Pharmacology Biochemistry and Behavior, Vol. 47, pp. 1-4, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Possible Anxiolytic Effects of Chrysin, a Central Benzodiazepine Receptor Ligand Isolated From *Passiflora Coerulea*

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Received 16 July 1992

WOLFMAN, C., H. VIOLA, A. PALADINI, F. DAJAS AND J. H. MEDINA. *Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from* Passiflora coerulea. PHARMACOL BIOCHEM BEHAV 47(1) 1-4, 1994.—The pharmacological effects of 5,7-dihydroxyflavone (chrysin), a naturally occurring monoflavonoid that displaces [^{3}H]flunitrazepam binding to the central benzodiazepine (BDZ) receptors, were examined in mice. In the elevated plus-maze test of anxiety, diazepam (DZ, 0.3-0.6 mg/kg) or chrysin (1 mg/kg) induced increases in the number of entries into the open arms and in the time spent on the open arms, consistent with an anxiolytic action of both compounds. The effects of chrysin on the elevated plus-maze was abolished by pretreatment with the specific BDZ receptor antagonist Ro 15-1788 (3 mg/kg). In the holeboard, diazepam (1 mg/kg) and chrysin (3 mg/kg) increased the time spent head-dipping. In contrast, high doses of DZ (6 mg/kg) but not of chrysin produced a decrease in the number of head dips and in the time spent head-dipping. In the horizontal wire test, diazepam (6 mg/kg) had a myorelaxant action. In contrast, chrysin (0.6-30 mg/kg) produced no effects in this test. These data suggest that chrysin possesses anxiolytic actions without inducing sedation and muscle relaxation. We postulate that this natural monoflavonoid is a partial agonist of the central BDZ receptors.

Benzodiazepine receptor Anxiolytic Chrysin Natural compound

NATURALLY occurring compounds acting on the central benzodiazepine (BDZ) receptor have been isolated and identified from plants used in folkloric medicine (7,9-11). These natural products, which are not structurally related to BDZs, inhibit [^{3}H]flunitrazepam binding to central BDZ receptors with IC_{50}s ranging from 6 nM to 30 μM. In this context, using BDZ receptor binding displacement for a bioassay-guided isolation we recently identified 5,7-dihydroxyflavone (chrysin) from *Passiflora coerulea* L. (9). This monoflavone was found to be a competitive ligand for the central BDZ receptor with a K_i of 3 μM and displayed anticonvulsant properties in mice (9).

In the present work, we examined in more detail the pharmacological effects of acute IP administration of chrysin in mice. Our results demonstrated that chrysin possesses anxiolytic effects but does not induce sedation and muscle relaxation, behaving as a partial agonist for the central BDZ receptor.

METHOD

Animals

Male CF1 mice from our breeding stock weighing 28-35 g were used. Animals were placed in groups of 10-12 with free access to water and food and maintained on a 12 L : 12 D cycle.

Experimental Devices

Elevated plus-maze. The elevated plus-maze consisted of four perpendicularly disposed wood arms (20 × 5 cm; two had 35-cm high wood walls and two were open) linked by a central 10 × 10-cm square. The maze was suspended 50 cm from the room floor. Animals were placed on the central part of the maze facing a closed arm. This test has been widely validated to measure anxiety in rodents (8,12). The number of entries into and the time spent on the open and closed arms were counted during 5 min (13). A selective increase in the parameters corresponding to open arms reveals an anxiolytic effect (12,13). Total exploratory activity (number of entries in both arms) was also determined (14).

Holeboard test. The holeboard consisted of a wood box (60 × 60 × 30 cm) with four 2-cm diameter holes equidistant in the floor box. The number of head dips and the time spent head-dipping were counted during 5 min (3). An increase in the number and the time spent head-dipping implies a greater

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exploratory activity (3). A decrease of both parameters reveals a sedative behavior.

Locomotor activity test. We used an OPTO-VARIMEX apparatus consisting of a glass box (36 × 15 × 20 cm) and two lateral bars with 15 light beams (0.32 cm diameter, beam spacing 2.65 cm). The apparatus detects automatically all the mouse movements and discriminates between total and ambulatory activity. The locomotor activity (number of movements across the beams) was counted during 5 min. An increase in the number of transitions through the beams reflects an augmented locomotor activity.

Horizontal wire test. This test consisted of a horizontally strung wire (1 mm diameter, 15 cm long) placed 20 cm from the table. Mice were lifted by the tail, allowed to grasp the wire with their forepaws, and released. The number of mice out of 10 that did not grasp the wire with their forepaws or actively grasped the wire with at least one hindpaw within 3 s was determined. After two trials, performed at 5-min intervals, the test took place (1). A myorelaxant drug, like diazepam (DZ) at high doses, will impair mice to grasp the wire. Generally, this state of muscle relaxation is commonly associated with sedation.

The general procedure for all the tests is as follows: Mice were injected with vehicle or the drug solution 20 min before the beginning of the test and put into another home cage. Ro 15-1788, a specific BDZ receptor antagonist (5), was injected 7 min before chrysin. All injections were given IP (5,6). Control mice were tested in each session, in parallel with those animals receiving chrysin or diazepam. Testing was carried out "blind." All data were submitted to analysis of variance (ANOVA). Posthoc comparisons between individual treatments and controls were made using Dunnett's *t*-test.

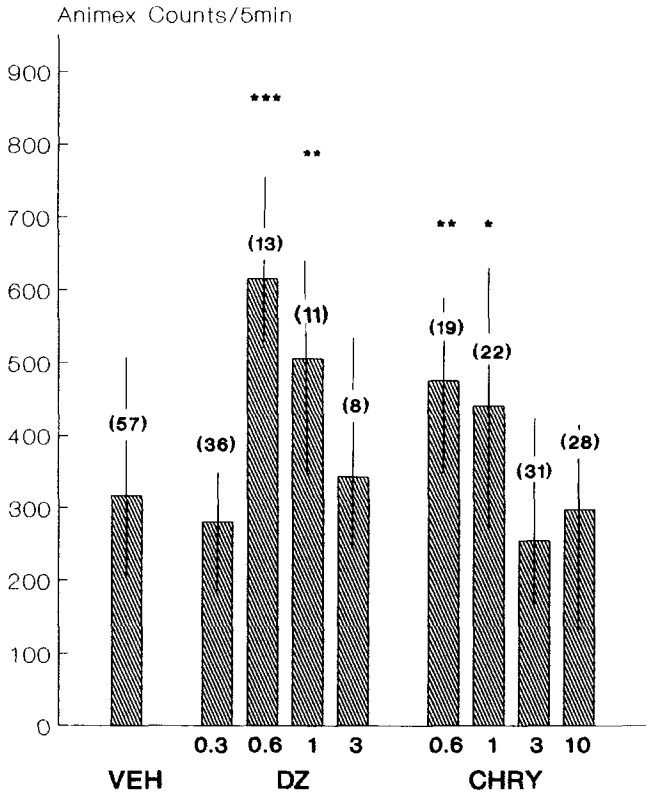

FIG. 1. Ambulatory locomotor activity counts during a 5-min test session in a OPTO-VARIMEX apparatus 20 min after IP injection with diazepam (DZ, 0.3–3 mg/kg) or chrysin (CHRY, 0.6–10 mg/kg). Data are expressed as medians (interquartile range) of (*n*) number of animals. $*p < 0.05$, $**p < 0.02$, $***p < 0.002$, significantly different from controls (Mann–Whitney test).

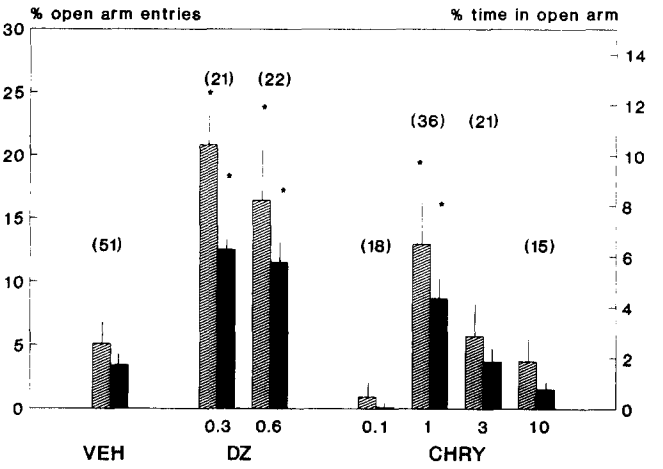

FIG. 2. Mean (± SEM) percentage of open arm entries (closed bars) and percentage of time (seconds) spent in the open arms (hatched bars) in mice given a 5-min test in the elevated plus-maze 20 min after IP injection with diazepam (DZ, 0.3 and 0.6 mg/kg) or CHRY (0.1–10 mg/kg). $*p < 0.01$, significantly different from controls (two-tailed Dunnett's *t*-test after analysis of variance).

Drugs

DZ (Hoffman-La Roche, Nutley, NJ) and chrysin (Sigma Chemical Co., St. Louis, MO) were dissolved in DMSO 40%, NaOH 0.1 N (7 : 3; v/v) at pH 8.2. Control animals were injected with the same vehicle. Ro 15-1788 (Hoffmann-La Roche) was suspended in DMSO 10%, propylenglycol 10%, in distillate water.

RESULTS

In all experiments, DZ (0.3–6 mg/kg) was used as reference drug. Figure 1 shows the typical pharmacological profile of

TABLE 1

TOTAL NUMBER OF ARM ENTRIES MADE BY MICE DURING 5-min TEST IN THE ELEVATED PLUS-MAZE 20 min AFTER DRUG INJECTION

Drugs (mg/kg)	*n*	Total Arm Entries
Vehicle	53	8.7 ± 0.6
DZ		
0.3	21	10.5 ± 0.8
0.6	22	12.4 ± 1.9
Chrysin		
1	36	9.9 ± 0.8
3	21	8.7 ± 1.1
10	15	10.2 ± 1.1

Data are expressed as means ± SEM of n = number of animals. Analysis of variance, $F(5, 160) = 2.27, p > 0.05$.

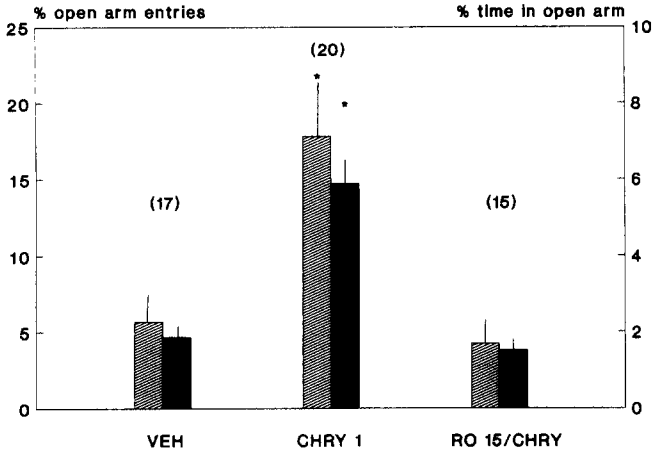

FIG. 3. Mean (±SEM) percentage of open arm entries (closed bars) and percentage of time (seconds) spent in the open arms (hatched bars) in mice given a 5-min test in the elevated plus-maze 20 min after IP injection with vehicle (VEH), chrysin (CHRY, 1 mg/kg), or CHRY + Ro 15-1788 (3 mg/kg) administered IP 7 min before chrysin. *$p < 0.01$, significantly different from controls (two-tailed Dunnett's t-test after analysis of variance). No significant differences were found in the total arm entries, $F(2, 49) = 3.18$.

increasing locomotor activity by DZ. Similarly, there was a significant increase in locomotor activity with equipotent doses of chrysin (0.6–1 mg/kg).

Performance of mice following IP administration of vehicle, DZ, or chrysin on the elevated plus-maze is shown in Fig. 2. DZ (0.3 and 0.6 mg/kg) increased the percentage of entries

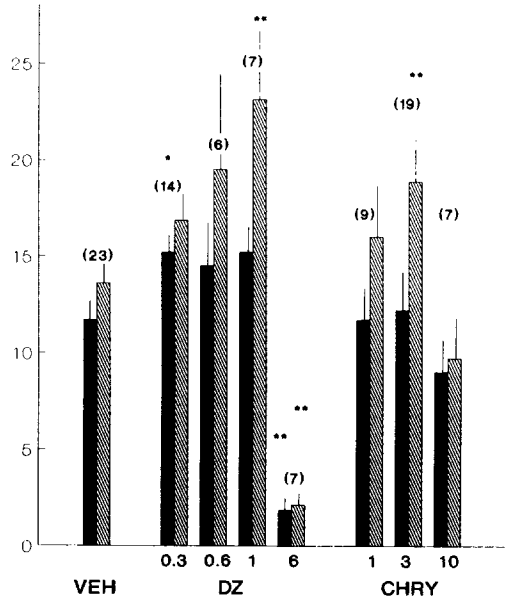

FIG. 4. Mean (±SEM) number of head dips (closed bars) and time (seconds) spent head-dipping (hatched bars) for mice given a 5-min test in the holeboard 20 min after an IP injection with diazepam (DZ, 0.3–6 mg/kg) or chrysin (CHRY, 1–10 mg/kg). *$p < 0.05$, **$p < 0.01$, significantly different from controls (two-tailed Dunnett's t-test after analysis of variance).

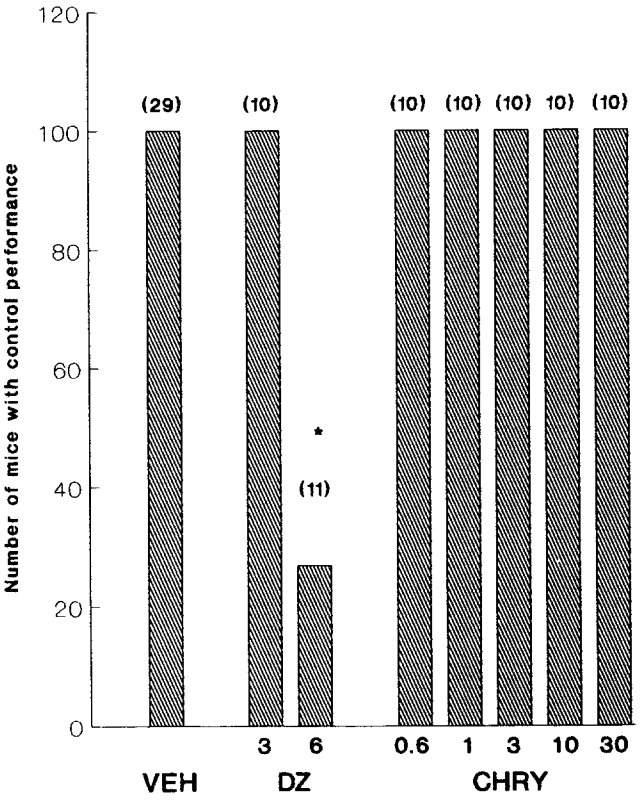

FIG. 5. Performance of mice in the wire test 20 min after an IP injection with diazepam (DZ, 3 and 6 mg/kg) or chrysin (CHRY, 0.6–30 mg/kg). The test took place after two trials, executed after a 5-min interval.

in the open arms ($p < 0.01$) and the percentage of the time spent on the open arms ($p < 0.01$). Chrysin (1 mg/kg) produced also an increase in both parameters ($p < 0.01$). No differences were observed in the total arm entries (Table 1). The effect of chrysin (1 mg/kg) on the number of entries into and the time spent on the open arms was prevented by the prior administration of Ro 15-1788, a central BDZ receptor antagonist (Fig. 3).

Confirming previous findings in the holeboard (4), DZ (0.3 mg/kg) increased the number of head dips ($p < 0.05$) and at 1 mg/kg increased the time spent head-dipping ($p < 0.01$). As expected, DZ (6 mg/kg) induced a decrease in both the number of head dips and in the time spent head-dipping ($p < 0.01$; Fig. 4).

Chrysin (3 mg/kg) produced a significant increase in the time spent head-dipping but did not elicit sedative effects at high doses (10 mg/kg; Fig. 4).

At 6 mg/kg, DZ significantly decreased the percentage of animals grasping the horizontal wire (Fig. 5). On the other hand, chrysin (0.6–30 mg/kg) was ineffective in the same test (Fig. 5).

DISCUSSION

The main finding of the present study is that chrysin had anxiolytic effects in the elevated plus-maze test without inducing sedation and muscle relaxation (Figs. 4 and 5). It is unlikely that the anxiolytic action of this naturally occurring

ligand for central BDZ receptors was due to sedative effects because there was no concomitant reduction in motor activity in this test and because chrysin showed no decline in exploratory head-dipping in the holeboard (Fig. 4). It is important to mention that the holeboard has been shown to be sensitive to potentially sedative effects (2,14).

Also like DZ, chrysin (0.6–1 mg/kg) induced an increase in locomotor activity (Fig. 1). However, chrysin (up to 30 mg/kg), unlike DZ (6 mg/kg), provoked no reduction in muscle tone (Fig. 5). These data suggested that at low doses (less that 3 mg/kg, IP) both chrysin and DZ have anxiolytic effects without generating sedation or muscle relaxation. At higher doses, DZ, but not chrysin, possesses sedative and myorelaxant effects.

The anxiolytic effect of chrysin was prevented by the prior administration of Ro 15-1788 at a dose (3 mg/kg) at which this specific BDZ receptor antagonist has no action per se (5).

The results support the contention that chrysin is a partial agonist at the BDZ receptor. Partial agonists at the BDZ receptor are highly promising agents because they retain several highly desired effects of BDZs but do not produce CNS depression. To further substantiate the hypothesis that chrysin possesses a partial agonist profile, it would be interesting to determine whether chrysin is able to antagonize the effects of classic BDZs in pharmacological test procedures. These types of experiments are currently underway in our laboratory.

In conclusion, our results support the suggestion that chrysin, unlike DZ, can reduce anxiety without inducing sedation and muscle relaxation.

ACKNOWLEDGEMENTS

This work was supported by the National Council of Scientific Research (Argentina) and International Foundation for Science (Sweden). The authors also thank Nadia Hardy for excellent secretarial assistance.

REFERENCES

1. Bonetti, E. P.; Pierri, L.; Cumin, R.; Schaffner, M.; Gamzu, E. R.; Müller, R. K. M. and Haefely, W. Benzodiazepine antagonist Ro 15-1788: Neurological and behavioral effects. Psychopharmacology (Berl.) 78:8–18; 1982.
2. File, S. E. What can be learned from the effects of benzodiazepines on exploratory behavior? Neurosci. Biobehav. Rev. 9:45–54; 1985.
3. File, S. E.; Pellow, S. The effects of triazolobenzodiazepines in two animal tests of anxiety and on the holeboard. Br. J. Pharmacol. 86:729–735; 1985.
4. File, S. E.; Pellow, S. No cross-tolerance between the stimulatory and depressant actions of benzodiazepines in mice. Behav. Brain. Res. 17:1–7; 1985.
5. File, S. E.; Pellow, S. Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788. Psychopharmacology (Berl.) 88: 1–11; 1986.
6. Gherezghiher, T.; Lal, H. Ro 15-1788 selectively reverses antagonism of pentylenetetrazol-induced discriminative stimuli by benzodiazepine but not by barbiturates. Life Sci. 31:2955–2960; 1982.
7. Lee, C. M.; Wong, H. N. C.; Chui, K. Y.; Choang, T. F.; Hon, P. M.; Chang, H. M. Miltirone, a central benzodiazepine receptor partial agonist from a Chinese medicinal herb *Salvia miltiorrhiza*. Neurosci. Lett. 127:237–241; 1991.
8. Lister, R. G. The use of plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl.) 92:180–185; 1987.
9. Medina, J. H.; Paladini, A. C.; Wolfman, C.; Levi de Stein, L.; Calvo, D.; Diaz, L. E.; Peña, C. Chrysin, a naturally occurring monoflavonoid, recognizes benzodiazepine receptors and possesses anticonvulsant properties. Biochem. Pharmacol. 40:2227–2232; 1990.
10. Medina, J. H.; Peña, C.; Piva, M.; Paladini, C. A.; De Robertis, E. Presence of benzodiazepine-like molecules in mammalian brain and milk. Biochem. Biophys. Res. Comm. 152:534–539; 1988.
11. Nielsen, M.; Frokjaer, S.; Braestrup, C. High affinity of naturally occurring biflavonoid, amentoflavone to brain benzodiazepine receptors in vitro. Biochem. Pharmacol. 37:3285–3287; 1988.
12. Pellow, S.; Chopin, P.; Fil, S.; Briley, M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Meth. 14:149–167; 1985.
13. Pellow, S.; File, S. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525–529; 1986.
14. Pellow, S.; File, S. E. Evidence that the β-carboline, ZK 91296, can reduce anxiety in animals at doses below those causing sedation. Brain Res. 363:174–177; 1986.