Pharmacology Biochemistry & Behavior, Vol. 36, pp. 139–143. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Motor Performance Decrement by Midazolam: Antagonism by Ro 15-1788 and CGS 8216

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Received 6 December 1989

LAU, C. E., J. L. FALK AND M. TANG. *Motor performance decrement by midazolam: Antagonism by Ro 15-1788 and CGS 8216.* PHARMACOL BIOCHEM BEHAV **36**(1) 139–143, 1990.—Rats were trained in a fine motor control performance that required operation by a paw of a force transducer so that it remained between upper and lower limits of a force band for a continuous 1.5-sec period to deliver each food pellet. Acute doses of midazolam (0.75–3.0 mg/kg, SC) impaired indices of motor performance in a graded, dose-related fashion. When administered alone, Ro 15-1788 (0.1–5.0 mg/kg, SC) had no effect on motor behavior while CGS 8216 (0.5 and 1.0 mg/kg, IP) alone had small effects. In general, the motor performance decrements produced by midazolam were antagonized in a dose-related fashion by both Ro 15-1788 and CGS 8216.

Motor performance Midazolam Benzodiazepine antagonism Ro 15-1788 CGS 8216

IN a previous study (17), we reported that acute doses of midazolam (0.75–3.0 mg/kg, SC) produced impaired performance on a discriminative motor control task in rats. In a chronic administration phase of the experiment, animals received daily doses of 3 mg/kg midazolam and the resulting performance impairment was antagonized by doses of the benzodiazepine antagonist agent Ro 15-1788 (5 and 10 mg/kg). The purposes of the present study were to determine: (a) if much lower doses of Ro 15-1788 would antagonize the acute effects of midazolam on this fine motor control task, and (b) whether the benzodiazepine blocker CGS 8216 also was able to antagonize the effects of midazolam.

Ro 15-1788 has been used in clinical settings to reverse the effects of benzodiazepines in humans. It has been employed to alleviate benzodiazepine intoxication, the amnesic effects of these agents, and to terminate their sedative effects (9). The Ro 15-1788 doses administered in these contexts are usually much lower than those used in studies of animal behavior. It is of interest, then, to determine whether low doses of benzodiazepine blocking agents can alleviate the impaired fine motor control produced by an agent such as midazolam which is used extensively as a sedative and anesthetic agent in surgical procedures.

METHOD

Animals

Three male, albino, adult rats of the Holtzman strain (Q4, Q6 and N10) with initial body weights of 384, 373 and 410 g, respectively, were used. They were housed individually in stainless-steel cages in a temperature-regulated room with a daily cycle of illumination from 0700–1900 hr. Water was available in these cages at all times.

Drugs

Midazolam maleate and Ro 15-1788 (flumazanil) were obtained from Hoffmann-La Roche, Nutley, NJ and CGS 8216 was supplied by Ciba-Geigy, Summit, NJ. Midazolam was dissolved in distilled water. The vehicle for Ro 15-1788 and CGS 8216 was a suspension of Agent K (Bio Serv, Inc., Frenchtown, NJ): 1 mg of Agent K in 1 ml of distilled water. All drug solutions were prepared immediately before injections.

Apparatus

Discriminative motor control was evaluated in a Plexiglas chamber ($25 \times 30 \times 30$ cm) with stainless-steel front and rear panels and a floor consisting of parallel-mounted, spaced, stainless-steel rods. The operandum was a stainless-steel lever mounted 2.5 cm from the floor. It was surrounded by a Plexiglas shield with a 1 cm wide × 4 cm high slot so that access to the lever was limited to a single paw. The front edge of the operandum was recessed 1.2 cm from the front surface of the shield to prevent 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 UC 3 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,

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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 msec. When the force applied by the animal's paw was within the 0.147 to 0.265 N band, an audio feedback signal (Sonalert SC648H, P. R. Mallory, Indianapolis, IN) was turned on.

Procedure

Discriminative motor control training. Animals were gradually reduced to 80% of their initial body weights over a 2-week period by limiting daily food rations. After weights had stabilized, animals were trained initially to hold the operandum for 0.5 sec within a wide force band (10–30 g) by delivering a 45-mg Noyes Lab Rat food pellet for each such lever hold. The holding duration was gradually lengthened and the force-band limit decreased over several sessions. The final behavior shaped required the continuous application of force within a 15–27 g force band for 1.5 sec for the delivery of each food pellet. A session terminated after the delivery of 50 pellets or if a 0.5-hr pause in performance occurred. When final performance baselines were attained, animals were exposed to sessions every other day. Body weights were maintained at the 80% level by adjusting daily food rations.

Discriminative motor control measures. Raw measures of motor behavior were accumulated in each session: Session Time (the time taken to earn 50 food pellets), Total Response Time (the amount of the session time that the transducer was held operated above the minimum recognition threshold of 0.015 N), In-Band Time (the amount of the session time that the transducer was held operated within the force-band window) and Entrances (the number of times during a session that applied force entered the band from either the lower or upper set limits). Except in the case of Entrances, these raw measures are not useful characterizations of motor performance. For example, the In-Band Time measure is more informative when compared with the minimum total In-Band Time that would produce the delivery of 50 food pellets (1.5 sec/pellet × 50 pellets = a Minimum Possible In-Band Time of 75 sec). Therefore, a measure of In-Band Efficiency is calculated by taking the ratio of these two values:

$$\text{In Band Efficiency} = \frac{\text{Minimum Possible In-Band Time}}{\text{In-Band Time}}$$

Also, raw In-Band Time can be viewed in relation to the Total Response Time in a session. Thus, Tonic Accuracy measures the proportion of the total response time of a session that is spent in band:

$$\text{Tonic Accuracy} = \frac{\text{In-Band Time}}{\text{Total Response Time}}$$

Work Rate is simply the proportion of the Session Time that the animal spent operating the transducer:

$$\text{Work Rate} = \frac{\text{Total Response Time}}{\text{Session Time}}$$

As indicated above, the Entrances measure is simply a count of the number of times during a session that the applied force enters the band from either its upper or lower limit. Entrances = total number of entrances into the force band.

A perfectly efficient performance would yield an In-Band Efficiency of 1.00. The Tonic Accuracy approaches 1.00 as the total time spent responding 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. 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. Although they often covary, Entrances and In-Band Efficiency are independent measures. For example, relative inefficiency could indicate that the in-band holding times often fall just short of the appropriate hold time; such a performance would not yield a high Entrances measure.

Drug assessment. After steady baseline performance sessions were attained, the effects of acute presession doses of midazolam (0.0, 0.75, 1.5 and 3.0 mg/kg, SC) were assessed. Drug doses were given in a random order and the effect of each dose was evaluated at least twice. Following the initial midazolam dose-response determination, the combined effect of Ro 15-1788 (0.0, 0.1, 0.5 and 5.0 mg/kg, SC) and midazolam (0.0, 0.75, 1.5 and 3.0 mg/kg) was evaluated. For animals Q4 and Q6, the effects of combining these doses of midazolam with CGS 8216 (0.5 and 1.0 mg/kg, IP) were also studied. Midazolam doses are given in terms of the salt. For drug-combination evaluations, midazolam was always given 30 min presession, while Ro 15-1788 and CGS 8216 were administered 15 and 30 min presession, respectively. A minimum of 7 days occurred between injections.

RESULTS

Midazolam and Discriminative Motor Control: Effects of Antagonism by Ro 15-1788

Figure 1 shows that all doses of midazolam sharply reduced In-Band Efficiency and Tonic Accuracy, although the effect was not graded with respect to dose for rats Q4 and N10. Entrances increased for all doses of midazolam, although again the effect was not graded for Q4 and N10. Work Rate increased at all doses for Q6, but was relatively unaffected for Q4 and N10 except at the highest dose which decreased it. The baseline level of performance is shown at point B (± SD) and was calculated as the mean of all the values from 3 consecutive sessions that immediately preceded each session in which a drug was injected. For all animals, administration of neither vehicle nor all dose levels of Ro 15-1788 used affected any of the motor measures. Rat N10 became sick before the 0.1 and 0.5 mg/kg doses of Ro 15-1788 alone could be administered and was terminated from the study at that time.

The largest dose of Ro 15-1788 used (5.0 mg/kg) antagonized the adverse effects of midazolam on all measures of motor performance. It had this palliative effect for all dose levels of midazolam in all animals. The intermediate (0.5 mg/kg) and low (0.1 mg/kg) doses of Ro 15-1788 were less efficacious in antagonizing the effects of midazolam, particularly at the largest midazolam dose. The largest dose of Ro 15-1788 antagonized the decrement in Work Rate produced in the performances of Q4 and N10 by the largest dose of midazolam. The intermediate and low dose levels of Ro 15-1788, when combined with the intermediate and largest doses of midazolam, often produced elevations in Work Rate similar to those associated with the low dose of midazolam.

Midazolam and Discriminative Motor Control: Effects of Antagonism by CGS 8216

Figure 2 shows that, for the most part, CGS 8216 antagonized

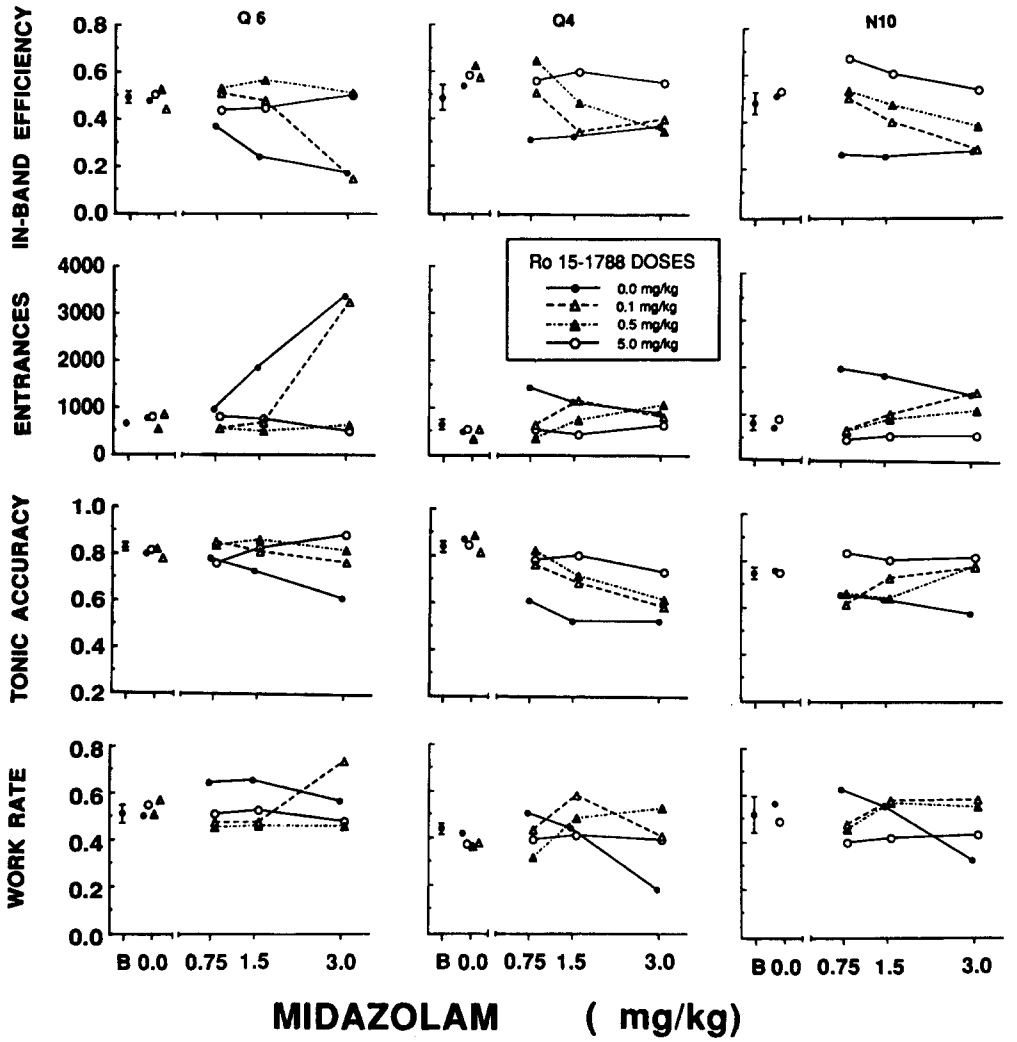

FIG. 1. Dose-effect relations for midazolam (SC, 30 min presession) and for midazolam plus Ro 15-1788 (SC, 15 min presession) for 3 rats (Q6, Q4, N10) for 4 measures of motor performance. Each dose-effect point mean of at least two values. B = baseline performance (± SD). 0.0 = vehicle.

the effects of midazolam in a dose-related fashion. On occasion, however, CGS 8216 alone had small effects on the motor indices that were not consistent between animals. For Q6, the combination of the low dose of CGS 8216 with the low dose of midazolam decreased Tonic Accuracy, although that dose of CGS 8216 alone did not. For Q4, both dose levels of CGS 8216 in combination with 3.0 mg/kg midazolam actually increased Entrances, while Work Rate values were relatively elevated.

DISCUSSION

Ro 15-1788 is an imidazodiazepine which specifically antagonizes the effects of benzodiazepines in behavioral, electrophysiological and binding-site preparations, while usually displaying little intrinsic activity (8). CGS 8216 is a pyrazoloquinoline with many of the same properties: it blocks the pharmacological effects of agents such as diazepam and is devoid of benzodiazepine-like activity (2,5). In the present study, both of these agents had dose-related blocking effects on the motor-control impairments produced by midazolam. The impairments occasioned by midazolam were similar to those reported in a previous study (17).

Only a few studies have reported the effects of Ro 15-1788 on motor deficits produced by benzodiazepines. Bonetti and his associates (3) found that Ro 15-1788 reversed a number of the motor changes and impairments produced by the administration of benzodiazepines to animals. Ro 15-1788 antagonized benzodiazepine ataxia in dogs, horizontal-wire test impairments in rats and mice, locomotor decreases in rats, and anticonvulsive effects in mice. While most of these tests employed intermediate to large doses of Ro 15-1788 (about 3–30 mg/kg), it is interesting to note that for mice and rats deficits in the horizontal-wire test produced by 3 mg/kg diazepam were antagonized by quite low doses of Ro 15-1788: The ED_{50} for mice was 0.2 mg/kg (oral), while the value for rats was 0.06. This order of sensitivity to antagonist action is comparable to that found in the present study. A rather large ED_{50} of 40.1 was reported for the protective effect of Ro 15-1788 against a 30 mg/kg PO diazepam dose in rats evaluated with the rotorod test (2). Bonetti and his associates (3) found that Ro 15-1788 had no benzodiazepine-like effects, as reflected in the above tests, when administered in the dose range producing potent antagonist effects. This agrees with our present results in which Ro 15-1788 had no effect on the indices of motor performance in the

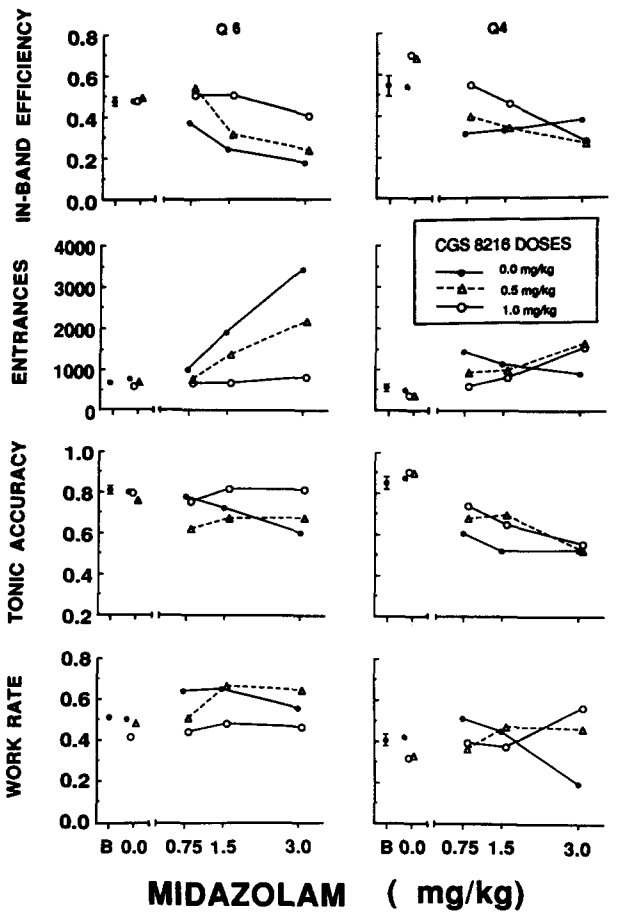

FIG. 2. Dose-effect relations for midazolam (SC, 30 min presession) and for midazolam plus CGS 8216 (IP, 30 min presession) for 2 rats (Q6, Q4) for 4 measures of motor performance. Each dose-effect point mean of at least two values. B = baseline performance (± SD). 0.0 = vehicle.

dose range administered. In human volunteers, a Ro 15-1788 dose of approximately 3 mg/kg blocked the disruption produced by a 40 mg dose of diazepam in tests of psychomotor performance (6).

Ro 15-1788 also blocks the effects of benzodiazepines on behaviors other than motor control. In rats, Ro 15-1788 at 10 mg/kg antagonized the effects of diazepam on fixed-ratio behavior (13) and the effects of chlordiazepoxide on DRL performance (10). In squirrel monkeys, Ro 15-1788 (1–10 mg/kg) antagonized the effects of chlordiazepoxide on punished behavior and on food- and shock-presentation scheduled behavior in a dose-related manner (1). Also in squirrel monkeys, Ro 15-1788 (1 and 3 mg/kg) reduced the rate-increasing effect of diazepam on fixed-interval schedule performance, and at the high dose (3 mg/kg) antagonized the rate decreasing effect of 10 mg/kg diazepam (20). With respect to ingestive behavior in rats, Ro 15-1788 antagonized the hyperphagic effect of chlordiazepoxide, but the blocker was effective only at rather high doses: 20 and 40 mg/kg (4). Ro 15-1788 (2.5–10 mg/kg) blocked the increased intake of 1.5% NaCl solution in rehydrating rats produced by midazolam administration (7). With respect to drug-discrimination behavior, the midazolam discriminative stimulus produced by the administration of 0.4 mg/kg (16) and by 1.0 mg/kg (22) to rats was significantly antagonized by an Ro 15-1788 dose of approximately 3 mg/kg.

In clinical use, Ro 15-1788 has proven efficacious in reversing sedation and hypnosis produced by midazolam (9). Patients undergoing transurethral resection of prostate or bladder tumors were given regional anesthesia followed by incremental doses of midazolam IV until the desired degree of sedation was attained (patient asleep, but arousable on command) (11). At the end of surgery, IV administration of 0.1 mg/kg of Ro 15-1788 produced a significant reversal of sedation compared to patients receiving placebo administration. In patients undergoing induced abortion even smaller doses of Ro 15-1788 given postoperatively were effective in reversing the general anesthesia produced by midazolam (21).

Few studies have addressed the efficacy CGS 8216 might possess in blocking motor effects produced by the benzodiazepines. Impaired rotorod performance in rats produced by diazepam was antagonized by 1 mg/kg of CGS 8216 ($ED_{50} = 1.45$ mg/kg) (2). A dose of 5 mg/kg of CGS 8216 blocked the reduction produced by diazepam in the spontaneous tonic activity of the gastrocnemius-soleus muscle electromyogram in mutant Han-Wistar rats (19).

While Ro 15-1788 typically acts as a competitive antagonist possessing a modest agonist action, the status of CGS 8216 is less clear. CGS 8216 itself at 1 and 3 mg/kg doses decreased response rates in squirrel monkeys on a fixed-interval schedule and did not increase the rate-decreasing effect of diazepam (20). In dogs, the rate-decreasing effects of diazepam on the fixed-ratio component of a multiple fixed-ratio, fixed-interval schedule was increased by 1 mg/kg of CGS 8216, PO (12). Although the rate-increasing effect of diazepam on the fixed-interval component was decreased by 30 mg/kg of CGS 8216, that dose alone also decreased fixed-interval rates. In rats, CGS 8216 (3–30 mg/kg) decreased fixed-ratio 10 behavior (13). This effect was not antagonized by diazepam, but CGS 8216 at 10 mg/kg did antagonize the rate-decreasing effect of a 30 mg/kg dose of diazepam. Also at 10 mg/kg, CGS 8216 antagonized the rate-increasing effect of chlordiazepoxide on DRL behavior (10). With respect to ingestive behavior in rats, a 5 mg/kg dose of CGS 8216 was able to antagonize the hyperphagic effect of 5 mg/kg of chlordiazepoxide without itself producing a decrease in food intake (4). However, CGS 8216 (3–30 mg/kg) failed to block the increased intake of 1.5% NaCl solution in rehydrating rats produced by midazolam administration (7), although at 10 mg/kg CGS 8216 decreased ingestive responses that were increased by chlordiazepoxide administration (18). With respect to drug-discrimination behavior, the discriminative stimulus produced in rats by the administration of 1 mg/kg diazepam was antagonized by 1 and 3 mg/kg doses of CGS 8216 (14) and by doses as low as 0.3 mg/kg (15).

In the above discussion of the literature, it is clear that although most investigations using Ro 15-1788 as an antagonist for the effects of benzodiazepines have used rather large doses, there is evidence from both rodent motor and human sedation studies that Ro 15-1788 is an effective antagonist at doses of 0.1 mg/kg and lower. The present study is consistent with this lower dose range. With respect to CGS 8216, again most studies have used much larger doses than those employed in the present study. While studies with this agent suggest that it has properties in addition to those of a competitive benzodiazepine antagonist, nevertheless the present study found only minor effects when CGS 8216 was administered alone, and dose-related antagonism in a rather low dose range.

ACKNOWLEDGEMENTS

This research was supported by grant DA 03117 from the National Institute on Drug Abuse. The authors wish to thank Dr. Peter F. Sorter of Hoffmann-La Roche, Inc., Nutley, NJ for generous supplies of diazepam and Ro 15-1788, and Charles F. Brownley, Jr., Ciba-Geigy Corp., Summit, NJ for a generous supply of CGS 8216.

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