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Interactions of Diazepam and Pentobarbital With RO 15-4513 on Intracranial Self-Stimulation Discrimination Behavior in Rats

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SCHAEFER, G J AND R P MICHAEL *Interactions of diazepam and pentobarbital with RO 15-4513 on intracranial self-stimulation discrimination behavior in rats* PHARMACOL BIOCHEM BEHAV **34**(1) 23–27, 1989 —Rats implanted with electrodes in the lateral hypothalamus were trained in a discrete trial procedure to make a differential response (right or left lever press) in the presence or absence of brain stimulation When a high level of accuracy (95% correct) was attained in the discrimination, testing with vehicle, RO 15-4513, diazepam (1.0–10 mg/kg), diazepam plus RO 15-4513 (1 0 mg/kg), pentobarbital (1 0–17 5 mg/kg) and pentobarbital plus RO 15-4513 began Diazepam, at 10 mg/kg, disrupted the discrimination behavior, and it also decreased the total number of lever-presses and increased the time to complete the session These effects were blocked by the coadministration of 1 0 mg/kg RO 15-4513 Pentobarbital produced effects similar to those of diazepam, but these effects were only reversed to a limited extent by RO 15-4513 By itself, however, RO 15-4513 also decreased the total number of lever presses and increased the time to complete the session Results were consistent with our previous findings with alcohol and RO 15-4513, and supported the notion that diazepam and alcohol have some similar effects at the GABA-benzodiazepine receptor complex

RO 15-4513 Diazepam Pentobarbital Brain self-stimulation Discriminative stimulus properties

ALCOHOL, benzodiazepines and barbiturates may be classified as sedative-hypnotic agents that depress the central nervous system and disrupt behavior These drugs also have discriminative stimulus effects, that is, animals can be trained to perform a different response depending upon the presence or absence of the drug However, there is no good agreement as to whether these compounds produce similar internal cues (1–3, 8, 9, 16, 19) While these three types of drugs share some discriminative stimulus, and presumably subjective effects, there are also differences between them which may, in part, be due to differences in their interactions at the GABA-benzodiazepine receptor complex (18) There is much current interest in RO 15-4513, a partial inverse benzodiazepine agonist, as a specific antagonist of alcohol (4, 6, 18) Rees and Balster (11) recently demonstrated, in rats trained in a food-reinforced task, that RO 15-4513 blocked the discriminative stimulus effects of alcohol and benzodiazepines, while not blocking those of the barbiturates We also have observed that RO 15-4513 can counteract some of the actions of alcohol, but that it has prominent behavioral effects of its own For example, when animals were trained to discriminate the presence or absence of intracranial self-stimulation (ICSS), 1 0 g/kg alcohol both altered the performance of the response and the animal's ability to make the appropriate choice in the discrimination task (14) The coadministration of 1 0 mg/kg RO 15-4513 together with 1 0 g/kg alcohol counteracted the latter's effects on performance and choice behavior. In the present study, we have used this procedure to study the effects of diazepam and of pentobarbital both alone and in the presence of RO 15-4513 The results indicate that the antagonism was not restricted to alcohol, but applied to the effects of diazepam also, while the effects of pentobarbital were not blocked These findings suggest that alcohol and diazepam may have actions in common at the GABA-benzodiazepine receptor complex, which may differ from those of pentobarbital

METHOD

Animals

The animals used were five adult male Sprague-Dawley rats (Charles-River, Wilmington, MA) These animals weighed 420–

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530 g when electrodes were implanted and, when not in test chambers, they were housed in group cages and maintained in a colony room on a 12-hr light-dark cycle with lights on at 0700 hr All housing and experimental procedures were conducted according to the Institutional regulations and the *NIH Guide for the Care and Use of Laboratory Animals* (NIH publication No 85-23, revised 1985)

Apparatus

The test chamber measured 30 5 by 31 by 45 cm high It contained two conventional levers ("choice levers") on one wall 11 cm apart, separated by a 6 2 cm floor-to-ceiling Plexiglas partition On the opposite wall, an omnidirectional lever ("initiating lever") was suspended from the ceiling The experiment was controlled and data were collected by a Timex/Sinclair 1000 computer using a 16 K RAM module and two interface boards A constant-current biphasic stimulator produced two different outputs When a choice lever was pressed, a 500-msec train of pulses with a pulse duration of 0 2 msec and a frequency of 100 Hz was generated Currents varied from 250 to 500 μA The stimulus produced by the initiating lever was always a proportion (0–100%) of that produced by the choice levers The stimulus current was randomly determined by the computer for each trial For a more detailed description of the apparatus, see Schaefer and Michael (12,13)

Surgery and Histology

The animals were deeply anesthetized with 50 mg/kg of sodium pentobarbital IP and also given 0 25 mg atropine sulfate IM to reduce respiratory discomfort Following placement in a stereotaxic instrument, a small burr hole was drilled in the exposed skin and the dura was incised A bipolar platinum electrode (tip diameter = 0 125 mm, Plastic Products Co , Roanoke, VA) was lowered into the brain and was aimed at the medial forebrain bundle-lateral hypothalamus (MFB-LH) using Pellegrino *et al* (10) coordinates (AP 5 2, L 1 7, H −2 2) The electrode assembly was held rigid with cranioplastic cement covering the top of the electrode and 4–5 stainless steel screws also fixed to the skull Animals were then administered 100,000 U of benzathine penicillin G and procaine penicillin G IM and given postoperative care When experiments were completed, the animals were given a large overdose of sodium pentobarbital and perfused through the heart with 10% formalin Following fixation, frozen sections of brain were cut at 50 μm Alternate sections were stained with cresyl violet and Weil's stain to locate the electrode tips

Procedure

When the animals had recovered completely from surgery, they were trained in a discrete trial procedure to make a differential response (right or left lever-press) in the presence or absence of brain stimulation At the beginning of each trial the animal was required to press the initiating lever, and then one of the two choice levers During training sessions one choice lever produced a strongly reinforcing stimulus train (previously determined for each animal), while the other choice lever did not activate the stimulator The first response on the initiating lever produced a brief tone from a Sonalert speaker and on 50% of the trials, a reinforcing stimulus train When stimulation was obtained, the animal was required to press the left-sided choice lever to obtain a second reinforcing stimulus train and terminate the trial The left-sided choice lever was called the ICSS choice lever A press on the right-sided choice lever terminated the trial without further stimulation When no ICSS occurred with the first lever-press on the initiating lever, the animal was required to press the right-sided choice lever to obtain stimulation and terminate the trial The right-sided choice lever was called the NO-ICSS choice lever Pressing the left-sided choice lever in this condition terminated the trial without stimulation For two of the five animals used in this study, the ICSS choice lever was on the right and the NO-ICSS choice lever was on the left A trial was terminated either by the completion of the two-response behavioral chain or by the end of the session The intertrial interval was 5 sec, and during this time the chamber was dark The animals were trained until they reached 95% accuracy (95 out of 100 trials) on four consecutive days For further details of the training procedure see Schaefer and Michael (12,13)

In the next phase of the study, testing with vehicle and drugs was begun Test sessions differed in two respects from training sessions First, the initiating lever produced a stimulus current that ranged from 0 to 100% of the training current Second, a response on either choice lever produced a reinforcing stimulus train, regardless of the current on the initiating lever Ten trials occurred at each of 12 stimulus currents, and these ten trials were randomly interspersed during the 120-trial session The animal's discrimination was determined by the stimulus current at which 50% of the trials were completed on the ICSS choice lever, and this was designated the ED_{50} current Two measures of performance were also obtained 1) total time to complete the test session (maximum = 2700 seconds), and 2) total number of lever presses on all three levers While only the first response in each trial on the initiating and the choice lever had programmed consequences, all lever presses during the trials and between trials were counted and this served as a general measure of lever pressing On Mondays and Thursdays, the animals were tested with vehicle, and on Tuesdays and Fridays with drugs On Wednesdays, the animals were given a 100-trial training session The experiments with pentobarbital and RO 15-4513 were conducted first, followed by tests with diazepam and RO 15-4513 RO 15-4513 was tested alone twice, at the beginning and at the end of these experiments and, since the results did not differ, they were combined

Drugs

RO 15-4513 was generously provided by Dr W E Haefely, Hoffmann-La Roche Inc , Basel, Switzerland, diazepam was a gift of Hoffmann-La Roche Inc , Nutley, NJ, and sodium pentobarbital was purchased from Sigma Chemical Co , St Louis, MO RO 15-4513 and diazepam were suspended in a 1 1 ratio of 15% propylene glycol and 1% Tween 80 (Sigma Chemical Co) and dispersed by ultrasound prior to injection Pentobarbital was dissolved in 0 9% saline and doses are expressed as the acid All drugs were administered intraperitoneally in a volume of 1 0 ml/kg RO 15-4513 was administered 5 min before the start of the test session, while diazepam was given 30 min and pentobarbital was given 15 min before testing

Data Analysis

The data consisted of the number of trials out of ten completed on the ICSS choice lever at each stimulus current To evaluate these data, trials completed on the ICSS choice lever at each current step were determined for vehicle and each dose of drug or drug combination A log-probit transformation was performed and the resulting regression lines were evaluated for parallelism and for the stimulus current that produced 50% responding on the ICSS

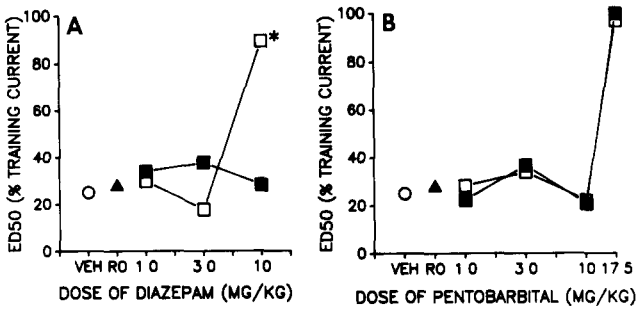

FIG 1 (A) Effects of vehicle (VEH) (○), 1 0 mg/kg RO 15-4513 (RO) (▲), graded doses of diazepam (□) and graded doses of diazepam plus 1 0 mg/kg RO 15-4513 (■) on the current (percentage of the training current) required to complete 50% of the trials on the lever appropriate for brain stimulation in animals trained to discriminate between the presence and absence of brain stimulation Each point is the mean of five animals *RO 15-4513 blocked the increase in the ED_{50} current produced by 10 mg/kg diazepam alone (B) Effects in the same animals of vehicle (VEH) (○), 1 0 mg/kg RO 15-4513 (RO) (▲), graded doses of pentobarbital (□) and graded doses of pentobarbital plus 1 0 mg/kg RO 15-4513 (■) on the current required to complete 50% of the trials on the lever appropriate for brain stimulation

choice lever (ED_{50}) the method of Litchfield and Wilcoxon (7) was used in the computerized version of Tallarida and Murray (17) Analyses of variance for repeated measures were used to test the dose-dependent changes produced by diazepam and pentobarbital alone and in combination with RO 15-4513 on the performance measures (SPSS/PC+, SPSS Inc , Information Analysis Systems, Chicago, IL) Post hoc testing was performed by using Dunnett's procedure (two-tailed) Repeated measures analyses of variance were also used to evaluate the effects of RO 15-4513 when animals were administered it alone

RESULTS

All animals acquired the discrimination and showed graded increases in responding on the ICSS-choice lever as the stimulus current on the initiating lever was increased. Doses of 1.0 and 3 0 mg/kg diazepam did not alter the discrimination When animals were administered 10 mg/kg diazepam, a nonparallel shift in the regression line indicated disruption of the discrimination, and this was reversed by 1 0 mg/kg RO 15-4513 (data not shown) Figure 1A shows the effects of vehicle, diazepam, RO 15-4513 alone and RO 15-4513 with graded doses of diazepam on the ED_{50} values It can be seen that RO 15-4513 alone did not alter the ED_{50}, nor did 1 0 and 3 0 mg/kg diazepam alone When animals were administered 10 mg/kg diazepam, however, the ED_{50} value was increased about 3-fold, and this increase was completely blocked by the administration of 1.0 mg/kg RO 15-4513 Figure 1B similarly shows the effects on the ED_{50} of pentobarbital At a dose of 17 5 mg/kg, pentobarbital disrupted the discrimination but, in contrast to the results with with diazepam, RO 15-4513 did not block this effect.

Changes in measures of operant performance during the discrimination procedure are shown in Table 1 For diazepam, an overall ANOVA for the total number of lever presses showed a significant main effect of dose, $F(3,32) = 5\ 38$, $p<0\ 005$, and a drug combination-by-dose interaction, $F(3,32) = 7\ 39$, $p<0.001$ There was a graded decrease in numbers of lever presses with dose, and at 10 mg/kg the decrease was significant ($p<0\ 01$, Dunnett's test) The combination of diazepam with 1 0 mg/kg RO 15-4513 produced no significant changes from vehicle values, although there was a tendency for a reduction at the lowest dose of diazepam This may have been due to the significant reduction in

TABLE 1

EFFECTS OF DIAZEPAM, PENTOBARBITAL AND RO 15-4513 ON OPERANT BEHAVIOR DURING DISCRIMINATION FOR INTRACRANIAL SELF-STIMULATION

Parameter	Vehicle	Drug	Dose (mg/kg) 1 0	3 0	10	17 5	F-ratio	p[3]
Total Presses[1]	839 ± 64	Diazepam	882 ± 59	757 ± 60	336 ± 126†		9 3	<0 001
Total Presses	828 ± 73	Diazepam + 1 0 mg/kg RO 15-4513	670 ± 88	710 ± 21	777 ± 38		1 3	n s
Total Time[2]	1285 ± 11	Diazepam	1251 ± 10	1706 ± 219†	2669 ± 31†		35 4	<0 001
Total Time	1272 ± 25	Diazepam + 1 0 mg/kg RO 15-4513	1496 ± 100	1391 ± 52	1406 ± 55		2 1	n s
Total Presses	843 ± 55	Pentobarbital	856 ± 72	945 ± 58	597 ± 155	181 ± 86†	11 1	<0 001
Total Presses	823 ± 65	Pentobarbital + 1 0 mg/kg RO 15-4513	694 ± 60	772 ± 94	762 ± 57	343 ± 180*	3 6	<0 025
Total Time	1318 ± 22	Pentobarbital	1252 ± 21	1227 ± 13	2251 ± 275†	2700 ± 0†	30 3	<0 001
Total Time	1321 ± 43	Pentobarbital + 1 0 mg/kg RO 15-4513	1544 ± 163	1342 ± 44	1701 ± 117	2609 ± 91†	26 7	<0 001
Total Presses	836 ± 64	RO 15-4513	669 ± 56				55 0	<0 005
Total Time	1236 ± 87	RO 15-4513	1582 ± 160				7 4	n s

[1]Total number of lever presses during the test session
[2]Total time in seconds to complete the test session
[3]Analysis of variance
Significantly different from vehicle at *$p<0\ 05$ and at †$p<0\ 01$ by Dunnett's test

rates (vehicle = 836 ± 64 presses vs RO 15-4513 = 669 ± 56 presses), $F(1,4) = 55\ 0$, $p<0\ 005$, produced by RO 15-4513 alone Similar results for time to complete the test session (maximum = 2700 seconds) occurred There were significant main effects of drug combination, $F(3,32) = 27\ 6$, $p<0\ 001$, and dose, $F(3,32) = 27\ 6$, $p<0\ 001$, and a drug combination-by-dose interaction, $F(3,32) = 27\ 2$, $p<0\ 001$ The middle and high doses of diazepam increased the time to complete the session, and these changes were reversed by 1 0 mg/kg RO 15-4513 By itself, RO 15-4513 increased the time to complete the session (vehicle = 1236 ± 87 sec vs RO 15-4513 = 1582 ± 160 sec), and this trend approached significance

Performance data for pentobarbital are also given in Table 1 The overall ANOVA for total number of lever presses revealed only a significant main effect of dose, $F(4,40) = 12\ 5$, $p<0\ 001$ A graded decrease in response rates occurred over the dose range 3 0–17 5 mg/kg This decrease was not reversed by RO 15-4513, although the decrease at the highest dose was somewhat less with the two drugs combined A similar effect was seen with time to complete the session The overall ANOVA resulted in a significant main effect of dose, $F(4,40) = 53\ 8$, $p<0\ 001$, and drug combination-by-dose interaction, $F(4,40) = 3\ 9$, $p<0\ 01$ When pentobarbital was administered alone, a graded increase in time to complete the session occurred When it was combined with 1 0 mg/kg RO 15-4513, the increase in total time produced by the intermediate dose of 10 mg/kg pentobarbital was reversed by RO 15-4513

Histological analysis revealed that the five electrodes terminated in or near the lateral hypothalamus Of these five, three were located on the border between the lateral hypothalamus and the zona incerta, and the two remaining electrode tips were approximately 1 mm deeper in the lateral hypothalamus

DISCUSSION

In this study, diazepam impaired the performance of a complex operant task A modest dose of RO 15-4513 blocked all of the diazepam-produced changes Similar changes in behavior produced by pentobarbital were very little blocked by RO 15-4513 In a previous study (15), RO 15-4513 alone produced a graded increase in locomotor activity and, when 1 0 mg/kg was given together with alcohol, activity was also increased In contrast to the stimulation of locomotor activity, operant responding for ICSS was decreased The extent of the decreases in response rate depended upon the schedule of reinforcement, greater decreases were observed in the fixed ratio paradigm than in the fixed interval paradigm In the present discrete trial procedure, which has certain similarities to a fixed interval paradigm, 1 0 mg/kg RO 15-4513 reduced the total number of lever presses by about 20%, which was rather similar to the 26% decrease found in the fixed interval 15-second schedule (15) Despite its own rate-decreasing effect, RO 15-4513 reversed the decreases in lever-pressing produced by a high dose of diazepam

RO 15-4513 consistently blocks the behavioral effects of benzodiazepines, including diazepam, oxazepam and chlordiazepoxide (4) Changes in locomotor activity as well as changes in operant response rates are antagonized by the coadministration of RO 15-4513 The present data showed that changes in more complex discrimination behavior were also antagonized by RO 15-4513 Changes in behavior produced by the barbituates, however, are not always antagonized by RO 15-4513 In particular, changes in operant response rate and changes in discrimination performance were not blocked (11) This is consistent with the findings of the present study in which RO 15-4513 had little effect on the disruption produced by pentobarbital Only a single dose of RO 15-4513 was used, and antagonism with a higher dose of RO 15-4513 might occur For example, while a dose of 10 mg/kg did not block motor impairment in mice, 20 mg/kg did so (5) However, since a dose-dependent decrease in operant rates occurs with RO 15-4513 alone (11,15), the rate-decreasing effects of the drug itself may have out-weighed its capacity to block the actions of the barbiturate

The previous report (14), together with the present study, demonstrated that three CNS depressant drugs produce similar changes in the performance of an ICSS discrimination task Alcohol, diazepam and pentobarbital all reduced operant response rates and increased the time to complete the test session However, RO 15-4513, while blocking the effects of alcohol and diazepam, largely failed to do so in the case of pentobarbital This is consistent with the view that alcohol and diazepam have similar effects on the GABA-benzodiazepine receptor complex in the brain and that pentobarbital may act differently There are clear differences between these compounds, and these became very apparent in the differential effects of RO 15-4513 on their actions in an ICSS discrimination task

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