Pharmacology Biochemistry & Behavior, Vol. 39, pp. 351–359. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Midazolam Withdrawal and Discriminative Motor Control: Effects of FG 7142 and Ro 15-1788

MICHAEL VIGORITO,[1] CHYAN E. LAU, MAISY TANG[2] AND JOHN L. FALK[3]

Department of Psychology—Busch, Rutgers University, New Brunswick, NJ 08903

Received 31 January 1991

VIGORITO, M., C. E. LAU, M. TANG AND J. L. FALK. *Midazolam withdrawal and discriminative motor control: Effects of FG 7142 and Ro 15-1788.* PHARMACOL BIOCHEM BEHAV **39**(2) 351–359, 1991.—Rats chronically drank either water or midazolam solution (0.1 mg/ml) in daily, 3-h schedule-induced polydipsia sessions and were evaluated in daily motor control sessions after polydipsia when midazolam metabolite levels had fallen to zero (withdrawal). Under midazolam polydipsia, animals orally self-administered between 21 and 38 mg/kg daily. The effect of acute drug administration [midazolam (0.75–3 mg/kg, SC), FG 7142 (1–8 mg/kg, IP), Ro 15-1788 (10–20 mg/kg, IP)] on motor control performance was similar after either chronic water or midazolam polydipsia. Thus chronic, oral midazolam self-administration did not lead to tolerance to the motor impairment produced by SC midazolam, nor did the daily discontinuation lead to impaired motor performance, nor had these performances, which occurred after daily elevated midazolam metabolite levels had reached zero (withdrawal), become sensitized to the effects of either the benzodiazepine inverse agonist FG 7142 or the antagonist Ro 15-1788.

Midazolam self-administration Benzodiazepine rebound Midazolam pharmacokinetics
Schedule-induced polydipsia Motor performance Behavioral tolerance Rat

IN the last decade, increasing attention has been given to a complication associated with the therapeutic use of long-acting benzodiazepines: physical dependence development (24). Recently, a syndrome of rebound anxiety and insomnia upon abrupt drug discontinuation has been related to the use of shorter-acting benzodiazepines (13). This syndrome suggests that a physical dependence can develop with the use of these agents despite their relatively brief duration of action. Most of the relevant animal experiments, while indicating that physical dependence can be produced with short-acting benzodiazepines, administered the drugs so that the total daily dose was distributed evenly over each 24-h cycle (15, 16, 30). Midazolam is an ultrashort-acting benzodiazepine (25) that is administered as a single dose at bedtime for the treatment of insomnia (9,23). In order to determine whether once-a-day exposure to an ultrashort agent could produce a physical dependence in animals, analogous to the rebound state (early-morning insomnia) reported in humans (12), rats drank a midazolam solution in daily, 3-h schedule-induced polydipsia sessions and were periodically tested 90 min postsession for withdrawal signs (8). Physical dependence, as evidenced by susceptibility to audiogenically induced seizures, developed after 12 weeks of exposure and increased in severity with continued exposure.

Usually, measures of drug dependence have been based on the observation of behavioral disruptions after withdrawal from repeated drug administration. These disruptions typically consist of items such as tremors, activity changes, convulsions and a variety of autonomic signs. Such changes in reflexive and other unlearned kinds of behavior constitute the definition of physical dependence. Recently, Balster (2) proposed that a wider notion of behavioral dependence be adopted which encompasses more subtle behavioral disruptions that may occur where the classical syndrome of physical dependence is not observed. Operant behavior baselines are sensitive to drug withdrawal under circumstances in which signs of physical dependence were not evident (2). In previous research, we have used the disruption of operant discriminative motor control performance after drug discontinuation to assess behavioral dependence on ethanol, diazepam and midazolam, and cross-tolerance to phenobarbital in ethanol-dependent animals (5, 19, 26, 31, 33). In these studies, animals either received or self-administered daily drug doses just prior to motor control evaluation sessions, and then, daily drug dosing was discontinued to evaluate withdrawal effects. In the present research, schedule-induced polydipsic intake of midazolam occurred in a daily session, but the ensuing, daily motor control session took place after a delay period such that midazolam and its major metabolite were no longer present in the serum. This regimen allows the results of chronically self-administered midazolam to be evaluated daily at the point of drug disappearance. This is the point at which the animal analogue of the state described as "rebound anxiety and insomnia" in humans should be evident. It is tantamount to daily drug discontinuance. As indicated above, physical dependence did develop in association with daily schedule-induced midazolam intake sessions (8). One

[1]Present address: Department of Psychology, Seton Hall University, South Orange, NJ 07079.
[2]Present address: Department of Neuropathology (Neuroscience), Harvard Medical School, Boston, MA 02115.
[3]Requests for reprints should be addressed to John L. Falk, Department of Psychology—Busch, Rutgers—The State University, P.O. Box 6836, Piscataway, NJ 08855-6836.

purpose of the present study was to use motor control performance measures, rather than seizure susceptibility, to evaluate the state after daily drug elimination (the putative rebound period).

Although the daily, 3-h, polydipsic self-administration of midazolam solution led to the development of physical dependence, the appearance of withdrawal signs required the use of a precipitating audio stimulus (8). Thus we considered that, although discriminative motor control performance is sensitive to drug withdrawal, clear evidence of behavioral disruption during a rebound period might require that the state be unmasked or synergized by the administration of an inverse agonist or antagonist agent during this period. Accordingly, the effects of acute doses of midazolam (agonist), FG 7142 (a partial inverse agonist) (4), and Ro 15-1788 (an antagonist) (3) on discriminative motor control performance were evaluated in sessions occurring after water polydipsia or after animals had been exposed to chronic midazolam polydipsia and were in the putative rebound state.

METHOD

Animals

Eight male albino adult rats of the Holtzman strain were used. They were divided into two groups of 4 animals, a *Midazolam Group* (mean initial body weight = 383 g; range = 380–388 g) and a *Water Group* (mean initial body weight = 385 g; range = 380–394 g). They were housed individually in Plexiglas chambers (30 × 26 × 23 cm) in a temperature-regulated room with continuous illumination. Animals were reduced to 80% of their ad lib body weights by limiting daily food rations. Food supplements necessary for maintaining these weights were made available in the living cages immediately after the completion of each animal's daily experimental sessions. Body weights were maintained at 80% for the first 5 months of the experiment and then allowed to increase slowly over the next 7 months so that, during the last 2 of these months, body weights were at 86%.

Drugs

Midazolam maleate (Ro 21-3981) solutions were prepared daily by dissolving the drug in distilled water. Midazolam doses are expressed in terms of the salt. Midazolam solutions were made available under a schedule-induced polydipsia condition to the Midazolam Group and were also given by SC injection in a distilled water vehicle. The partial inverse agonist agent FG 7142 (N-methyl-β-carboline-3-carboxamide) and the benzodiazepine antagonist agent Ro 15-1788 were administered by IP injection in a 10% solution of Cremophor EL vehicle (Sigma Chemical, St. Louis).

Serum and Brain Analysis of Midazolam and 4-Hydroxymidazolam

Blood samples (100 μl) were taken from the tail tip. Clear serum was analyzed for midazolam and its major metabolite, 4-hydroxymidazolam, with HPLC, using a reversed phase column and a UV detector. The method was a modification of that developed for the analysis of diazepam (17). The whole brain was removed, weighed and homogenized in cold, nanopure water (1 g tissue:4 g water) and centrifuged. The supernatant (100 μl) was used for extraction as for serum samples.

Apparatus

Schedule-induced polydipsia. Each living chamber was equipped with a stainless steel pellet receptacle and a drinking fluid reservoir which consisted of a stainless steel, ball-bearing spout attached to a 250-ml Nalgene graduated cylinder. There were daily 3-h sessions during which a 45-mg food pellet (Bio Serv, Frenchtown, NJ) was delivered automatically into the food receptacle every 60 s (FT 1-min schedule), thus giving a total of 180 pellets during each feeding session.

Discriminative motor control. The experimental space was a Plexiglas chamber (25 × 30 × 30 cm) with stainless steel front and rear panels and a floor consisting of parallel-mounted, spaced, stainless steel rods. Discriminative motor control was measured using a force-sensitive, stainless steel operandum mounted on the front panel 2.5 cm from the floor. The operandum was surrounded by a thick Plexiglas shield fashioned with a 1.0-cm wide × 4.0-cm high slot so that access to it was limited to a single paw. The front edge of the operandum was recessed 1.2 cm from the front surface of the shield. This prevented lever-biting, nose-poking or behavior other than paw actuation from operating the lever. The operandum was suspended by a phosphor-bronze leaf spring (0.20 mm thick), and its shaft rested on a drive rod connected to a force transducer (Model UC3 strain gauge, Statham Instruments, Oxnard, CA) through a load cell (Statham Model UL4). The voltage output from the force transducer was conveyed to a customized signal control box (Tri-Tech Services, Hamilton Square, NJ) and sorted into one of three signal regions: above, below or within a window defined by preset lower and upper voltage limits. These limits corresponded to applied forces of 0.147 N (15 g force) and 0.265 N (27 g force), respectively, incident at the paw-placement region of the operandum. A buffer was set so that a minimum force of 0.015 N (1.5 g force) was required for signal recognition. A Commodore Pet 4016 microcomputer was programmed in assembly language to sample signal input once every 10 ms. When the force applied by the animal was within the 0.147 to 0.265 N band, an audio feedback signal (Sonalert SC648H, P. R. Mallory, Indianapolis, IN) was turned on.

Discriminative Motor Control Measures

A continuously applied in-band force lasting 1.5 s was required for the delivery of a 45-mg food pellet (Bio Serv). If the applied force went above or below the band before 1.5 s had elapsed, then this timer was reset. Thus the behavior reinforced by food pellet delivery was holding the force transducer steadily operated within the force band for a continuous, set period of time. Ordinarily, a session was terminated when the 50th pellet had been delivered, but a session was also terminated if 30 min had elapsed without operation of the transducer. The latter occurrences were rare and were associated with a high drug dose. They are indicated in the relevant figures in the results.

The raw measures of motor behavior taken for each session were: the *session time* (the time taken to earn 50 pellets), the *total response time* (amount of the session time that the transducer was held operated above the minimum recognition threshold of 0.015 N), the *in-band time* (amount of the session time that the transducer was held operated within the force band, i.e., between 0.147 and 0.265 N), and the *entrances* (the total number of times during a session that the applied force entered the band from either the lower or upper set limits). Except in the case of the entrances measure, these raw measures in isolation are not useful characterizations of motor performance. For example, the in-band time measure is best interpreted in relation to how it compares with the minimum total in-band time that would satisfy the contingencies set for a particular experiment (e.g., in the present case, this value is 1.5 s/pellet for a total of 50 pellets, which yields a minimum possible in-band time of

75 s). Similarly, raw session in-band time is difficult to interpret unless viewed in relation to total response time.

Four measures of motor behavior were calculated from each session:

$$\text{In-band efficiency} = \frac{\text{minimum possible in-band time}}{\text{in-band time}}$$

$$\text{Tonic accuracy} = \frac{\text{in-band time}}{\text{total response time}}$$

$$\text{Work rate} = \frac{\text{total response time}}{\text{session time}}$$

Entrances = total number of entrances into the force band

The in-band efficiency measure has a fixed numerator (50 pellets × 1.5 s), making the minimum possible time in-band to deliver all pellets 75 s. A perfectly efficient performance would yield an efficiency measure of 1.00. The measure of tonic accuracy approaches 1.00 as the total time spent responding (i.e., more than 0.015 N applied to the transducer) approaches the time spent in-band. It measures an aspect of discriminative motor control that is somewhat different than that measured by in-band efficiency. Although a high proportion of session operandum holding might be within the appropriate force band, if the holding times are frequently of too short a duration to produce pellet delivery, then tonic accuracy could be high although in-band efficiency is low. Work rate is simply the proportion of the session time that the animal spends operating the transducer. Because work rate can approach a value of 1.00 or zero, the previous measures can approximate 1.00 or zero in complete independence of work rate. The entrances measure is simply the number of times the applied force enters the appropriate band, with a high count indicating difficulty maintaining steady in-band holding. It is a different measure than in-band efficiency, in which relative inefficiency could indicate that the in-band hold times often fall just short of the appropriate hold time; such a performance would not yield a high entrances measure.

Procedure

The training sequence for producing the final discriminative motor control performance has been described previously (5). After 4 months of daily training, all animals were started on the additional, daily schedule-induced polydipsia procedure (FT 1-min schedule) with water as the available fluid. This 3-h session occurred from 0800 h to 1100 h, followed by a 5-h delay, and then the motor control session occurred. After 2 months on this regime, both polydipsic intake and motor performance daily values were stable, and the effects of midazolam, Ro 15-1788 and FG 7142 injections on motor performance were evaluated. All drug doses were given in an ascending dose order, and doses were separated by at least 5 days. First, midazolam injections were given 30 min before the motor performance session (0.75, 1.5 and 3.0 mg/kg, SC), then Ro 15-1788 at 15 min presession (10 and 20 mg/kg, IP) and finally FG 7142 at 15 min presession (1, 2, and 4 mg/kg, IP). Either 1 or 2 doses of the appropriate vehicle were administered at the beginning or end of the injection series for each drug.

Upon completion of the evaluation of these drug effects on motor performance, the animals continued their daily sessions but were divided into two groups. One group (N = 4) continued to drink distilled water during the 3-h polydipsia sessions, and the other (N = 4) had midazolam solution substituted for water. At all other times, distilled water was freely available to both groups in their living cages. The concentration of the midazolam solution available to the Midazolam Group during the daily polydipsia session was slowly increased over 45 days from 0.02 to a final concentration 0.1 mg/ml. (A particular concentration was available for 2–4 days before being increased.) The final concentration was available for 202 days.

It was deemed necessary that the session polydipsic intakes of the two groups be approximately equal. Therefore, a few animals that did not acquire a strong polydipsic response to the FT 1-min schedule had their session fluid contents adjusted by the addition of a minimal amount of sodium saccharin to maintain the required reliable increase in session intake. In the Water Group, animals G4 and G9 drank water throughout the study, but G14 and G15 drank 0.04% saccharin. In the Midazolam Group, E8 and G17 drank midazolam solution throughout the study. For animal G2, when the midazolam concentration was increased to its final value of 0.1 mg/ml, it was necessary to offer the midazolam solution in a 0.02% saccharin solution for 10 days to attain a satisfactory level of polydipsia. Thereafter, the saccharin component was withdrawn, and G2 drank the standard 0.1 mg/ml midazolam solution for the remainder of the study. After about 2 months of exposure to the final midazolam concentration, the polydipsic intake of animal G1 began to decrease, and it was necessary to adjust its solution so that it contained 0.08% saccharin for the remainder of the study.

After the Midazolam Group had been drinking the final concentration (0.1 mg/ml) for about 2 weeks, tail-tip blood samples were taken for analysis immediately after the polydipsic session and again at 1, 2, 4 and 5 h postsession.

After an additional 2 weeks, daily individual intakes had stabilized, and the evaluation of drug effects on motor performance was again determined for both groups. Midazolam and FG 7142 dose-effect relations were determined as before, except an 8-mg/kg dose of FG 7142 was added. Then, in the light of results obtained with FG 7142 (see the Results section), the delay period between the end of the polydipsia session and the time of the motor performance session was increased from 5 to 19 h. Consequently, the 3-h polydipsia sessions were begun at 1030 h and motor performance sessions at 0830 h the next day. The FG 7142 dose-effect relation was redetermined under this regimen, and then the scheduling of the sessions was returned to the original 5-h delay period for the evaluation of Ro 15-1788.

RESULTS

Schedule-Induced Fluid and Midazolam Intakes

Tables 1 and 2 show the schedule-induced session intakes for each animal in the Midazolam and Water Groups. Each value in the tables is the mean (± SE) of values occurring in the phase of the experiment denoted by the names of the drugs (left column) that were being evaluated in the ensuing discriminative motor control sessions. As indicated in the procedure section, for animal G1, a 0.08% saccharin solution was introduced as the vehicle for midazolam at the start of FG 7142 evaluation during drug polydipsia (cf. Table 1, middle row of 2nd panel). Also, the values shown in Table 2 for G14 and G15 are for 0.04% saccharin as the drinking solution. Animal G17 developed an anterior pituitary tumor and had to be sacrificed during the evaluation of the FG 7142 dose-effect relation (19-h post-polydipsia delay condition). Consequently, half the data for this condition and the ensuing evaluation of Ro 15-1788 are missing

TABLE 1

FLUID (ml) AND MIDAZOLAM INTAKES (mg/kg) DURING 3-H SCHEDULE-INDUCTION SESSIONS FOR MIDAZOLAM-GROUP ANIMALS DURING WATER AND MIDAZOLAM (0.1 mg/ml) POLYDIPSIA PHASES

	Animals							
	E 8		G 1		G 2		G 17	
	ml	mg/kg	ml	mg/kg	ml	mg/kg	ml	mg/kg
During Water Polydipsia (5 h Post)								
Midazolam	117.0	—	138.0	—	99.2	—	122.2	—
	(±4.1)	—	(±6.2)	—	(±4.8)	—	(±2.7)	—
Ro15-1788	121.7	—	127.3	—	97.7	—	120.0	—
	(±7.5)	—	(±0.8)	—	(±11.0)	—	(±1.4)	—
FG 7142	129.3	—	129.5	—	114.5	—	121.8	—
	(±3.6)	—	(±2.5)	—	(±5.5)	—	(±2.6)	—
During Drug Polydipsia (5 h Post)								
Midazolam	125.8	38.0	72.2	21.7	93.2	27.8	94.6	28.7
	(±4.9)	(±1.4)	(±14.0)	(±4.2)	(±6.0)	(±1.8)	(±13.1)	(±4.1)
FG 7142	103.3	31.6	94.8	28.6	93.2	27.9	84.4	25.8
	(±5.7)	(±1.8)	(±3.7)	(±1.4)	(±2.0)	(±0.8)	(±2.9)	(±0.9)
Ro15-1788	125.0	38.1	118.7	35.5	126.7	37.8	—	—
	(±13.9)	(±3.9)	(±2.9)	(±0.9)	(±6.4)	(±1.8)	—	—
During Drug Polydipsia (19 h Post)								
FG 7142	87.0	26.7	104.1	31.0	98.8	29.5	68.0	20.6
	(±7.1)	(±2.2)	(±8.2)	(±2.3)	(±9.3)	(±2.9)	(±5.7)	(±3.1)

Drug names (left column) refer to stages of experiment during which those agents were being evaluated in the ensuing discriminative motor control sessions.

TABLE 2

FLUID (ml) INTAKES DURING 3-H SCHEDULE-INDUCTION SESSIONS FOR WATER-GROUP ANIMALS DURING ALL POLYDIPSIA PHASES

	Animals			
	G 9	G 14	G 15	G 4
During Water Polydipsia (5 h Post)				
Midazolam	86.4	80.2	101.6	87.6
	(±3.8)	(±4.8)	(±2.4)	(±5.5)
Ro15-1788	79.0	71.0	105.3	70.3
	(±3.0)	(±13.0)	(±2.2)	(±7.0)
FG 7142	76.8	69.0	98.7	69.25
	(±4.0)	(±3.8)	(±15.7)	(±4.5)
During 2nd Water Polydipsia (5 h Post)				
Midazolam	104.8	96.4	82.6	76.6
	(±4.6)	(±2.2)	(±5.2)	(±2.7)
FG 7142	103.2	91.6	72.4	76.6
	(±3.6)	(±3.6)	(±4.3)	(±3.31)
Ro15-1788	88.3	89.0	75.0	71.3
	(±2.9)	(±4.6)	(±3.7)	(±6.53)
During 2nd Water Polydipsia (19 h Post)				
FG 7142	91.0	106.2	67.0	57.2
	(±10.5)	(±11.7)	(±7.1)	(±4.2)

Drug names (left column) refer to stages of experiment during which those agents were being evaluated in the ensuing discriminative motor control sessions.

for this animal in Table 1 and the relevant figures. In general, polydipsia was well maintained for the duration of the study. The associated intakes of midazolam were between 21 and 38 mg/kg/session (Table 1).

Initial Dose-Effect Relations After Water Polydipsia Sessions

In the initial phase of drug effect evaluation by motor control performance, both groups were water polydipsic in the sessions preceding each motor performance session. Figures 1 and 2 show the midazolam (SC) results for this period (open circles). The decreases in In-Band Efficiency, Tonic Accuracy and Work Rate, and increases in Entrances, indicate impaired discriminative motor control performance. A dose-related aspect is not apparent. The drug effect appears quantal rather than graded, while, with the largest dose, there are instances of uncompleted sessions (pausing greater than 30 min).

Figures 3 and 4 show the corresponding results for Ro 15-1788 (open circles) and FG 7142 (open squares). Animals G4 and G17 yield evidence of equivalent impairment by both doses of Ro 15-1788; G9 was affected only by the lower dose, while the other animals were little affected by this agent. About one-half of the animals show some impairment with doses of FG 7142, with G2 and E8 ceasing work at the highest dose used in this initial series (4 mg/kg).

Dose-Effect Relations After Polydipsic Sessions Exposing Groups to Either Water or Midazolam Solutions

Figures 1 and 2 show the second midazolam dose-effect determination (filled circles) in groups currently continued on water polydipsia (Fig. 1) or polydipsic on midazolam solution (Fig.

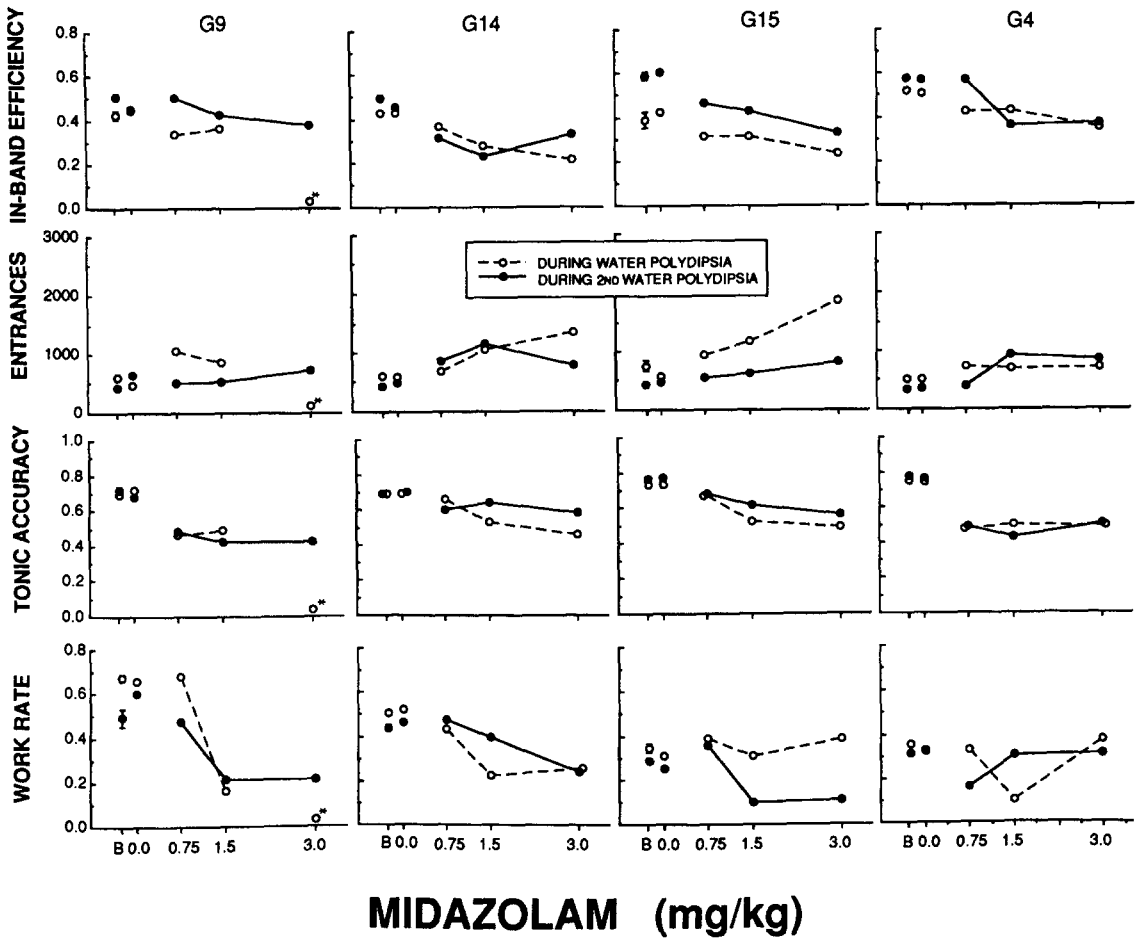

FIG. 1. Water polydipsic group. Mean (SE) discriminative motor control indices as functions of midazolam dose (SC) for individual animals measured 5 h after 3-h water polydipsia sessions. B = baseline; 0.0 = vehicle injection. *Session not finished.

2). Although there was a 5-month period between the end of the 1st midazolam dose-effect determination and the beginning of the 2nd determination, the baseline levels (B) and effects of vehicle injection (0.0) were remarkably reproducible. Only G15 showed a baseline shift (increased In-Band Efficiency and decreased Entrances), indicating that motor control performance had improved between determinations. In general, the 2nd midazolam dose-effect determination was similar to the 1st for the Water Group (Fig. 1). Likewise, the current high daily intake of midazolam in the Midazolam Group had little appreciable effect on the redetermination of the dose-effect relation (Fig. 2). The most notable difference was that animals in either group that did not complete a drug session on the 1st determination did so on the 2nd.

Redetermination of the FG 7142 dose-effect relation was done first with the same 5-h delay time between sessions, and then with a 19-h delay. Both redeterminations included a dose higher than that employed in the 1st determination, viz., 8 mg/kg. The 5-h delay redetermination (filled squares) overall showed no remarkable change from the initial FG 7142 determination (Figs. 3 and 4). In the Midazolam Group, animal G1 (increased In-Band Efficiency and Tonic Accuracy, and a decreased Entrances compared to the 1st determination) and G2 (which now completed the session under the 4-mg/kg dose) were, contrary to the hypothesis, somewhat less impaired by the drug on the 2nd determination. The results of the 19-h delay redetermination (solid triangles) were not appreciably different from those of the 5-h delay in both groups.

Redetermination of the Ro 15-1788 dose-effect relation yielded no appreciable differential effect between the two groups, nor were the results different for individual animals for the two determinations. Unfortunately, the animal most affected by Ro 15-1788 on the initial determination (G17) was the one that developed the anterior pituitary tumor and was eliminated from the experiment prior to this redetermination.

Serum and Brain Drug Profile

Figure 5 shows the serum concentration-time profile of 4-hydroxymidazolam for the Midazolam Group animals. Midazolam, which is rapidly metabolized, was not detected. Peak concentration for 4-hydroxymidazolam was attained between 1 and 2 h postsession. For 3 of the 4 animals, the metabolite concentration was zero by 5 h postsession, and it had fallen to a low level for G2. After the last midazolam polydipsia session, the animals were sacrificed at 5 h postsession and the brains removed for drug analysis. Brain midazolam and 4-hydroxymidazolam levels were not detected.

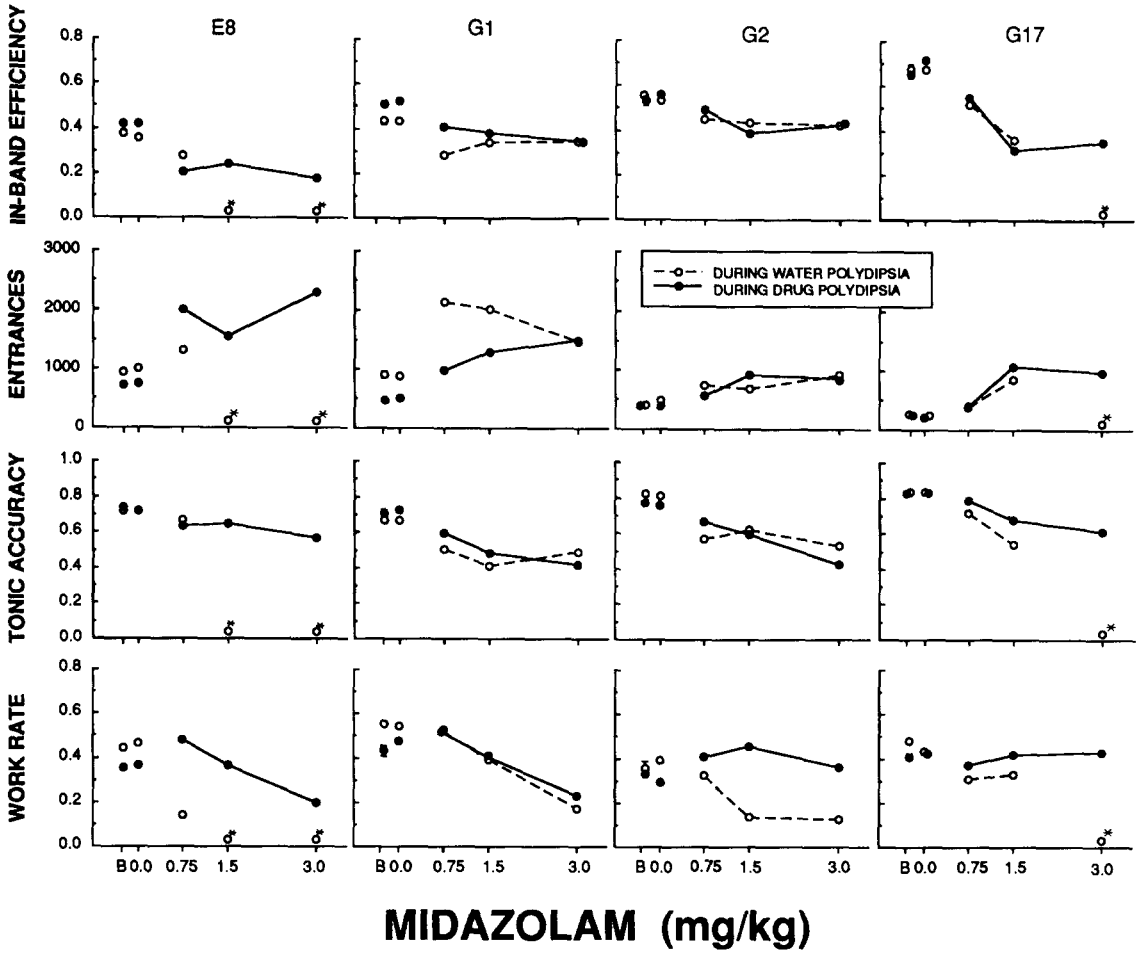

FIG. 2. Midazolam polydipsic group. Mean (SE) discriminative motor control indices as functions of midazolam dose (SC) for individual animals measured 5 h after 3-h water polydipsia or 3-h midazolam solution polydipsia sessions. B = baseline; 0.0 = vehicle injection. *Session not finished.

DISCUSSION

The daily 3-h, schedule-induced oral intake of midazolam was greater (Table 1) than the intakes obtained in our previous studies, which were approximately 17 mg/kg (7,8), mainly because the concentration available was 0.1 mg/ml rather than 0.05 mg/ml. As described in the Introduction, physical dependence was shown to develop when animals self-administered the lower concentration (8). Inasmuch as the midazolam intakes of the present animals were notably greater, their dependence status would be at least comparable. The 4-hydroxymidazolam metabolite reached its peak serum concentration at 1–1.5 h postsession (Fig. 5), as it did in our previous study (8). This bitonic profile was unlike that for another rapidly metabolized agent, cocaine and its metabolites (6), or for ethanol (32), in which concentrations were highest immediately after the 3-h schedule-induced polydipsia session and decayed thereafter.

During the initial water polydipsia phase, the acute effects of midazolam (Figs. 1 and 2) and of Ro 15-1788 (Figs. 3 and 4) on discriminative motor control performance were entirely consistent with results presented in our previous reports (18,33).

During the second polydipsic phase, during which groups drank either water again or midazolam solution, little change was evident in the redetermined midazolam dose-effect functions, which again were obtained after a 5-h postpolydipsia delay. The only notable change was that those animals that originally had not finished certain motor performance sessions (cf. asterisks, Figs. 1 and 2), usually owing to the highest dose of SC midazolam, now completed all sessions. But groups were not different in this regard. This tolerance to the suppressive effects of midazolam on Work Rate reflects a tolerance to the sedative effect of midazolam, a tolerance that is complete after only a few doses of the drug (33). As only a few doses are required to effect a complete tolerance to Work Rate suppression by midazolam, the lack of a group difference is not unexpected. Thus tolerance to the suppression of Work Rate was not a function of midazolam polydipsia; rather, it was due to the SC midazolam doses administered in obtaining these functions.

In previous research, we found that, although some tolerance developed to the motor impairment produced by presession doses of SC midazolam, this tolerance, as measured by the motor performance indices other than Work Rate, was not complete (33). For the present Midazolam Group, daily presession midazolam self-administration was completed 5 h presession, and serum and brain midazolam and 4-hydroxymidazolam levels were zero or very low by the time motor performance was evaluated. Hence, these animals were functionally an "after group" in the usual

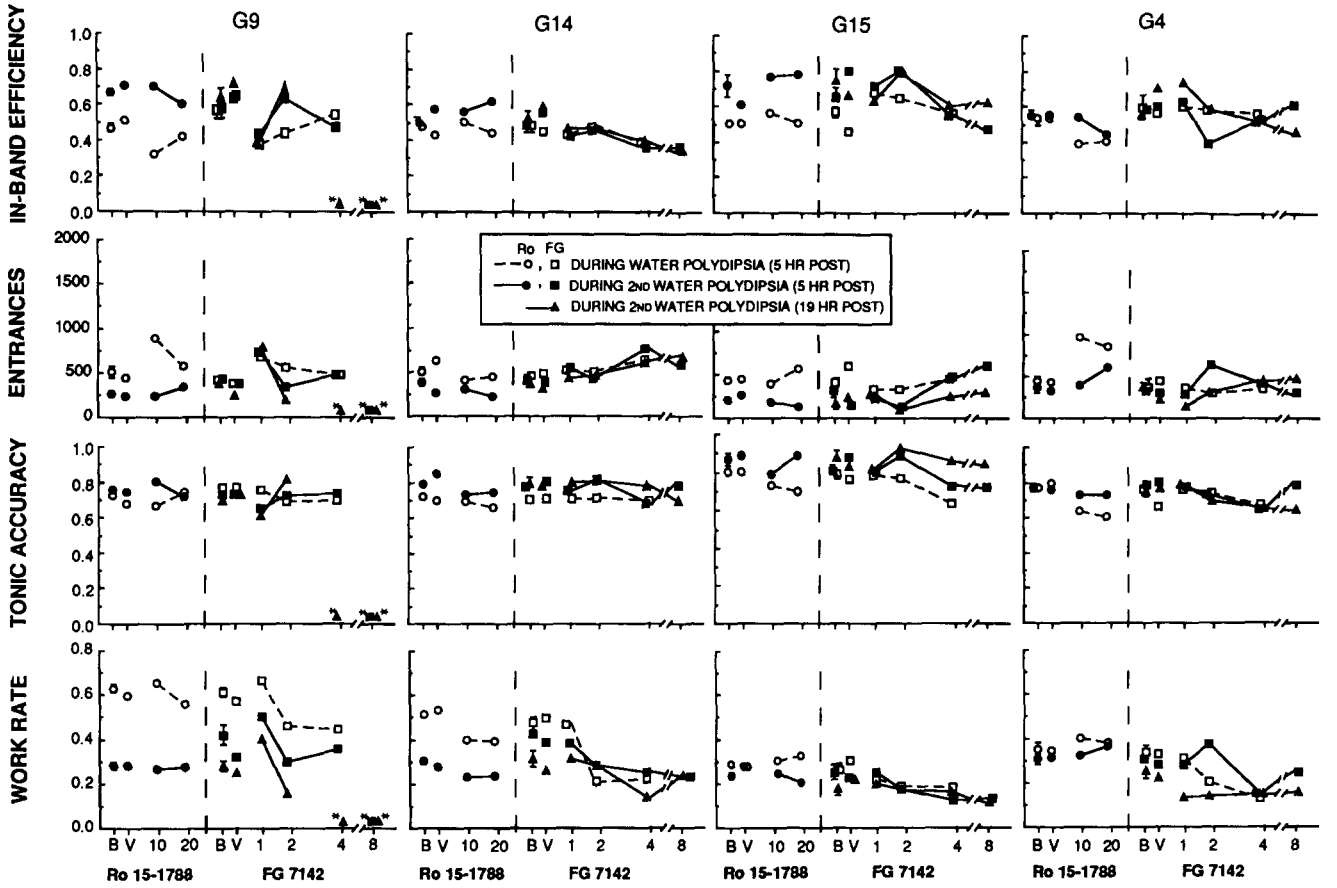

FIG. 3. Water polydipsic group. Mean (SE) discriminative motor control indices as functions of doses (mg/kg) of the benzodiazepine antagonist Ro 15-1788 and partial inverse agonist FG 7142 (IP) for individual animals measured either 5 or 19 h after 3-h water polydipsia sessions. B = baseline; V = vehicle injection. *Session not finished.

"before" versus "after" drug-injection design for evaluating behavioral tolerance (34). Insofar as the midazolam dose-effect relations did not change as a result of chronic exposure to midazolam, this group's results were consistent with those we reported for a discriminative motor performance "after" group receiving chronic midazolam doses (33). Further, discontinuing chronic SC midazolam injections in that experiment did not yield any withdrawal effect (motor performance disruption) in the "after" group, but did disrupt "before" group performance (33). Again, these two findings are consistent with: a) the present Midazolam Group functioning like an "after" group, and b) demonstrating that this discriminative motor control procedure can detect midazolam withdrawal effects.

If Midazolam Group animals were in a rebound state either 5 or 19 h after midazolam self-administration, when their serum and brain midazolam and metabolite levels had returned to zero, then it might be expected that FG 7142 administration would exacerbate the rebound, and that exacerbation would manifest as a disruption in motor performance. Figures 3 and 4 yield no evidence of this. In fact, as indicated in the Results section, two animals in the Midazolam Group gave some evidence of improved performance under the 5-h delay condition. Several studies have demonstrated that mice discontinued from chronic administration of flurazepam, diazepam or lorazepam were more likely to convulse after a 40-mg/kg IP dose of FG 7142 (20, 21, 28, 29). It is difficult to compare seizure incidence in mice with the motor control measures used with rats in the present experiment. Also, the FG 7142 dose used with mice was much greater than the upper range of doses we could use: Three animals failed to complete motor performance sessions at doses of 4 and 8 mg/kg (Figs. 3 and 4).

The effects of Ro 15-1788 on motor performance were not appreciably different as a function of either dose-effect redetermination or a history of chronic, oral midazolam self-administration. Again, the possible induction of a rebound or dependence state was not manifest as a changed reaction to this benzodiazepine antagonist after the imposed 5-h delay period. In a recent study, rats given diazepam doses (5 mg/kg) twice per day showed decreases in fixed-ratio behavior when injected with Ro 15-1788 at 1 h (10 mg/kg) or 3 h (33 mg/kg) after the last diazepam dose, but not after a delay of 18 h (22). This is of interest since the plasma half-life of diazepam (5 mg/kg) is 0.88 h for the rat (10) and a comparable 0.92 h for serum midazolam (33). The respective metabolites are also rapidly eliminated (8,10). These results suggest that the lack of effect of Ro 15-1788 in the present experiment might have been due to the single, daily midazolam dose regimen [in comparison with twice-daily dosing (22)] or too long a postdosing delay time (5 h) relative to the magnitude of the highest antagonist dose administered (10 mg/kg). Baboons exposed to a single daily dose of midazolam (5.6 mg/kg) for 5 days and then given a 5-mg/kg dose of Ro 15-1788 1 h after the 5th dose showed low but unmistakable with-

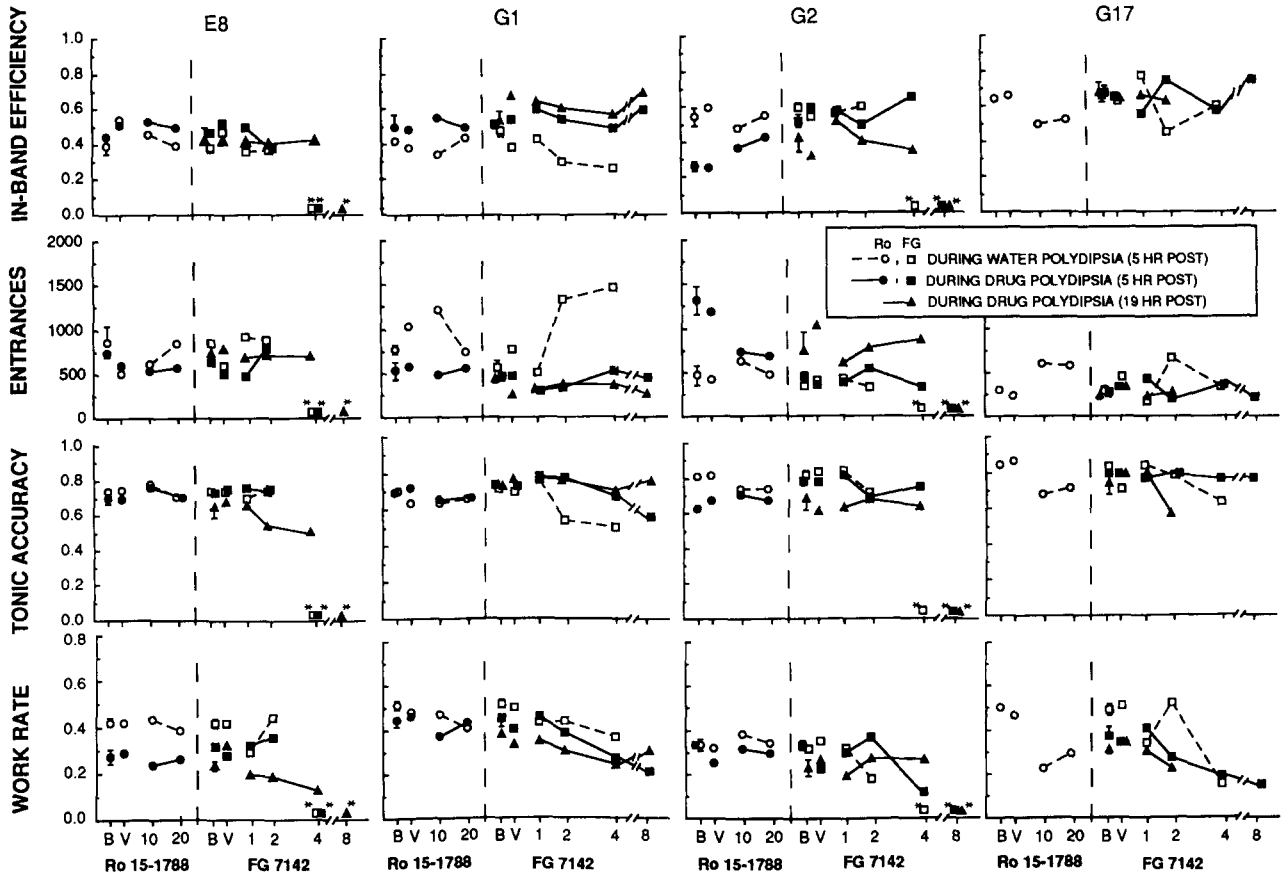

FIG. 4. Midazolam polydipsic group. Mean (SE) discriminative motor control indices as functions of doses (mg/kg) of the benzodiazepine antagonist Ro 15-1788 and partial inverse agonist FG 7142 (IP) for individual animals measured either 5 or 19 h after 3-h water polydipsia or 3-h midazolam solution polydipsia sessions. B = baseline; V = vehicle injection. *Session not finished.

drawal signs (27). Again, the relatively short delay (1 h) between the last midazolam dose and the antagonist injection may have permitted the detection of the withdrawal signs.

In designing this study, our aim was to produce the rodent equivalent of the early morning insomnia rebound state occurring in humans who remain exposed subchronically to a once/day regimen of an ultrashort-acting benzodiazepine. The state is reported to consist in a worsening of sleep in the last third of the night and increased anxiety throughout the day (11). Recent studies found midazolam to be therapeutically efficacious in promoting sleep in insomniacs but failed to find evidence of the early morning rebound state (1,14). These investigators suggest that therapeutically adequate doses do not give rise to the early morning rebound state, but that doses greater than those required for hypnotic efficacy can do so (1,14).

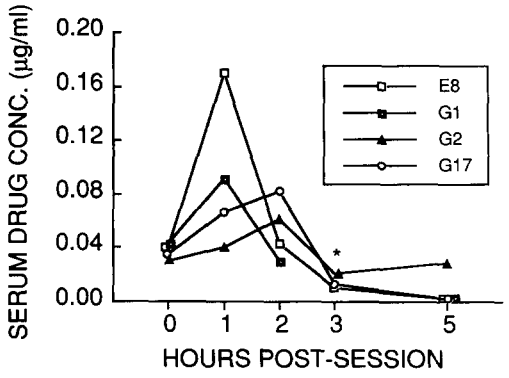

FIG. 5. Serum time-concentration profile for 4-hydroxymidazolam for individual animals after 3-h midazolam solution (0.1 mg/ml) polydipsia session. *Value missing for animal G1.

We have previously presented evidence for the induction of physical dependence on midazolam by the chronic oral self-administration of midazolam solution in daily 3-h sessions (8). The dependence was measured as the incidence of audiogenically induced seizures 90 min after the end of a midazolam self-administration session. The present study used a more precise measure of motor control disturbance and specific pharmacologic precipitating stimuli but failed to yield evidence for a state homologous to the early morning rebound insomnia sometimes observed in humans. In humans, evidence indicates that it is a relatively high-dose phenomenon. In the light of literature reviewed, our animals probably did not manifest the rebound state owing to kinetic-profile parameters. Exposure to a single daily midazolam dose orally self-administered over a 3-h period, even though it was a large total dose, did not produce the drug peak concentration elevation seen with administration by injection (33) or by

oral capsule. The postsession delay time (5 h), set to permit 4-hydroxymidazolam to disappear completely prior to motor performance evaluation, also may have allowed any induced rebound state to dissipate as well.

ACKNOWLEDGEMENTS

This research was supported by Grants DA 03117 and DA 05305 from the National Institute on Drug Abuse. John L. Falk is the recipient of Research Scientist Award K05 00142 from the National Institute on Drug Abuse. We thank Dr. Peter F. Sorter of Hoffmann-La Roche, Inc., Nutley, NJ, for generous supplies of midazolam maleate and Ro 15-1788. We are grateful to Dr. David N. Stephens of Schering AG, Berlin, for a generous supply of FG 7142 (ZK 39 106).

REFERENCES

1. Allen, R. P.; Mendels, J.; Nevins, D. B.; Chernik, D. A.; Hoddes, E. Efficacy without tolerance or rebound insomnia for midazolam and temazepam after use for one to three months. J. Clin. Pharmacol. 27:768–775; 1987.
2. Balster, R. L. Behavioral studies of tolerance and dependence. In: Seiden, L. S.; Balster, R. L., eds. Behavioral pharmacology: The current status. New York: Alan R. Liss; 1985:403–418.
3. Bonetti, E. P.; Pieri, L.; Cumin, R.; Schaffner, R.; Pieri, M.; Gamzu, E. R.; Muller, R. K. M.; Haefely, W. Benzodiazepine antagonist Ro 15-1788: Neurological and behavioral effects. Psychopharmacology (Berlin) 78:8–18; 1982.
4. Braestrup, C.; Nielsen, M.; Honore, T.; Jensen, L. H.; Petersen, E. N. Benzodiazepine receptor ligands with positive and negative efficacy. Neuropharmacology 22:1451–1457; 1983.
5. Culberson, J. W.; Tang, M.; Lau, C. E.; Falk, J. L. Diazepam and discriminative motor control: Acute, chronic and withdrawal effects. Pharmacol. Biochem. Behav. 35:419–427; 1990.
6. Falk, J. L.; Ma, F.; Lau, C. E. Chronic oral cocaine self-administration: Pharmacokinetics and effects on spontaneous and discriminative motor functions. J. Pharmacol. Exp. Ther.; 257:457–465; 1991.
7. Falk, J. L.; Tang, M. Midazolam oral self-administration. Drug Alcohol Depend. 15:151–163; 1985.
8. Falk, J. L.; Tang, M. Development of physical dependence on midazolam by oral self-administration. Pharmacol. Biochem. Behav. 26:797–800; 1987.
9. Fischbach, R. Efficacy and safety of midazolam and Vesparax in treatment of sleep disorders. Br. J. Clin. Pharmacol. 16:167S–171S; 1983.
10. Friedman, H.; Abernethy, D. R.; Greenblatt, D. J.; Shader, R. I. The pharmacokinetics of diazepam and desmethyldiazepam in rat brain and plasma. Psychopharmacology (Berlin) 88:267–270; 1986.
11. Kales, A.; Kales, J. D. Sleep laboratory studies of hypnotic drugs: Efficacy and withdrawal effects. J. Clin. Psychopharmacol. 3:140–150; 1983.
12. Kales, A.; Soldatos, C. R.; Bixler, E. O.; Kales, J. D. Early morning insomnia with rapidly eliminated benzodiazepines. Science 220: 95–97; 1983.
13. Kales, A.; Soldatos, C. R.; Vela-Bueno, A. Clinical comparison of benzodiazepine hypnotics with short and long elimination half-lives. In: Smith, D. E.; Wesson, D. R., eds. The benzodiazepines: Current standards for medical practice. Boston: MIT Press; 1985:121–147.
14. Kripke, D. F.; Hauri, P.; Ancoli-Israel, S.; Roth, T. Sleep evaluation in chronic insomniacs during 14-day use of flurazepam and midazolam. J. Clin. Psychopharmacol. 10:32S–43S; 1990.
15. Lamb, R. J.; Griffiths, R. R. Precipitated and spontaneous withdrawal in baboons after chronic dosing with lorazepam and CGS9896. Drug Alcohol Depend. 14:11–17; 1984.
16. Lamb, R. J.; Griffiths, R. R. Effects of repeated Ro 15-1788 administration in benzodiazepine-dependent baboons. Eur. J. Pharmacol. 110:257–261; 1985.
17. Lau, C. E.; Dolan, S.; Tang, M. Microsample determination of diazepam and its three metabolites in serum by reversed-phase high-performance liquid chromatography. J. Chromatogr. 416:212–218; 1987.
18. Lau, C. E.; Falk, J. L.; Tang, M. Motor performance decrement by midazolam: Antagonism by Ro 15-1788 and CGS 8216. Pharmacol. Biochem. Behav. 36:139–143; 1990.
19. Lau, C. E.; Tang, M.; Falk, J. L. Cross-tolerance to phenobarbital following chronic ethanol polydipsia. Pharmacol. Biochem. Behav. 15:471–475; 1981.
20. Lewin, E.; Peris, J.; Bleck, V.; Zahniser, N. R.; Harris, R. A. Diazepam sensitizes mice to FG 7142 and reduces muscimol-stimulated $^{36}Cl^-$ flux. Pharmacol. Biochem. Behav. 33:465–468; 1989.
21. Little, H. J.; Nutt, D. J.; Taylor, S. C. Kindling and withdrawal changes at the benzodiazepine receptor. J. Psychopharmacol. 1:35–46; 1987.
22. Lucki, I.; Kucharik, R. F. Increased sensitivity to benzodiazepine antagonists in rats following chronic treatment with a low dose of diazepam. Psychopharmacology (Berlin) 102:350–356; 1990.
23. Lupolover, R.; Ballmer, U.; Helcl, J.; Escher, J.; Pavletic, B. Efficacy and safety of midazolam and oxazepam in insomniacs. Br. J. Clin. Pharmacol. 16:139S–143S; 1983.
24. Owen, R. T.; Tyrer, P. Benzodiazepine dependence: A review of the evidence. Drugs 25:385–398; 1983.
25. Pieri, L.; Schaffner, R.; Scherschlict, R.; Polc, P.; Sepinwall, J.; Davidson, A.; Mohler, H.; Cumin, R.; DaPrada, M.; Burkard, W. P.; Keller, H. H.; Muller, R. K. M.; Gerold, M.; Pieri, M.; Cook, L.; Haefely, W. Pharmacology of midazolam. Drug Res. 31:2180–2201; 1981.
26. Samson, H. H.; Falk, J. L. Ethanol and discriminative motor control: Effects on normal and dependent animals. Pharmacol. Biochem. Behav. 2:791–801; 1974.
27. Sannerud, C. A.; Cook, J. M.; Griffiths, R. R. Behavioral differentiation of benzodiazepine ligands after repeated administration in baboons. Eur. J. Pharmacol. 167:333–343; 1989.
28. Schatzki, A.; Lopez, F.; Greenblatt, D. J.; Shader, R. I.; Miller, L. G. Lorazepam discontinuation promotes "inverse agonist" effects of benzodiazepines. Br. J. Pharmacol. 98:451–454; 1989.
29. Schneider, H. H.; Stephens, D. N. Co-existence of kindling induced by the β-carboline, FG 7142, and tolerance to diazepam following chronic treatment in mice. Eur. J. Pharmacol. 154:35–45; 1988.
30. Stockhaus, K.; Bechtel, W. D. Physical dependence capacity of brotizolam in rhesus monkeys. Drug Res. 36:597–600; 1986.
31. Tang, M.; Falk, J. L. Ethanol withdrawal and discriminative motor control: Effect of chronic intake level. Pharmacol. Biochem. Behav. 11:581–584; 1979.
32. Tang, M.; Falk, J. L. Production of physical dependence on ethanol by a short drinking episode each day. Pharmacol. Biochem. Behav. 19:53–55; 1983.
33. Tang, M.; Lau, C. E.; Falk, J. L. Midazolam and discriminative motor control: Chronic administration, withdrawal and modulation by the antagonist Ro 15-1788. J. Pharmacol. Exp. Ther. 246:1053–1060; 1988.
34. Wolgin, D. L. The role of instrumental learning in behavioral tolerance to drugs. In: Goudie, A. J.; Emmett-Oglesby, M. W., eds. Psychoactive drugs: Tolerance and sensitization. Clifton, NJ: Humana Press; 1989:17–114.