Pharmacology Biochemistry and Behavior, Vol. 59, No. 1, pp. 19–25, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00309-2

Rohypnol ("Roofies") Control of Drug Discrimination: Effect of Coadministered Ethanol or Flumenazil

MARTIN D. SCHECHTER

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

Received 2 October 1996; Revised 24 February 1997; Accepted 7 March 1997

SCHECHTER, M. D. *Rohypnol ("roofies") control of drug discrimination: Effect of coadministered ethanol or flumenazil.* PHARMACOL BIOCHEM BEHAV **59**(1) 19–25, 1998.—The benzodiazepine flunitrazepam (Rohypnol®) was employed to control differentially discriminative performance in 10 Sprague–Dawley rats on a food-motivated FR10 schedule. The training dose was 2.5 mg/kg, and 20 min was employed between intraperitoneal administration and training; both values were employed in this study, which, in reality, is the first time in the drug discrimination literature for the training of this drug. Dose–response experiments indicated decreasing discriminative performance in concert with decreasing time to reach FR10 lever selection as the dose tested decreased from 2.5 to 0.04 mg/kg. The calculated ED_{50} for discriminative performance, i.e., lever selection of the drug-correct lever, was 0.076 mg/kg. The relatively few sessions needed to reach discrimination criterion, and the fact that the ED_{50} value was 1/33 of the training dose, suggests that a lower dose of Rohypnol may be used in the future to train rats in this paradigm. Time course experiments indicate decreasing discriminative performance from 20–240 min postadministration with a calculated half-life of 162.3 min. Administration of 450, 600, and 900 mg/kg ethanol (10% w/v) IP produced saline-like discriminative responding, whereas the combination of these doses with the 0.08 mg/kg Rohypnol dose produced increasing discriminative performance with the highest ethanol dose producing 72.2% Rohypnol-appropriate lever selections in a mean time to attain lever selection on the FR10 schedule of 12.8 s. These results suggest that a lower training dose of Rohypnol may allow for testing of a smaller ED_{50} Rohypnol dose with ethanol to produce a more complete generalization. The ability of flumenazil (Ro 15-1788) to dose dependently block the discrimination of Rohypnol suggests that this benzodiazepine produces its action by its agonistic efficacy at these receptors. The coadministration of Rohypnol and ethanol as a popular drug combination in humans is discussed, and evidence is offered as to their synergistic interactions in rat discrimination. © 1998 Elsevier Science Inc.

Drug discrimination Rohypnol Flunitrazepam Ethanol Generalization Flumenazil Dose–response Time course Rats

ROHYPNOL® is the brand name of the anxiolytic flunitrazepam manufactured by the large Roche Pharmaceutical holdings in, and used throughout, Europe, Asia, and South America. Street names for this compound include but are not limited to: "roofie" or "rophy," "rufinol" (perhaps a combination of the brand name and the street name), "roopies," "rope," "circles," "Mexican valium," "rib," "roach-2" and "R-2." Medically, Rohypnol (R) is a rather typical benzodiazepine and, as it is commercially available in 1 and 2 mg tablets, acts as an effective short-term sedative-hypnotic used in this capacity for insomnia or, in special clinical use, as a preanesthetic medication (13). Rohypnol is neither manufactured nor sold licitly in the United States, and yet there is a tremendous increase in the number of DEA drug seizures, especially in southern states from California to Florida, of what appears to be commercially produced Rohypnol as evidenced by its confiscation in commercially available "bubblepacks."

Even though R taken alone rarely leads to death, combining it with alcohol reduces this safety margin and the combination of R and alcohol has allowed for self-intoxication reported to lead to "blackouts" that last 8 to 24 h, depending on the dose (7). Another troublesome effect of this combination is behavioral disinhibition coupled with amnesia, allowing this drug to be added to drinks at social gatherings or bars. When given to unsuspecting females in hopes of lowered inhibition, the combination has proven capable of facilitating sexual "conquest." With the added ability to cause anterograde amnesia, the combination of "roofie" plus alcohol has received a justifiable notoriety as "the new date-rape drug of choice" in the lay press [e.g., (1,19,20)]. Due to the increase in seizures of

supplies and the growing abuse potential seen in the United States, the DEA has recently placed R into Schedule I of the Controlled Substance Act of 1970, allowing punishment for manufacture and distribution of this compound to be as severe as it is for other illegal drugs such as heroin and LSD.

In the area of scientific investigation, there is a paucity of animal experimentation regarding the behavioral effects of R, as well as R in combination with ethanol. To this end, the present study intends to use the well-documented (14,22) behavioral paradigm known as drug discrimination to train animals to differentially respond to administration of a dose of R vs. its vehicle in an effort to see if this centrally psychoactive drug is capable of controlling differential discriminative responding; to evaluate the dose–effect relationship as a discriminative performance gradient; to determine the discriminative time course of this drug; to allow for experiments to indicate any possible additive or potentiating effects when this drug, in low dose, is combined with ethanol; and to explore if R, like most other benzodiazepines used to train discriminative performance in rats, is attenuated by pretreatment with the benzodiazepine receptor blocker flumenazil, aka Ro 15-1788 [e.g., (6,21,24)].

METHOD

Subjects, Dose, and Time Course

For the present experimentation, the readily available, commercially bought (Zivic-Miller Laboratories, Allison Park, PA) Sprague–Dawley male rats were chosen as this is the first attempt in the behavioral pharmacology literature to train R as a drug capable of controlling discrimination responding and the need for replicability is evident. The choice of drug dose was based on the very limited number of published animal behavioral experiments using this agent. The dose selection aimed to employ a dose high enough to be discriminable, but not too high as to cause excessive sedation (5). The time between drug administration and conditioning/testing of discrimination was sought to allow for maximal availability of the drug entry into the brain and, once again, reports of R experiments in rats were limited [(5), using a postadministration time of 10 min]. Nonetheless, as a result of published data analyzing plasma concentrations of flunitrazepam, as given to humans by various routes of administration (2), a 20-min interval between administration and training was chosen.

The 10 male Sprague–Dawley rats arrived at this site weighing 125–150 g. At the onset of a week of quarantine, they were placed into individual wire cages in a Vivarium facility with an ambient temperature of 20–22°C and maintained on a 12:12 light:dark cycle with lights on at 0600 h. Behavioral training/testing was conducted in a room separate from the animal colony. Water was available ad lib in their home cages and daily rationing of approximately 16 g of commercial rat chow allowed maintenance of their body weights at 85–90% of that determined by free-feeding control rats of the same age and sex. This procedure was in place to facilitate motivation of operant performance for food reward.

Apparatus

Ten standard rat-operant chambers (Lafayette Instruments Corp., Lafayette, IN), each containing two levers situated 7 cm apart and 7 cm above a metal grid floor, were the experimental apparatus. Equidistant between the levers was placed a food receptacle into which a 45 mg (P. J. Noyes Co., Lancaster, NH) food pellet was delivered. Each operant chamber was enclosed in a sound-attenuating enclosure with an exhaust fan and a 9-W house light. Solid-state programming equipment (Med Associates, St. Albans, VT), located in an adjacent room, was used to control and record the discrimination sessions.

Training to Lever Press

The food-restricted rats were administered 1 ml/kg saline (0.9% NaCl in distilled water) by intraperitoneal (IP) injection 20 min prior to being placed into the apparatus. They were trained by the successive approximation method to press one of the two levers at which time one press produced one food pellet (fixed-ratio 1; FR1). For half of the animals, this lever was designated to be the one to the left of the food magazine and for the other half it was the lever to the right of the food magazine. It was this lever on subsequent days that was defined as the "saline-appropriate" lever and, after administration of saline, was the only functional lever. Presses on the opposite lever produced no programmed consequence. The animals were trained to lever press with a gradual incrementing fixed-ratio schedule going from 1 to 10 over 8 consecutive training days. Once all rats attained an FR10 on the saline-appropriate lever, the next training day was used to administer IP an equal volume (1 ml/kg) of (saline) vehicle containing 2.5 mg/ml flunitrazepam. The rats were placed, 20 min later, into the apparatus and required to press the second lever for food reinforcement on an FR1 schedule. This procedure was continued with the animals' schedule of reinforcement gradually incremented to an FR10 requirement over 6 days.

Discrimination Training

Once all animals were capable of FR10 responding on both levers according to saline or drug (Rohypnol; R) administration on that particular day, discrimination training commenced. This training began 20 min after the daily administration of either 2.5 mg/kg R or saline (S) using a pseudorandom schedule with the following sequence: S,R,R,S,S; R,S,S,R,R. The first lever upon which 10 responses were accumulated at the beginning of each daily training session was considered the "selected" lever for that session. At the time of the tenth response, presses on both the selected and unselected lever were recorded. However, the session was continued for a maximum of 10 min, regardless of the correctness of the selected lever, or until 400 responses were made on the correct lever for that session and, therefore, until 40 reinforcements (on the FR10 schedule) were received. Presses on the incorrect lever produced no programmed consequence. The intent was that animals would be required to select the correct lever appropriate for the substance injected on that day in 8 of 10 consecutive training sessions. This criterion of performance, noted as session-to-criterion, is most often used in the literature and it is defined as the "number of training sessions before the beginning of criterion performance, with criterion defined as 'A out of B consecutive sessions' with a correct choice on the first trial of the session" (15).

Dose–Response Tests

Following the establishment of criterion performance in all animals, the administration regimen was limited to every other day to maintain discrimination. Thus, on every second day, either 2.5 mg/kg R or S was tested 20 min after IP administration. If any animal was seen to fall below the criterion of eight correct lever selections on 10 consecutive sessions during these maintenance-day tests, the data on their dose–response responding was to be

precluded from the results. This, however, did not occur. On maintenance days following lever selection, the animals were given reinforcement on the FR10 schedule for either 10 min or after 400 responses on the injection-appropriate lever, whichever came first. Intervening days were used to test doses of R different than the 2.5 mg/kg training dose. Each test dose was administered twice—once following an R maintenance session and once following an S maintenance session. The counterbalancing procedure was used to control for any possible residual influences from the previous day's maintenance session. Doses of 0.04, 0.08, 0.16, 0.32, 0.63, and 1.25 mg/kg R were each tested on two occasions and the rats were immediately removed, on test days, without receiving reinforcement for pressing one lever 10 times. During this series of, and in all following, experiments, the animals' time to attain the FR10 criterion for lever selection, i.e., the time in seconds from pressing the lever for the first time until 10 responses were made on either lever, was measured with stop watches (see Measurements, below).

Time Course of R Discrimination

After the dose–response experimentation, in which the dose was varied and the time between administration and placement into the experimental chamber was constant at 20 min, the 2.5 mg/kg training dose of R was kept constant and the postinjection interval prior to testing was varied. Thus, 2.5 mg/kg R was injected IP, the animal returned to its home cage for 60, 120, 180, or 240 min, at which time it was placed into the experimental chamber and allowed to accumulate 10 responses on either of the two levers. Each time period was tested once following an S maintenance day and once following a 2.5 mg/kg R maintenance day at 20 min postadministration. As with the dose–response experiments, the instant that the rat accumulated 10 responses on one of the two levers it was removed from the experimental chamber without receiving reinforcement. This was done to preclude the possibility of training at either a dose or at a postadministration latency time different than the 2.5 mg/kg R training dose at 20 min postinjection.

Measurement and Data Analysis

The data collected in the drug discrimination session were expressed as a quantal measurement, which indicates the percentage of rats that chose the R-appropriate lever as their selected lever, i.e., accumulated 10 presses first on this lever. In addition, the moment that the rat started to press one of the levers in the experimental chamber to the moment that it accumulated 10 responses on either lever was timed by stop watch. This indicator of rate of responding, therefore, consisted of two factors: first, the time that the animal started to press a lever and, second, the time it took to accumulate 10 presses on either lever. This was used as a measurement of the time, in seconds, to an FR10 lever selection, be it correct or incorrect as to the drug/dose administered in that day's session/test.

The computer-generated formulation of the Litchfield–Wilcoxon procedure (23), which employs probits vs. log-dose effects, was used to yield ED_{50} values for R, with 95% confidence limits from the quantal responses. Likewise, the half-life ($t_{1/2}$) was generated from the time course data.

Ethanol Administration

Following the time course experiments, test days were employed to test three IP doses of ethanol, i.e., 450, 600, and 900 mg/kg (10% w/v). Because there was a need to increase the volume of administration to 5 ml/kg (at the highest ethanol dose used) so as to avoid any pain/necrosis with this dose of ethanol, two additional test days were used to ascertain the discrimination effects of a larger volume (5 ml/kg) of 0.9% saline than used in training and maintenance sessions (1 ml/kg). Each dose of ethanol was then tested on two occasions—once following a saline (1 ml/kg) and once following a 2.5 R maintenance session in a random order with animals immediately removed upon accumulating 10 responses on one or the other lever. The dose of the 10% w/v ethanol solution was varied from 450 to 900 mg/kg by administration of different volumes.

Once trials with ethanol by itself were completed, the large volume of 5.0 ml/kg saline was coadministered with the calculated ED_{50} value for R (see below; 0.08 mg/kg) on two occasions. Likewise, the 450, 600, and 900 mg/kg doses of ethanol were coadministered with the same dose of R on two occasions. These experimental sessions aimed to indicate the effects of doses of ethanol upon the dose of R that produced 50% quantal responding in animals trained to discriminate 2.5 mg/kg R from its saline vehicle.

Pretreatment with Flumenazil (Ro 15-1788)

Following the coadministration experiments with R and three doses of ethanol, the rats were coadministered three doses of the benzodiazepine antagonist flumenazil at the same time as the training dose of R and tested 20 min after the coinjection. Flumenazil (at 8 mg/kg) was also administered with saline on two occasions and all tests were done once following an R maintenance day and once following a saline-maintenance day. Test days, as always, had the animals timed by stop watch from the onset of lever pressing to the accumulation of 10 responses on any lever and instantaneously removed upon that 10th response without receiving food reinforcement.

Drugs

Flunitrazepam [5-(2-fluorophenyl)-1,3 dihydro-1-methyl-7-nitro-1,4-benzodiazepine-2-one] was purchased from Research Biochemical International (Natick, MA), whereas flumenazil (Ro 15-1788) was supplied by Hoffmann–LaRoche (Basel, Switzerland); both dissolved daily in 0.9% NaCl in distilled water. Doses were calculated as base and injection volumes were constant at 1 ml/kg IP Ethanol was calculated as a 10% w/v solution in the same vehicle with administration IP at a maximal volume of 5 ml/kg. The 10% w/v solution appeared to produce no discomfort or tissue necrosis because of the large volume of administration.

RESULTS

Discrimination Learning

As stated in the Method section, the aim of differential reinforcement after drug and saline administration, is to arrive at a performance level previously set at eight correct lever selections in 10 consecutive drug and saline administrations. The pseudorandom administration schedule employed precludes more than 2 consecutive days of either drug or saline administration and allows for five saline and five drug administrations in a 10-day (2 working weeks) time frame. This biweekly schedule is generally repeated until the 8 of 10 correct lever selection criterion is met. In most cases, anywhere from 40–60 days of training are needed (15). In the case of 2.5 mg/kg R vs. saline discrimination training, the 8 of 10 sessions-to-criterion was achieved almost immediately. The data for the animals on each of the 14 days required for training are presented as

Table 1. On the first discrimination training day, following 6 consecutive days to reach FR10 training to R, the administration of saline produced 6 of the 10 animals selecting the R-appropriate lever (designated as "S_1," the first saline session in Table 1). According to the administration schedule, the next 2 days were R,R trials and resulted in all selected lever responses being made on the R-appropriate lever. On the fourth day of discrimination training, as well as the fifth day, rats never selected the R-appropriate lever after saline administration; thus, all selections were on the saline-appropriate lever after saline ("S_2" and "S_3"). This extremely efficient and reliable discriminative performance was maintained so that all 10 rats reached the criterion of eight correct lever selections in 10 consecutive training sessions by the 14th training session; seven after each of R and S.

Dose–Response Relationship to Lower R Doses

The interspersed maintenance schedule with 2.5 mg/kg R or saline continued to show high levels of responding with 2.5 mg/kg R producing errorless responding (100%), whereas saline produced 94.5% of all selected lever choices on the saline-correct lever (Fig. 1). As the dose of R on two occasions each was tested at 1.25, 0.63, and 0.32, discriminative performance continued to remain at 90%. It was only when the dose tested was decreased to 0.16 mg/kg that the discriminative responding began to decrease (to 66.7%). A further dilution of this dose to 0.08 mg/kg produced 61% selected R-lever responses, whereas the lowest dose tested, 0.04 mg/kg R, produced 38.9% of R-lever selections in the nine remaining rats (one rat died of unrelated causes during the conduct of the dose–response experiments and, therefore, the data for 0.16 mg/kg and lower doses represents an $n = 9$). The time in seconds to complete the FR10 on one of the two levers is represented as the right ordinate and indicates that as the discriminative responding decreased with decreasing R doses, the time to reach FR10, in seconds, generally increased. Thus, at the lower discrimination levels seen after 0.16 mg/kg or lower R doses, the greater the amount of time needed to attain FR10 responding on one lever; this duration approached the 85 s needed after saline administration (found as open square on left Y axis).

TABLE 1

SELECTED LEVER DURING DISCRIMINATION TRAINING IN RATS (n = 10) TREATED WITH 2.5 mg/kg ROHYPNOL (R) OR SALINE (S)

Day	Treatment (No.)	Number of Rats Selecting R-Appropriate Lever
1	S_1	6
2	R_1	10
3	R_2	10
4	S_2	0
5	S_3	0
6	R_3	9
7	S_4	1
8	S_5	0
9	R_4	9
10	R_5	8
11	S_6	0
12	R_6	9
13	R_7	10
14	S_7	0

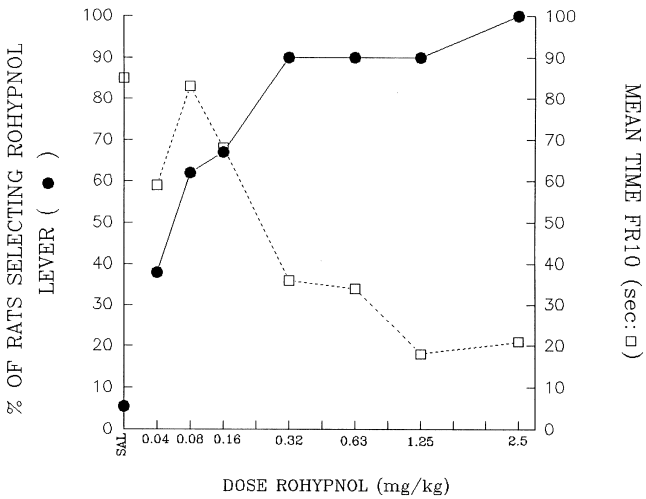

FIG. 1. Dose–response relationship between Rohypnol (R) administered on two occasions in doses of 0.0 (saline), 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, and 2.5 mg/kg to nine male rats trained to discriminate 2.5 mg/kg R from saline. Closed circles indicate selection (pressing R-correct lever 10 times first on an FR10 schedule without receiving reinforcement) after each dose administered twice; once following drug maintenance and once following saline maintenance day. Open squares indicate the time (in s) to complete an FR10 selection on one of the two available levers from the time of placement into the experimental chamber to the finish of the FR10. Each point is a mean of 18 trials (two trials in each of nine animals).

Application of the Litchfield–Wilcoxon procedure (23) to the dose–response experiments indicate an ED_{50} value of 0.076, with a 95% confidence limit range of 0.0443–0.131 mg/kg. Thus, it appears that the training dose used in the discrimination was 33 times greater than the ED_{50} value for discriminative performance.

Time Course of Effects

The use of various postinjection intervals prior to testing of 2.5 mg/kg R in nine rats is presented in Fig. 2. The high level of discriminative performance at 20 min postinjection was maintained at greater than 90% (17 R choices of a possible 18 trials) at 60 min postadministration. This discriminative performance decreased gradually until 240 min postadministration where 3 of 18 or 16.7% of the animals chose the R-appropriate lever. Analysis (23) indicates a half-life for the IP 2.5 mg/kg R dose of 162.3 (129.9–202.7) min or approximately 2.7 h. On the right Y axis, the mean time in seconds to reach the FR10 on one of the two levers was, again, seen to generally increase as the discrimination performance decreased, with the lowest discriminable performance at 240 min requiring a mean of 88 s to reach an FR10. This value is closest to the mean of 115 s required after saline during interspersed maintenance trials (open square on left Y axis).

Administration of Ethanol Alone and with the Calculated ED_{50} of R

The administration of 5 ml/kg saline as a control for the larger volume required to administer ethanol (900 mg/kg) by itself was shown to produce all-saline appropriate lever selections (data not shown).

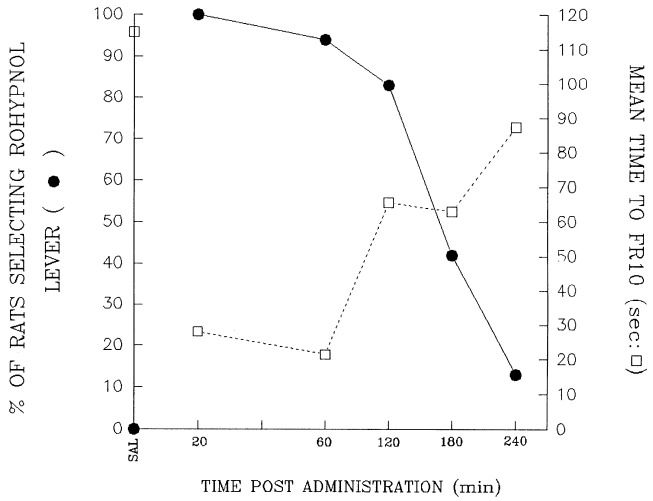

FIG. 2. Time course relationship of Rohypnol (R) discrimination in rats trained to 2.5 mg/kg R at 20 min postadministration. Abscissa: Time, in min, of testing post-R administration; left ordinate: percent R discrimination; right ordinate: time to complete FR10.

Increasing the dosage of ethanol (10% w/v) administered in 5 ml/kg from 450 to 600 to 900 mg/kg, by itself, produced 0, 5.6 and 5.6% of the lever choices upon the R-appropriate lever, respectively (Fig. 3) . Thus, no dose of ethanol administered by itself produced greater than saline-like responding. Administration of this 5 ml/kg saline with the calculated ED_{50} of 0.08 mg/kg R produced 16.7% of lever selections upon the Rohypnol-appropriate lever. This was below the 50% level calculated to be the ED_{50} probably as a result of dilutional factors since both injections were given IP at the same time. When 450 mg/kg ethanol was given with the 0.08 mg/kg dose of R, the animals chose the Rohypnol-appropriate lever on 8 of a possible 18 trials (two trials × nine rats) or 44.4%. The coadministration of 600 mg/kg produced 55.5% and the highest dose of ethanol coadministered with 0.08 mg/kg produced 72.2% of all selected lever responses on the Rohypnol lever. Unfortunately, as the dose of ethanol used in coadministration with 0.08 mg/kg R increased, so did the mean time to reach FR10 with the highest dose of 900 mg/kg in combination yielding FR10 discrimination performance after a mean of 768.6 s on two trials in nine animals. This suggests that it took an average of 12.8 min for the animals to reach an FR10 selection on one of the two levers and, although in over 70% of the time this lever selection was the R-lever, one would, at this point, say that the combination of 900 mg/kg ethanol and 0.08 mg/kg Rohypnol was producing behavioral disruption in the animal. This precluded higher doses from being used.

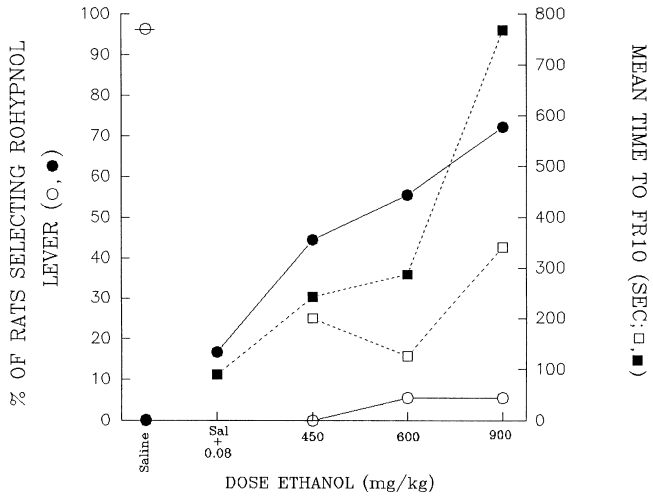

FIG. 3. The effects of coadministration of 450, 600, and 900 mg/kg ethanol (10% w/v solution) in rats trained to discriminate 2.5 mg/kg Rohypnol (open circles) and mean time to finish this FR10 selection (open squares). In addition, the effects of administration of these same ethanol doses and saline with 0.08 mg/kg R coadministered 20 min prior to testing upon discrimination (closed circles) and the mean time in s to select one of the two levers (closed squares). Left-most figure indicates the discrimination performance after saline (closed circle) or R (open circle with horizontal line) during interspersed maintenance day trials.

Coadministration of Flumenazil and Rohypnol

The administration of increasing doses of flumenazil from 2 to 8 mg/kg with the training dose of Rohypnol produced a progressive decrease in the rats' ability to discriminate R (Fig. 4), whereas the highest dose of 8 mg/kg flumenazil administered with saline produced exclusively saline-like responding. During the course of this experimentation, one additional rat died of unrelated causes and the data is indicative of $n = 8$. Analysis (23) of the data indicate that the flumenazil dose caused a 50% reduction in R discrimination, i.e., the ID_{50} (with 95% confidence limits) was 4.098 (3.051–5.504) mg/kg. Lastly, the administration of doses of 2–8 mg/kg flumenazil did not greatly reduce the mean time to attain FR10 responding on one or the other lever as compared to when 8 mg/kg of flumenazil was administered with saline (open square on left ordinate; Fig. 4).

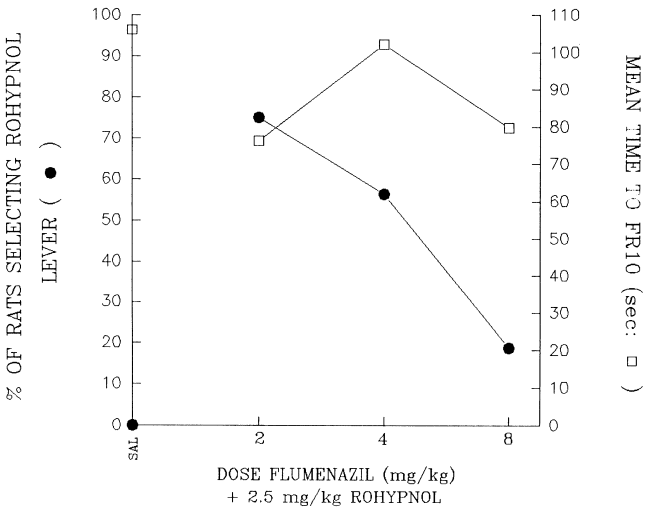

FIG. 4. The effect of coadministration of 2, 4, or 8 mg/kg flumenazil with 2.5 mg/kg Rohypnol in rats trained to discriminate 2.5 mg/kg Rohypnol ($n = 8$). Abscissa: Dose of flumenazil coadministered with the training dose of Rohypnol. Left ordinate: percentage of rats selecting the R-lever after saline + 8 mg/kg flumenazil (open circle on axis) or 2.5 mg/kg R. Right ordinate: time, in seconds, to complete FR10 after each coadministration tested in two sessions.

DISCUSSION

Although a large number of benzodiazepines have been successfully used and shown capable of controlling differential responding in a drug discrimination paradigm (21), the present report is the first one in the literature using Rohypnol (R) to train rats. Thus, both the training dose (2.5 mg/kg) and the time course postinjection (20 min) to be used were "educated guesses," although previous studies in rats trained to discriminate the benzodiazepine diazepam (18,25) or midazolam (8,24) reported the ability of R to substitute for the training drug at low doses. As it turns out, the 2.5 mg/kg R dose appeared to be higher than actually needed to gain control of differential discrimination. This is seen both in the extremely fast discriminative learning (Table 1), as well as by the observation, in dose–response experiments, indicating that the training dose was 33 times the ED_{50} value generated during these experiments. It is, therefore, suggested that continued work employing R as a drug capable of controlling discriminative performance may be best served by a dose lower than 2.5 mg/kg. This will not compromise the animals' ability to discriminate R while, at the same time, it will increase their sensitivity to the interoceptive cues produced by this compound. The ability of any drug to produce differential responding in a drug discrimination task is based upon its state-dependent nature. Rohypnol has previously been shown to produce state-dependent learning deficits in humans as a dose of 2 mg R in volunteer subjects was reported to have disruptive effects inherent in anterograde memory loss; this was suggested to involve state-dependent learning (11). Thus, benzodiazepines appear to produce performance deficits in humans in going from the drug-state during learning to the nondrug state during testing or from the nondrug state during learning to the drug-state during testing. The effect of benzodiazepines, in general, upon human memory has been reviewed (3,4) with a most-recent evidence for the memory deficit occurring with R in healthy human volunteers (10).

An unexpected finding was the stimulatory effect of R at doses (Fig. 1) and postadministration time intervals (Fig. 2) that allowed for the greatest amount of discriminative performance. Thus, in the former case, doses of 0.32–2.5 mg/kg were shown to require a mean time to reach FR10 responding on one lever between 20 and 40 s after placement into the experimental chamber. This rate of response was seen to approach that after saline administration as the R doses fell below 0.32 with 0.08 mg/kg allowing for a mean time to achieve the FR10 selection close to that seen with saline. In the time course experiments, the 2.5 mg/kg R dose tested at 20 and 60 min postadministration also produced a mean time to FR10 selection between 20 and 50 s. This increased to approximately 65 s at 120 and 160 min postadministration and only approached the mean time required to FR10 after interspersed saline maintenance sessions (of 115 s; Fig. 2) at the postadministration time of 240 min. The possibility, therefore, exists that, at least at this training dose and postadministration training interval, there is an increase in stimulation as indicated by rate of FR10 lever responding. This has previously been reported to occur in animals trained to discriminate other depressive drugs, such as ethanol (16,17). In relation to the human condition, there have been reports of increased irritability and sudden outbursts of aggression following intranasal administration of R (12).

The time-course of R discrimination was shown to be maximal at 20–60 min and to return to saline-like levels by 240 min. The calculated $t_{1/2}$ of 2.7 h after IP administration was also determined. These observations may be compared with the reported time to reach peak concentrations after the intranasal administration of 0.5 mg R of 41.7 min (2) and an intravenous $t_{1/2}$ of 60 min in humans administered in a constant infusion of 2 mg/2 ml over 90 s (9).

The results indicate that ethanol by itself in IP doses of 450, 600, and 900 mg/kg produced saline-like responding, whereas when coadministered with the calculated ED_{50} R of 0.08 mg/kg, there was a synergistic effect upon Rohypnol discrimination with increasing doses producing increased discriminative performance. In concert with this increased discriminative selection on the Rohypnol lever, there was a decrease in activity, as indicated by increasing mean time to reach the FR10; the combination of 600 mg/kg ethanol plus 0.08 mg/kg R required a mean of 287 s, whereas the largest dose of ethanol with 0.08 mg/kg R produced a prohibitively long mean time to FR10 and, therefore, higher doses of ethanol were precluded. Nonetheless, it does appear that there is a synergistic effect of ethanol upon Rohypnol discrimination when coadministered. Future research using a lower training dose, e.g., 1 mg/kg Rohypnol, will, most assuredly, allow for a lower ED_{50} dose to be coadministered with like doses of ethanol and, perhaps, allow for at least 80% Rohypnol-like responding when the ED_{50} dose is administered with 900 mg/kg ethanol without the excessive behavioral disruption. This work is currently underway in this laboratory.

As early as 1986 (21), Ro 15-1788 (presently known as flumenazil) was shown to dose dependently block the discriminative cue produced by a centrally active benzodiazepine. This ability to block the benzodiazepine receptor has been shown in many subsequent experiments involving the rat [e.g., (24,25); for review, see (22)] and it is, therefore, once again suggested that Rohypnol, like other benzodiazepine agonists, produce its drug discriminative properties via an affinity for benzodiazepine binding in the rat cerebral cortex (18).

In summary, Rohypnol has been shown to be capable of controlling discriminative responding in the rat. This first attempt also allowed for two parametric manipulations of the discriminative effects of Rohypnol that suggest that future research using this drug not only employ a lower dose, but also a slightly later postadministration time for training. This continued research should be aimed at defining the exact mechanism by which Rohypnol works in the central nervous system in its apparent interactive capacity with ethanol as seen in human abusers/victims.

ACKNOWLEDGEMENTS

The author would like to express his sincere gratitude to Denise McBurney for her continued expertise in both the behavioral and graphical aspects of this work and to Martha Hilgert and Sheila Formick for their tenacious word processing skills.

REFERENCES

1. Associated Press Release, Victims of 'date rape' drug testify, July 17, 1996.
2. Bond, E.; Seijas, D.; Dawling, S.; Lader, M.: Systemic absorption and abuse liability of snorted flunitrazepam. Addiction 89:821–830; 1994.
3. Curran, H. V.: Tranquilizing memories: A review of the effects of benzodiazepines on human memory. Biol. Psychol. 23:179–213; 1986.
4. Curran, H. V.: Benzodiazepines, memory and mood: A review. Psychopharmacology (Berlin) 105:1–8; 1991.

5. De St. Hilaire-Kafi, Z.; Vallet, P. G.; Gaillard, J. M.: Hypnotic actions of flunitrazepam is reversed by proglumide in rats. Prog. Neural Psychopharmacol. Biol. Psychiatry 15:817–823; 1991.
6. DeVry, J.; Slangen, J. L.: Effects of chlordiuzepoxide training dose on the mixed agonist-antagonist properties of benzodiazepine receptor antagonist Ro 15-1788, in a drug discrimination procedure. Psychopharmacology (Berlin) 88:177–183; 1986.
7. Drummer, O. H.; Syrjanew, M.L.; Cordner, S. M.: Deaths involving the benzodiazepine flunitrazepam. Am. J. For. Med. 14: 230–243; 1993.
8. Garcha, H. S.; Rose, I. C.; Stolerman, I. P.: Midazolam cue in rats: Generalization tests with anxiolyties and other drugs. Psychopharmacology (Berlin) 87:233–237; 1985.
9. Gentil, V.; Tavares, S.; Gorenstein, C.; Bello, C.; Mathias, L.; Gronich, G.; Singer, J.: Acute reversal of flunitrazepam effects by Ro 15-1788 and Ro 15-3505 inverse agonism, tolerance and rebound. Psychopharmacology (Berlin) 100:54–59; 1990.
10. Ingum J.; Beylic, H. K.-M.; Morland, J.: Amnesic effects and subjective ratings during repeated dosing flunitrazepam to healthy volunteers. Eur. J. Clin. Pharmacol. 45:235–240; 1993.
11. Jensen, H. H.; Paulsen, J. C.: Amnesic effects of diazepam: "Drug dependence" explained by state-dependent learning. Scan. J. Psychol. 23:107–111; 1982.
12. Maddaleno, M.; Florenzano, R.; Santa Cruz, X.; Vidal, R.: Consumo de flunitrazepam via nasal en adolescentes marginales de Santiago de Chile. Rev. Med. Chile 116:691–694; 1988.
13. (The) Merck Index: An encyclopedia of chemical, drug and biologicals, 11th ed., Budavari, S.; O'Neil, M. J.; Smith A.; Heckelman, P.E., eds. No. 4072; Rahway, NJ: Merck & Co., Inc.; 1989:4070.
14. Overton, D. A.: A historical perspective on drug discrimination. NIDA Res. Monogr. 116:5–24; 1991.
15. Overton, D. A.; Leonard, W. R.; Merkle, D. A.: Methods for measuring the strength of discriminable drug effects. Neurosci. Biobehav. Rev. 10:251–263; 1986.
16. Schechter, M. D.: Locomotor activity but not conditioned place preference is differentially affected by a moderate dose of ethanol administered to P and NP rats. Alcohol 9:185–188; 1992.
17. Schechter, M. D.: Cocaethylene produces discriminative stimulus properties in the rat: Effect of cocaine and ethanol coadministration. Pharmacol. Biochem. Behav. 51:285–289; 1995.
18. Shannon, H. E.; Herling, S.: Discriminative stimulus effects of diazepam in rats: Evidence for a maximal effect. J. Pharmacol. Exp. Ther. 227:160–166; 1983.
19. Smith, D. E.; Wesson, D. R.; Calhoun, S. R.: Rohypnol (Flunitrazepam) fact sheet. Haight Ashbury Free Clinics, Inc., San Francisco, CA; May 6, 1996.
20. Staten, C.: Roofies called date rape drug of choice. Am. Rep. 2:Jan. 8, 1996.
21. Stolerman, I. P.; Garcha, H. S.; Rose, I. C.: Midazolam cue in rats: Effects of Ro 15-1788 and picrotoxin. Psychopharmacology (Berlin) 89:183–188; 1986.
22. Stolerman, I. P.; Rasul, F.; Shine, P. J.: Trends in drug discrimination research analysed with a cross-indexed bibliography, 1984–1987. Psychopharmacology (Berlin) 98:1–19; 1989.
23. Tallarida, R. J.; Murray, R. B.: Manual of pharmacologic calculations with computer programs, 2nd ed. New York: Springer Verlag; 1986.
24. Woudenberg, F.; Slangan, J. L.: Discriminative stimulus properties of midazolam: Comparison with other benzodiazepines. Psychopharmacology (Berlin) 97:466–470; 1989.
25. Young, R.; Glennon, R. A.: Stimulus properties of benzodiazepines: Correlations with binding affinities, therapeutic potency, and structure activity relationships (SAR). Psychopharmacology (Berlin) 93:529–533; 1987.