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The discriminative stimulus effects of midazolam are resistant to modulation by morphine, amphetamine, dizocilpine and γ -butyrolactone in rhesus monkeys

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Abstract

Rationale—Although abuse of benzodiazepines alone is uncommon, it is high in polydrug abusers, including those who abuse primarily opioids or stimulants.

Objectives—This study investigated whether drugs that are abused (e.g., amphetamine) or have mechanisms of action similar to abused drugs (e.g., morphine) alter the discriminative stimulus effects of the benzodiazepine midazolam.

Methods—Three rhesus monkeys discriminated 0.56 mg/kg of midazolam while responding under a fixed ratio 10 schedule of food presentation. Dose-effect curves were determined for midazolam alone and in the presence of morphine (opioid receptor agonist), amphetamine (dopamine receptor indirect agonist), dizocilpine (N-methyl-D-aspartic acid receptor antagonist), or γ -butyrolactone (prodrug of γ -hydroxybutyrate, which acts primarily at GABA_B receptors).

Results—Doses of midazolam larger than 0.32 mg/kg produced $\geq 80\%$ midazolam-lever responding. When administered alone, morphine, amphetamine, dizocilpine and γ -butyrolactone did not produce midazolam-lever responding, although large doses of each drug eliminated responding; when administered in combination with midazolam, they did not alter the discriminative stimulus effects of midazolam up to doses that markedly decreased response rates.

Conclusions—The current study demonstrates a lack of modulation of the discriminative stimulus effects of midazolam by morphine, amphetamine, dizocilpine, and γ -butyrolactone. Other effects of benzodiazepines, such as their reinforcing effects, might be altered by these other drugs, or benzodiazepines might modulate the discriminative stimulus or reinforcing effects of the other drugs, which might contribute to the relatively high incidence of benzodiazepine abuse among polydrug abusers.

Keywords

drug abuse; polydrug abusers; midazolam; drug discrimination; monkeys

Benzodiazepines are positive modulators of γ -aminobutyric acid_A (GABA_A) receptors and are commonly prescribed for anxiety, insomnia and seizure disorders. Despite their large margin of safety, adverse effects can limit their clinical use. For example, benzodiazepines are abused, and their nonmedical use appears to be increasing. From 1992 to 2002,

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admissions for treatment of primary benzodiazepine abuse increased 79%; during that period, overall admissions for substance abuse treatment increased 22% (The DASIS Report, 2005). Despite these increases, the incidence of exclusive benzodiazepine abuse remains relatively low, and benzodiazepines are most commonly abused with other drugs. For example, benzodiazepine abuse is high among heroin abusers (Gelkopf et al. 1999; San et al. 1993) and patients in methadone (Gossop et al. 1999; Peles et al. 2006; Stitzer et al. 1981; Woody et al. 1975) or buprenorphine maintenance programs (Lavie et al., 2009). Benzodiazepine use by patients receiving methadone predicts poorer treatment outcomes, and those patients are more likely to engage in risky behaviors (Ghitza et al. 2008; Bleich et al. 1999). Drug abusers report using benzodiazepines with opioids because they enhance the effects of opioids (Gelkopf et al. 1999; Iguchi et al. 1993; Stitzer et al. 1981), and these observations are supported by laboratory studies. When a combination of methadone and diazepam was given to humans, subjective effects and pupil constriction increased, as compared to effects of either drug administered alone (Preston et al. 1984). In baboons self administering the benzodiazepine flunitrazepam, methadone appears to enhance the reinforcing effects of small doses of flunitrazepam, shifting the dose-effect curve upward or to the left, although that curve did not shift back to the right after discontinuation of methadone treatment (Ator et al. 2005). Nevertheless, both epidemiologic and laboratory studies suggest that benzodiazepines enhance some effects of opioids, which might account for the concurrent abuse of these drugs.

Abusers of amphetamines or cocaine commonly use benzodiazepines (Darke et al. 1994; Degenhardt and Topp 2003), although the drugs are generally not taken simultaneously. Instead, abusers report taking benzodiazepines to reduce the unwanted effects of stimulants, such as paranoia and anxiety, that can occur as an episode of drug use is being discontinued (Degenhardt and Topp 2003; Gelkopf et al. 1999). Benzodiazepines have also been shown to modulate some effects of stimulants in the laboratory. For example, alprazolam decreased the discriminative stimulus and subject-rated effects of amphetamine in humans discriminating amphetamine (Rush et al. 2004), and triazolam attenuated the discriminative stimulus effects of cocaine in some monkeys discriminating cocaine (Negus et al. 2000). Also, alprazolam significantly decreased cocaine self administration at doses that did not alter responding for food in rats (Goeders et al. 1993). Thus, benzodiazepines are coabused with a variety of drugs, and the reasons for taking benzodiazepines with other drugs depend on their mechanism of action; reportedly, benzodiazepines are used with opioids to enhance the desired effects, and with amphetamines and cocaine to decrease unwanted effects.

Finally, benzodiazepines, particularly flunitrazepam, are among a diverse group of drugs that are commonly used at bars, clubs, parties and concerts. In these settings, multiple drugs are sometimes used concurrently (Halkitis and Palamar 2006; Wu et al. 2006), with coabuse likely to occur among readily available drugs, including ketamine, γ -hydroxybutyrate and benzodiazepines. There are few studies on interactions between benzodiazepines and either ketamine or γ -hydroxybutyrate. The effects of ketamine appear to be modified by benzodiazepines in the laboratory. For example, lorazepam reduced ketamine-associated emotional distress in humans and exacerbated the sedative and amnesic effects of ketamine (Krystal et al. 1998). Although reports describing interactions between benzodiazepines and γ -hydroxybutyrate are rare, their acute subjective effects are comparable. Triazolam and γ -hydroxybutyrate produced similar subjective effects in humans, although their profile of effects did not completely overlap (Carter et al. 2009). Nevertheless, these similarities suggest that benzodiazepines might also enhance the effects of γ -hydroxybutyrate. Presumably, the ability of benzodiazepines to alter the effects of other abused drugs contributes to their popularity among drug abusers.

Benzodiazepines moderate the actions of other drugs, and other drugs might alter the effects of benzodiazepines. The goal of the current study was to determine whether the discriminative stimulus effects of the benzodiazepine midazolam were changed by drugs with mechanisms of action similar to those that are reportedly coabused with benzodiazepines. Midazolam dose-effect curves were determined alone and in the presence of other drugs; a cumulative-dosing procedure was used so that the entire midazolam dose-effect curve was obtained in a single session. Time-course studies conducted in these monkeys demonstrated short durations of action for some drugs that are abused concurrently with benzodiazepines (data not shown), indicating that these drugs would not last long enough to complete the midazolam dose-effect curve in a single session. Consequently, drugs with similar mechanisms and longer durations of action were used in place of drugs abused by humans. Specifically, morphine was studied in place of heroin, dizocilpine was studied in place of ketamine, and γ -butyrolactone was studied in place of γ -hydroxybutyrate.

Methods

Subjects

Two male (KI, GI) and 1 female (JA) rhesus monkeys (6.5–8.0 kg) were housed individually in a room with controlled temperature and humidity and maintained on a 14/10-h light/dark schedule. While in their home cage, monkeys had free access to water. Monkeys received food pellets (Bio Serv, Inc, Frenchtown, NJ) during sessions with fresh fruit and primate chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI) provided in the home cage in sufficient quantities to allow the two young adult monkeys (KI and JA) to gain 1 kg each over the course of the study and to maintain an older monkey (GI) at a relatively constant weight throughout the study (i.e., no increasing or decreasing trend). Each monkey was experimentally naïve before they were trained to discriminate midazolam, although monkey GI had a history of discriminating midazolam while responding under a fixed-ratio 10 schedule of stimulus-shock termination. All three monkeys responded under the current schedule for at least 2 years before this study began. Monkeys used in the study were maintained in accordance with the Institutional Animal Care and Use Committee, University of Texas Health Sciences Center at San Antonio, and guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council [Department of Health, Education and Welfare, publication No. (NIH) 85-23, revised 1996].

Apparatus

During experimental sessions, monkeys were seated in chairs (Primate Products, Miami, FL) that provided restraint at the neck. Chairs were placed in sound-attenuating chambers equipped with two response levers, two stimulus lights, ventilating fans, and a trough to which food pellets could be delivered from a dispenser located outside of each chamber. White noise was present in the chamber to mask extraneous noise. Chambers were connected to a computer through a commercially available interface (MED Associates Inc., East Fairfield, VT); the computer controlled experiments and recorded data using MED-PC/Medstate Notation software (MED Associates Inc., East Fairfield, VT).

Procedure

Monkeys discriminated 0.56 mg/kg of midazolam while responding under a fixed ratio 10 schedule of food presentation. Experimental sessions were divided into 2–8 discrete cycles. Each cycle was 15 minutes in duration and began with a 10-minute timeout period during which chambers were dark and responding had no programmed consequence; injections were administered during the first minute of each cycle. The timeout was followed by a 5-minute response period during which stimulus lights were illuminated and the 10th consecutive response on the lever designated correct by the injection given at the beginning

of the cycle resulted in the delivery of a food pellet. Responses on the injection-inappropriate lever reset the response requirement on the appropriate lever. Lever designation varied among monkeys. Lights were extinguished and response periods ended after 5 minutes or the delivery of 10 pellets, whichever occurred first. Any time remaining between the end of the response period and the beginning of the next cycle was a timeout. Sessions were conducted 7 days/week.

Training sessions began with an injection of either 0.56 mg/kg of midazolam or saline. For some training sessions, monkeys received midazolam in the first cycle followed by a sham injection (i.e., monkeys were handled but did not receive an injection) in the second cycle; only responding on the midazolam-appropriate lever resulted in delivery of food pellets during response periods for both cycles. For other training sessions, saline or sham injections were administered for 2–8 cycles, and only responding on the saline-appropriate lever resulted in delivery of food pellets. For still other training sessions, saline or sham injections were given during the first 1–4 cycles, midazolam was given on the second to last cycle and a sham injection was given on the last cycle; monkeys could respond on the saline-appropriate lever at the beginning of the session and on the midazolam-appropriate lever during the last 2 cycles to receive food pellets. Tests were conducted when the following criteria were satisfied: $\geq 80\%$ of the total responses for the session emitted on the injection-appropriate lever and fewer than 10 responses emitted on the injection-inappropriate lever prior to delivery of the first food pellet. Test sessions were conducted every third day as long as monkeys satisfied these criteria during each cycle for the 2 intervening training sessions; otherwise, training continued until the criteria were satisfied for 2 consecutive sessions.

During test sessions, 10 consecutive responses on either lever resulted in delivery of food pellets, and test compounds were administered before sessions during which monkeys received sham injections at the beginning of each cycle or midazolam dose-effect curves were determined. The time course for each of the 4 test compounds (morphine, amphetamine, dizocilpine and γ -butyrolactone) was determined by administering a single dose of a test compound on the first cycle followed by 5 cycles during which sham injections were given. On a separate occasion, saline was administered on the first cycle of a 6-cycle session. Because the rate-decreasing effects of γ -butyrolactone were not evident until 60 minutes after the injection, additional time points were studied by administering γ -butyrolactone 60 minutes before sessions comprising 6 sham cycles. Each of the test compounds was also administered prior to sessions during which midazolam dose-effect curves were determined. Results from the time course studies were used to select intervals between administration of a test compound and sessions. Morphine, dizocilpine and γ -butyrolactone were administered 30, 15 and 60 min, respectively, before the first cycle which began with an injection of saline; when amphetamine was studied, it was given on the first cycle of sessions. Based on the time course data, these intervals between administration of the largest dose of each drug and the session would be expected to decrease mean response rates to at least 50% of control during the first cycle of the session. If response rates on the first cycle were $< 20\%$ of control, midazolam was not administered and sessions ended after the first cycle; if rates were $> 20\%$ of control, midazolam dose-effect curves were determined using a cumulative-dosing procedure. A dose of 0.032 mg/kg of midazolam was administered on the second cycle and the dose increased across cycles in $\frac{1}{2}$ log unit increments. Administration of midazolam continued until $\geq 80\%$ of the total responding during the cycle occurred on the midazolam lever or until rates decreased to $< 20\%$ of control. Because of the substantial and long-lasting rate-decreasing effects of γ -butyrolactone, the time course for 320 mg/kg of γ -butyrolactone was determined only in 2 monkeys (KI and JA) and doses of γ -butyrolactone larger than 178 mg/kg were not studied in combination with midazolam. Midazolam dose-effect curves were determined in the

absence of other treatment at the beginning and end of these studies as well as periodically throughout the studies.

Drugs

Midazolam hydrochloride (Bedford Laboratories, Bedford, OH) was purchased as a commercially prepared solution and diluted with sterile water. Morphine sulfate (Research Technology Branch, National Institute on Drug Abuse, Rockville, MD), d-amphetamine sulfate and dizocilpine hydrogen maleate (Sigma-Aldrich Co., St. Louis, MO) were dissolved in sterile water. γ -Butyrolactone was purchased from a commercial source (Sigma-Aldrich Co., St. Louis, MO). Drugs were administered s.c. in volume ranging from 0.2 ml to 2.5 ml. Doses were expressed in the form listed above in mg/kg of body weight.

Data analyses

Control (no drug) response rates were obtained for individual monkeys by first averaging rates across cycles within sessions during which vehicle or sham was administered and monkeys satisfied the testing criteria; these rates were then averaged across 5 training sessions (mean $\pm$ 1 SEM). Response rates obtained during test sessions were expressed as a percentage of the control (no drug) rate for each monkey and averaged across monkeys. Mean response rates when saline or sham injections were administered were compared to rates obtained when 0.56 mg/kg midazolam was administered using a two-tailed t-test. Discrimination data were not included in the analyses when response rates were less than 20% of control for an individual monkey, and mean discrimination data were not included in the figures or analyses unless rates were greater than 20% of control for at least 2 of the 3 monkeys. To obtain a time course for the rate-decreasing effects of each of the 4 test compounds, rates of responding (% of control $\pm$ SEM) were averaged across monkeys and plotted as a function of time after administration of each test compound. Because γ -butyrolactone was given immediately and 60 minutes before separate sessions comprising 90 minutes, the effects of γ -butyrolactone at two of the time points (75 min and 90 min) were obtained twice; data for each of those two time points were first averaged for each monkey across the 2 sessions and then averaged among monkeys. In addition, saline was administered at the beginning of 90-min sessions, and 95% confidence intervals were determined. A test compound was considered to change response rates significantly when the mean rate after drug administration was outside of the 95% confidence intervals obtained after saline. When drugs were studied in combination with midazolam, the percentage of responses on the drug-appropriate lever (% midazolam-lever responding $\pm$ SEM) and rates (% of control $\pm$ SEM) were plotted as a function of midazolam dose. Rates were considered significantly changed when the mean rate after drug administration was outside of the 95% confidence intervals obtained after saline, which occurred on the first cycle of sessions in which midazolam dose-effect curves were determined in the absence of other drugs.

Midazolam dose-effect curves obtained in the absence or presence of a test compound were compared by simultaneously fitting straight lines to the dose-effect curves for each monkey using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). Straight lines were fit to the linear portion of the dose-effect curves which included one data point below 25%, one data point above 75% and all data points in between. To determine the simplest model that best fit the data, slopes obtained in the absence of other treatment were compared to those obtained in the presence of one of the test compounds using an F-ratio test. When slopes were significantly different, a more complex model was needed to fit the data; however, when slopes were not different, the simple model was used by selecting a common slope. Dose-effect curves could then be further compared by determining whether the data were best fit by a common intercept. Significance was set at $P < 0.05$. To examine further any differences between midazolam dose-effect curves determined in the absence

and presence of other drugs, potency ratios were calculated for each monkey by dividing ED_{50} values for midazolam determined in the presence of a test compound by ED_{50} values for midazolam determined in the absence of other treatment. Significant changes in potencies were detected when the 95% confidence intervals of the potency ratios averaged among monkeys did not include 1.

Results

Response rates (mean $\pm$ 1 SEM) obtained when saline or sham was administered (1.21 ± 0.24 responses/sec) were not significantly different from those obtained when 0.56 mg/kg midazolam was administered (1.21 ± 0.04 responses/sec). Morphine, amphetamine, dizocilpine and γ -butyrolactone produced $\leq 20\%$ responding on the midazolam lever (data not shown) up to doses that markedly decreased response rates. Rate-increasing effects were evident 45 and 60 min after administration of 1 mg/kg of morphine and 15 min after 5.6 and 10 mg/kg. The larger doses decreased rates to $<60\%$ of control; these effects were not evident until 45 min after morphine administration with rates remaining significantly decreased for the remainder of the 90-min session (upper left panel, Fig. 1). Amphetamine dose dependently decreased response rates, and this effect was evident within 15 min of administration of doses larger than 0.1 mg/kg. The duration of the rate-decreasing effects varied with the dose of amphetamine; a dose of 0.56 mg/kg of amphetamine decreased response rates to $<10\%$ of control for the entire 90-minute session (upper right panel, Fig. 1). Dizocilpine had little effect on response rates 15 min after administration and dose dependently decreased rates at later time points. Rates were significantly reduced 30 and 45 min after administration of 0.0178 mg/kg of dizocilpine; for the larger doses, rates were significantly decreased for the remainder of the 90-min session (lower left panel, Fig. 1). Doses of 100 and 178 mg/kg of γ -butyrolactone produced small but significant decreases in response rates. A dose of 320 mg/kg of γ -butyrolactone eliminated responding beginning 60 min after administration and lasting until the end of the session (lower right panel, Fig. 1).

Midazolam dose dependently increased midazolam-lever responding with doses larger than 0.32 mg/kg producing $\geq 80\%$ midazolam-lever responding; response rates were not markedly altered by those doses of midazolam (closed diamonds, Fig. 2–5). When administered 30 minutes before sessions, 1 mg/kg of morphine did not alter the discriminative stimulus effects of midazolam. There was no significant difference in either the slope [$F(1,14)=0.030$, $p>0.05$] or intercept [$F(1,15)=0.00087$, $p>0.05$] of the midazolam dose-effect curve determined after 1 mg/kg morphine, as compared to the midazolam dose-effect curve determined alone (upper panel, Fig. 2). Moreover, ED_{50} values for midazolam were similar in the absence (0.28 mg/kg) or presence of 1 mg/kg of morphine (0.27 mg/kg), resulting in a potency ratio of 1.13 and a 95% confidence interval that included 1, further indicating that morphine did not significantly alter the potency of midazolam (Table 1). A combination of 3.2 mg/kg of morphine and small doses of midazolam (0.1 and 0.32 mg/kg) produced, on average, $<30\%$ midazolam-lever responding. Although 3.2 mg/kg of morphine did not alter response rates (squares above V, lower panel, Fig. 2), that dose of morphine combined with 0.1 mg/kg of midazolam significantly increased rates; a larger dose of midazolam (1 mg/kg) with morphine significantly decreased rates (squares, Fig. 2). Doses of 5.6 mg/kg and 10 mg/kg of morphine, studied in combination with midazolam, significantly decreased response rates, as compared with rates obtained when only saline was administered (lower panel, Fig. 2). Line analyses of midazolam dose-effect curves determined in the presence of the 3 largest doses of morphine indicated that, while slopes were not different between these 3 curves [$F(2,21)=1.36$, $p>0.05$], intercepts were significantly different [$F(2,23)=5.75$, $p<0.01$], indicating a leftward shift in the rate-decreasing effects of midazolam with increasing doses of morphine.

Amphetamine also did not alter the discriminative stimulus effects of midazolam. Neither the slopes [$F(2,21)=1.01$, $p>0.05$] nor intercepts [$F(2,23)=1.35$, $p>0.05$] of dose-effect curves for midazolam determined in the presence of 0.1 or 0.178 mg/kg of amphetamine were significantly different from those determined in the absence of amphetamine (upper panel, Fig. 3). Although a 2.6-fold larger ED_{50} value was obtained for midazolam following administration of 0.1 mg/kg of amphetamine, as compared to the ED_{50} value for midazolam alone, this difference was not significant (i.e., 95% confidence intervals for the potency ratio included 1; Table 1). In contrast to the effects obtained when morphine was studied in combination with midazolam, amphetamine did not enhance the effects of midazolam on response rate up to the dose of amphetamine (0.56 mg/kg) that eliminated responding (lower panel, Fig. 3).

When given 15 minutes before sessions, doses of 0.0178 mg/kg and 0.032 mg/kg of dizocilpine did not alter the discriminative stimulus effects of midazolam (upper panel, Fig. 4), as indicated by slopes [$F(2,18)=0.86$, $p>0.05$] and intercepts [$F(2,20)=2.95$, $p>0.05$] of midazolam dose-effect curves that were not different in the presence or absence of dizocilpine and by no change in the potency of midazolam (Table 1). These 2 doses of dizocilpine produced small but significant decreases in response rates when administered alone; increasing doses of midazolam did not further modify rates (lower panel, Fig. 4). A larger dose of dizocilpine (0.056 mg/kg) given 15 min before sessions decreased mean response rates to <20% of control (triangles above V, Fig. 4).

Up to the largest dose that could be safely studied, γ -butyrolactone did not alter the discriminative stimulus effects of midazolam. When given 60 minutes before sessions, 100 mg/kg of γ -butyrolactone did not change the discriminative stimulus effects of midazolam (upper panel, Fig. 5), as evidenced by slopes [$F(1,13)=0.0084$, $p>0.05$] and intercepts [$F(1,14)=1.08$, $p>0.05$] that were not significantly changed by γ -butyrolactone and by no difference in the potency of midazolam (Table 1). This dose of γ -butyrolactone significantly decreased response rates, and these rate-decreasing effects were not enhanced by increasing doses of midazolam (lower panel, Fig. 5). When 178 mg/kg of γ -butyrolactone was studied in combination with midazolam, monkeys responded predominantly on the saline lever up to a dose of midazolam (1 mg/kg) that decreased mean response rates to 10% of control rates (Fig. 5).

Discussion

Benzodiazepine use remains high among polydrug abusers who report using benzodiazepines to modulate the effects of other drugs (Darke et al. 2010; Degenhardt and Topp 2003; Gelkopf et al. 1999). Few studies have examined whether drugs that are commonly coabused with benzodiazepines alter the behavioral effects of benzodiazepines in a manner that might contribute to their increased abuse in this population. This study examined whether morphine, amphetamine, dizocilpine and γ -butyrolactone modulate the discriminative stimulus effects of midazolam.

The reasons for coabusing benzodiazepines with other drugs vary depending on the mechanism of action of the other drug. For example, benzodiazepines are often used to “boost” the effects of opioids (Gelkopf et al. 1999; Stitzer et al. 1981); this enhancement is supported by results obtained in human laboratory studies demonstrating that physiologic and subjective effects of methadone were greater when it was combined with diazepam (Preston et al. 1984). In addition, one study in baboons suggested that methadone enhanced the reinforcing effects of flunitrazepam (Ator et al. 2005), perhaps indicating that not only do opioids alter the effects of benzodiazepines but benzodiazepines alter the effects of opioids. In contrast to those effects, morphine did not alter the discriminative stimulus

effects of midazolam in the current study, which is consistent with a previous study reporting that benzodiazepines did not alter the discriminative stimulus effects of morphine in morphine-deprived monkeys or the discriminative stimulus effects of naltrexone in morphine-treated monkeys (Gerak et al. 1998). In the current study, morphine enhanced the rate-decreasing effects of midazolam. Thus, opioids modulate some but not all effects of benzodiazepines.

Abusers of amphetamines and cocaine also use benzodiazepines, although for different reasons. Whereas benzodiazepines seem to enhance the effects of opioids, they attenuate some effects of amphetamines or cocaine and are often used by polydrug abusers to diminish unwanted effects that emerge as abusers discontinue an episode of stimulant use (Degenhardt and Topp 2003; Gelkopf et al. 1999). Although attenuation of the discriminative stimulus effects of amphetamine and cocaine by benzodiazepines has been reported (Barrett et al. 2005; Druhan et al. 1991; Goeders et al. 1993; Negus et al. 2000; Nencini and Woolverton 1988), amphetamine did not alter the discriminative stimulus effects of midazolam in the current study. These asymmetrical interactions between amphetamine and benzodiazepines in drug discrimination studies suggest that increased use of benzodiazepines in stimulant abusers is related to alterations of effects of stimulants by benzodiazepines but not vice versa.

The concomitant abuse of benzodiazepines and multiple other drugs that are available at clubs and parties is not well understood (Halkitis and Palamar 2006). One possible reason for coabuse might be indiscriminate use of a wide variety of drugs, including benzodiazepines, in these settings where many different drugs are readily available; another possibility is that specific drug combinations might involve pharmacodynamic interactions between benzodiazepines and other drugs, which could contribute to their coabuse. Benzodiazepines reduced ketamine-associated emotional distress (Krystal et al. 1998), suggesting an interaction between these drugs. Moreover, some similarities in behavioral effects of γ -hydroxybutyrate and benzodiazepines might suggest that it would enhance the effects of benzodiazepines (Carter et al. 2009). This study used drugs that have mechanisms of action similar to those of ketamine and γ -hydroxybutyrate to examine interactions with benzodiazepines; dizocilpine and γ -butyrolactone were used because they have longer durations of action than ketamine and γ -hydroxybutyrate, respectively. Neither drug altered the discriminative stimulus effects of midazolam. Little is known regarding coabuse of benzodiazepines with these other drugs, although the lack of effect observed in the current study suggests that benzodiazepines are not taken with these other drugs because of an enhancement of their discriminative stimulus effects.

Although the test compounds examined in the current study did not alter the discriminative stimulus effects of benzodiazepines, other drugs can modify those effects. For example, drugs that act at benzodiazepine sites on GABA_A receptors can shift the midazolam dose-effect curve to the left (e.g., triazolam; McMahon and France, 2005) or to the right (e.g., flumazenil; Lelas et al., 1999), depending on their relative efficacy. Drugs acting at other modulatory sites on GABA_A receptors, such as the positive modulators pregnanolone and pentobarbital, also shift the midazolam dose-effect curve leftward (McMahon and France, 2005). Moreover, drugs acting at different receptor systems can also alter the discriminative stimulus effects of midazolam with valproic acid producing leftward shifts in the midazolam dose-effect curve and diphenhydramine producing rightward shifts (McMahon and France, 2003). Thus, the stability of the midazolam dose-effect curve in the current study appears to be due to a lack of interaction between midazolam and each of the other drugs, rather than an inability to detect changes in the discriminative stimulus effects of midazolam.

The types of drugs that are used by polydrug abusers depend on personal preference, local availability, and even on local prescribing practices if drugs are obtained by diversion (Gossop et al. 1998). Polydrug abuse is common (Darke and Hall 1995; Darke et al. 2010) with the reasons for combining drugs varying among individuals and classes of drugs. Results of the current study suggest that increased abuse of benzodiazepines in polydrug abusers is not due to changes in the discriminative stimulus effects of benzodiazepines caused by other drugs of abuse. Although a lack of interaction between benzodiazepines and other drugs has also been demonstrated using different dependent variables, such as respiratory depression (e.g., Gerak et al. 1998), reinforcing properties are very important for assessing abuse potential of psychoactive drugs (Ator and Griffiths 2003), and self-administration studies might further elucidate reasons for increased benzodiazepine use in polydrug abusers.

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Fig. 1.

Time course for the effects of morphine (upper left), amphetamine (upper right), dizocilpine (lower left) and γ -butyrolactone (lower right) on rates of responding in three monkeys discriminating 0.56 mg/kg midazolam. Abscissae: time (min) after drug administration. Ordinates: average rate expressed as a percentage of control response rate (± 1 S.E.M.).

Fig. 2. Discriminative stimulus effects of midazolam determined alone and in combination with morphine in three monkeys discriminating midazolam. Abscissae: dose of midazolam in mg/kg body weight. Points above V represent the effects of vehicle. Ordinates: top panel, percentage of total responses emitted on the midazolam lever; bottom panel, average rate expressed as a percentage of control response rate (± 1 S.E.M.).

Fig. 3. Discriminative stimulus effects of midazolam determined alone and in combination with amphetamine in three monkeys discriminating midazolam. See Fig. 2 for other details.

Fig. 4. Discriminative stimulus effects of midazolam determined alone and in combination with dizocilpine in three monkeys discriminating midazolam. See Fig. 2 for other details.

Fig. 5. Discriminative stimulus effects of midazolam determined alone and in combination with γ -butyrolactone in three monkeys discriminating midazolam. See Fig. 2 for other details.

Table 1

Mean ED₅₀ values and potency ratios with 95% confidence intervals (CI) for the discriminative stimulus effects of midazolam determined after administration of morphine, amphetamine, dizocilpine and γ -butyrolactone.

	ED ₅₀ (mg/kg)		Potency ratio ^a	
	Mean	95% CI	Mean	95% CI
Midazolam	0.28	0.21–0.39		
+1 mg/kg morphine	0.27	0.063–1.14	1.13	–1.00–3.26
+0.1 mg/kg amphetamine	0.50	0.042–5.91	2.60	–1.96–7.17
+0.178 mg/kg amphetamine	0.31	0.084–1.17	1.06	–0.75–2.86
+0.0178 mg/kg dizocilpine	0.15	0.016–1.37	0.64	–0.35–1.64
+0.032 mg/kg dizocilpine	0.16	0.076–0.32	0.58	0.04–1.12
+100 mg/kg γ -butyrolactone	0.41	0.21–0.830	1.53	0.01–3.06

^a Potency ratio of ED₅₀ values for midazolam determined in the presence of another drug divided by ED₅₀ values of midazolam determined in the absence of other drugs.