Flumazenil Discrimination by Humans under a Two-Response and a Novel-Response Procedure

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ABSTRACT

In this study we assessed the discriminative stimulus, self-reported, and performance effects of flumazenil in humans. The first group (n = 6) was trained to discriminate flumazenil (0.56 mg/70 kg i.v.) from saline and tested with flumazenil (0.10, 0.32, 0.56, and 1.0 mg/70 kg) under a two-response drug discrimination procedure. The second group (n = 8) was trained to discriminate flumazenil (0.56 mg/70 kg i.v.) from saline and tested with flumazenil (0.32, 0.56, and 1.0 mg/70 kg), midazolam (0.10, 0.56, and 1.0 mg/70 kg), and caffeine (75 mg/70 kg) under a novel-response drug discrimination procedure. In both groups, flumazenil was acquired and maintained as a discriminative stimulus. Flumazenil dose-dependently increased flumazenil-appropriate responding and ratings of strength of drug effect and sedation, and decreased ratings of stimulant effects and psychomotor performance. Under the novel-response procedure, midazolam produced dose-dependent increases in flumazenil-appropriate responding. However, midazolam produced 43 and 25% novel responding at the intermediate and highest test doses, respectively. Midazolam dose-dependently increased ratings of strength of drug effect and sedation, and decreased ratings of stimulant effects and psychomotor performance. The magnitude of effects on ratings of strength of drug effect and sedation were comparable after flumazenil and midazolam, but psychomotor performance effects were greater after midazolam than after flumazenil. Caffeine produced mostly saline-appropriate responding. The results indicate that flumazenil has agonist effects similar to those of midazolam; however, novel responding after midazolam, and the greater performance decrement after midazolam, suggest that flumazenil does not act as a traditional benzodiazepine agonist.

Flumazenil (Romazicon) is a benzodiazepine receptor antagonist. Benzodiazepine receptor antagonism was first demonstrated by the ability of flumazenil to displace tritiated benzodiazepines from receptors and by its ability to block the behavioral and physiological effects of benzodiazepines (Hunkeler et al., 1981). Clinically, flumazenil (1 mg/70 kg i.v.) has been used to reverse the effects of benzodiazepine overdose and speed recovery time after benzodiazepines have been administered for medical procedures (Trevor and Way, 1998).

Early reports of the effects of flumazenil showed that, although this compound demonstrated affinity for benzodiazepine receptors, it lacked intrinsic activity, and for this reason, flumazenil was considered a “pure” antagonist and was often used as a control in experiments characterizing benzodiazepines (Hunkeler et al., 1981; Herling and Shannon, 1982; Ator and Griffiths, 1983; Darragh et al., 1983; File and Pellow, 1986). However, increasing evidence suggests that flumazenil has agonist effects. Six studies with nonhuman subjects used drug discrimination procedures and provided evidence for agonist effects (Dantzer and Perio, 1982; De Vry and Slangen, 1985a,b; Woudenberg and Slangen, 1990; Rowan and Lucki, 1992; Wong et al., 1993; France and Gerak, 1997). The first of these studies demonstrated that flumazenil fully substituted for clorazepate, suggesting benzodiazepine-like discriminative stimulus effects of flumazenil alone (Dantzer and Perio, 1982). Taking this finding a step further, De Vry and Slangen (1985a,b) and Woudenberg and Slangen (1990) demonstrated that rats readily acquired flumazenil (10 or 15 mg/kg i.p.)-saline discriminations. In addition, substitution tests in these flumazenil-trained animals demonstrated full substitution of benzodiazepines (clordiazepoxide, flunitrazepam, and clonazepam), and partial substitution of barbiturates (pentobarbital and pheno-
barbital), and the benzodiazepine receptor inverse agonist pentylentetrazol (De Vry and Slangen, 1985b). Using a conditioned taste aversion procedure in rats, Rowan and Lucki (1992) showed that a flumazenil-saline discrimination was readily acquired, but neither full agonists (i.e., diazepam, alprazolam) nor full inverse agonists (i.e., pentylenetetrazol) fully substituted for flumazenil. A more recent study demonstrated that flumazenil could be trained as a discriminative stimulus in monkeys dependent on clordiazepoxide (France and Gerak, 1997). The discriminative stimulus effects of flumazenil in this study were thought to result from precipitated withdrawal; however, when clordiazepoxide was discontinued, monkeys did not show clear signs of benzodiazepine withdrawal, and flumazenil still controlled flumazenil-appropriate responding. Thus, the initial discrimination may have been based, in part, on the intrinsic effects of flumazenil. Finally, pigeons were trained to discriminate flumazenil (100 μg/kg) from vehicle; however, in contrast to most findings with rodents, classic benzodiazepine agonists did not substitute for flumazenil, and ligands with high affinity for diazepam-insensitive receptors did substitute for flumazenil (Wong et al., 1993). Collectively, these drug discrimination studies from nonhumans indicate that flumazenil can be trained as a discriminative stimulus, but they do not demonstrate a clear profile of benzodiazepine agonist effects.

In contrast to these studies, other studies in which rats, monkeys, or baboons were trained to discriminate either a benzodiazepine or a barbiturate from saline found that flumazenil (1–100 mg/kg) produced predominantly saline-appropriate responding (Herling and Shannon, 1982; Ator and Griffiths, 1983; Schechter, 1984; Willets and Balster, 1989; Woolverton and Nader, 1995), suggesting that flumazenil lacked benzodiazepine-like discriminative stimulus effects.

The purpose of the present study was to characterize the discriminative stimulus, self-reported, and performance effects of flumazenil in humans and to compare these effects to a traditional benzodiazepine (i.e., midazolam) with a novel-response drug discrimination procedure. A previous series of studies in humans trained to discriminate triazolam, a short-acting benzodiazepine, from placebo demonstrated that the novel-response procedure is useful for making distinctions among sedative/hypnotic drugs that cannot be made under standard two-response procedures (Kamien et al., 1997; Smith and Bickel, 1999). In the first group of participants, we studied whether flumazenil could be trained as a discriminative stimulus in humans and whether flumazenil’s effects were dose dependent. The training dose of flumazenil (0.56 mg/70 kg i.v.) was chosen from pilot data as the lowest dose that antagonized the discriminative stimulus effects of triazolam (0.32 mg/70 kg p.o.). Because it did not include testing of drugs other than flumazenil, this part of the study was conducted under a standard two-response procedure. With the second group of participants, 0.56 mg/70 kg flumazenil was again trained as a discriminative stimulus, and substitution tests with midazolam and caffeine were conducted under the novel-response procedure. Midazolam was chosen because it can be administered i.v. and has similar pharmacokinetics to flumazenil. Caffeine (75 mg/70 kg) was chosen as a negative control.

Materials and Methods

Participants

Participants were 28 healthy volunteers recruited through advertisements in local newspapers and posters placed around our university campus. Potential participants were screened to ensure good health with no prior histories of psychiatric illness or drug or alcohol abuse according to medical histories provided by the participants, as well as physical assessments, EKGs, and routine laboratory screening. As a safety precaution against adverse reactions, the inclusion criterion for participation was 2 to 20 alcohol drinks per week. In previous studies, this criterion has been effective in providing cross-tolerance to the effects of benzodiazepines (Kamien et al., 1994, 1997). Exclusion criteria were 1) current or chronic diagnosed medical conditions; 2) pregnancy, plans to become pregnant, or inadequate birth control; 3) medical contraindication to or adverse effect from the drug class(es) to be tested; 4) history of drug or alcohol abuse (other than caffeine or nicotine) or major psychiatric disorder; 5) presently or recent use of over-the-counter or prescribed psychoactive drug(s) that would have an interaction with any of the drugs to be tested; 6) avoidance of caffeine or caffeinated beverages; and 7) plans to significantly change alcohol, cigarette, caffeine, exercise, or dietary habits.

Current abstinence from amphetamine, barbiturates, benzodiazepines, cocaine, opioids, and cannabinoids was confirmed via urinalysis testing before participation. Participants were instructed to refrain from caffeine and solid food for 4 h and alcohol for 24 h before an experimental session. Participants also were told to refrain from all illicit drug use for the duration of the study and urine samples were screened randomly once per week to test compliance. This study was approved by the Institutional Review Board at the University of Vermont. Each participant gave written informed consent before participating. Participants received monetary compensation for their participation at the rate of $4/h and earned up to an additional $12/session, depending upon their discriminative performance each session.

Fourteen participants (3 females, 11 males) completed the study. Characteristics of these participants are summarized in Table 1. Of the 14 participants who did not complete the study, 7 were dismissed for failing to learn the discrimination. Of the seven other participants who did not complete the study, two were dismissed before acquisition of the discrimination could be determined (one for high blood pressure and one for a drug-positive urine screen). The other five participants had learned to discriminate flumazenil from saline, but did not complete the study for other reasons (two were dismissed for drug-positive urine screens, two quit before finishing, and one was dismissed for poor attendance).

Apparatus

Sessions were conducted in a 4.8 m × 3.7 m room at the General Clinical Research Center at Fletcher Allen Hospital (Burlington, VT). This room contained two experimental stations. Participants reclined on a standard hospital bed, adjusted to the sitting position.

### TABLE 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>22 yr</td>
<td>18–28 yr</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>100 kg</td>
<td>59–115 kg</td>
</tr>
<tr>
<td><strong>Alcohol consumption</strong></td>
<td>6 Drinks/week</td>
<td>2–16 Drinks/week</td>
</tr>
<tr>
<td><strong>Nicotine consumption</strong></td>
<td>83 Cigarettes/week</td>
<td>35–140 Cigarettes/week</td>
</tr>
<tr>
<td><strong>Caffeine consumption</strong></td>
<td>648 mg/week</td>
<td>60–1680 mg/week</td>
</tr>
</tbody>
</table>

*Twelve of 14 volunteers reported current use of caffeine. The two remaining volunteers reported that although they did not currently consume caffeine, they did not actively avoid it.*
All questionnaires and performance tests were presented on a Macintosh PowerBook 520 computer in a prearranged and timed sequence. Participants used the track-pad mouse and an externally attached keypad to make their responses.

**Experimental Session**

Sessions began at either 9 AM or 1 PM and participants remained at the laboratory for ~3 h. Sessions were always conducted at the same time of day for each participant. Before each session, sobriety tests (i.e., tests of balance, hand coordination, and simple arithmetic) were completed as a baseline for comparison before release. Pregnancy tests were completed for all female participants before each session. At the beginning of each session, participants had an i.v. catheter placed in one arm, and blood pressure, breath alcohol level, and heart rate were recorded. A pulse oximeter finger clasp was then attached to the nondominant ring finger of each participant to monitor oxygen saturation for the duration of the experimental session.

Assessment of presession baseline measures followed. These measures included the Addiction Research Center Inventory (ARCI) short form, an adjective rating scale, and the Digit Symbol Substitution Test (DSST). Once these presession measures were completed, participants were administered a drug. Drug was infused over 5 min. Five minutes after completion of the drug infusion, participants completed the ARCI, the adjective rating scale; visual analog scales (VAS); the DSST; and a fixed-interval (FI) 1-s discrimination task (described below). A sealed envelope that contained the letter-code identity of the administered drug, or the information that it was a test session, was opened at the end of this set of tasks for each participant. The i.v. catheter was removed, and after having their heart rate and blood pressure checked, participants were released to a recovery area. In recovery, participants ate and engaged in activities such as watching TV or reading. Beginning at 11:30 AM or 3:30 PM, blood pressure, heart rate, sobriety tests, and a recall task were completed at 15-min intervals until participants satisfied release criterion and were allowed to leave the laboratory.

**Design**

After an initial nondrug training session was conducted to familiarize each participant with the computer tasks and the routine of the laboratory procedure, the study proceeded in three phases, with sessions conducted three to five times/week. The training and test-of-acquisition phases were identical for both groups I and II. The test phase for group I was conducted under a two-response procedure and for group II under a novel-response procedure as described below.

**Training (Phase 1).** During the training phase (4 sessions), participants received either 0.56 mg/70 kg flumazenil (e.g., drug A) or saline (e.g., drug B). Participants were informed of the letter code appropriate for the drug at the time of drug administration. The training doses of flumazenil and saline were administered on alternate days. Even though participants were informed of the letter code associated with the administered drug, correctly identifying the drug by appropriate letter code on the discrimination task increased participants’ monetary compensation.

**Test-of-Acquisition (Phase 2).** During the test-of-acquisition phase (4–8 sessions), participants were not informed of the letter code associated with the drug at the time of administration so that discriminative control by the training drugs could be tested. Participants identified which drug they received on the discrimination task (described below). At the completion of the session, the identity of the correct drug code was revealed to the participant. Correctly identifying the administered drug by letter code increased monetary compensation. To meet the criterion for acquisition of the discrimination, participants had to respond ≥80% appropriately to the drug condition for four consecutive sessions (two training drug and two saline administrations in mixed order) within a maximum of eight sessions (see **Discrimination Measure**).

**Test Phase (Phase 3): Two-Response Procedure (Group I).** During the test phase, group I received various doses of flumazenil (0, 0.10, 0.32, 0.56, and 1.0 mg/70 kg) under the two-response procedure. Under the two-response procedure, participants were instructed to respond on the discrimination task with the two buttons corresponding to the letter codes associated with the training drugs (e.g., A and B). They were not told how to respond if they received a drug unlike either of the two training drugs. On test sessions, participants were not informed of the letter code associated with the drug they received, but were instead informed at the end of the session that they had completed a “test” session. Participants were instructed that the accuracy of their responding on test sessions would be disclosed at the completion of the study. In fact, upon completion of the study, participants were compensated $12 for every test session completed, independent of their performance.

**Test Phase (Phase 3): Novel-Response Procedure (Group II).** During the test phase, group II received doses of flumazenil (0, 0.32, 0.56, and 1.0 mg/70 kg), midazolam (0, 0.10, 0.56, and 1.0 mg/70 kg), and caffeine (75 mg/70 kg) under the novel-response procedure. Under the novel-response procedure, participants were told to respond on the discrimination task with the three buttons corresponding to the letter codes associated with the training drugs (e.g., A and B) and the novel response (i.e., N). They were told to respond on the N button if they received a drug with effects unlike those of either of the training drugs. As in group I, during test sessions, participants were not informed of the letter code associated with the drug they received, but were instead informed at the end of the session that they had completed a test session. Participants were instructed that the accuracy of their responding on test sessions would be disclosed at the completion of the study. In fact, upon completion of the study, participants were compensated $12 for every test session completed, independent of their performance.

**Test Phase (Phase 3): Both Groups I and II.** To ensure that the discrimination was maintained during the test phase, test-of-acquisition sessions were interspersed between test sessions. During these sessions, participants received either flumazenil (0.56 mg/70 kg) or saline. At the completion of these sessions, the identity of the correct drug code was revealed to the participant. Participants had to respond with ≥80% of their responses toward the administered drug to move to a test session. If they did not meet this criterion, additional test-of-acquisition sessions were added until two consecutive sessions met the discrimination criterion.

**Dependent Measures**

**Discrimination Measure.** The FI 1-s schedule of point presentation is a 3-min task in which participants used the mouse and cursor to select a button associated with a drug code. The first response following each 1-s interval resulted in an increase of one point on the counter, which was displayed on the computer screen. A 10-s changeover delay occurred if participants switched from one letter code to another. Each button press was worth $0.067. Thus, if a participant earned the total number of points (i.e., 180) on the correct letter code, earnings equaled $12.

**Self-Reports.** The ARCI consisted of 49 true/false questions that were scored as five subscales: a morphine-benzodiazepine (MBG) group, a pentobarbital-chlorpromazine-alcohol group (PCAG), a lysergic acid diethylamide (LSD) group, a benzodrine group (BG), and an amphetamine (A) group (Martin et al., 1971; Jasinski 1977). The adjective rating scale presented 32 adjectives that participants rated on a 5-point scale from 0 (not at all) to 4 (extremely). The items were grouped into two subscales: a sedative scale consisting of adjectives describing sedative effects (Kamien et al., 1994) and a stimulant scale consisting of adjectives describing stimulant effects (Hughes et al., 1991). The VAS consisted of 100-point horizontal lines anchored with “not at all” on one end and “extremely” on the other. Participants rated the strength of drug effect, drug-liking, good drug effects, bad drug effects, drug-induced high, drug-induced anxiety, and the similarity of the drug to each training condition. During the test
phase, Group II also rated the degree of “novelty” of the administered drug.

DSST. A computerized version of the DSST was used (McLeod et al., 1982). Participants used the keypad to reproduce a geometric pattern associated with a digit according to the code presented continuously across the top of the screen. Participants were told to complete as many patterns as possible, as accurately as possible, in the allotted time (90 s). Data collected were the number of trials correctly completed and the total number of trials completed.

End-of-Study Questionnaire. After the experimental session on their last day of participation, participants completed a paper and pencil end-of-study questionnaire. Participants were asked the open-ended question of what cues they used to discriminate the two drugs, and then were asked to circle which class of drug they thought each drug came from (sedative, stimulant, placebo, or other).

Drugs

All drugs were administered i.v. over 5 min. Each syringe contained the same volume on each session for each participant (prepared by the Fletcher Allen Health Care Investigational Pharmacy). Intravenous flumazenil has a quick onset of action (~1 min) and a short elimination half-life (0.8–1.16 h; Trevor and Way, 1998). Intravenous midazolam and i.v. caffeine also have rapid onsets of action (1–2 min), but longer elimination half-lives (1.2–12.3 and 3–5 h, respectively; Trevor and Way, 1998). For groups I and II, the order of drug testing of flumazenil during the test phase was randomized across participants. For group II, all doses of each drug were assessed before the next drug and the order of drug testing was mixed across participants. Because midazolam can cause significant respiratory depression, midazolam sessions were scheduled such that one of the two lower doses of midazolam was administered before the highest dose. This dosing requirement allowed for some variability in dose order, but importantly, also allowed each participant’s reaction to midazolam to be assessed before the highest test dose. All participants tolerated all doses of midazolam.

Data Analysis

Data from the 14 participants who completed the study were included in the analyses. FI responding on the drug discrimination task was averaged across groups and for each group separately and is reported as the percentage of responses on the flumazenil-appropriate button for group I and on the flumazenil-appropriate and novel buttons for group II. Consistent with previous studies in drug discrimination, ≥80% drug-appropriate responding was interpreted as full substitution, between 25 and 79% drug-appropriate responding was interpreted as partial substitution, and <25% drug-appropriate responding was interpreted as no substitution. Scores on the adjective rating scales and the ARCI are reported as the change from baseline; scores on the visual analog scales and the DSST are reported as the postdrug scores. Self-report and DSST data were analyzed statistically. All statistical analyses were done with BMDP statistical software (University of California, Berkeley, CA) except where noted. For all statistical analyses, \( p \leq .05 \) was used to infer significance. The results from the end-of-study questionnaire were not analyzed statistically.

Test-of-Acquisition. First, the self-report and DSST data collected during the test-of-acquisition phase were averaged across the two saline and the two flumazenil exposures for those four sessions for each participant that met the criterion for acquisition of the discrimination (groups I and II; \( n = 14 \)). These data were then analyzed using repeated measures ANOVA testing for a main effect of drug (flumazenil versus saline).

Because only one dose of caffeine (75 mg/70 kg) was tested in group II, these scores were compared with the flumazenil and saline scores from the test-of-acquisition phase (group II only; \( n = 8 \)) using ANOVA. In cases in which a significant result occurred, pairwise comparisons were conducted with Fischer’s protective least-significant difference procedure to determine where differences occurred.

Dose-Effect Curves. The self-report and DSST data for group I (\( n = 6 \)) and group II (\( n = 8 \)) were analyzed with separate repeated measures ANOVA with dose of flumazenil (0.10, 0.32, 0.56, and 1.0 mg/70 kg) as the within-participant factor or with dose of flumazenil (0.32, 0.56, and 1.0 mg/70 kg) or midazolam (0.10, 0.56, and 1.0 mg/70 kg) as the within-participant factor, respectively. Placebo points from each dose-effect curve were excluded from these analyses because the primary goal was to examine the effects across active doses. These analyses tested the overall dose effects as well as linear, quadratic, and for group II only, cubic contrasts. In addition, each active drug dose from each dose-effect curve was compared with saline using Dunnett’s post hoc test.

Results

Groups I and II: Test-of-Acquisition

Discrimination. Of the 28 participants who began the study, 26 participated in the test-of-acquisition phase. Nineteen of these 26 participants (73%) met the discrimination criterion. For the 14 participants who completed the study, the discrimination criterion was met in an average of five sessions (range, 4–8). Ten of these participants met the criterion for acquiring the discrimination in the minimum four sessions.

Self-Reports and DSST. Table 2 summarizes the results of statistical analyses testing for a main effect of drug condition (0.56 mg/70 kg flumazenil versus saline) for the 14 participants who completed the study. On the ARCI, flumazenil significantly increased scores on the PCAG (Fig. 1 and Table 2) and significantly decreased scores on the BG subscales (Fig. 1). The training conditions did not differentially alter ratings on the other subscales of the ARCI (Table 2).

On the adjective rating scales, flumazenil significantly increased sedative ratings relative to saline and significantly decreased stimulant ratings relative to saline. As shown in Fig. 2, flumazenil significantly increased VAS measuring strength of drug effect, drug-liking, drug-induced high, good drug effects, similarity to saline, and similarity to flumazenil.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>F values and associated significance from ANOVA testing for main effects of drug condition from the test-of-acquisition phase for self-report measures and the DSST for groups I and II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Measures</td>
<td>Flumazenil (0.56 mg/70 kg) versus Saline ( ^{df} )</td>
</tr>
<tr>
<td>PCAG</td>
<td>14.3**</td>
</tr>
<tr>
<td>BG</td>
<td>6.01*</td>
</tr>
<tr>
<td>MBG</td>
<td>0.34</td>
</tr>
<tr>
<td>A</td>
<td>1.00</td>
</tr>
<tr>
<td>LSD</td>
<td>1.05</td>
</tr>
<tr>
<td>Sedative subscale</td>
<td>20.6**</td>
</tr>
<tr>
<td>Stimulant subscale</td>
<td>8.65**</td>
</tr>
<tr>
<td>Strength of drug effect</td>
<td>40.4**</td>
</tr>
<tr>
<td>Drug-liking</td>
<td>5.57*</td>
</tr>
<tr>
<td>Drug-induced high</td>
<td>25.4**</td>
</tr>
<tr>
<td>Good effects</td>
<td>5.46*</td>
</tr>
<tr>
<td>Bad effects</td>
<td>1.91</td>
</tr>
<tr>
<td>Drug-induced anxiety</td>
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</tr>
<tr>
<td>Similar to flumazenil</td>
<td>196***</td>
</tr>
<tr>
<td>Similar to saline</td>
<td>119***</td>
</tr>
<tr>
<td>Number completed</td>
<td>4.74*</td>
</tr>
<tr>
<td>Number completed</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Nonsignificant F values are not followed by an asterisk. \( ^{*} p < .05 \), \( ^{**} p < .01 \), and \( ^{***} p < .001 \).
The training conditions did not produce significant differences on the other visual analog scales (Table 2).

Flumazenil significantly decreased performance on the total number of patterns completed on the DSST (Fig. 2). This effect was of a small magnitude. Flumazenil did not significantly affect the number of patterns correctly completed relative to saline (Table 2).

**Group I: Flumazenil Dose-Effect Curve Determinations under Two-Response Procedure**

**Discrimination.** The effects of different doses of flumazenil on FI responding on the drug discrimination task for the six participants in group I are shown in Fig. 3. The lowest dose of flumazenil (0.10 mg/70 kg) produced primarily saline-appropriate responding. The percentage of flumazenil-appropriate responding was increased in a dose-related manner, with both the training dose of flumazenil and the highest test dose (1.0 mg/70 kg) of flumazenil producing >90% flumazenil-appropriate responding (Fig. 3).

**Self-Reports and DSST.** The results of ANOVA of flumazenil dose on the self-reports and DSST are summarized in Table 3. On the ARCI, flumazenil dose-dependently increased scores on the PCAG and dose-dependently decreased scores on the BG (Fig. 3). Flumazenil dose did not significantly affect the other subscales of the ARCI (Table 3). Flumazenil did not significantly affect either of the subscales of the adjective rating scale (Table 3).

On the VAS, flumazenil significantly increased ratings of strength of drug effect and similarity to flumazenil, and significantly decreased similarity to saline (Fig. 3). Additionally, flumazenil produced a marginally significant decrease on the rating of drug-liking (Table 3). Flumazenil did not significantly affect ratings on the other VAS (Table 3). On the DSST, flumazenil significantly decreased scores on the number of patterns correctly completed (Table 3 and Fig. 3) but ANOVA did not reveal a significant affect on the total number of patterns completed (Table 3).

In addition to these ANOVA, Dunnett’s post hoc testing revealed that responses to one or both of the higher doses of flumazenil were significantly different from responses after saline on several measures, including the PCAG, BG, strength of drug effect, similarity to flumazenil, similarity to saline, and drug-induced high (Fig. 3, open circles).

**Group II: Flumazenil and Midazolam Dose-Effect Curve Determinations under Novel-Response Procedure**

**Discrimination.** As shown in Fig. 4, flumazenil and midazolam both increased flumazenil-appropriate responding. Flumazenil-appropriate responding increased to 100% after the training dose but decreased to 75% after the highest test dose. This dose also produced 12.5% saline-appropriate and 12.5% novel responding. Midazolam produced dose-dependent increases in flumazenil-appropriate responding, with an average of 0% after the lowest test dose, 41% after the intermediate test dose, and 75% after the highest test dose. Midazolam also produced a maximum of 43% novel responding after the intermediate dose, and 25% novel responding after the highest test dose.

**Self-Reports and DSST.** The results of ANOVA of flumazenil dose and midazolam dose on self-reports and the DSST...
for group II are shown in Table 3. Flumazenil, unlike midazolam, did not produce any significant dose-related effects on any of these measures (Table 3).

On the ARCI, midazolam produced a significant dose-related increase on the PCAG and a significant dose-related decrease on the BG subscale (Fig. 5). Midazolam did not produce any other significant effects on the ARCI (Table 3). On the adjective rating scale, midazolam significantly increased ratings on the sedative subscale in a dose-dependent manner, but did not significantly decrease scores on the stimulant subscale (Fig. 5 and Table 3). On the VAS, midazolam produced significant dose-related increases on measures of strength of drug effect, drug-induced high, good drug effects, and similarity to flumazenil (Fig. 6). In addition, midazolam also produced a significant dose-related decrease on the measure of similarity to saline (Fig. 6). Midazolam did not significantly affect scores on the other VAS (Table 3). On the DSST, midazolam produced a significant dose-related decrease on both measures of performance (Fig. 6).

In addition to these findings from ANOVA, Dunnett’s post hoc testing revealed that the three active doses of flumazenil were significantly different from saline on several measures. These measures include the VAS rating strength of drug effect, drug-induced high, good drug effects, drug-liking, similarity to flumazenil, and similarity to saline (Fig. 6). By contrast, only the two higher active doses of midazolam were significantly different from saline on VAS ratings strength of drug effect, drug-induced high, similarity to flumazenil, and similarity to saline (Fig. 6). In addition, scores after the highest dose of midazolam were significantly different from
saline on the PCAG subscale of the ARCI, the stimulant subscale of the adjective rating scale, and on both measures of DSST performance (Figs. 5 and 6).

**Group II: Effects of Caffeine Compared with Flumazenil and Saline.** Caffeine (75 mg/70 kg) produced 72% saline-appropriate, 13% flumazenil-appropriate, and 15%

**Table 3**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>PCAG</td>
<td>7.86***</td>
<td>0.08</td>
<td>4.66*</td>
</tr>
<tr>
<td>BG</td>
<td>4.02*</td>
<td>0.72</td>
<td>6.08*</td>
</tr>
<tr>
<td>MBG</td>
<td>0.63</td>
<td>0.32</td>
<td>0.00</td>
</tr>
<tr>
<td>A</td>
<td>1.22</td>
<td>0.72</td>
<td>0.39</td>
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<tr>
<td>LSD</td>
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<td>0.77</td>
</tr>
<tr>
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<td>1.11</td>
<td>8.26*</td>
</tr>
<tr>
<td>Stimulant subscale</td>
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<td>0.07</td>
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</tr>
<tr>
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<td>1.51</td>
<td>7.52**</td>
</tr>
<tr>
<td>Drug-liking</td>
<td>2.79</td>
<td>0.19</td>
<td>2.09</td>
</tr>
<tr>
<td>Drug-induced high</td>
<td>2.26</td>
<td>0.62</td>
<td>4.32*</td>
</tr>
<tr>
<td>Good effects</td>
<td>2.30</td>
<td>0.54</td>
<td>1.39</td>
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<tr>
<td>Bad effects</td>
<td>1.83</td>
<td>0.17</td>
<td>5.06*</td>
</tr>
<tr>
<td>Drug-induced anxiety</td>
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<td>0.82</td>
<td>0.14</td>
</tr>
<tr>
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<td>3.57</td>
<td>10.56**</td>
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<tr>
<td>Similar to saline</td>
<td>10.9***</td>
<td>3.34</td>
<td>31.52**</td>
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<tr>
<td>Number correct</td>
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<td>0.19</td>
<td>7.25**</td>
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<tr>
<td>Number completed</td>
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<td>0.50</td>
<td>9.48**</td>
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<tr>
<td>Similar to novel</td>
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<td>1.88</td>
<td></td>
</tr>
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</table>

* Quadratic effect of dose. * p < .05, ** p < .01, and *** p < .001.

**Fig. 3.** Effects of flumazenil (0.10–1.0 mg/70 kg) on discriminative performance and several self-reported measures for group I (n = 6). Data are expressed as the mean percentage of flumazenil-appropriate responding (upper left), change from baseline (lower left), and postdrug score (other). Points above "S" represent responding after saline; ○, points significantly different from saline. Vertical bars are S.E. The significance of the F values from the ANOVA were: *, p < .05, **, p < .01, and (***, p < .001.

**Fig. 4.** Effects of flumazenil (0.32–1.0 mg/70 kg) and midazolam (0.10–1.0 mg/70 kg) on the FI 1-s schedule of point presentation for group II (n = 8). Discriminative performance is expressed as the percentage of flumazenil-appropriate (circles), and novel responding (squares). Vertical bars are S.E. All other details as in Fig. 3.
novel responding, and on most self-report measures did not produce effects significantly different than either flumazenil or saline. However, on six self-reports, ANOVA indicated that significant differences occurred among saline, flumazenil, and 75 mg/70 kg caffeine. These measures were the sedative subscale of the adjective rating scale, the VAS measuring strength of drug effect, similarity to flumazenil and similarity to saline, and the number of patterns correctly completed and the total number of patterns completed on the DSST (Fig. 7). Pairwise comparisons revealed that these differences occurred as a function of flumazenil-saline, flumazenil-caffeine, and saline-caffeine differences, as shown in Fig. 7.

**Group I and Group II: End-of-Study Questionnaire.**
Eight of the 14 participants completed the end-of-study questionnaire. When asked what cues were used to differentiate the training drugs, participants used the following words to describe flumazenil: “heavy head” ($n = 2$), “disoriented” ($n = 1$), “sweating” ($n = 1$); and “dizzy” ($n = 2$). Seven participants identified flumazenil as a sedative, one identified flumazenil as a stimulant. None identified flumazenil as placebo. Five participants identified saline as placebo, two identified saline as stimulant, and one identified saline as sedative.

**Discussion**

This study is the first to demonstrate that humans can acquire and maintain a flumazenil (0.56 mg/70 kg) versus saline discrimination. The results of this study support previous studies with rodents that showed flumazenil discriminations could be readily acquired (de Vry and Slangen, 1985a,b; Woudenberg and Slangen, 1990; Rowen and Lucki, 1992). Further, the discriminative stimulus, self-reported, and performance effects of flumazenil were demonstrated to be dose-dependent; midazolam produced a dose-dependent increase in flumazenil-appropriate responding as well as some novel responding, and midazolam had a greater magnitude of effect on performance than flumazenil. These results support other evidence demonstrating agonist effects of flumazenil in humans (Higgit et al., 1986; Kapczinski et al., 1994; Saxon et al., 1997).

Overall, 73% of participants who began the study were able to acquire the flumazenil-saline discrimination. These results are similar to previous studies in which participants were trained to discriminate triazolam p.o. from placebo (Oliveto et al., 1992, 1994; Bickel et al., 1993; Kamien et al., 1994, 1997). In these studies, an average of 75% of participants acquired the discrimination in an average of five to seven sessions across studies. The major difference between the previous triazolam discrimination studies and the present study was the route of administration. Whether the discriminative stimulus effects of flumazenil would be as salient orally is not known.

The results from the tests-of-acquisition revealed that relative to saline, the training dose of flumazenil significantly increased measures related to the strength of drug effect, positive mood effects, and measures of sedation, and de-
increased performance on the DSST. This profile of effects is similar to that observed in past studies in which participants were trained to discriminate triazolam from placebo (Oliveto et al., 1992; Bickel et al., 1993). Indeed, a similar profile of effects has been noted with other commonly prescribed sedative/hypnotics such as diazepam, zolpidem, and secobarbital (Kamien et al., 1994, 1997).

Lower doses of flumazenil were tested to determine the pharmacological sensitivity of the flumazenil discrimination. Under both the two-response (group I) and the novel-response (group II) procedures, flumazenil produced dose-dependent increases in flumazenil-appropriate responding. Consistent with previous studies, the novel-response procedure did not disrupt the dose-dependent increase in training drug-appropriate responding when doses lower than the training dose were tested (Bickel et al., 1993; Kamien et al., 1997). In addition to lower test doses, a dose higher than the training dose was tested in both groups. This dose produced mostly flumazenil-appropriate responding in both groups I and II (in 6 of 6 participants and 6 of 8 participants, respectively). In group II, the highest dose of flumazenil produced novel responding in one participant, and saline-appropriate responding in another participant. This degree of novel responding to a dose higher than the training dose is consistent with a previous study of triazolam discrimination (Kamien et al., 1994). The dose-dependent increase in flumazenil-appropriate responding after midazolam administration, with nearly full substitution (75%) at the highest test dose, is similar to data from nonhumans demonstrating full substitution (>80% flumazenil-appropriate responding) of flunitrazepam and clonazepam for flumazenil (De Vry and Slangen, 1985a,b; Woudenberg and Slangen, 1990), and partial substitution of chlordiazepoxide and midazolam for flumazenil (Woudenberg and Slangen, 1990; Rowan and Lucki, 1992). Collectively, the results of the present study and the previous studies suggest that the effects produced by flumazenil are similar to traditional benzodiazepines in both rodents and humans.

Both our study and the studies in rodents are in contrast to a previous study of flumazenil discrimination in pigeons.
In that study, only ligands with high affinity for diazepam-insensitive receptors substituted for flumazenil. The classic benzodiazepine agonists midazolam and chlordiazepoxide produced only ~2% flumazenil-appropriate responding. These results may be due to a species difference in the relative abundance of diazepam-insensitive receptors in pigeons (Wong et al., 1993).

The previous studies in which flumazenil discriminations were trained in rodents also demonstrated partial substitution of inverse agonists, such as PTZ (De Vry and Slangen, 1985b). This finding provided additional evidence for activity at the benzodiazepine receptor. Tests with inverse agonists were not conducted in our study due to the risk of seizure associated with these compounds. However, findings on the self-report measures suggest flumazenil has a profile of effects consistent with sedative/hypnotics. Increases on measures of anxiety and stimulation, which would be expected of an inverse agonist (Baldwin and File, 1988), were not demonstrated in our study. Although inverse agonist activity was not evident on self-reports, this may not necessarily indicate that an inverse agonist, acting at the benzodiazepine receptor, would not substitute for flumazenil because discriminative stimulus effects and self-reports do not always covary (Chait et al., 1989; Lamb and Henningfield, 1994).

The novel-response drug discrimination procedure was used to assess the similarity of midazolam’s discriminative stimulus effects to those of flumazenil. Midazolam produced both novel responding and a dose-dependent increase in flumazenil-appropriate responding. These results suggest that, although the discriminative stimulus effects of midazolam are similar to flumazenil, they are not identical. Research with other selective and nonselective drugs for the benzodiazepine receptor needs to be conducted in a flumazenil discrimination study to determine the specificity of flumazenil’s discriminative stimulus effects in humans.

Testing flumazenil in a benzodiazepine discrimination study in humans also may be useful. Past research indicates that assessing cross-substitution profiles is useful for determining differences in selectivities within drug classes (Ator and Griffiths, 1983, 1997). In these studies with baboons responding under a two-response procedure, lorazepam substituted for a pentobarbital training stimulus, but pentobarbital did not substitute for a lorazepam training stimulus. This asymmetrical substitution profile is not typical under a two-response procedure, and suggested that lorazepam may be selective for subtypes of benzodiazepine receptors (Ator and Griffiths, 1983). These data are consistent with the finding that under the novel-response procedure lorazepam produced ~20% novel responding in humans trained to discriminate triazolam from placebo (Kamien et al., 1994). Thus,
lorazepam did not fit the profile of traditional benzodiazepines, such as diazepam (Oliveto et al., 1994), which produced no novel responding in triazolam-trained participants. The congruent findings across baboon and human studies support the utility of using the novel-response procedure in future investigations of flumazenil’s selectivity in humans.

On several self-report measures, flumazenil and midazolam dose-dependently increased ratings of strength of drug effect and sedative effects. A wider range of doses of flumazenil were tested in group I than in group II, and this may account for the greater number of statistically significant linear trends in group I. Data from the two groups were not compared statistically, but in terms of the absolute magnitudes of effects, midazolam increased ratings of sedation more compared with flumazenil in participants who received both drugs (group II), but not compared with the participants who only received flumazenil (group I). Midazolam decreased performance scores more than flumazenil. Thus, the self-report and performance data indicate that on most measures, flumazenil and midazolam produced similar effects, and only on a subset of measures (liking/disliking of drug, DSST), did the effects differ.

The profile of self-reported effects of flumazenil from this study is not similar to other studies that assessed the self-reported effects of flumazenil in humans, which generally reported no increases on standard measures of sedation (Darra\(g\) et al., 1983; Nutt et al., 1990; Griffiths et al., 1993). When increases were noted, the magnitudes of effects were not comparable with those observed with traditional benzodiazepines (e.g., increased dizziness; Nutt et al., 1990; Griffiths et al., 1993), despite the testing of doses equivalent to or higher than those tested in the present study (i.e., 2–3 mg i.v.; 600 mg p.o.). One major difference between these studies and our study was the inclusion of the discrimination component. Indeed, one of the advantages of training drug discriminations is that participants can learn to discriminate relatively low doses. Previous research on caffeine discrimination in humans demonstrated that doses that are not normally behaviorally active (e.g., 18 mg) can be trained as discriminative stimuli and alter self-reports (Griffiths et al., 1993; Silverman and Griffiths, 1992). Thus, the contingencies associated with discrimination training may increase participants’ sensitivity to the effects of drugs, and may explain the more robust effects of flumazenil in this study compared with previous studies in humans that did not include discrimination.

Caffeine (75 mg/70 kg) was tested as a negative control in our study, but produced mostly saline-appropriate responding, indicating that the chosen dose was too low and relatively inactive. For this reason, caffeine was not useful in this study as a control for novel responding, even though on some measures (e.g., DSST), the effects of caffeine were significantly different from saline and/or the training dose of flumazenil. The lack of an adequate negative control limits the conclusions that can be made regarding novel responding in this study. Despite the failure of caffeine to control novel responding, the concern that participants were biased against responding on the novel response is not valid. Six of the eight participants in group II used the novel response at least once, after either caffeine, the highest test dose of flumazenil, or to one of the two higher doses of midazolam.

In summary, our results demonstrate that flumazenil can be trained as a discriminative stimulus in humans. The results support previous research suggesting that flumazenil has benzodiazepine-like activity. The novel responding after midazolam indicates that the discriminative stimulus effects of flumazenil are not identical to those of a classic benzodiazepine agonist. The novel-response procedure may be useful in future experiments on the specificity of flumazenil in which the effects of other selective and nonselective benzodiazepine and nonbenzodiazepine anxiolytic drugs can be tested.

References


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