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β-Carboline and Pentylenetetrazol Effects on Conflict Behavior in the Rat

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HILL, T. J., D. J. FONTANA, T. C. McCLOSKEY AND R. L. COMMISSARIS. *β-Carboline and pentylenetetrazol effects on conflict behavior in the rat.* PHARMACOL BIOCHEM BEHAV **42**(4) 733–736, 1992.—The β-carbolines and the convulsant agent pentylenetetrazol (PTZ) have been reported as "anxiogenic" in several animal models for anxiety. The present study examined the effects of the β-carboline noreleagnine (NOR) and PTZ, administered alone and in combination with the benzodiazepine antagonist, Ro 15-1788, on behavior in the conditioned suppression of drinking (CSD) conflict procedure. In daily 10-min sessions, water-deprived female SD rats were trained to drink from a tube that was electrified (0.25 mA). Electrification was signaled by a tone. Acute (20-min) treatment with NOR or PTZ resulted in a dose-dependent decrease in both punished responding (shocks received) and unpunished responding (water intake). Both NOR and PTZ decreased punished responding only at doses that also depressed unpunished responding. Coadministration of Ro 15-1788 (2 mg/kg) reduced the effects of NOR on punished, but not unpunished, responding; this Ro 15-1788 cotreatment reduced the effects of PTZ on both punished and unpunished responding. These data suggest that both PTZ and NOR produce benzodiazepine receptor-mediated anxiogenic-like effects on conflict behavior.

Anxiogenic agents Conflict behavior β-Carboline Noreleagnine Pentylenetetrazol Anxiety Ro 15-1788

MEMBERS of the β-carboline chemical family have been shown to display a wide range of pharmacological actions. Perhaps the most prominent of these actions is as an "inverse agonist" at benzodiazepine receptors (29). This inverse agonist effect is characterized by increased alertness, attentive behavior, and fearfulness in the cat (23), a decrease in punished responding in rats (2,5,20) and mice (24), and an increase in anxiety in man (4). Although noreleagnine (NOR) is the parent compound of the β-carboline class (18), the inverse agonist effects of this agent have not been extensively studied. In the only reports to date, Shekhar and coworkers found NOR to exhibit a proconflict effect that is blocked by pretreatment with Ro 15-1788 (28) and Emmanouil and Quock reported anxiogenic-like effects in mice in a staircase paradigm; this effect also was blocked by Ro 15-1788 (7).

Pentylenetetrazol (PTZ) has been used as an agent to activate latent epileptogenic foci in the diagnosis of epilepsy (11). In subconvulsive doses, PTZ also has been reported to cause anxiety in man (25,26). Using the discriminative stimulus paradigm, Lal and Emmett-Oglesby characterized the interoceptive cues of subconvulsive doses of PTZ as an "anxiogenic" stimulus [for review, see (21)], while Giusti et al. (15) have shown a proconflict effect with PTZ using Vogel-like (31) conflict paradigm.

The conditioned suppression of drinking (CSD) conflict procedure, a modification of the Geller-Seifter conditioned conflict test (12–14) and the Vogel acute conflict task (31), has been shown to be an effective animal model for the study of anxiety and antianxiety agents in rats (1,10,19,22). Although the CSD procedure has been used in numerous studies examining different benzodiazepine anxiolytic agents, there are no reports on the effects of NOR or any other inverse agonists in the CSD paradigm.

The present studies were designed to determine the effects of NOR and PTZ on behavior in the CSD paradigm. In addition, the ability of the benzodiazepine antagonist Ro 15-1788 to antagonize these effects was examined.

METHOD

Animals

Female Sprague-Dawley rats (200–225 g at the start of the experiment), purchased from Charles River Farms Inc. (Cambridge, MA), were used in these experiments. Rats were housed two to four/cage in a climate-controlled room with a 12 L : 12 D cycle (lights on 0700–1900 h). Rats were given ad lib access to food with restricted water (see below).

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Apparatus

Conditioned suppression testing was conducted in an apparatus similar to that described by McCloskey et al. (22). The testing chamber was a rectangular box with Plexiglas® sides and a metal floor and top. Recessed in one wall was a metal drinking tube to which a calibrated (0.5-ml units) length of polyethylene tubing was attached for measuring the volume of water consumed. Programming for the test session was controlled by solid-state modular programming equipment (Coulbourn Instruments Co., Inc., Lehigh Valley, PA).

General Procedure

For the first few sessions, water-restricted (24 h deprivation) subjects were placed in the experimental chamber and allowed to consume water freely without the shock contingency. After 1 week of nonshock sessions, the tone/shock contingency was initiated. The 7-s tone periods were presented at regular (22 s ISI) intervals to subjects. During the latter 5 s of these tone periods, contact between the floor and the metal drinking tube completed a circuit that resulted in a 0.25-mA shock to the rat. The duration of the shock was equal to the duration of the tube contact (less than 200 ms). Shocks were delivered by a Coulbourn Instruments Two Pole Small Animal Shocker (Model #E13-02).

Initially, the shock inhibited fluid consumption in the test chamber. After several days, however, all subjects learned to consume stable volumes of water during the silent periods and made relatively few and very brief contacts with the tube during the tone.

In all experiments, subjects were tested individually in 10-min sessions at the same time of day (0700–0900 h). All subjects achieved stable control values (day-to-day coefficients of variation of approximately 30% for individual rats) for punished and unpunished responding by the end of the second week of CSD sessions with the alternating tone:no tone periods. Baseline (i.e., nondrug) CSD testing was continued for 2 additional weeks before drug testing was initiated. For baseline determinations and throughout each experiment, CSD testing was conducted 4 days per week (Tuesday–Friday) and free access to water was provided on nontest days (Friday p.m.–Monday a.m.).

Following CSD training as described above, the effects of NOR were determined in the presence or absence of Ro 15-1788 coadministration. In these studies, subjects received Ro 15-1788 or its vehicle on both the Thursday and Friday test days, while NOR or its vehicle were administered on alternate days. Thus, the antagonist or its vehicle treatment was held constant for a given test week but varied from week to week. NOR or its vehicle were administered 20 min prior to CSD testing with Ro 15-1788 or its vehicle being administered 10 min prior to CSD testing (10 min after NOR/Veh). Thus, all subjects received all combinations of NOR (2.8, 4, 5.6, and 8 mg/kg) ±2.0 mg/kg Ro 15-1788 in a randomized manner across 8 weeks of CSD testing. The results of pilot studies had suggested that 2.8 mg/kg NOR was an inactive dose on CSD behavior. Upon examination of the data from the present experiment, this was found not to be the case. Therefore, the effects of 2.0 mg/kg NOR alone or following pretreatment with 2.0 mg/kg Ro 15-1788 were determined in a separate group of subjects.

In a third group of animals, the effects of various doses of PTZ alone and following Ro 15-1788 coadministration were determined. The procedure used was similar to that described above, with all subjects receiving all combinations of PTZ (7.1, 10, 14.2, and 20 mg/kg) ±2.0 mg/kg Ro 15-1788 in a randomized manner across 8 weeks of CSD testing. In this experiment, Ro 15-1788 or its vehicle were administered 20 min prior to CSD testing with PTZ or its vehicle being administered 10 min prior to CSD testing (10 min after Ro 15-1788).

Drugs

NOR free base (Sigma Chemical Co., St. Louis, MO) was suspended in 0.5% methylcellulose. PTZ sodium (Sigma) was dissolved in distilled water. Ro 15-1788 was received as a gift from Hoffman LaRoche (Nutley, NJ) and was prepared in a 0.5% methylcellulose suspension. All drugs were injected IP in a volume of 1 ml/kg.

Statistical Analyses

The effects of single doses of NOR or PTZ on CSD performance were compared to vehicle using *t*-test for paired values. The effect of Ro 15-1788 (2.0 mg/kg) vs. vehicle coadministration on the response to NOR or PTZ were analyzed using 2 × 4 factorial analyses of variance (ANOVAs) with repeated measures (main effects: Ro 15-1788/Veh pretreatment, NOR/PTZ doses). Post hoc comparisons were made using the least-significant differences (LSD) test. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance (30).

RESULTS

Control (i.e., nondrug) CSD behavior was characterized by a stable number of shocks accepted (65 ± 11) and stable volume of water consumed (14.6 ± 0.3 ml) in each session. It should be noted that nearly all water intake occurred during the unpunished periods. Thus, the volume of water consumed accurately reflects unpunished responding in the CSD.

Figure 1 illustrates the effects of NOR on CSD behavior when administered alone or in combination with Ro 15-1788. NOR decreased both punished responding (shocks received) and unpunished responding (water intake) in a dose-related manner. The effect of NOR doses (2.8–8.0 mg/kg) on the change in shocks received was not significant, $F(3, 54) = 1.16$, n.s., whereas the effect of this range of NOR doses on the change in water intake was dose dependent, $F(3, 54) = 19.10$, $p < 0.05$. At no dose did NOR produce a selective proconflict effect (i.e., a decrease in punished responding without a concomitant decrease in unpunished responding). Ro 15-1788 coadministration significantly antagonized the effects of NOR on the number of shocks received, $F(1, 18) = 10.9$, $p < 0.05$, but not water intake, $F(1, 18) < 1$, n.s.. The interaction between NOR and ± Ro 15-1788 was not significant for either punished responding, $F(3, 54) < 1$, n.s., or unpunished responding, $F(3, 54) < 1$, n.s..

Figure 2 illustrates the effects of PTZ on CSD behavior when administered alone or in combination with Ro 15-1788. PTZ decreased both punished and unpunished responding. The effect of PTZ doses on the number of shocks received was significant, $F(3, 30) = 6.65$, $p < 0.05$, as was the effect of PTZ doses on the change in water intake, $F(3, 30) = 12.40$, $p < 0.05$. Similar to the effects of NOR, PTZ treatment failed to produce a selective proconflict effect at any dose administered. Although there was considerable variability

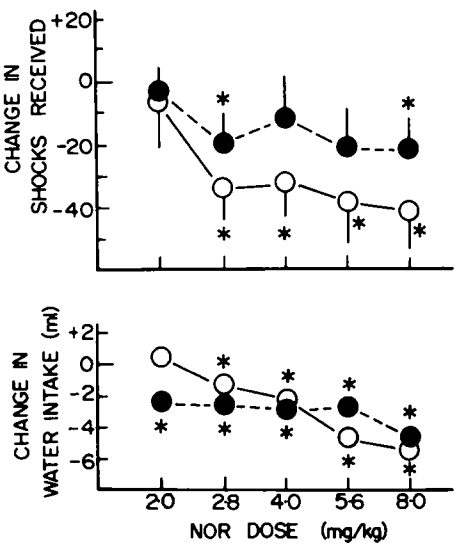

FIG. 1. Effects of noreleagnine (NOR) alone and with Ro 15-1788 coadministration on conflict behavior. Plotted are the mean ± SEM (n = 19) change in shocks received (top panel) and water consumed (bottom panel) produced by NOR alone following a 20-min pretreatment (○) or NOR with coadministration of Ro 15-1788, 10 min after NOR (●). Note that when SEM bars are not apparent the SEM falls within the confines of the symbol. *NOR significantly different from vehicle control at the indicated dose, t-test for paired values, $p < 0.05$. Ro 15-1788 coadministration significantly antagonized the effects of NOR on the change in shocks received, but not on the change in water intake (see text for details).

across the different PTZ doses, Ro 15-1788 coadministration significantly antagonized the effects of PTZ on the number of shocks received [$F(1, 10) = 3.40$, $p < 0.05$, one-tailed] and water intake, $F(1, 10) = 15.25$, $p < 0.05$. The PTZ dose × ± Ro 15-1788 interaction effect was not significant for punished responding, $F(3, 30) < 1$, n.s.; however, for the PTZ-induced change in water intake there was a significant PTZ dose × ± Ro 15-1788 interaction, $F(3, 30) = 3.61$, $p < 0.05$.

DISCUSSION

As expected, acute treatment with either the β-carboline NOR or PTZ resulted in a decrease in punished responding in the CSD. These data are consistent with the findings of other investigators suggesting that these compounds produce anxiogenic-like effects (9,20,27). Although the benzodiazepine antagonist Ro 15-1788 has no effect on CSD behavior when administered alone (6), this agent antagonized both the decrease in punished and unpunished responding produced by PTZ. In contrast, Ro 15-1788 selectively antagonized the effects of NOR only on punished responding.

The fact that the anxiogenic-like effects of PTZ can be blocked by anxiolytic compounds such as diazepam and phenobarbital is well documented (21,27). However, the effects of Ro 15-1788 on the anxiogenic-like response produced by PTZ are not as well understood. The Ro 15-1788 antagonism of this PTZ-induced anxiogenic-like response can be addressed in the following manner. The decrease in punished and unpunished responding caused by PTZ may be mediated in part through a benzodiazepine receptor. Ro 15-1788 has been reported to have weak intrinsic activity (3,8), which may cause a decrease in the anxiogenic effects of PTZ. Further support for this hypothesis is provided in the work of Hantraye and coworkers (16), who reported that administration of 20–30 mg/kg PTZ induced an immediate increase in Ro 15-1788 binding.

The finding that NOR produces a decrease in both punished and unpunished responding and that Ro 15-1788 antagonizes only the effect on punished responding is consistent with the behavioral profile of Ro 15-1788 when administered in combination with other compounds in this class. Koob et al. (20) reported that Ro 15-1788 reversed the effects of FG-7142 on punished but not unpunished responding in a conflict procedure. In addition, Hindley et al. (17) showed that microinjections of Ro 15-1788 into the dorsal raphe blocked the decrease in social interaction produced by intraperitoneal methyl β-carboline-3-carboxylate; however, this Ro 15-1788 treatment did not antagonize the decrease in locomotor activity caused by methyl β-carboline-3-carboxylate. Together, these findings suggest that the effects of the β-carbolines on punished and unpunished responding can be selectively identified and studied using the coadministration of Ro 15-1788. Alternatively, it has been proposed that the impairment of unpunished responding produced by the β-carbolines is actually a part

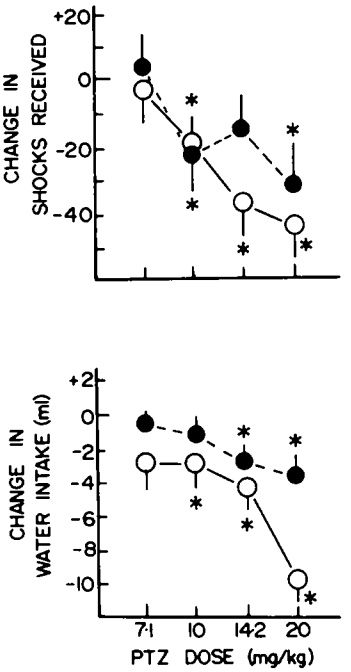

FIG. 2. Effects of pentylenetetrazole (PTZ) alone and with Ro 15-1788 coadministration on conflict behavior. Each value represents the mean ± SEM obtained from 11 subjects. Plotted are the mean ± SEM (n = 11) change in shocks received (top panel) and water consumed (bottom panel) produced by PTZ alone following a 10-min pretreatment (○) or PTZ with coadministration of Ro 15-1788, 10 min prior to PTZ (●). *PTZ significantly different from vehicle control at the indicated dose, t-test for paired values, $p < 0.05$. Ro 15-1788 coadministration significantly antagonized the effects of PTZ on the change in shocks received and the change in water intake (see text for details).

of the anxiogenic-like effect of these agents (24). According to this hypothesis, Ro 15-1788 is able to only partially antagonize the anxiogenic-like effects produced by the β-carbolines.

In summary, acute treatment with NOR or PTZ resulted in an anxiogenic-like effect on conflict behavior. Furthermore, the anxiogenic-like effect of either agent was at least partially antagonized by Ro 15-1788, suggesting that the effect is benzodiazepine receptor-mediated for both NOR and PTZ.

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REFERENCES

1. Commissaris, R. L.; Harrington, G. M.; Ortiz, A. M.; Altman, H. J. Maudsley reactive and non-reactive rat strains: Differential performance in a conflict task. Physiol. Behav. 29:631-634; 1986.
2. Corda, M. G.; Baker, W. D.; Mendelson, W. B.; Guidotti, A.; Costa, E. Beta-carbolines enhance shock induced suppression of drinking in rats. Proc. Natl. Acad. Sci. USA 80:2072-2076; 1983.
3. Dantzer, R.; Perio, A. Behavioral evidence for partial agonist properties of Ro 15-1788, a benzodiazepine receptor antagonist. Eur. J. Pharmacol. 81:655-658; 1982.
4. Dorow, R.; Horowski, R.; Paschelke, G.; Amin, M.; Braestrup, C. Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors. Lancet 2:98-99; 1983.
5. Drugan, R. C.; Maier, S. F.; Skolnick, P.; Paul, S. M.; Crawley, J. N. An anxiogenic benzodiazepine receptor ligand induces learned helplessness. Eur. J. Pharmacol. 113:453-457; 1985.
6. Ellis, D. M.; Fontana, D. J.; McCloskey, T. C.; Commissaris, R. L. Chronic anxiolytic treatment effects on conflict behavior in the rat. Pharmacol. Biochem. Behav. 37:177-186; 1990.
7. Emmanouil, D. E.; Quock, R. M. Effects on benzodiazepine agonist, inverse agonist and antagonist drugs in the mouse staircase test. Psychopharmacology (Berl.) 102:95-97; 1990.
8. File, S. E.; Lister, R. G.; Nutt, D. J. Intrinsic actions of benzodiazepine antagonist. Neuropharmacology 21:1033-1037; 1982.
9. File, S. E.; Pellow, S.; Braestrup, C. Effects of the beta-carboline, FG 7142, in the social interaction test of anxiety and the holeboard: Correlations between behavior and plasma concentrations. Pharmacol. Biochem. Behav. 22:941-944; 1985.
10. Ford, R. D.; Rech, R. H.; Commissaris, R. L.; Mayer, L. Effects of acute and chronic interactions of diazepam and *d*-amphetamine on punished and unpunished behavior of rats. Psychopharmacology (Berl.), 65:197-204; 1979.
11. Franz, D. N. Central nervous system stimulants. In Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F., eds. The pharmacological basis of therapeutics. New York: Macmillan Publishing; 1985:585.
12. Geller, I. Relative potencies of benzodiazepines as measured by their effects on conflict behavior. Arch. Int. Pharmacodyn. Ther. 149:243-247; 1964.
13. Geller, I.; Kulak, J. T.; Seifter, J. Effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. Psychopharmacologia 3:347-385; 1962.
14. Geller, I.; Seifter, J. The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482-492; 1960.
15. Giusti, P.; Guidetti, G.; Costa, E.; Guidotti, A. The preferential antagonism of pentylenetetrazole proconflict responses differentiates a class of anxiolytic benzodiazepines with potential antipanic action. J. Pharmacol. Exp. Ther. 257:1062-1068; 1991.
16. Hantraye, P.; Brouillet, E.; Guibert, C.; Chavoix, C.; Fududa, C.; Prenant, C.; Crouzel, M.; Naquet, R.; Maziere, M. Pentylenetetrazol-induced seizure is not mediated by benzodiazepine receptors in vivo. Neuropharmacology 26:1509-1512; 1987.
17. Hindley, S. W.; Hobbs, A.; Paterson, I. A.; Roberts, M. H. T. The effects of methyl beta-carboline-3-carboxylate on social interaction and locomotor activity when microinjected into the nucleus raphe dorsalis of the rat. Br. J. Pharmacol. 86:753-761; 1985.
18. Ho, B. T.; Fritchie, G. E.; Kralik, P. W.; Tansey, L. W.; Walker, K. E.; McIsaac, W. M. Inhibitors of monoamine oxidase V: Effects of substitution on the transport of tetrahydro-beta-carboline analogs to mouse brain. J. Pharmacol. Sci. 58:1423-1425; 1969.
19. Kilts, C. D.; Commissaris, R. L.; Rech, R. H. Comparison of anti-conflict drug effects in three experimental models of anxiety. Psychopharmacology (Berl.) 74:290-296; 1981.
20. Koob, G. F.; Braestrup, C.; Thatcher Britton, K. The effects of FG7142 and Ro 15-1788 of the release of punished responding produced by chlordiazepoxide and ethanol in the rat. Psychopharmacology (Berl.) 90:173-178; 1986.
21. Lal, H.; Emmett-Oglesby, M. W. Behavioral analogues of anxiety: Animal models. Neuropharmacology 22:1423-1441; 1983.
22. McCloskey, T. C.; Paul, B. K.; Commissaris, R. L. Buspirone effects in an animal conflict procedure: Comparison with diazepam and phenobarbital. Pharmacol. Biochem. Behav. 27:171-175; 1987.
23. Ongini, E.; Barzaghi, C.; Marzanatti, M. Intrinsic and antagonistic effects of beta-carboline FG 7142 on behavioral and EEG actions of benzodiazepines and pentobarbital in cats. Eur. J. Pharmacol. 95:125-129; 1983.
24. Prado de Carvalho, L.; Grecksch, G.; Chapouthier, G.; Rossier, J. Anxiogenic and non-anxiogenic benzodiazepine antagonists. Nature 301:64-66; 1983.
25. Rodin, E. A. Metrazol tolerance in a "normal" and volunteer population. Electroenceph. Clin. Neurophysiol. 10:433-446; 1958.
26. Rodin, E. A.; Calhoun, H. D. Metrazol in a volunteer population. J. Nerv. Ment. Dis. 150:438-450; 1970.
27. Shearman, G. T.; Lal, H. Generalization and antagonism studies with convulsants, GABAergic and anticonvulsant drugs in rats trained to discriminate pentylenetetrazol from saline. Neuropharmacology 19:473-479; 1980.
28. Shekhar, J.; Hingtgen, N.; DiMicco, J. A. Anxiogenic effects of noreleagnine, a water soluble beta-carboline in rats. Neuropharmacology 28:539-542; 1989.
29. Skolnick, P.; Ninan, P.; Insel, T.; Crawley, J.; Paul, S. A novel chemically induced model of human anxiety. Psychopathology 17:25-36; 1984.
30. Steele, R. G.; Torrie, J. H. Principles and procedures of statistics. New York: McGraw-Hill; 1985.
31. Vogel, R. A.; Beer, B.; Clody, D. E. A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacology (Berl.) 21:1-7; 1971.