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Effects of Ro 15-4513 on Schedule-Controlled Responding of Pigeons

DAWN DELANEY[1]

Department of Psychology, Western Michigan University, Kalamazoo, MI 49008

HENRY SCHLINGER

Department of Psychology, Western New England College, Springfield, MA 01119

AND

ALAN POLING[2]

Department of Psychology, Western Michigan University, Kalamazoo, MI 49008

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DELANEY, D., H. SCHLINGER AND A. POLING. *Effects of Ro 15-4513 on schedule-controlled responding of pigeons.* PHARMACOL BIOCHEM BEHAV **33**(4) 777–780, 1989.—The partial inverse benzodiazepine agonist Ro 15-4513 has been found to antagonize some of the behavioral and physiological effects of ethanol, but relatively little is known about the behavioral effects of the drug alone. In the present study, pigeons responding under a multiple fixed-ratio 25 interresponse-time-greater-than-6-sec schedule of food delivery were exposed acutely and chronically to Ro 15-4513. Acute administrations of the drug (1.0, 1.8, 3.2, and 5.4 mg/kg) reduced response rates under the fixed-ratio component at some doses, although two birds were more sensitive to the drug than the third subject. Response rates under the interresponse-time-greater-than-6-sec component were not affected by acute administrations of Ro 15-4513. When 5.4 mg/kg Ro 15-4513 was administered prior to 15 consecutive sessions, tolerance developed to the rate-reducing effects of the drug under the fixed-ratio component. These findings, in contrast to those of early investigations in which gross measures of behavior were employed, suggest that Ro 15-4513 is behaviorally active at relatively low doses.

Ro 15-4513 Schedule-controlled behavior Fixed-ratio schedule Interresponse-time-greater-than-*t* schedule Pigeons

IN VITRO investigations of the experimental drug Ro 15-4513, the azido analogue of the benzodiazepine antagonist Ro 15-1788, have revealed that the drug (a) is a partial inverse agonist at the benzodiazepine receptor site (1) and (b) acts at GABA-benzodiazepine receptors to block ethanol-stimulated chloride uptake (12). Early in vivo investigations demonstrated that pretreatment with Ro 15-4513 blocked the motor impairment induced by diazepam, phenobarbital, and ethanol (1,2), and the antipunishment effects and sedation produced by low-to-moderate doses of ethanol (12). The drug also antagonized ethanol-stimulated chloride uptake (12). These findings raised questions concerning the potential utility of Ro 15-4513 in the treatment of ethanol abuse [e.g., (6)], and fostered research directed toward discovering the range of ethanol effects antagonized by the drug.

Results of recent studies have been generally consistent with the notion that Ro 15-4513 antagonizes some of the behavioral and physiological effects of ethanol. Ro 15-4513 reportedly blocks ethanol-induced general intoxication (2, 5, 7, 12). It also reduces the antipunishment (3,12) and positive reinforcing (11) effects of ethanol. Ethanol-induced hypothermia apparently is not blocked by pretreatment with Ro 15-4513 (5). The lethal effects of ethanol in rats were blocked by Ro 15-4513 in one study (4), but not in another (10).

The behavioral effects of Ro 15-4513 alone have not been examined in depth. Early investigations found that Ro 15-4513 is a relatively nontoxic drug, with an LD_{50} of over 5000 mg/kg (2). Administered alone, Ro 15-4513 does not significantly affect rotarod performance (5) or locomotor activity (7), although it does

[1]The reported data were collected as part of the Ph.D. thesis of the senior author.
[2]Requests for reprints should be addressed to Alan Poling.

reduce both the number and duration of head dips evidenced during a holeboard test (7). In one study, the drug reportedly produced no effect on punished and nonpunished operant responding of rats (12). In a later investigation, however, Ro 15-4513 was found to decrease punished and nonpunished responding (3). A very recent investigation with rats (13) also revealed that Ro 15-4513 reduced responding under FR 10 and tandem variable-interval 40-sec fixed-ratio 10 schedules of food delivery.

In an attempt to explore further the effects of Ro 15-4513 alone on operant responding, the present study examined the acute and chronic effects of the drug on the responding of pigeons under a multiple fixed-ratio 25 interresponse-time-greater-than-6-seconds (mult FR 25 IRT >6-sec) schedule of food delivery. This schedule characteristically engenders high rates under one component (i.e., the FR) and low rates under the other, and has a demonstrated sensitivity to many kinds of drugs [e.g., (9)]. To date, nothing has been reported concerning (a) the behavioral effects of Ro 15-4513 in pigeons, (b) the behavioral effects of chronic administrations of Ro 15-4513, and (c) the effects of Ro 15-4513 under schedules of reinforcement that engender low rates of responding. The reported study provides information in each of these areas.

METHOD

Subjects

Three adult female White Carneau pigeons, maintained at 80% of free-feeding weights, served as subjects. All subjects had experience responding under multiple schedules of food delivery, but had no drug history. During periods outside experimental sessions, they were individually housed with unlimited access to grit and water.

Apparatus

Experimental testing was conducted in three computer-controlled operant conditioning chambers, measuring 32 cm long, 36 cm high, and 35 cm wide. In each chamber, three response keys 2.5 cm in diameter were located 23 cm from the bottom of the front wall, approximately 5.5 cm apart. Only the left and right keys, each of which could be illuminated in red or blue-green, were lighted and operative in this study. A minimum of 0.2 g pressure was required for key operation. An aperture centered horizontally on the front wall 7.5 cm above the floor allowed access to a hopper filled with mixed grain when the hopper was raised. When raised, the hopper was illuminated by a 7-W white bulb. A 7-W white bulb (houselight) centrally mounted 33 cm above the chamber floor provided ambient illumination and a white noise generator provided masking sound. Programming of experimental events and data collection were accomplished through the use of a Digital Equipment Corp. (Maynard, MA) PDP8/A computer using interfacing and software (SUPERSKED) supplied by State Systems Inc. (Kalamazoo, MI).

Behavioral Procedure

Because all subjects had histories of food-maintained key pecking, initial training was not required and subjects were exposed from the onset of the study to the mult FR 25 IRT >6-sec schedule of food delivery. Under this schedule, red key illumination was correlated with the FR 25 component and blue-green key illumination was correlated with the IRT >6-sec component. Food was delivered for 3 sec following every 25th keypeck under the FR 25 component, and following the first response emitted at least 6 sec from the previous food delivery or the onset of blue-green key illumination under the IRT >6-sec component. Components alternated at 4-min intervals, with the initial component selected at random each session. Red key illumination was always presented on the left key, whereas blue-green illumination was always presented on the right key. A single 24-min session was conducted for each bird 6 days per week at about the same time each day.

Pharmacological Procedure

Subjects were exposed to the mult FR 25 IRT >6-sec schedule until the response rates of the individual birds were stable under both components. The criterion for stability was three consecutive sessions in which the response rate in each individual session was within 10% of the mean rate of responding across those three sessions. Once this criterion was met, acute dose-response determinations were begun. Four doses of Ro 15-4513 (1.0, 1.8, 3.2, and 5.4 mg/kg), suspended in a vehicle of distilled water with Tween 80 (2 drops/10 ml) added, were evaluated. In all phases of the study, drug (and vehicle control) injections were administered intramuscularly (IM) at an injection volume of 1 ml/kg, 15 minutes prior to testing. During acute dose-response determinations, each subject received all doses on two occasions, in random order. All drug administrations were separated by a minimum of three sessions in which responding was stable as defined above; one of these sessions was preceded by a control (vehicle) injection.

Following completion of the acute dose-response determinations, all subjects received control injections prior to at least five consecutive sessions. Subjects then received 5.4 mg/kg Ro 15-4513 prior to each of 15 consecutive sessions. Following the fifteenth day of chronic exposure, subjects were injected with a challenge dose of 10 mg/kg.

RESULTS

Figure 1 shows overall response rates for individual subjects under all experimental conditions. In the absence of drug, all subjects responded at a much higher rate under the FR 25 component than under the IRT >6-sec component. In all subjects, mean response rates under the FR component were below the control value at all doses of Ro 15-4513. However, drug rates differed very little from the control value at 1 mg/kg for all subjects and at 1.8 mg/kg for subject 2. At all other doses, rates under the FR differed from control rates by more than 15%. Overall, subject S2 appeared to be less sensitive to Ro 15-4513 than the other two pigeons. For all subjects, IRT >6-sec response rates were not systematically affected by acute administrations of any of the doses.

The two subjects that were most sensitive to Ro 15-4513 (S1, S3) developed tolerance to the rate-reducing effects of the drug when it was administered chronically at a dose of 5.4 mg/kg. For those subjects, response rates under the FR schedule were much higher during the final session of chronic exposure than during acute exposure. The FR 25 response rate for S2 at the end of chronic exposure to 5.4 mg/kg was comparable to the rate observed when this dose was given acutely. Administration of a dose of 10 mg/kg following the chronic regimen revealed FR response rates that approximated those seen at chronic 5.4 mg/kg for all subjects. This finding suggests that tolerance developed to the rate-decreasing effect of Ro 15-4513. For all subjects, chronic exposure to 5.4 mg/kg produced no detectable change in IRT >6-sec response rates.

Table 1 shows mean food deliveries per session under all experimental conditions. Because rate of reinforcement is proportional to (i.e., 4% of) rate of responding under the FR 25 component, the effects of Ro 15-4513 on mean food deliveries per session under this component were equivalent to its effects on

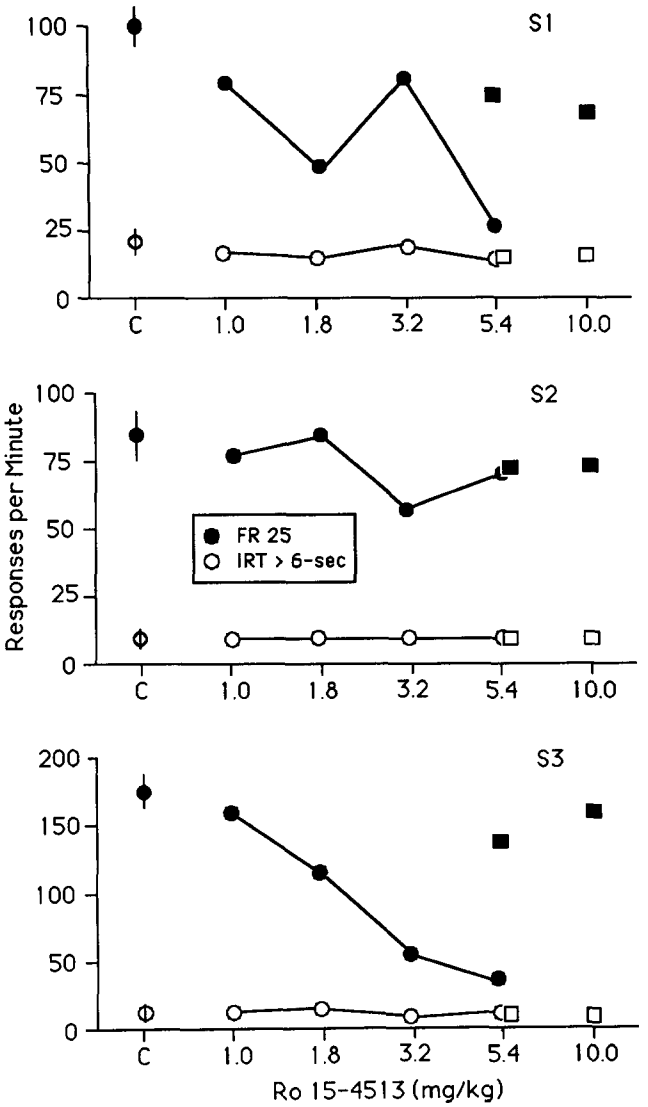

FIG. 1. Response rates of individual subjects under all experimental conditions. Data at C represent mean response rates across all control sessions; vertical lines represent plus and minus 1 standard deviation. Connected drug data points (circles) represent the mean of two acute administrations of the listed dose. The unconnected points (squares) at 5.4 mg/kg indicate performance on the fifteenth session of chronic exposure to that dose. The 10.0 mg/kg dose was given as a challenge dose after chronic exposure to 5.4 mg/kg.

response rates. There is no simple relation between rate of responding and number of food deliveries under the IRT >6-sec schedule, and the effects of Ro 15-4513 under this component were highly variable across the three subjects. For subject S1, mean number of reinforcers earned per session under the IRT >6-sec component increased when Ro 15-4513 was administered. For subject S2, this measure decreased as a function of Ro 15-4513 administration. For subject S3, it was generally unaffected by the drug.

DISCUSSION

Previous observations with rats have revealed that Ro 15-4513 does not produce changes in behavior that are evident in gross observations (5,12). Nonsystematic observations of subjects in the present study also failed to reveal gross changes in behavior when the drug was administered. Prior to, during, and after experimental sessions, the pigeons did not appear to be ataxic or unresponsive to environmental stimuli. They were, however, affected by the drug, which substantially reduced response rates under the FR component. That the drug did so indicates that Ro 15-4513 is behaviorally active in pigeons at relatively low doses. A previous report has demonstrated similar sensitivity in rats, in which doses of Ro 15-4513 comparable to those used in the present study reduced response rates under a tandem variable-interval 40-sec fixed-ratio 10 schedules of food delivery (13). That schedule, like the FR component in the present study, engendered relatively high response rates in the absence of drug.

In contrast, the IRT >6-sec component employed in the present study engendered low response rates in the absence of drug. Response rates under this component were unaffected by doses of Ro 15-4513 that substantially reduced FR responding. That this occurred suggests that the effects of Ro 15-4513 on schedule-controlled responding may be related to response rate. The drug appears to reduce high-rate operant responding at doses that leave low-rate operant responding intact. Because the FR and IRT components differ in several regards other than the rates engendered, further research will be required to determine if the effects of Ro 15-4513 are actually rate dependent.

The neuropharmacological mechanism through which Ro 15-4513 affects operant behavior is uncertain. A plausible mechanism

TABLE 1

MEAN NUMBER OF REINFORCERS EARNED PER SESSION

Drug Dose (mg/kg)	FR 25 Component	IRT >6-sec Component
Subject 1		
0	49 (3.2)*	4 (2.5)*
1.0	41	10
1.8	25	18
3.2	41	0
5.4	14	23
5.4 (chronic)	37†	9†
10.0	35	5
Subject 2		
0	42 (4.0)	50 (5.6)
1.0	39	38
1.8	42	38
3.2	31	39
5.4	35	35
5.4 (chronic)	36	55
10.0	37	53
Subject 3		
0	77 (3.6)	33 (5.1)
1.0	71	36
1.8	55	34
3.2	30	30
5.4	20	28
5.4 (chronic)	64	49
10.0	72	24

*These values represent the mean of all control sessions; the value in parentheses is one standard deviation.

†These values represent the final (fifteenth) session of chronic exposure.

involves the ability of the drug to act as a partial inverse agonist at the benzodiazepine receptor site (12). However, the response-suppressing effects of Ro 15-4513 (5 mg/kg) on schedule-controlled behavior in rats were not antagonized by the selective benzodiazepine antagonist Ro 15-1788 (13). This suggests that the response suppression was not mediated by benzodiazepine receptors.

The chronic effects of Ro 15-4513 have been essentially ignored. In the present study, tolerance appeared to develop to the rate-reducing effects of the drug. Whether this involved a change in the rate of drug metabolism (i.e., metabolic tolerance) or some other mechanism is unknown.

Within the past five years, much has been learned concerning Ro 15-4513. Although Ro 15-4513 does block some of the behavioral and physiological effects of ethanol, it is not a selective amethystic agent. Not all of the effects of ethanol are blocked by Ro 15-4513, and the drug blocks the effects of certain other drugs with GABAergic mechanisms of action. Moreover, Ro 15-4513 has proconvulsant effects (7) and is behaviorally active. Once envisioned as a potentially useful tool for treating alcohol abuse [e.g., (6)], Ro 15-4513 now appears to be an interesting research tool with complex actions in its own right, as well as when combined with ethanol.

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