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A Behavioral Examination of Convulsant Benzodiazepine and GABA Antagonist, Ro 5-3663, and Benzodiazepine-Receptor Antagonist Ro 15-1788

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FELDON, J., T. LERNER, D. LEVIN AND M. MYSLOBODSKY. *A behavioral examination of convulsant benzodiazepine and GABA antagonist, Ro 5-3663, and benzodiazepine-receptor antagonist Ro 15-1788.* PHARMACOL BIOCHEM BEHAV **19**(1) 39–41, 1983.—The potential "anxiogenic" effects of convulsant benzodiazepine and GABA-antagonist, Ro 5-3663 and specific antagonist of benzodiazepine receptors, Ro 15-1788 were compared in the Geller-Seifter conflict paradigm. Chlordiazepoxide (CDP) (5 mg/kg) was used as a "positive" control. Both Ro 5-3663 (1 mg/kg) and Ro 15-1788 (10 mg/kg) antagonized the anticonflict effect of CDP. However, while Ro 15-1788 had a modest anticonflict potency, Ro 5-3663 had an anxiogenic effect in its own right.

Anxiety GABA Convulsant benzodiazepine Benzodiazepine antagonist

IT HAS been reported [4] that convulsant benzodiazepine and GABA antagonist, Ro 5-3663, may act agonistically to chlordiazepoxide (CDP) and show modest anxiolytic properties in its own right. The nature of this effect is ill understood, as Ro 5-3663 has been predicted to act as anxiety-activating rather than anxiolytic compound [14].

Ro 5-3663 is a weak inhibitor of [^{3}H]-flunitrazepam [16] and [^{3}H]-diazepam [14] binding. However, since only a small proportion of benzodiazepine receptors need be occupied to produce their typical response profile [15], a possibility that Ro 5-3663 acts at the allosteric GABA-benzodiazepine unit should be considered. In fact, the specific antagonist of the benzodiazepine receptor, Ro 15-1788 [8] also showed antiaversive properties in the aversive stimulation test [9]. In the present study the effects of the two drugs, Rq 5-3663, and Ro 15-1788, and their interaction with CDP, were compared in the classical conflict paradigm of food-rewarded behavior depressed by a punished contingency [5].

METHOD

Subjects and Materials

Experimentally naive male 220–280 g Charles-River rats were housed in a standard laboratory environment with water ad lib. Night-day cycle (12 hr darkness/12 hr light) was maintained by artificial lighting. Experiments were conducted between 8:30 a.m. and 4:30 p.m.

The following compounds were used: Chlordiazepoxide HCl (CDP, Hoffman-LaRoche, Inc.), dissolved in saline, Ro 5-3663 and Ro 15-1788 (Hoffman-LaRoche, Inc.). The former was dissolved in saline, the latter was suspended in distilled water to which Tween 80 (2 drops/10 ml) was added. CDP, Ro 5-3663, and Ro 15-1788 were administered IP, 10, 5, and 1 minute, respectively, prior to the daily session.

Procedure and Apparatus

Rats were exposed gradually to a 23 hr food-deprivation regime, and tested in standard rodent operant test chambers (25×23×23 cm; Campden Instruments Ltd.). The rats were shaped to press the left-hand lever to obtain 45 mg precision food pellets (Campden Instruments Ltd.). Each testing session lasted 57 minutes, and the central stimulus light was on throughout. For three weeks the rats were kept on a 60 sec variable interval (VI) schedule. Thereafter, the session was composed of 5 periods of 9 minutes VI, intermingled with 4 intrusion periods of 3 minutes. During the intrusion period the house light in the center of the ceiling flashed on and off at 2 Hz. During these periods (conflict) the rats were on a continuous reinforcement (CRF) schedule where each lever-press resulted in one food pellet as well as a 0.5 sec scrambled electric shock delivered by a Campden Instruments shock source. The shock level was increased gradually from 0.15 mA individually for each rat so as not to disturb their stable responding during the VI periods. The final shock levels ranged between 0.15 mA and 0.5 mA and the restriction for shock adjustment was that the total number of responses during the 4 CRF periods would range from 4 to 12. This method assured that there were no baseline disruptions during the VI periods or total suppression during the CRF periods. The rats were run for 60 stabilization sessions before drug treatments started. The animals were tested throughout the experiment once daily (Sun–Fri) and each

TABLE 1

THE INTERACTION OF CDP WITH Ro 5-3663 AND Ro 15-1788 IN THE GELLER-SEIFTER PARADIGM

Test Drugs	Dose (IP)	N	Non-Conflict Responses (Mean ± SEM) Vehicle	Non-Conflict Responses (Mean ± SEM) Drug	Conflict Responses (Mean ± SEM) Vehicle	Conflict Responses (Mean ± SEM) Drug
CDP	5 mg/kg	12	994 ± 188	1409 ± 280	7.80 ± 1.59	51.00 ± 11.30†
Ro 15-1788	10 mg/kg	12	1092 ± 236	1549 ± 304*	9.30 ± 2.08	13.10 ± 2.79
CDP+ Ro 15-1788	5 mg/kg 10 mg/kg	12	1915 ± 357	1595 ± 346*	69.90 ± 13.20	27.00 ± 5.70*
CDP	5 mg/kg	16	1512 ± 176	1845 ± 267	11.03 ± 1.80	34.80 ± 6.40†
Ro 5-3663	1 mg/kg	13	1499 ± 183	1161 ± 165*	14.70 ± 3.80	4.70 ± 0.87†
CDP+ Ro 5-3663	5 mg/kg 1 mg/kg	24	1625 ± 157	1713 ± 193	66.30 ± 8.07	31.00 ± 6.98‡

CDP, Ro 5-3663, Ro 15-1788, or a combination of CDP with one of the antagonists was tested. Each drug was tested against its own vehicle, while each combination of drugs (CDP + Ro 15-1788, or CDP + Ro 5-3663) was compared to its CDP baseline. After injection, rats were placed in the skinner box and allowed to bar press for food in the non-conflict (VI) and conflict (CRF) segments of the session.

Values represent the means (±SEM) of the total number of responses during the VI and CRF segments of the paradigm with number of animals specified. A two-way ANOVA for repeated measurements composed of drug effects (drug vs. vehicle) and replications was carried out. In no instance was the interaction of drug × replication significant. (*$p<0.05$ †$p<0.01$ ‡$p<0.001$).

animal was used as its own control. Drugs were administered on Mondays and Thursdays. The examination of the effects of Ro 5-3663 and Ro 15-1788 was carried out on two different groups of rats. Each received the following schedule: Week 1: Examination of CDP (5 mg/kg) on Monday and Thursday; Week 2: Examination of Ro 5-3663 (1 mg/kg) or Ro 15-1788 (10 mg/kg) on Monday and Thursday; Week 3 and 4: On Mondays the rats were injected with 5 mg/kg of CDP and on Thursdays with 5 mg/kg of CDP together with either 1 mg/kg of Ro 5-3663 or 10 mg/kg of Ro 15-1788. The examination of Ro 5-3663 was continued for an additional third week. This drug schedule allowed a separate evaluation of the effects of CDP and each of the two antagonists as well as a comparison of the effects of CDP + Ro 5-3663 or CDP + Ro 15-1788 with the effects of CDP alone. On the no-drug days, rats were administered with 1 ml/kg of the vehicle.

Statistical treatment was performed using a two-way ANOVA for repeated measurements.

RESULTS

Data summerized in Table 1 show the effects of CDP and antagonists on conflict and non-conflict behaviors in the rat. CDP significantly increased the rate of responding in the conflict segment of the paradigm, and elevated, although non-significantly, non-conflict responding. Both Ro 5-3663 and Ro 15-1788 reversed considerably the release of suppressed responding induced by CDP in the conflict period. Ro 15-1788, but not Ro 5-3663 also significantly reversed the effect of CDP in the non-conflict period. Administered alone, Ro 5-3663 reliably suppressed responding in both the conflict and non-conflict periods. To the contrary, Ro 15-1788 elevated significantly non-conflict responding and also increased the rate of conflict responding at about 52% (albeit this result was short of significance).

DISCUSSION

While both Ro 5-3663 and Ro 15-1788 reversed the anticonflict effects of CDP, they showed pronounced differences in their intrinsic behavioral effects as well as in their patterns of reversal. Ro 5-3663 and Ro 15-1788 have been demonstrated to reverse diazepam-induced suppression of wave-spike shaped photically evoked sensory afterdischarges in rats [10]. Given that this is a rather proconvulsive (petit mal activating) response [11], and that anxiety is conceptualized as a state of diminished GABAergic neurotransmission [13], one might anticipate that these drugs have anxiogenic properties. However, Ro 15-1788 seems to have a modest anticonflict effect in its own right. This is suggested by the effect of Ro 15-1788 in both the conflict period, where there was some release from suppression of punished responding, (though nonsignificant), and by its effect in the non-conflict period, where a general elevation of responding was observed. The effect was obtained with a rather low dose of Ro 15-1788, and it strikingly differed from the effects of Ro 5-3663. It has been previously shown in numerous studies (for review see Gray, [7]) that one of the typical findings following administration of anxiolytic drugs is an increase in response rate under intermittent schedules of food reinforcement. Indeed, in the present study such a tendency was observed, though it failed to attain the acceptable level of significance. The effect mentioned above, of the anxiolytic drugs is considered to reflect their tendency to increase food appetite. The findings of Lloyd *et al.* [9] who noted that Ro 15-1788 delayed the escape response to aversive stimulation of the periaqueductal gray, is also in agreement with the suggested anticonflict properties of Ro 15-1788.

To the contrary, Ro 5-3663 in its own right showed a rather pronounced anxiogenic effect. This compound significantly suppressed responding in both the conflict and non-

conflict periods of the paradigm. These changes in behavior were not correlated with any observable symptoms of the drug epileptogenicity, nor did Ro 5-3663 induce symptoms reminiscent of sedation. The present result is consistent with previous observations that Ro 5-3663 antagonizes postictal analgesia and enhances flight response to tail pressure [12]. However, it is at variance with our previous findings with the water-lick paradigm where anticonflict properties of Ro 5-3663 have been noted [4]. Drug/pain and drug/food motivation interactions on which the present conflict protocol is based may have contributed to the variance. It has been suggested that GABA is involved in antinociception [18]. It is therefore tempting to conjecture that Ro 5-3663 attenuates shock-induced analgesia as may be gleaned from the findings where this compound reduced postictal analgesia [12]. This would explain the suppression of responding in the Geller-Seifter paradigm but would contradict the effects of the drug in the water-lick test. If this result were to be replicated, a possibility might be considered that Ro 5-3663 produces hyperanalgesia during foot shock rather than mouth shock, an effect which should considerably diversify the results of conflict paradigms based on pain punishment. This possibility is not a remote one if it is recalled that the neurochemical system controlling shock-induced analgesia shows dependency upon the body region shocked [17].

Another important source of variance is the differences between the rewarding properties of food and water. It is not excluded that liquid may antagonize pain more effectively than solid food. This conjecture is supported by findings that liquid but not solid food produces synchronized EEG [1,6] similar to "post-reinforcement synchronization" [2] or "pleasure waves" [17].

Whatever the nature of this variance, it cautions that benzodiazepines-GABA antagonistic effects of some drugs may vary with the paradigm employed. Morag (unpublished) has noted recently that in a test of exploratory activity between a brightly-lit vs. a small dark compartment [3], both Ro 5-3663 and Ro 15-1788 showed antiaversive properties.

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