Pharmacology Biochemistry and Behavior, Vol. 41, pp. 859–862, 1992
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0091-3057/92 $5.00 + .00
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BRIEF COMMUNICATION

Conditioned Place Preference Induced by Ro 16-6028, a Benzodiazepine Receptor Partial Agonist

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Received 5 July 1991

DI SCALA, G., P. OBERLING, B. ROCHA AND G. SANDNER. *Conditioned place preference induced by Ro 16-6028, a benzodiazepine receptor partial agonist.* PHARMACOL BIOCHEM BEHAV 41(4) 859–862, 1992.—A place conditioning situation was used to assess the putative affective properties of benzodiazepine receptor ligands in the rat. The benzodiazepine receptor partial agonist Ro 16-6028 induced a conditioned place preference, suggesting that this compound has rewarding properties. The benzodiazepine receptor antagonist Ro 15-1788 induced neither place preference nor aversion, but prevented the place preference induced by Ro 16-6028, suggesting that the rewarding effects of Ro 16-6028 are due to its action on the benzodiazepine receptor. The benzodiazepine receptor full agonist diazepam did not induce a conditioned place preference in our hands, in contrast with previous studies. The sensitivity of place conditioning with benzodiazepine ligands to situational factors, such as the existence of a preconditioning preference, is discussed.

Place conditioning Reward Benzodiazepine receptor ligands Ro 16-6028 Ro 15-1788 Diazepam *d*-amphetamine

PLACE conditioning has become a widely used situation for studying the affective effects of drugs [for reviews, see (2,12,25)]. In this situation, it is held that an increased preference for a drug-associated environment is indicative of a rewarding effect of the drug under study (2,21). Besides a large body of research on the affective properties of dopaminergic and opioidergic compounds, some interest has arisen for other classes of drugs such as the ligands of the benzodiazepine receptor (BZR). For several years, a variety of compounds have been synthesized that exert novel behavioral effects through an action on this receptor. Briefly, the BZR ligands have been classified along a functional continuum (9,11) ranging from agonists with the classical anxiolytic, anticonvulsant, sedative, and amnesic effects (e.g., diazepam) to inverse agonists with anxiogenic, convulsant, and nootropic effects (e.g., β-CCE). Between are found antagonists that can inhibit the effects of both the agonists and inverse agonists (e.g., Ro 15-1788) and compounds that have been called partial agonists or partial inverse agonists as they do not share all the properties of the full agonists or inverse agonists (e.g., FG 7142, a partial inverse agonist, is anxiogenic and nootropic but only proconvulsant) (13). In the place conditioning situation, full agonists (diazepam, lorazepam) were found to produce conditioned place preferences (CPP's) (7,22,24). In contrast, on the opposite end of the continuum, conditioned place aversions (CPA's) were obtained with full inverse agonists (B-CCE) (27) and with partial inverse agonists (CGS 8216 and FG 7142) (5,7,28). To our knowledge, the effects of partial agonists have not yet been studied in the place conditioning situation, and the present study was aimed at investigating the affective properties of one of these compounds: Ro 16-6028 (10). Ro 16-6028 is presented as a clinically promising anxiolytic compound (16), displaying a potent anticonflict and anticonvulsant activity in animals, but with much weaker motor impairing activity (15) and much reduced physiological dependence liability relative to classical BZR full agonists (14,17). The

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putative affective properties of the BZR antagonist Ro 15-1788 were studied, and this compound was later used to antagonize the effects of Ro 16-6028 to ascertain that Ro 16-6028-induced effects are due to an action on the BZR. For sake of comparison, we verified whether diazepam and *d*-amphetamine, which have been previously shown to induce CPP (2,3,7,12,22-24), would do so in our experimental design.

METHOD

Animals

Subjects were 120 male Long-Evans rats (Janvier, France) weighing 270-400 g. They were housed two per cage in a colony room maintained on a 12 L:12 D cycle (light on at 8:00) with food and water provided ad lib.

Apparatus

Each rat was tested and conditioned in one of four wooden place conditioning apparatus. The apparatus consisted of three compartments. Two large ones (A and B: 45 × 45 × 30 cm) separated by a wooden partition had a Plexiglas front and distinctive roof, walls, and floor. One had a white roof, black and white vertical striped walls, and a Plexiglas floor covered by wooden chips; the other had a black roof, black walls, and a wire grid floor. The third compartment (C: 36 × 18 × 30 cm) was a side compartment and was adjacent to both compartments A and B. It was painted grey and had a removable wooden partition. When this partition was in place, the rat could be confined in one of the large compartments. When the partition was removed, the rat could move freely between the two large compartments (via compartment C). An infrared detector (TALCO, IRP124) at the roof of each compartment was used to detect the presence of the animal through the heat emitted. The signal was fed into a programmable controller (Omron, Sysmac C20) that computed the time spent in each compartment. In this apparatus, no initial preference for a given compartment before conditioning has been reported (5,6).

Procedure

Behavioral testing and conditioning started after three daily handling sessions and was always conducted during the same time of the day: during the light phase of the cycle. The procedure was divided into three consecutive phases.

Preconditioning test. On the first day of the experiments, the shuttle compartment was open. Each rat was put in the shuttle compartment and allowed to move freely throughout the apparatus for 15 min. The time spent by rats in each compartment was recorded.

Conditioning. The conditioning phase lasted 8 days and consisted of four pairings of the drug with one compartment and four pairings of the vehicle with the other compartment. During this phase, the shuttle compartment was closed. Each rat was injected with the conditioned drug on one day and with the same volume of vehicle on the other day. After a 10-min postinjection delay, rats were confined for 30 min in compartment A or B. The number of animals experiencing the drug in A was counterbalanced with the number of animals experiencing it in B. Rats were randomly assigned to 1 of 10 groups receiving the following treatments (the doses used were selected from previous studies):

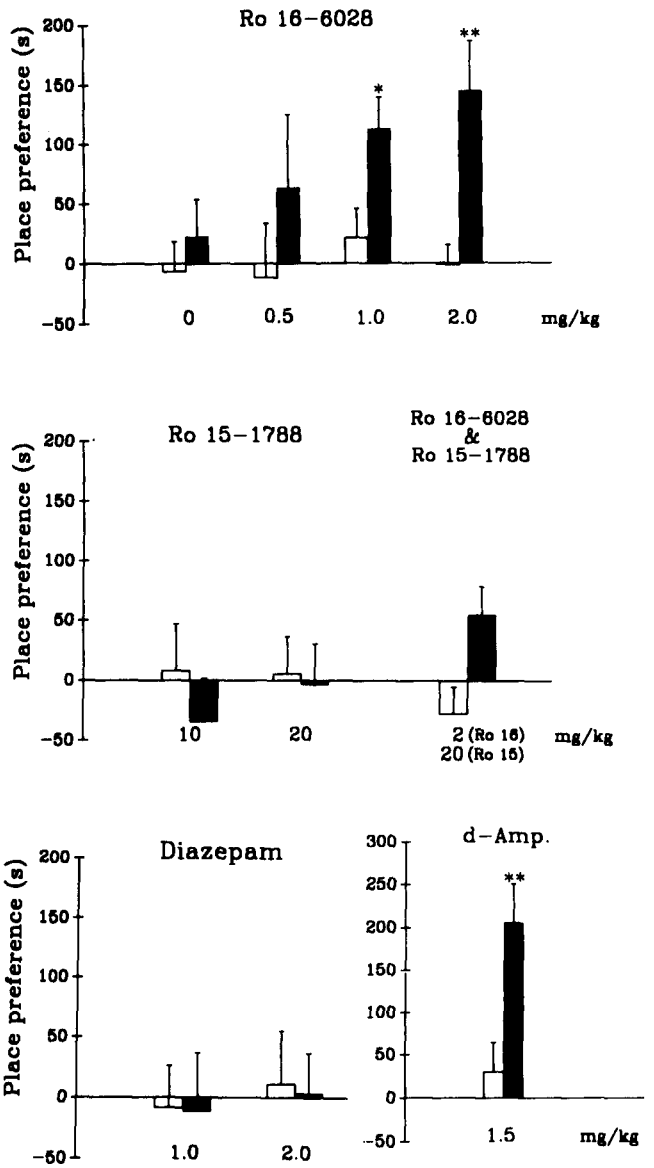

FIG. 1. Place conditioning with Ro 16-6028, Ro 15-1788, diazepam, and *d*-amphetamine. Histograms represent the difference of time spent in the drug- and vehicle-paired compartments (drug − vehicle) during tests. Open bars, preconditioning test; filled bars, postconditioning test. Asterisks indicate significant differences between the pre- and postconditioning tests (*t*-test): $*p < 0.05$, $**p < 0.02$.

- Ro 16-6028 (Roche) at the doses of 0.5, 1, and 2 mg/kg (20) (respectively, $n = 10$, 20, and 10).
- Ro 15-1788 (Roche) at the doses of 10 and 20 mg/kg (8,28) ($n = 10$ in each group).
- Ro 16-6028 (2 mg/kg) followed by Ro 15-1788 (20 mg/kg) with a 30-s delay ($n = 10$).
- Diazepam (Valium, Roche) at the doses of 1 and 2 mg/kg (7,22,24) ($n = 20$ and 10, respectively).
- *d*-Amphetamine (Sigma) at the dose of 1.5 mg/kg (3,23) ($n = 10$).

Ro 16-6028, Ro 15-1788, and diazepam were dissolved in saline added with Tween-80 (Polyethylene Sorbitan Mono-

Oleate, Sigma; 1 drop every 2 ml) and continuously stirred. *d*-Amphetamine was dissolved in saline. Each drug was injected at a volume of 1 ml/kg (IP). The last group of rats (n = 10) received vehicle injections (saline + Tween, 1 ml/kg) paired with both compartments; the compartment considered as the "drug-paired" compartment was randomly assigned before experimentation.

Postconditioning test. On day 10, rats were placed in the shuttle compartment with the partition opened and the time they spent in each compartment was measured over a 15-min period.

Statistical Analysis

The difference of the time spent in the drug-paired compartment and in the vehicle-paired compartment was computed for each test (drug − vehicle). These differences, called place preferences, were used as the dependent variable in Student's bilateral matched t-tests.

RESULTS

Figure 1 depicts the results obtained with the various treatments. During the preconditioning tests, no preference for a given compartment was observed in the various groups (i.e., the mean time spent in the drug- or vehicle-paired compartments were roughly similar and close to 330 s).

The top graph in Fig. 1 depicts the results obtained with Ro 16-6028. Animals conditioned with vehicle (dose 0) showed no change of preference after conditioning. Conversely, groups of animals conditioned with Ro 16-6028 displayed a dose-related preference for the drug-paired compartment during the postconditioning test. Matched t-tests revealed that the difference of time spent in both compartments during the postconditioning test significantly differed from that observed during the preconditioning test for groups treated with 1 and 2 mg/kg [respectively: $t(19) = 2.24$, $p < 0.05$, and $t(9) = 2.95$, $p < 0.02$].

The middle graph in Fig. 1 shows that conditioning animals with high doses of Ro 15-1788 (10 or 20 mg/kg) did not result in CPP or CPA. It also shows that the CPP produced by injections of 2 mg/kg Ro 16-6028 was prevented by the subsequent injection of 20 mg/kg Ro 15-1788.

The bottom graph in Fig. 1 shows that diazepam at the doses of 1 or 2 mg/kg did not result in a CPP. For the sake of comparison, it also shows that conditioning animals with 1.5 mg/kg *d*-amphetamine resulted in a significant place preference, $t(9) = 4.36$, $p < 0.01$.

DISCUSSION

The main finding of the present study was that the BZR partial agonist Ro 16-6028 induced a CPP, suggesting that this compound has rewarding properties in the rat. Ro 16-6028 has potent anxiolytic activities in man and animals (15,16), but the dependence of Ro 16-6028-induced CPP on its anxiolytic activity may be questioned since using our procedure the prototypical anxiolytic diazepam failed to produce CPP. This may suggest that the rewarding effects of Ro 16-6028, as assessed in place conditioning, is due to an activity not (or only partly) related to its anxiolytic activity. An alternative explanation is that Ro 16-6028 is devoid of some of the effects of diazepam that prevented the latter drug from inducing CPP in our experimental situation. As a matter of fact, Ro 16-6028 has much weaker sedative effects than diazepam, a property that has been related to its partial agonist action on the BZR (15). Further studies with other BZR partial agonists are therefore needed to determine whether CPP is due to their anxiolytic activity or to an effect distinct from their anxiolytic activity. In this regard, it is noteworthy that in a conditioned burying paradigm Ro 16-6028 produced behavioral effects that differed markedly from those produced by anxiolytic drugs such as diazepam or the adenine derivative BW A78U (4,20).

The BZR antagonist Ro 15-1788 induced neither preference nor aversion, but prevented the CPP induced by Ro 16-6028. The lack of affective properties of Ro 15-1788 is consistent with previous results showing that Ro 15-1778 is devoid of intrinsic activity (11,20,26), although some evidence exists of agonist or inverse agonist activities, depending on the dose and the test situation (8). The antagonism of Ro 16-6028-induced CPP by Ro 15-1788 is consistent with the known antagonistic action of Ro 15-1788 on the BZR (9,11) and suggests that the rewarding effects of Ro 16-6028 are due to its action on the BZR. Taken together, these results suggest that in the rat Ro 16-6028 produces rewarding effects through an action on the BZR, but it is unsure that these rewarding effects depend on the anxiolytic activity of this compound.

The preceding conclusion partly rests on two results (obtained with diazepam and Ro 15-1788) that differ from previously published studies, and it may be useful to consider the possible causes of these discrepancies. In our hands, diazepam did not induce a CPP, in contrast with previous studies (7,22,24). A poor sensitivity of our situation or procedure to the rewarding effects of drugs is yet unlikely since it is shown that *d*-amphetamine, a drug consistently shown to have rewarding effects in place conditioning (3,19,23), produced a CPP in our situation. Similarly, our results show that Ro 15-1788 had no intrinsic effects, whereas one report shows that it can produce a CPA (28). Here again, a poor sensitivity of our situation to the aversive effects of drugs is unlikely since previous studies of ours have shown that our situation could reveal the aversive effects of a BZR partial inverse agonist (FG 7142) (5), as well as the aversive effects of intracranial microinjections of drugs (1,6). A major difference between the experimental situation used by other authors (7,22,24,28) and ours that could account for these discrepancies resides in the preconditioning status of each compartment since in previous studies "unbalanced" situations [with a preconditioning preference for one of the compartments, see (2)] were used. Our situation is a "balanced" situation (with no clear preconditioning preference) and the present results are germane to those obtained elsewhere with such in a situation, showing that the structural analogue of diazepam, triazolam, did not produce a CPP, whereas *d*-amphetamine did so (18). It is hence suggested that the existence of a strong preference (or aversion) for one compartment before conditioning may interact in a complex way with the affective properties of the drug under study. Furthermore, this interaction may be more important for drugs acting on the BZR than for psychostimulants such as *d*-amphetamine since positive results were obtained in balanced and unbalanced situations with this drug (2,18,23).

ACKNOWLEDGEMENTS

This study was supported by grants from the French Fondation pour la Recherche Médicale. Ro 16-6028 and Ro 15-1788 were generously provided by Drs. J. L. Moreau and F. Jenck (Hoffman LaRoche, Basle, Switzerland). B. R. was supported by the Conselho Nacional de Pesquisa and the Universidade Federal do Rio de Janeiro, Brazil.

REFERENCES

1. Bagri, A.; Di Scala, G.; Sandner, G. Conditioned place aversion produced by blockade of GABAergic neurotransmission within the inferior colliculus of rats (in preparation).
2. Carr, G. D.; Fibiger, H. C.; Phillips, A. G. Conditioned place preference as a measure of drug reward. In: Liebman, J. M.; Cooper, S. J., eds. The neuropharmacological basis of reward. Oxford: Clarendon Press; 1989:264–319.
3. Di Scala, G.; Martin-Iverson, M. T.; Phillips, A. G.; Fibiger, H. C. The effects of progabide (SL 76002) on locomotor activity and conditioned place preference induced by d-amphetamine. Eur. J. Pharmacol. 107)271–274; 1985.
4. Di Scala, G.; Misslin, R.; Guyon, A.; Bourguignon, J. J. Anxiolytic-like effects of the adenine derivative BW A78U in mice and rats. Psychopharmacology (Berl.) 101(suppl.):S15; 1990.
5. Di Scala, G.; Sandner, G. Conditioned place aversion produced by FG 7142 is attenuated by haloperidol. Psychopharmacology (Berl.) 99:176–180; 1989.
6. Di Scala, G.; Sandner, G. Conditioned place aversion produced by microinjections of semicarbazide into the periaqueductal gray of the rat. Brain Res. 483:91–97; 1989.
7. File, S. E. Aversive and appetitive properties of anxiogenic and anxiolytic agents. Behav. Brain Res. 21:189–194; 1986.
8. File, S. E.; Pellow, S. Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788. Psychopharmacology (Berl.) 88: 1–11; 1986.
9. Gardner, C. R. Functional in vivo correlates of the benzodiazepine agonist-inverse agonist continuum. Prog. Neurobiol. 31: 425–476; 1988.
10. Haefely, W. E. Pharmacological profile of two benzodiazepine partial agonists: Ro 16-6028 and Ro 17-1812. Clin. Neuropharmacol. 7:S1; 1984.
11. Haefely, W. E. The benzodiazepine receptor and its clinically useful ligands. Clin. Neuropharmacol. 9:398–400; 1986.
12. Hoffman, D. C. The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res. Bull. 23: 373–387; 1989.
13. Little, H.; Nutt, D. J.; Taylor, S. C. Acute and chronic effect of the benzodiazepine receptor ligand FG 7142: Proconvulsant properties and kindling. Br. J. Pharmacol. 83:951–958; 1984.
14. Martin, J. R.; Kuwahara, A.; Horii, I.; Moreau, J. L.; Jenck, F.; Sepinwall, J.; Haefely, W. E. Evidence that the benzodiazepine receptor partial agonist Ro 16-6028 has minimal abuse and physical dependence liability. Soc. Neurosci. Abstr. 16:1104; 1990.
15. Martin, J. R.; Pieri, L.; Bonetti, E. P.; Schaffner, R.; Burkard, W. P.; Cumin, R.; Haefely, W. E. Ro 16-6028: A novel anxiolytic acting as a partial agonist at the benzodiazepine receptor. Pharmacopsychiatry 21:360–362; 1988.
16. Merz, W. A. Partial benzodiazepine agonists: Initial results in man. Clin. Neuropharmacol. 7(suppl. 1):672–673; 1984.
17. Moreau, J. L.; Jenck, F.; Pieri, L.; Schoch, P.; Martin, J. R.; Haefely, W. E. Physical dependence induced in DBA/2J mice by benzodiazepine receptor full agonists, but not by partial agonist Ro 16-6028. Eur. J. Pharmacol. 190:269–273; 1990.
18. Pettit, H. O.; Batsell, W. R.; Mueller, K. Triazolam attenuates amphetamine but not morphine place preferences. Psychopharmacology (Berl.) 98:483–486; 1989.
19. Reicher, M. A.; Holman, E. W. Location preference and flavor aversion reinforced by amphetamine in rats. Anim. Learn. Behav. 5:343–356; 1977.
20. Rohmer, J. G.; Di Scala, G.; Sandner, G. Behavioral analysis of the effects of benzodiazepine receptor ligands in the conditioned burying paradigm. Behav. Brain Res. 38:45–54; 1990.
21. Rossi, N. A.; Reid, L. D. Affective states associated with morphine injections. Physiol. Psychol. 4:269–274; 1976.
22. Spyraki, C.; Fibiger, H. C. A role for the mesolimbic dopamine system in the reinforcing properties of diazepam. Psychopharmacology (Berl.) 94:133–137; 1988.
23. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Dopaminergic substrates of amphetamine place preference conditioning. Brain Res. 253:185–193; 1982.
24. Spyraki, C.; Kazandjian, A.; Varonos, D. Diazepam-induced place preference conditioning: Appetitive and antiaversive properties. Psychopharmacology (Berl.) 87:225–232; 1985.
25. Swerdlow, N. R.; Gilbert, D.; Koob, G. F. Conditioned drug effects on spatial preference. Critical evaluation. In: Boulton, A. A.; Baker, G. B.; Greenshaw, A. J., eds. Neuromethods—psychopharmacology, chap. 13. Clifton, NJ: Humana; 1989:399–446.
26. Treit, D. Ro 15-1788, CGS 8216, picrotoxin and pentylenetetrazol: Do they antagonize anxiolytic drug effects through an anxiogenic action? Brain Res. Bull. 19:401–405; 1987.
27. Tsuda, A.; Ida, Y.; Nishimura, H.; Tanaka, M. Anxiogenic effects of beta-CCE as measured in two different conditioning paradigms. Psychobiology 17:202–206; 1989.
28. Wagner, J. A.; Katz, R. J. Anxiogenic action of benzodiazepine antagonists Ro 15-1788 and CGS 8216 in the rat. Neurosci. Lett. 48:317–320; 1984.