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THE Ro 15-1788 CUE: EVIDENCE FOR BENZODIAZEPINE AGONIST AND INVERSE AGONIST PROPERTIES

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Rats discriminating Ro 15-1788 (10 mg/kg, i.p.) from vehicle completely generalized this cue to typical benzodiazepines, and partially generalized it to barbiturates, pentylenetetrazol, CGS 8216, β-CCM and PK 8165. CL 218 872, Ro 5-4864, phenytoine, progabide, propranolol, yohimbine and various CNS stimulants predominantly induced vehicle-appropriate responding. Chlordiazepoxide, pentobarbital, CL 218 872, PK 8165, pentylenetetrazol, CGS 8216 and β-CCM failed to block the Ro 15-1788 cue. Generalization to both anxiolytics/anticonvulsants and anxiogenics/(pro) convulsants suggests that Ro 15-1788 has benzodiazepine agonist and inverse agonist properties.

Benzodiazepine agonist | Benzodiazepine antagonist | Benzodiazepine inverse agonist | Drug discrimination
Rat | Ro 15-1788 cue

1. Introduction

The imidazodiazepine Ro 15-1788 was initially characterized as a benzodiazepine (BDZ) receptor antagonist, devoid of intrinsic behavioral activity (Haefely et al., 1983). Subsequently however, partial BDZ agonist properties (e.g. Lloyd et al., 1981), as well as inverse agonist properties (effects which are opposite to the typical BDZ effects, e.g. File et al., 1982) have been reported. Schöpf et al. (1984) found central activating effects of Ro 15-1788 on the human EEG pattern; these effects were partially comparable with changes induced by psychogeriatrics and psychostimulants.

BDZ agonist properties of Ro 15-1788 were also found in drug discrimination studies. In particular, rats discriminating either clorazepate (Dantzer and Pério, 1982) or chlordiazepoxide (De Vry and Slangen, 1984; and unpublished results) from saline, generalized the BDZ cue to Ro 15-1788. The ability of Ro 15-1788 to induce drug stimulus control in the rat has recently been demonstrated (De Vry and Slangen, 1985). In order to further characterize the cue properties of Ro 15-1788 (10 mg/kg), we now investigated to what extent presumably related compounds could substitute for, or block the Ro 15-1788 cue.

2. Materials and methods

Twelve male Wistar rats were trained to discriminate Ro 15-1788 from vehicle in a two-lever food-reinforced procedure. The procedure and the acquisition of drug discrimination have been reported elsewhere (De Vry and Slangen, 1985). In short, rats were injected with either Ro 15-1788 (10 mg/kg i.p., 15 min) or vehicle (distilled water + Tween-80) and, depending on the injection conditions reinforcement (45 mg Noyes pellets) could be obtained according to a tandem VI 40 s FR 10 schedule, by pressing either the drug or vehicle-appropriate lever. Sessions were run 5 days a week and lasted 15 min. Extinction periods, consisting of the time interval needed to accumulate 10 re-

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sponses on either level (lever selection) increased by 2 min, were introduced at the beginning of control sessions and yielded 2 measurements: (1) number of responses (total number of responses on both levers); (2) percentage injection-appropriate lever responses ((number of responses on the appropriate lever/number of responses) × 100). Control sessions were run twice a week, under both sets of training conditions and provided an index of discriminative performance.

When discriminative performance had stabilized, generalization or antagonism tests were run on Wednesdays and Fridays, while training (including control sessions) continued on the remaining days. During test sessions, the rats were allowed to respond during a time interval covering lever selection plus 2 min. The sessions ended after this interval or after 15 min, whichever came first. No reinforcement could be obtained. Testing yielded 2 measurements: (1) number of responses; (2) percentage drug lever responses. At least 10 responses were required to make the second measurement valid. The drugs to be tested were selected on the basis of the suggested relationship between their effects and the effects of Ro 15-1788 and included anxiolytics and/or anticonvulsants, anxiogenics and/or (pro)convulsants and psychostimulants. Generalization tests were run 15 min after injection of the test drug, except with phenobarbital, progabide, PK 8165 and Ro 5-4864, which were tested after 30 min. In antagonism tests, the drug was injected 15 min before Ro 15-1788 (10 mg/kg) and testing was done 15 min after the injection of Ro 15-1788. All drugs were injected i.p. Drugs were suspended in the Ro 15-1788 vehicle, except for pentobarbital, phenobarbital, chlordiazepoxide, dl-propranolol, d-amphetamine, fencamfamine, cocaine and caffeine which were dissolved in 0.9% NaCl (saline).

3. Results

Test results were obtained after 95 training sessions. The mean control results for the test phase were 90.0% and 87.9% injection-appropriate lever responses for the drug and vehicle training conditions, respectively.

The BDZs chlordiazepoxide, flunitrazepam and clonazepam completely generalized with the Ro 15-1788 cue (table 1; maximum extent of generalization: $\geqslant 85\%$). The barbiturates pentobarbital and phenobarbital, the non-specific or specific BDZ antagonists pentylenetetrazol, β-CCM and CGS 8216 (Czernik et al., 1982; Haefely et al. 1983) and the quinoline derivative PK 8165 (LeFur et al., 1981) induced partial generalization with Ro 15-1788. Generalization was more than 40% with these substances but failed to reach the maxima obtained with the BDZs even at the highest doses which could be tested (table 1). The generalization maxima obtained with other drugs (dose range in mg/kg is indicated in parentheses) were: Ro 5-4864 (30): 9.5%; propranolol (10): 11.2%; phenytoin (10-30): 13.4%; cocaine (1-3): 15.0%; progabide (30-100): 16.8%; CL 218 872 (1-10): 22.6%; yohimbine (3): 24.8%; fencamfamine (0.1-1): 36.4%; amphetamine (0.1-1): 36.8%; and caffeine (3-30): 37.0% ($n = 4$-5). There was no statistical evidence for a difference between discriminative responding induced by the latter compounds and their vehicle (Student's t-test). Failure to generalize could not be attributed to behaviorally inactive doses, as the highest doses tested generally significantly decreased the response rate (data not shown).

Antagonism test results are shown in table 2. None of the compounds tested could block the Ro 15-1788 cue significantly (t-test for a difference between test drug and vehicle pretreatment conditions). Results obtained with CGS 8216 (56.8% at 10 mg/kg) and pentylenetetrazol (60.2% at 20 mg/kg) may indicate weak antagonism. However, higher doses of these compounds failed to increase the extent of antagonism and therefore the results may reflect partial antagonism at best.

Ro 15-1788 (10 mg/kg) significantly increased the response rate (table 1, $t = 3.64$, $df = 9$, $P < 0.01$, t-test for a difference in the mean number of responses after drug and vehicle treatment). The compounds generally decreased the response rate (statistically significant effects are indicated in table 1). Generalization with the Ro 15-1788 cue was obtained both under testing conditions which significantly affected response rate (e.g., flunitrazepam 0.3 mg/kg), and testing conditions which had

TABLE 1

Generalizaton test results obtained in rats trained to discriminate Ro 15-1788 (10 mg/kg) from vehicle. Only test compounds which induced more than 40% drug generalization are shown. n/N indicates the number of rats completing at least 10 responses versus the number of rats tested.

Treatment	Dose (mg/kg)	Mean % drug lever responses (S.E.M.)	Mean number of responses (S.E.M.)	n/N
Tween-80 vehicle	- [a]	12.1 (2.7)	133 (16)	10/10
Saline vehicle	-	11.4 (4.2)	133 (12)	10/10
Ro 15-1788	10 [a]	90.0** (3.3)	181** (19)	10/10
Chlordiazepoxide	0.1	35.1 (9.6)	139 (23)	10/10
	1	44.1** (12.0)	119 (25)	10/10
	10	85.9** (4.6)	140 (24)	10/10
Flunitrazepam	0.01	67.4* (12.5)	133 (25)	5/5
	0.3	93.0** (3.8)	71* (16)	5/5
Clonazepam	0.03	46.6* (9.6)	133 (28)	5/5
	0.3	75.4* (12.9)	68 (29)	5/5
	1	88.3	34 (32)	1/5
Pentobarbital	0.1	42.2 (14.2)	166 (32)	5/5
	1	57.0 (15.3)	111 (21)	5/5
	10	61.2* (9.7)	81** (17)	5/5
	15	60.0 (7.7)	51* (17)	4/4
Phenobarbital	10	20.4 (8.5)	146 (43)	5/5
	40	64.3 (14.7)	46** (19)	3/5
CGS 8216	1	45.4 (21.7)	128 (26)	5/5
	10	75.4** (10.5)	62** (15)	5/5
	30	42.0 (23.0)	13** (8)	2/5
Pentylenetetrazol	0.2	31.6 (16.0)	133 (28)	5/5
	2	75.2* (15.5)	117 (25)	5/5
	20	26.3 (13.7)	33* (9)	4/5
β-CCM	0.1	8.0 (4.6)	132 (30)	4/4
	1	33.0 (15.8)	156 (39)	4/4
	3	43.8 (24.0)	78* (41)	4/4
	10	53.0 (47.0)	57 (52)	2/4
PK 8165	0.1	25.3 (20.3)	80 (33)	3/4
	1	49.5 (25.6)	207 (76)	4/4
	10	8.5 (8.5)	39* (25)	2/4

[a] Averaged across the control sessions interspersed between test sessions. * $P < 0.05$; ** $P < 0.01$ difference between test drug and its vehicle (Student's t-test).

no effect on response rate (e.g., chlordiazepoxide 10 mg/kg). The response rate-decreasing effects of the test compounds tended to be reversed by pretreatment with Ro 15-1788 (tables 1, 2). A statistically significant effect was obtained when Ro 15-1788 was combined with CGS 8216 (10 mg/kg) and CL 218 872 (10 mg/kg) ($t = 3.07$, $df = 4$, $P < 0.05$ and $t = 3.99$, $df = 4$, $P < 0.02$, respectively).

4. Discussion

The generalization of the Ro 15-1788 cue to typical BDZs now described is in accordance with the suggestion that Ro 15-1788 may have BDZ agonist properties (e.g. Lloyd et al., 1981). It has previously been shown that rats, discriminating the effects of a BDZ, may generalize the BDZ cue to Ro 15-1788 and it was suggested that this finding reflected BDZ agonist properties of Ro 15-1788 (Dantzer and Pério, 1982; De Vry and Slangen, 1984). This suggestion is supported by the presently obtained generalization of Ro 15-1788 to BDZs. The Ro 15-1788 cue also generalized to the barbiturates pentobarbital and phenobarbital but to a lesser extent. This finding is not surprising as it is well known that the dis-

TABLE 2

Antagonism test results obtained in rats trained to discriminate Ro 15-1788 (10 mg/kg) from vehicle. Pretreatment was carried out 15 min before administration of 10 mg/kg Ro 15-1788.

Pretreatment	Dose (mg/kg)	Mean % drug lever responses (1 S.E.M.)	Mean number of responses (1 S.E.M.)	n/N
Vehicle	-	97.0 (2.0)	193 (21)	10/10
CGS 8216	1	89.0 (7.8)	148 (26)	5/5
	10	56.8 (23.0)	174 (38)	5/5
	30	67.8 (18.4)	65 (17)	5/5
Pentylenetetrazol	2	93.2 (6.6)	118 (23)	5/5
	20	60.2 (17.8)	76 (13)	5/5
	30	77.0 (6.4)	32** (14)	3/5
β-CCM	3	88.3 (6.4)	116 (31)	4/4
	10	99.3 (0.7)	89 (46)	3/4
Chlordiazepoxide	10	87.8 (5.3)	193 (59)	4/4
Pentobarbital	15	83.4 (6.4)	19** (5)	5/5
CL 218872	10	79.8 (18.0)	136 (32)	5/5
PK 8165	1	100.0 (0.0)	235 (70)	4/4
	10	89.5 (10.5)	12** (7)	2/4

**$P < 0.01$ difference between drug and vehicle pretreatment conditions (Student's t-test).

criminative effects of BDZs and barbiturates are closely similar (e.g. Herling and Shannon, 1982) and Ro 15-1788 induced weak barbiturate-like discriminative effects in rats trained to discriminate pentobarbital from saline (Herling and Shannon, 1982; De Vry and Slangen, submitted). The finding that BDZ receptor ligand PK 8165 (LeFur et al., 1981) generalized partially with Ro 15-1788, may be consistent with the suggested partial BDZ agonist properties of PK 8165 (Gee et al., 1983). However, a decrease in the extent of generalization was observed at the 10 mg/kg test dose of PK 8165, suggesting that the discriminative effects of Ro 15-1788 and PK 8165 may not be identical.

On the other hand, the Ro 15-1788 cue was found to generalize at least partially to pentylenetetrazol, β-CCM and CGS 8216. These compounds are able to produce behavioral effects which are opposite to typical BDZ effects (e.g. Pellow and File, 1984). These results therefore support the suggestion that Ro 15-1788 also may have BDZ inverse agonist properties (e.g. Schöpf et al., 1984). The observation that DMCM (BDZ receptor ligand with inverse agonist properties)-trained rats partially generalized this cue to Ro 15-1788 (Nielsen et al., 1985) has thus far been the only indication of possible BDZ inverse agonist properties of Ro 15-1788 in drug discrimination research. In contrast to the generalization results obtained with BDZs, higher test doses of pentylenetetrazol and CGS 8216 failed to increase the extent of drug generalization. This finding may indicate that the cue properties of pentylenetetrazol and CGS 8216 on the one hand, and of Ro 15-1788 on the other hand, are not identical. The failure of chlordiazepoxide, pentobarbital, pentylenetetrazol, CGS 8216 and β-CCM to block significantly the Ro 15-1788 cue, may perhaps be attributed to their Ro 15-1788-like discriminative effects.

Schöpf et al. (1984) suggested that Ro 15-1788 had weak stimulant effects partially similar to the effects of psychostimulants and psychogeriatrics. In the pesent experiment, weak stimulant effects of Ro 15-1788 may be suggested by the fact that it increased the response rate. However, as indicated by generalization test results, these effects of Ro 15-1788 can be differentiated from the effects of psychostimulant compounds such as amphetamine, cocaine, caffeine and fencamfamine.

It may be suggested that peripheral-type and micromolar BDZ binding sites do not mediate the Ro 15-1788 cue, as the respective ligands Ro 5-4864 (Braestrup and Squires, 1977) and phenytoin (Bowling and DeLorenzo, 1982) failed to generalize with Ro 15-1788. Also it may be suggested

that occupation of BDZ receptors (central-type BDZ binding sites) is not a sufficient condition to produce the Ro 15-1788 cue, as is indicated by the absence of significant generalization with the BDZ receptor ligand CL 218 872 (Lippa et al., 1979). Failure of generalization with progabide, a GABA receptor agonist, (Worms et al., 1982), similarly indicates that stimulation of GABA receptors is not a sufficient condition to produce the Ro 15-1788 cue. There was no generalization of the Ro 15-1788 cue to propranolol and yohimbine, compounds with suggested anxiolytic and anxiogenic properties, respectively, but with effects presumably not mediated by the BDZ receptor.

To conclude, it was found that the Ro 15-1788 cue generalized specifically to both anxiolytics/anticonvulsants and anxiogenics/(pro)convulsants acting at the BDZ receptor-GABA receptor-Cl^- ionophore complex. This result suggests that Ro 15-1788 (10 mg/kg) has BDZ agonist as well as inverse agonist properties. Further research is needed to elucidate the complex nature and mechanism of action of the Ro 15-1788 cue.

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