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Discriminative Stimulus Effects of Flumazenil in Rhesus Monkeys Treated Chronically With Chlordiazepoxide

CHARLES P. FRANCE[1] AND LISA R. GERAK

Department of Pharmacology, Louisiana State University Medical Center, 1100 Florida Avenue, New Orleans, LA 70119

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FRANCE, C. P. AND L. R. GERAK, *Discriminative stimulus effects of flumazenil in rhesus monkeys treated chronically with chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **56**(3) 447–455, 1997.—Discriminative stimulus effects of the benzodiazepine antagonist flumazenil were studied in two rhesus monkeys receiving 3.2 mg/kg/12 h of chlordiazepoxide while discriminating between vehicle and 0.056 mg/kg of flumazenil. In a drug discrimination component responding was maintained under a FR 10 schedule of stimulus-shock termination; in a non-discrimination component responding was maintained under a FR 10 schedule of food presentation. Flumazenil and Ro 15-4513 occasioned >80% flumazenil-lever responding at doses larger than 0.032 and 0.056 mg/kg, respectively. Pentylenetetrazole, ethyl-β-carboline-3-carboxylate (βCCE), ketamine and spiradoline failed to substitute for flumazenil although >80% drug-lever responding was observed for two of the compounds in one monkey. Flumazenil, Ro 15-4513, pentylenetetrazole, βCCE but not ketamine or spiradoline decreased rates of responding in the food component at doses that had little effect on rates in the stimulus-shock termination component. When chlordiazepoxide injections were discontinued and saline was administered before the session, monkeys did not respond on the flumazenil lever; when flumazenil was administered under the same conditions, monkeys responded on the flumazenil lever despite not having received chlordiazepoxide for nine days. Drug stimulus control was established with flumazenil in monkeys receiving chlordiazepoxide and substitution studies suggest that this effect of flumazenil might result from antagonist actions at benzodiazepine receptors; however, lack of withdrawal-related effects after termination of chlordiazepoxide treatment precludes validation of this procedure for studying benzodiazepine dependence.

Benzodiazepine Chlordiazepoxide Flumazenil Rhesus monkey Drug discrimination

BENZODIAZEPINES comprise one of the most widely prescribed classes of psychoactive drugs, in part, because of their well-established clinical effectiveness as well as their wide margin of safety as compared to other compounds (e.g., barbiturates) that also have sedative, hypnotic or anxiolytic actions. Despite their favorable margin of safety, one consequence of repeated exposure to benzodiazepines is the development of physical dependence as evidenced by the emergence of characteristic withdrawal signs and symptoms upon termination of long-term benzodiazepine treatment (9). Although physical dependence can develop to benzodiazepines, even under clinically-appropriate conditions, there is emerging evidence that in the absence of physical dependence some benzodiazepines (e.g., flunitrazepam) are used recreationally either alone or in combination with other psychoactive substances (5,34,47). In light of the potential risk associated with the use of benzodiazepines, there continues to be a need for a broader understanding of the conditions under which physical dependence develops to benzodiazepines. In this regard, studies in non-human species have been especially useful in providing descriptive information on the development and expression of dependence to and withdrawal from benzodiazepines (4,35, 39,44,50).

Among the many experimental approaches that have been used to study drug dependence in non-human species are conditioned behavioral disruption (often called behavioral de-

[1] To whom requests for reprints should be addressed.

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pendence) and drug discrimination. In the former case, subjects responding under a schedule of reinforcement are treated chronically with drug and, under appropriate conditions, termination of drug treatment produces a time- and dose-related disruption in patterns and rates of responding (48). This disruption is presumed to be a reflection of withdrawal because: for some classes of drugs a similar disruption can be elicited by pharmacological antagonists; and often the disruption is attenuated by re-administration of the drug that was administered during chronic treatment. Thus, behavioral dependence has been demonstrated for a wide variety of drugs, including some that do not appear to produce physical dependence by other methods of assessment (1,10,11,49,54).

A second approach that is used to study dependence utilizes drug discrimination procedures in subjects treated chronically with drug (17,19). Typically, subjects are trained to discriminate between a pharmacologic antagonist and vehicle and the antagonist discriminative stimulus is presumed to be related to withdrawal because: under some (21), but not all (30), conditions termination of agonist treatment results in a time- and dose-related increase in antagonist-appropriate responding; and re-administration of the drug that was administered during chronic treatment (or a pharmacologic equivalent) reverses antagonist-appropriate responding (22,23). These drug discrimination procedures have been used to study the effects of drugs in rhesus monkeys (22), rats (30) and pigeons (21), using a variety of compounds, including μ opioids (30), κ opioids (23) and non-opioids (14).

Physical dependence has been characterized in the laboratory for a range of benzodiazepines under a variety of dosing conditions (37,38,50). However, with a single exception (14), the drug discrimination procedures that have proven to be useful for studying opioid dependence and withdrawal (22,30) have not been applied to benzodiazepine dependence despite the availability of a wide variety of appropriate agonists, inverse agonists and antagonists. For example, there are several selective pharmacologic antagonists (e.g., flumazenil) that attenuate the effects of benzodiazepines (31,51) and precipitate withdrawal in benzodiazepine-dependent subjects (28,39); moreover, these antagonists appear to have potentially-interesting effects when administered alone in untreated pigeons (53).

The goals of the current study were: (i) to establish a drug discrimination between vehicle and flumazenil in rhesus monkeys treated chronically with chlordiazepoxide; (ii) to combine in the same procedure a drug discrimination component, in which responding is maintained by shock avoidance, and a non-discrimination component, in which responding is maintained by food, to see whether disruptions in either component are correlated with other presumed indices of withdrawal (e.g., responding on the flumazenil lever); (iii) to characterize the discriminative stimulus effects of flumazenil as well as other benzodiazepines and non-benzodiazepines; and (iv) to determine whether termination of chlordiazepoxide treatment produces effects that are qualitatively similar to the effects of flumazenil. The drugs that were studied included compounds that are known to have affinity for benzodiazepine binding sites on the gamma-aminobutyric acid (GABA)/benzodiazepine receptor complex (flumazenil, Ro 15-4513, βCCE), a compound that is thought to exert its actions at non-benzodiazepine binding sites on the GABA/benzodiazepine receptor complex (pentylenetetrazole) as well as pharmacologically unrelated compounds: the non-competitive *N*-methyl-D-aspartate receptor antagonist ketamine and the κ opioid receptor agonist spiradoline (52).

METHOD

Subjects

Two adult, female rhesus monkeys (Macaca mulatta) were housed individually in stainless steel cages. Both subjects had responded under FR schedules (stimulus-shock termination) and had received opioids in previous studies: monkey LU (unpublished) and monkey DI (19) had received opioid agonists and antagonists in a drug discrimination study; both monkeys also received opioids in other studies on the antinociceptive and respiratory effects of opioids (20,25). Throughout the current study monkeys had free access to water and received fresh fruit several times per week; for several years prior to as well as during most of the current study, monkeys were maintained at 90% of their free-feeding weights (6.3 kg for LU, 5.6 kg for DI); in the current study monkeys received food in sessions and supplemental feeding in the home cage (LabDiet monkey chow).

Apparatus

Monkeys were seated in chairs that provided restraint at the neck and feet; during sessions the chairs were located in sound-attenuating, ventilated chambers that were equipped with three response levers (model PRL-001; BRS/LVE, Inc., Laurel MD), stimulus lights and a food cup located directly below the center lever. A translucent 2.5 cm diameter window was centered 7.5 cm above each lever and could be transilluminated by red or green lights located behind the window. A food hopper (model PDC-040; BRS/LVE) located outside of the chamber could deliver 300 mg, banana-flavored food pellets to the food cup. Chairs were equipped with a pair of shoes that contained brass electrodes; electric shock (250 msec, 3 mA, 600 V) could be delivered to the shoes from an a.c. shock generator that was located outside of the chamber. Experimental events were controlled and data were recorded by a microprocessor (Dell 486SX) and a commercially-available interface (MedAssociates, Inc., E. Fairfield, VT).

Procedure

Monkeys received 3.2 mg/kg/12 h of chlordiazepoxide (s.c.) with one of the injections given 3 h prior to daily sessions. Prior to drug discrimination training, monkeys were trained to respond under a fixed-ratio (FR) schedule of stimulus-shock termination, followed by training sessions during which monkeys responded in different components under either the stimulus-shock termination schedule or a FR schedule of food presentation. The food component was discontinued and drug discrimination training commenced with vehicle and 0.056 mg/kg of flumazenil. Monkeys responded only under a schedule of stimulus-shock termination during drug discrimination training; injections of vehicle and flumazenil alternated under both single- and double-alternation schedules. Once drug stimulus control was established, the FR food component was reintroduced and training continued (with the multiple FR food, FR stimulus-shock termination [drug discrimination] schedule) until drug stimulus control was re-established. A single session was conducted each day using the following terminal experimental conditions: a 10-min timeout (TO) period, during which the experimental chamber was dark and lever presses had no programmed consequence; a 4-min response period, during which a green light was illuminated over the center lever and a FR 10 schedule of food presentation was in effect only on the center lever; a 2-min TO; and a 4-min

response period during which a red light was illuminated over each left and right lever and a FR 10 schedule of stimulus-shock termination was in effect on the left and right levers (i.e., drug discrimination). During the first 4-min response period, stimulus lights were extinguished after 4 min or 50 food presentations, whichever occurred first. During the second 4-min response period, brief electric shock was scheduled to be delivered every 15 sec; monkeys could postpone shock and extinguish stimulus lights for 30 sec by responding 10 times on the lever designated correct according to an injection administered during the first min of the 10-min TO (left = flumazenil, right = vehicle for one monkey and left = vehicle, right = flumazenil for the other monkey). Drug discrimination response periods ended after 4 min or the delivery of 4 shocks, whichever occurred first. Test sessions began when the following criteria were satisfied for 5 consecutive sessions: >80% responding on the correct lever and <10 responses on the incorrect lever prior to the first reinforcer.

Test sessions were identical to training sessions except that responding on either lever postponed shock and various doses of flumazenil or other drugs were administered during the first minute of the TO. Test sessions typically were conducted after monkeys satisfied the testing criteria (see above) for at least two consecutive training sessions, with the exception that on several occasions (i.e., with the smallest doses of some test drugs) tests were conducted over consecutive days. In general, the order in which drugs and doses were studied varied non-systematically between monkeys. For studies on the time course of discriminative stimulus effects of flumazenil, monkeys received the normal dose (3.2 mg/kg) of chlordiazepoxide 3 h prior to the beginning of the session; an injection of the training dose (0.056 mg/kg) of flumazenil was administered at varying times during or prior to the test session. For the shortest interval, flumazenil was administered 6 min after the beginning of the session (i.e., 4 min prior to the food component and 10 min prior to the stimulus-shock termination [drug discrimination] component). For the longest interval, and on a different day, flumazenil was administered 104 min prior to the session (i.e., 114 min prior to the food component and 120 min prior to the stimulus-shock termination [drug discrimination] component).

In a final study, vehicle was substituted for the twice daily injections of chlordiazepoxide for either 4 or 9 days. In the former case, monkeys also received a vehicle injection immediately prior to test sessions. In the latter case, monkeys received an injection of the flumazenil training dose (0.056 mg/kg) prior to test sessions.

Drugs

The drugs used in this study were: chlordiazepoxide hydrochloride (Sigma Chemical Co., St. Louis, MO); flumazenil (Hoffmann-LaRoche, Nutley, NJ); ketamine hydrochloride (Fort Dodge Laboratories, Fort Dodge, IA); spiradoline mesylate (The Upjohn Company, Kalamazoo, MI); Ro 15-4513 (ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a]-[1,4]benzodiazepine-3 carboxylate), ethyl beta-carboline-3-carboxylate (βCCE), and pentylenetetrazole (Research Biochemicals International, Natick, MA). Flumazenil, Ro 15-4513 and βCCE were dissolved in a vehicle comprising 40% propylene glycol, 50% saline and 10% ethanol. Chlordiazepoxide, spiradoline and pentylenetetrazole were dissolved in sterile water. A commercially-available solution of ketamine was diluted with sterile 0.9% saline. Drugs were administered s.c. (see RESULTS), typically in a volume of 0.1 ml/kg body weight. Chlordiazepoxide was typically prepared within 48 h of its administration; other drugs were prepared less frequently. Doses are expressed in terms of the forms listed above.

Data Analyses

Drug discrimination results are presented as the percentage of responses on the flumazenil-associated lever throughout the entire session as a function of either dose or time after drug administration. Other compounds were considered to have substituted for flumazenil when they produced ⩾80% responding on the flumazenil-associated lever. Rates of responding are expressed as a percentage of the vehicle (control) rates; for each condition, these rates are the average rates over 10 consecutive training sessions during which monkeys satisfied the testing criteria and were calculated separately for the stimulus-shock termination component (i.e., drug discrimination) and the food component for individual subjects. Differences in control response rates among training conditions were analyzed for individual subjects with a t-test or, when unequal variance was obtained, a Mann-Whitney Rank Sum test ($p < 0.05$ level of significance for both tests).

RESULTS

Under the initial drug discrimination training conditions (i.e., stimulus-shock termination schedule only), the two monkeys were under adequate drug stimulus control for testing after 210 and 263 training sessions. An additional 20 and 26 training sessions were required to re-establish drug stimulus control after the addition of the food component to the procedure. Rates of lever pressing for the two subjects in each of the two components are displayed in Table 1. For both monkeys under both schedules (food and stimulus-shock termination), rates of responding during vehicle training sessions were significantly ($p < 0.05$) higher than rates of responding during flumazenil training sessions. Moreover, for both monkeys under both training conditions (vehicle and flumazenil), rates of responding were significantly ($p < 0.05$) higher in the stimulus-shock termination (i.e., drug discrimination) component than in the food component.

Increasing doses of flumazenil occasioned responding on the flumazenil-appropriate lever in a dose-related manner with doses ⩾0.056 mg/kg (i.e., the training dose) producing >80% drug-lever responding in both monkeys (Fig. 1a). In monkey LU, flumazenil decreased response rates to a greater extent in the food component than in the stimulus-shock termination component (compare open and closed triangles, Fig. 1b); there was no apparent difference in the rate-decreasing effects of flumazenil in monkey DI.

The training dose (0.056 mg/kg) of flumazenil occasioned predominantly drug-lever responding for up to 40 min after s.c. injection; however, flumazenil-like discriminative stimulus effects were not observed for this dose 120 min post injection (Fig. 2a). For 35 min after administration of 0.056 mg/kg of flumazenil, there was a trend for rates of food-maintained responding to be decreased more than rates of stimulus-shock termination-maintained responding. For example, rates of food-maintained responding in monkey LU were ⩽63% of control rates in the food component for up to 35 min after the administration of flumazenil; over the same time period, rates of responding in the stimulus-shock termination component were at or above control rates (compare open and closed triangles, Fig. 2b). The discriminative stimulus and rate-altering effects of flumazenil had a similar time course in both

TABLE 1

RATES OF RESPONDING DURING VEHICLE AND FLUMAZENIL SESSIONS IN MONKEYS RECEIVING 3.2 mg/kg/12 h OF CHLORDIAZEPOXIDE

	Vehicle		0.056 mg/kg Flumazenil	
Subject	Food	SST	Food	SST
LU	0.33 ± 0.05[1]	2.61 ± 0.11	0.14 ± 0.03	2.06 ± 0.25
DI	2.00 ± 0.06	2.21 ± 0.06	0.28 ± 0.12	1.47 ± 0.15

[1]Each entry is the mean response rate (responses/sec) ± 1 SEM averaged over 10 training sessions during which monkeys satisfied the testing criteria.

monkeys insofar as the rate-decreasing effects of flumazenil that were evident in the food component dissipated by 120 min post injection.

Ro 15-4513 also occasioned responding on the flumazenil-associated lever in a dose-related manner with doses ⩾0.1 mg/kg (LU) or a dose of 0.178 mg/kg (DI) producing ⩾80% responding on the drug lever (Fig. 3a). Moreover, rates of responding in the food component were less than rates of responding in the stimulus-shock termination component; for monkey LU this difference was related to the rate decreasing effects of Ro 15-4513 only in the food component; for monkey DI this difference was related to a tendency for rates to be increased to a greater extent in the stimulus-shock termination component than in the food component (Fig. 3b).

While neither βCCE nor pentylenetetrazole substituted completely for flumazenil in both monkeys, substantial drug-lever responding was observed in at least one monkey for each compound (Fig. 4a and b). βCCE produced a maximum of 62% drug-lever responding in LU at a dose of 0.32 mg/kg and a maximum of 76% drug-lever responding in DI at a dose of 1.0 mg/kg. A dose of 17.8 mg/kg of pentylenetetrazole occasioned 98% drug-lever responding in monkey DI and <50% drug-lever responding in the second monkey. Doses of pentylenetetrazole smaller or larger than 17.8 mg/kg did not occasion >80% flumazenil-lever responding in either monkey. For both βCCE and pentylenetetrazole, there was a tendency for rates of responding in the food component to be decreased to a greater extent than rates of responding in the stimulus-shock termination component (Fig. 4c and d).

Up to doses that eliminated responding in both components, neither ketamine nor spiradoline substituted completely for flumazenil in both monkeys, although a dose of 0.32 mg/kg of ketamine did occasion complete drug-lever responding in monkey DI (Fig. 5a and b). Ketamine and spiradoline each had similar potencies in decreasing rates of responding in the food and stimulus-shock termination components; that is, with the exception of a small difference in the potency of spiradoline in decreasing responding in the two components in monkey LU (compare open and closed triangles, Fig. 5d), the same dose eliminated responding in the both components (Fig. 5c and d).

When daily chlordiazepoxide injections were discontinued and monkeys received vehicle injections twice daily, as well as a vehicle injection during the first minute of the session, responding occurred predominantly on the vehicle-associated lever (Fig. 6a). With the exception of 24 h after the last injection of chlordiazepoxide, when responding in the stimulus-shock termination component was decreased to 70% of control, rates of responding in the two components were at or above control (vehicle) response rates over the four days fol-

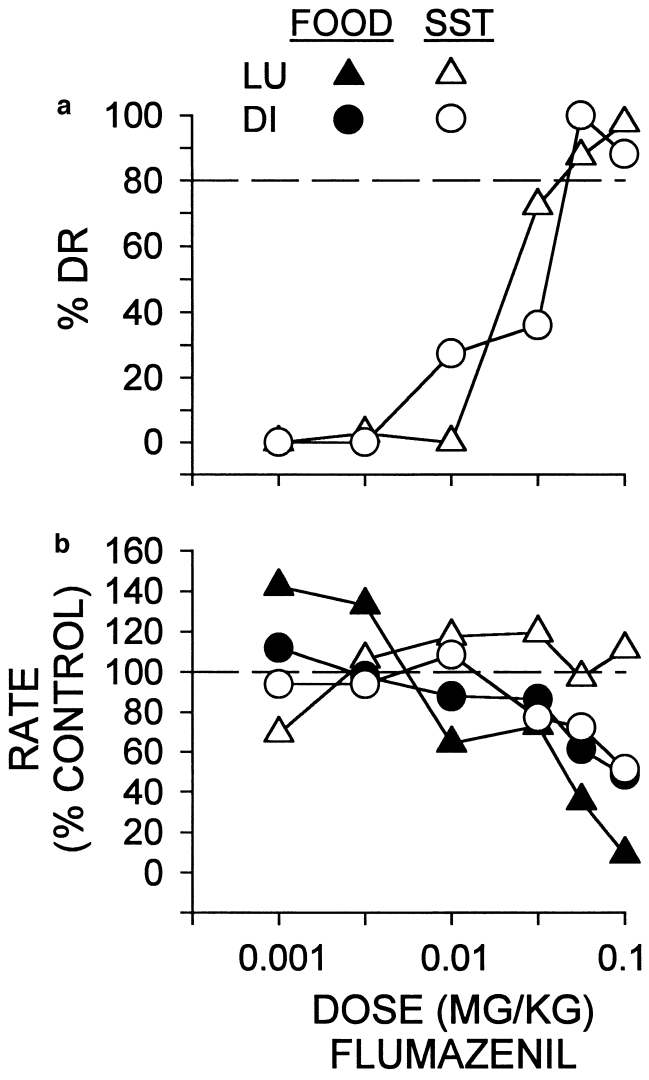

FIG. 1. Discriminative stimulus effects (a) and rate-decreasing effects (b) of flumazenil in two rhesus monkeys (LU, triangles; DI, circles) treated twice daily with 3.2 mg/kg of chlordiazepoxide and discriminating between 0.056 mg/kg of flumazenil and vehicle. Ordinates: a, percentage of responses on the flumazenil lever (% drug-lever responding [%DR]); b, response rate in the stimulus-shock termination (i.e., drug discrimination) component (open symbols) and in the food component (closed symbols) expressed as a percentage of response rates during vehicle (control) sessions. Abscissa: dose of flumazenil in mg/kg body weight.

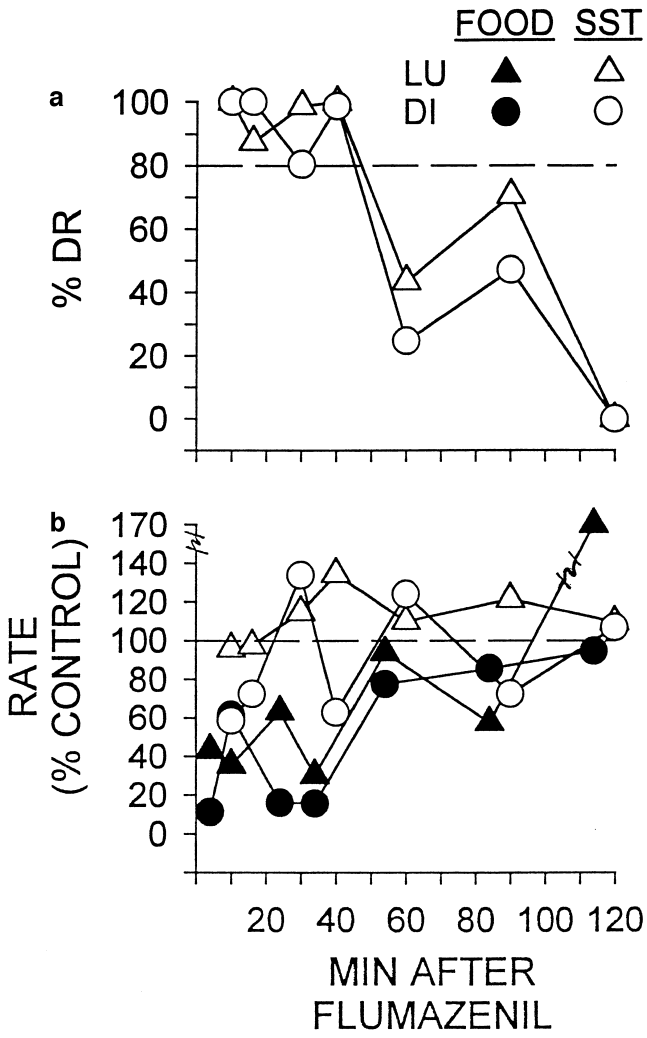

FIG. 2. Time course of the discriminative stimulus effects and rate-decreasing effects of the training dose of flumazenil in two monkeys discriminating between vehicle and 0.056 mg/kg of flumazenil. Abscissa: time after injection of 0.056 mg/kg of flumazenil. See Fig. 1 for other details.

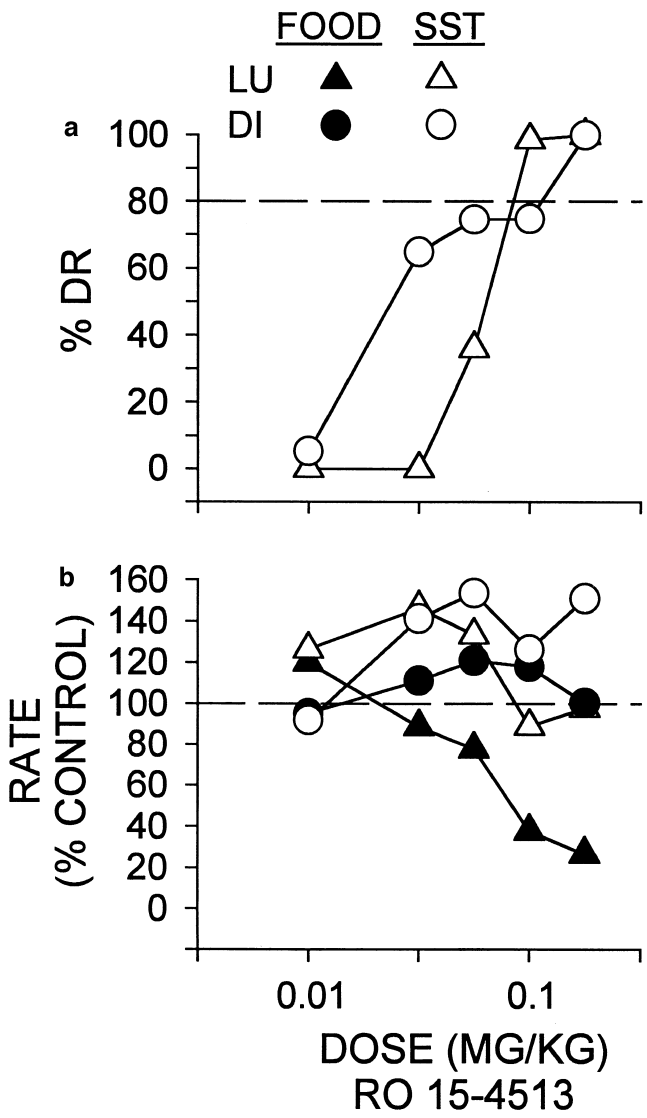

FIG. 3. Discriminative stimulus effects and rate-decreasing effects of Ro 15-4513 in two monkeys discriminating between vehicle and 0.056 mg/kg of flumazenil. See Fig. 1 for other details.

lowing discontinuation of chlordiazepoxide injections (Fig. 6c). On a different occasion, chlordiazepoxide injections were postponed for nine days and monkeys were tested daily with the training dose of flumazenil. With the exception of the fourth day of the experiment, when the average percentage of flumazenil-lever responding fell below 80% (79%), flumazenil continued to occasion predominantly drug-lever responding over a nine-day period during which monkeys did not receive chlordiazepoxide (Fig. 6b). Moreover, rates of responding were decreased slightly in the stimulus-shock termination component and markedly in the food component for 2-3 days following termination of chlordiazepoxide injections. Rates of responding in the stimulus-shock termination component were at or above control rates on days 5-9 of flumazenil testing without chronic benzodiazepine treatment whereas rates of responding in the food component remained low as compared to control (vehicle) rates (Fig. 6d).

DISCUSSION

It is well established that long-term exposure to any of a variety of benzodiazepine receptor agonists can produce physical dependence (4,35–38,44) and one typical consequence of physical dependence is an enhanced sensitivity to the effects of pharmacologic antagonists. This general relationship between sensitivity to antagonists and agonist exposure has been well-characterized for opioids (19,30) as well as for benzodiazepines (14,24). In the current study, monkeys treated twice daily with chlordiazepoxide reliably discriminated between 0.056 mg/kg of flumazenil and vehicle in the absence of any apparent adverse effects. Stimulus control has been established previously with flumazenil in untreated (13,43) and chlordiazepoxide-treated (14) rats and in untreated pigeons (53). The current study provides the first demonstration of stimulus control with flumazenil in non-human primates; however, systematic studies on the discriminative

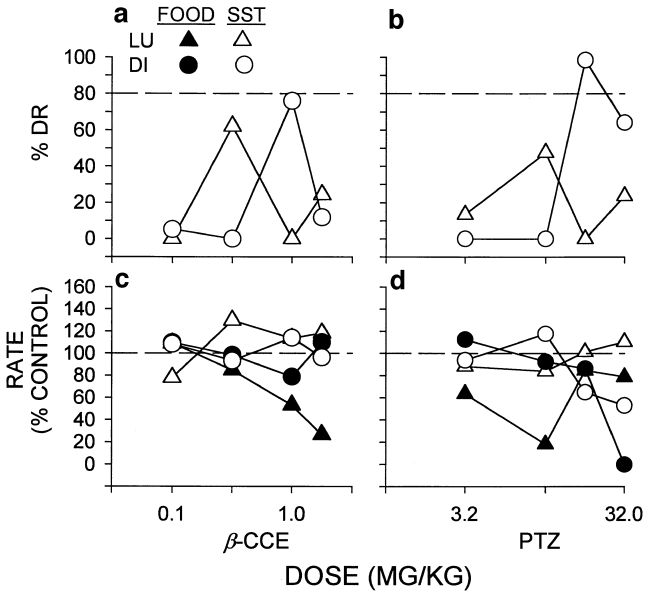

FIG. 4. Discriminative stimulus effects and rate-decreasing effects of βCCE (a and c) and pentylenetetrazole (PTZ; b and d) in two monkeys discriminating between vehicle and 0.056 mg/kg of flumazenil. See Fig. 1 for other details.

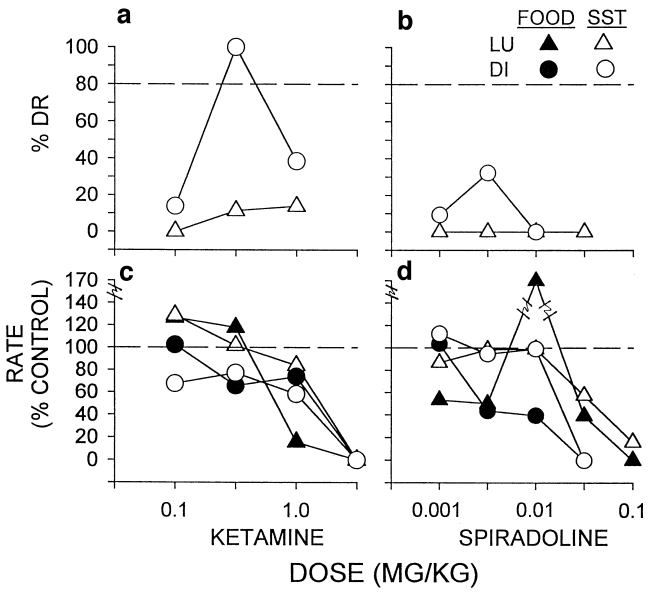

FIG. 5. Discriminative stimulus effects and rate-decreasing effects of ketamine (a and c) and spiradoline (b and d) in two monkeys discriminating between vehicle and 0.056 mg/kg of flumazenil. See Fig. 1 for other details.

stimulus effects of flumazenil in untreated monkeys are needed to determine whether monkeys treated daily with chlordiazepoxide are more sensitive than untreated monkeys to the discriminative stimulus effects of flumazenil.

The discriminative stimulus effects of flumazenil in chlordiazepoxide-treated monkeys appear to be pharmacologically selective insofar as the benzodiazepine receptor inverse agonist Ro 15-4513 (12,29) was the only compound that substituted completely for flumazenil in both monkeys. Full substitution for flumazenil by Ro 15-4513 is consistent with the ability of this partial inverse agonist to substitute for flumazenil under some (53) but not all (43) conditions and with its ability to attenuate many of the effects, including the discriminative stimulus effects (40,42), of benzodiazepine receptor agonists (8,41).

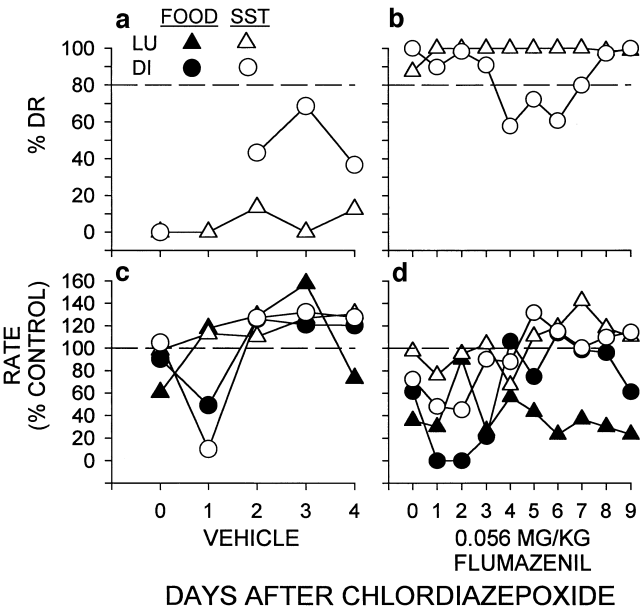

FIG. 6. Discriminative stimulus effects and rate-decreasing effects during daily tests when either vehicle (a and c) or 0.056 mg/kg of flumazenil (b and d) was administered immediately prior to the session. Throughout these studies monkeys received twice daily injections of saline, instead of the twice daily injections of 3.2 mg/kg of chlordiazepoxide that were administered during other studies, with one injection administered 3 h prior to the session. Abscissae: days after the last injection of 3.2 mg/kg of chlordiazepoxide. See Fig. 1 for other details.

Other compounds that are believed to act at the GABA/benzodiazepine receptor complex did not substitute fully for flumazenil in both monkeys. For example, β-carbolines are known to exert many of their effects by acting at GABA/benzodiazepine receptors (6,7), yet βCCE did not occasion flumazenil-lever responding in both monkeys. The β-carbolines βCCE and βCCM as well as pentylenetetrazole fail to substitute completely for flumazenil in untreated rats discriminating between flumazenil and vehicle (13,43), although pentylenetetrazole substitutes for flumazenil in rats treated chronically with chlordiazepoxide (100.0 mg/kg/day) and discriminating between flumazenil (2.5 mg/kg) and vehicle (14). Moreover, diazepam withdrawal, initiated either by the termination of diazepam treatment or by the administration of flumazenil, substitutes for pentylenetetrazole in rats (15,16,18). However, in chlordiazepoxide-treated monkeys, pentylenetetrazole fails to reliably substitute for flumazenil. It has been suggested that, under conditions where differences in efficacy do not account for differences in behavioral effects, variations in discriminative stimulus effects observed among benzodiazepine receptor agonists might be due to the various selectivities of agonists for different subtypes of benzodiazepine receptors (46). Similarly for antagonists, the discriminative stimulus effects of flumazenil might result from actions at a subset of benzodiazepine receptor subtypes, as has been demonstrated

in untreated pigeons (53), and antagonists or inverse agonists that do not act at the same receptor subtype(s) might not mimic the flumazenil discriminative stimulus. For example, in monkeys the flumazenil discriminative stimulus could result from actions at a receptor subtype at which chlordiazepoxide acts selectively (e.g., BZ_2 [(45)]); the reported selectivity of βCCE for a different receptor subtype (BZ_1 [(40,46)]) is consistent with the lack of full substitution for flumazenil by βCCE in chlordiazepoxide-treated monkeys. Collectively, these results are consistent with the view that some benzodiazepine receptor agonists, antagonists and inverse agonists might exert their actions by interacting with different subtypes of GABA/benzodiazepine receptors (e.g., [(33)]).

For some drugs (e.g., opioids [(26)]) discontinuation of chronic drug treatment or administration of an appropriate antagonist results in the emergence of pharmacologically-specific signs of withdrawal and often there is a strong positive relationship between the dose of agonist used during treatment and the magnitude of withdrawal that is observed (35–37,50). For other drugs, discontinuation of chronic treatment fails to produce any obvious behavioral signs (i.e., withdrawal) that would indicate the development of physical dependence (e.g., [(39)]). In some cases, when discontinuation of chronic drug treatment fails to produce obvious signs of withdrawal, other withdrawal-specific effects can be observed using more sensitive and sophisticated behavioral techniques (e.g., operant schedules). For example, often the withdrawal signs are either very modest or entirely absent after discontinuation of chronic treatment with cocaine or Δ-9 tetrahydrocannabinol (THC). However, when chronic treatment with either cocaine (54) or THC (3) is abruptly terminated in monkeys responding under a schedule of food presentation, rates and patterns of responding are markedly disrupted and recover only after several days. This general finding has been replicated with a wide variety of compounds and has been used to demonstrate more subtle behavioral consequences of chronic drug exposure (10,11,48,49).

One goal of the current study was to merge a traditional drug discrimination procedure with a behavioral dependence procedure in order to assess the feasibility of using this combined approach for studies on dependence and withdrawal. One supposition was that the addition of a non-discrimination (food) component might help to validate any relationship between discriminative stimulus effects of a drug and withdrawal since, in the absence of other (independent) indices of withdrawal, responding on the antagonist-associated lever can be interpreted as the absence of agonist (i.e., drug used for treatment [(17,19)]). Incorporation of the food component in the drug discrimination procedure did not appear to adversely affect performance in the drug discrimination component, since only 20-26 additional training sessions were required for monkeys to satisfy stimulus control (e.g., between flumazenil and vehicle) after the two components were combined. Moreover, responding in the food component appeared to be more sensitive than responding in the stimulus-shock termination component to disruption by either flumazenil or Ro 15-4513 (and, perhaps, by the discontinuation of chlordiazepoxide treatment). Although under some conditions the effects of benzodiazepines vary dramatically depending on the events that are used to maintain responding (2), the discriminative stimulus effects and the rate-decreasing effects of benzodiazepines appear to be similar between monkeys responding under a schedule of food presentation and monkeys responding under a schedule of stimulus-shock termination (51). In the current study, enhanced sensitivity to the disruptive effects of some drugs on food-maintained responding appeared to be pharmacologically selective insofar as there was no difference in the potency of two pharmacologically-unrelated compounds (ketamine and spiradoline) in decreasing rates of responding in the two components. Should this general relationship between enhanced sensitivity in the food component and benzodiazepine antagonist (or inverse agonist) actions be supported with a wider variety of benzodiazepines and non-benzodiazepines, then it would appear as though this combined schedule will both facilitate a validation of the drug discrimination procedure as an index of withdrawal and provide a highly-sensitive metric of drug dependence (withdrawal) that might be applied to the assessment of physical dependence for a variety of drugs in non-human species.

Under some conditions, the response to flumazenil diminishes with repeated exposure to the drug (24,35,37). In the two-year period during which the current study was conducted there was no evidence for a reduction in drug stimulus control or for a diminished effect of flumazenil on rates of responding during the food component. Nevertheless, the possibility remains that frequent exposure to flumazenil prevented the development of physical dependence to chlordiazepoxide and that the discriminative stimulus effects of flumazenil were unrelated to the precipitation of withdrawal.

It is well established that flumazenil can precipitate withdrawal in benzodiazepine-treated subjects (28) and in the current study a small dose of flumazenil was reliably discriminated from vehicle in monkeys treated daily with chlordiazepoxide. However, the lack of a reliable change in behavior after discontinuation of chlordiazepoxide treatment suggests that the effects of flumazenil did not depend upon chlordiazepoxide treatment, that these dosing conditions might not produce adequate physical dependence, or that the durations of action of chlordiazepoxide and its active metabolites (e.g., demoxepam) are so long as to preclude systematic measures of withdrawal over time periods that would be practical for well-controlled studies (27,32). Current studies are examining other dosing conditions with shorter-acting benzodiazepines in order to determine the necessary and sufficient conditions for mimicking the spontaneous withdrawal that occurs in humans after discontinuation of some benzodiazepine treatment.

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