor Effects.** Using a behavioral scoring system to quantify drug effects analogous to muscle relaxation and ataxia in monkeys, we found that both diazepam and the subtype selective benzodiazepine agonists induced reliable and comparable levels of muscle relaxation (Fig. 2a, Dunnett’s tests, P < 0.05). In contrast, maximum scores for ataxia were observed with diazepam and zolpidem only, whereas L-838,417 had no reliable ataxic effects (Fig. 2b). In addition, diazepam and zolpidem reliably suppressed locomotor activity (Fig. 3a, Dunnett’s tests, P < 0.05); whereas L-838,417 was ineffective up to 10 mg/kg (Fig. 3a). We recently have reported an observable measure of sedation in monkeys, characterized as an immobile and flaccid posture (10). As shown in Fig. 3b, diazepam and zolpidem induced reliable levels of sedation by using this measure, whereas L-838,417 did not induce sedation up to a relatively large dose of 10 mg/kg. Overall, these results suggest that in primates, stimulation of GABA<sub>A</sub>-preferring agonist zolpidem, like diazepam, substituted in monkeys trained to discriminate triazolam (Fig. 4), a prototypical benzodiazepine that is an agonist at all GABA<sub>A</sub> receptor subtypes. In contrast, L-838,417 did not substitute for triazolam up to a dose of 10 mg/kg (Fig. 4).

Because L-838,417 has very low efficacy at α<sub>1</sub>GABA<sub>A</sub> receptors, we next examined the extent to which this compound could antagonize the discriminative stimulus effects of triazolam. We found that pretreatments with L-838,417 shifted the dose–response function for triazolam to the right (Fig. 5). Therefore, over a dose range that did not substitute for triazolam, L-838,417 was a pharmacological antagonist of the subjective effects of a reference agonist.

**Self-Administration.** To date, very little information exists regarding the role of different GABA<sub>A</sub> receptor subtypes in mediating the addictive potential of benzodiazepine-type drugs. We evaluated the ability of zolpidem and the functionally selective α<sub>2,3,5</sub>GABA<sub>A</sub> agonist L-838,417 to maintain responding in a progressive-ratio schedule of i.v. methohexital delivery. Zolpidem maintained near maximum performance, with a mean number of injections per session of 17 (of 20) at a dose of 0.03 mg/kg per injection (Fig. 6a). L-838,417 also maintained self-administration reliably above vehicle levels, with a maximum mean number of injections per session of 8.0 (Dunnett’s tests,

---

**Fig. 3.** Effects of zolpidem (○), L-838,417 (□), and diazepam (●) on observable measures related to sedation, including locomotor activity (a) and procumbent posture (sedation, b), in squirrel monkeys (n = 4). Data are expressed as the mean frequency score (±SEM). *P < 0.05 vs. vehicle, Dunnett’s tests.

**Fig. 4.** Effects of selective and nonselective benzodiazepine agonists in squirrel monkeys (n = 5) trained to discriminate triazolam (0.03 mg/kg) from vehicle. Data are mean (±SEM) percentage of responding on the triazolam-associated lever. ▼, Triazolam; ●, diazepam; ○, zolpidem; □, L-838,417.

**Fig. 5.** Antagonism of the effects of triazolam by L-838,417 in squirrel monkeys (n = 4) trained to discriminate triazolam (0.03 mg/kg) from vehicle. Data are mean (±SEM) percentage of responding on the triazolam-associated lever. ▼, Triazolam alone; □, +0.1 L-838,417; △, +0.3 L-838,417; ○, +1.0 L-838,417.
indicate that similar to nonselective benzodiazepine agonists respectively (data not shown, see Fig. 8, which is published as (Bonferroni tests, obtained for zolpidem or the nonselective benzodiazepines whereas activity at cient, but not necessary, to maintain self-administration, which the two compounds is that L-838,417 is active at α5GABA receptors, whereas zolpidem is not (3). Although differences in the anxiolytic effects of L-838,417 and zolpidem might be attributable to differences in action at α5GABA receptors, this possibility seems unlikely because α5GABA receptors exist almost exclusively in the hippocampus (6), and mice with a point mutation rendering this receptor subtype insensitive to benzodiazepines show normal anxiolytic-like responses to diazepam (7, 8). Moreover, experiments with transgenic mice point to the α5GABA subtype in mediating anxiolytic effects. In this regard, transgenic mice with a point mutation rendering the α5GABA receptor insensitive to benzodiazepines showed no anxiolytic response to diazepam (5). On the contrary, a compound with inverse agonist effects at the α5GABA receptor but not the α5GABA receptor was anxiogenic in rodents (14). These latter findings raise the possibility that the exclusive role for α5GABA receptors in mediating anxiolytic effects in genetically modified mice might not generalize to nongenetically modified animals. Alternatively, the α5GABA and α5GABA receptor subtypes might interact in mediating benzodiazepine-induced anxiolysis. Regardless, our findings support a key role for α5GABA receptors in mediating the anxiolytic effects of benzodiazepine-type drugs in primates.

In addition to reducing anxiety, benzodiazepines are effective clinically as muscle relaxants. A unique property of L-838,417 reported here is its ability to engender muscle relaxation in the absence of other motor effects, a finding that we have not observed in this procedure with any other type of compound to date (including opiates, barbiturates, and dopamine antagonists). This finding is consistent with the observation that diazepam is ineffective as a muscle relaxant in mutant mice in which the α5GABA receptor is insensitive to diazepam because of a point mutation (15) and provides evidence in primates for a role of a specific GABA receptor in the muscle relaxation induced by conventional benzodiazepines.

It is well documented that the clinical use of benzodiazepines is restricted by their undesirable side effects. For example, treatment of anxiety disorders with benzodiazepines often is hampered by motor incoordination, which can prevent a patient from engaging in important activities such as driving an automobile. In the present study, both the α1GABA-preferring agonist zolpidem and the nonselective benzodiazepine diazepam induced a marked impairment in the ability of monkeys to balance on a pole, indicative of ataxia. In contrast, L-838,417, which lacks activity at α1GABA receptors, did not engender ataxia over the dose range tested. These results agree with our previous finding that benzodiazepine-induced ataxia in monkeys was blocked by a α1GABA receptor-selective antagonist (10). Thus, our findings suggest that this receptor subtype plays a key role in motor impairments often observed with clinical use of benzodiazepines. It should be noted, however, that L-838,417 possesses intermediate efficacy at α2GABA, α3GABA, and α5GABA receptors, raising the possibility that a lack of ataxia may reflect this compound’s relatively lower efficacy at GABA receptors compared with conventional benzodiazepines.

Another commonly observed effect that limits the use of benzodiazepines as anxiolytics and muscle relaxants is the occurrence of daytime drowsiness. Sedative effects are a documented property of α1GABA-preferring drugs, and our finding that zolpidem suppressed locomotor activity and induced procumbent posture in monkeys is consistent with the reported sedative properties of this drug. In contrast, L-838,417 lacked sedative effects over the doses receptors. In contrast, the reinforcing effects of these drugs might involve all receptor subtypes. These findings demonstrate GABA receptor subtype-specific behaviors of benzodiazepines in nonhuman primates.

Although the most notable difference between L-838,417 and zolpidem is their actions at α5GABA receptors, another difference between the two compounds is that L-838,417 is active at α5GABA receptors, whereas zolpidem is not (3). Although differences in the anxiolytic effects of L-838,417 and zolpidem might be attributable to differences in action at α5GABA receptors, this possibility seems unlikely because α5GABA receptors exist almost exclusively in the hippocampus (6), and mice with a point mutation rendering this receptor subtype insensitive to benzodiazepines show normal anxiolytic-like responses to diazepam (7, 8). Moreover, experiments with transgenic mice point to the α5GABA subtype in mediating anxiolytic effects. In this regard, transgenic mice with a point mutation rendering the α5GABA receptor insensitive to benzodiazepines showed no anxiolytic response to diazepam (5). On the contrary, a compound with inverse agonist effects at the α5GABA receptor but not the α5GABA receptor was anxiogenic in rodents (14). These latter findings raise the possibility that the exclusive role for α5GABA receptors in mediating anxiolytic effects in genetically modified mice might not generalize to nongenetically modified animals. Alternatively, the α5GABA and α5GABA receptor subtypes might interact in mediating benzodiazepine-induced anxiolysis. Regardless, our findings support a key role for α5GABA receptors in mediating the anxiolytic effects of benzodiazepine-type drugs in primates.

In addition to reducing anxiety, benzodiazepines are effective clinically as muscle relaxants. A unique property of L-838,417 reported here is its ability to engender muscle relaxation in the absence of other motor effects, a finding that we have not observed in this procedure with any other type of compound to date (including opiates, barbiturates, and dopamine antagonists). This finding is consistent with the observation that diazepam is ineffective as a muscle relaxant in mutant mice in which the α5GABA receptor is insensitive to diazepam because of a point mutation (15) and provides evidence in primates for a role of a specific GABA receptor in the muscle relaxation induced by conventional benzodiazepines.

It is well documented that the clinical use of benzodiazepines is restricted by their undesirable side effects. For example, treatment of anxiety disorders with benzodiazepines often is hampered by motor incoordination, which can prevent a patient from engaging in important activities such as driving an automobile. In the present study, both the α1GABA-preferring agonist zolpidem and the nonselective benzodiazepine diazepam induced a marked impairment in the ability of monkeys to balance on a pole, indicative of ataxia. In contrast, L-838,417, which lacks activity at α1GABA receptors, did not engender ataxia over the dose range tested. These results agree with our previous finding that benzodiazepine-induced ataxia in monkeys was blocked by a α1GABA receptor-selective antagonist (10). Thus, our findings suggest that this receptor subtype plays a key role in motor impairments often observed with clinical use of benzodiazepines. It should be noted, however, that L-838,417 possesses intermediate efficacy at α2GABA, α3GABA, and α5GABA receptors, raising the possibility that a lack of ataxia may reflect this compound’s relatively lower efficacy at GABA receptors compared with conventional benzodiazepines.

Another commonly observed effect that limits the use of benzodiazepines as anxiolytics and muscle relaxants is the occurrence of daytime drowsiness. Sedative effects are a documented property of α1GABA-preferring drugs, and our finding that zolpidem suppressed locomotor activity and induced procumbent posture in monkeys is consistent with the reported sedative properties of this drug. In contrast, L-838,417 lacked sedative effects over the doses

**Discussion**

The results of the present study provide important evidence for a differential role of GABA receptor subtypes in the anxiolytic, motor, subjective, and reinforcing effects of benzodiazepines in nonhuman primates. Specifically, the anxiolytic effects of benzodiazepine-like drugs appear to involve α2,3GABA receptors, whereas the subjective, ataxic, and sedative properties of benzodiazepine-like drugs likely are mediated by α5GABA receptors. In contrast, the reinforcing effects of these drugs might involve all receptor subtypes. These findings demonstrate GABA receptor subtype-specific behaviors of benzodiazepines in nonhuman primate species.

**Fig. 6.** Self-administration of benzodiazepine agonists by rhesus monkeys trained under a progressive-ratio schedule of i.v. drug delivery. (a) Dose-response functions for the mean number of injections per session (±SEM) maintained by zolpidem and L-838,417 (n = 5 monkeys). *P < 0.05 compared to saline availability, Dunnett’s tests. ○, Zolpidem; □, L-838,417. (b) Maximum BP irrespective of dose (BPmax). Data are mean ± SEM for n = 5 monkeys. Lines represent reliable differences from L-838,417, Bonferroni tests.

P < 0.05; Fig. 6a). For comparison, diazepam and the short duration of action benzodiazepine midazolam increased the mean number of injections per session to maximum averages (±SEM) of 12 (±1.5) and 13 (±1.0) injections per session, respectively (data not shown, see Fig. 8, which is published as supporting information on the PNAS web site). These findings indicate that similar to nonselective benzodiazepine agonists both zolpidem and L-838,417 had reinforcing effects. Thus, our results suggest that stimulation of α1GABA receptors is sufficient, but not necessary, to maintain self-administration, whereas activity at α5GABA, α3GABA, and/or α5GABA receptor subtypes might result in abuse potential.

To determine the extent to which zolpidem and L-838,417 differ in terms of their effectiveness as reinforcers, we compared the BPmax values (i.e., maximum BP irrespective of dose) among these compounds, as well as to the values obtained with nonselective agonists. Zolpidem maintained the highest BPmax values, followed by midazolam, diazepam, and L-838,417 (Fig. 6b). The mean BPmax value for L-838,417 was reliably lower than those obtained for zolpidem or the nonselective benzodiazepines (Bonferroni tests, P < 0.05; Fig. 6b). These findings suggest that although activation of α1GABA, α3GABA, and/or α5GABA receptors is sufficient to engender self-administration, activation of the α5GABA receptor might enhance the reinforcing effects of benzodiazepine ligands. Alternatively, the intermediate intrinsic efficacy of L-838,417 might account for this compound’s reduced reinforcing effectiveness compared with other benzodiazepine agonists.
intermediate efficacy at the lack of effects of L-838,417 might reflect this compound's benzodiazepines (11, 13), although, as with the ataxia measure, the idea that the sedative properties of benzodiazepine-type drugs tested. Thus, as with studies using mutant mice, our findings suggest a critical role for antagonistic of the effects of triazolam over dose ranges similar to lam-like discriminative stimulus effects and proved to be an antagonist of the effects of triazolam over dose ranges similar to those producing anxiolytic-like effects. These results are consistent with our previous findings that suggest a critical role for α₁GABA₁ receptors in the discriminative stimulus effects of benzodiazepines (11, 13), although, as with the ataxia measure, the lack of effects of L-838,417 might reflect this compound's intermediate efficacy at α₂GABA₂, α₃GABA₃, and/or α₁GABA₁ subtypes. To the extent that discriminative stimulus effects reflect subjective effects associated with the abuse of benzodiazepines, these findings suggest that unique subjective effects induced by selective stimulation of GABA₁ receptor subtypes might result in compounds with reduced abuse potential compared with conventional benzodiazepines.

To evaluate the abuse potential of subtype-selective benzodiazepine-type drugs directly, we conducted experiments in which the compounds were available for self-administration under a progressive-ratio schedule of drug delivery (12). L-838,417 maintained reliable self-administration, indicating that this compound can function as a positive reinforcer. Because this compound exhibits virtually no efficacy at α₁GABA₁ receptors in vitro, activity at GABA₁ receptors containing α₂-α₃- and/or α₁-subunits might be sufficient to engender reinforcing effects. Moreover, based on our analysis of BPs, the reinforcing effectiveness of L-838,417 was less than that of zolpidem and benzodiazepines such as diazepam and midazolam. This finding may be attributable to L-838,417 having no efficacy at α₁GABA₁ receptors and/or to the compound having intermediate efficacy at α₂GABA₂, α₃GABA₃, and/or α₁GABA₁ receptors. Interestingly, zolpidem maintained the highest levels of self-administration of any benzodiazepine-type drug tested (see ref. 1). Thus, selective stimulation of the α₁GABA₁ receptor, although not necessary for self-administration, might result in reinforcing effects that are greater than those induced by stimulation of α₂GABA₂, α₃GABA₃, and/or α₁GABA₁ receptor subtypes.

Anxiety disorders are some of the most frequently diagnosed disorders in psychiatric medicine worldwide. Although conventional benzodiazepines are effective anxiotics, their use is restricted in part because of the occurrence of undesirable side effects. The identification of α₂GABA₂ receptors as important for mediating the anxiolytic effects of benzodiazepines, in combination with the possibility that subjective and motor effects might influence action primarily at α₁GABA₁ receptors, provides an important framework for developing improved medications for the treatment of anxiety disorders. As a note of caution, however, our results suggest that intrinsic efficacy at α₂GABA₂, α₃GABA₃, and/or α₁GABA₁ receptor subtypes is sufficient for a benzodiazepine-type compound to possess some abuse potential.

We thank A. Martino and A. Duggan for technical assistance and Drs. R. D. Spealman and D. Reynolds for comments on the manuscript. This work was supported by U.S. Public Health Service Grants DA11792, DA13591, and RR00168.