Pharmacology Biochemistry & Behavior, Vol. 22, pp. 941–944, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Effects of the β-Carboline, FG 7142, in the Social Interaction Test of Anxiety and the Holeboard: Correlations Between Behaviour and Plasma Concentrations

SANDRA E. FILE, SHARON PELLOW AND CLAUS BRAESTRUP*

MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy University of London, 29-39 Brunswick Square, London, WC1N 1AX, U.K.
**NOVO, Pharma Research, Novo Alle, DK-2880 Bagsvaerd, Denmark*

Received 24 September 1984

FILE, S. E., S. PELLOW AND C. BRAESTRUP. *Effects of the β-carboline, FG 7142, in the social interaction test of anxiety and the holeboard: Correlations between behaviour and plasma concentrations.* PHARMACOL BIOCHEM BEHAV 22(6) 941–944, 1985.—The behavioural effects of the β-carboline FG 7142 were investigated in the social interaction test of anxiety and the holeboard test of exploration and locomotor activity. FG 7142 (5–20 mg/kg) produced a significant decrease in the time spent in social interaction by pairs of rats, without an accompanying decrease in motor activity. This anxiogenic effect was highly correlated with the plasma concentrations of FG 7142 for the rats receiving 5 and 10 mg/kg doses, but not for those receiving the 20 mg/kg dose. In the holeboard, FG 7142 had no effect on exploratory head-dipping at the doses tested, but selectively reduced locomotor activity and the number of rears. The profile of FG 7142 in these tests is compared with those of the β-carbolines, B-CCE and B-CCP.

FG 7142 β-Carbolines Anxiety Social interaction Exploration Locomotor activity Plasma concentrations

FG 7142 (β-carboline-3-carboxylic acid methyl amide, for structure see Fig. 1), like several other β-carboline-3-carboxylic acid derivatives, has high affinity for benzodiazepine binding sites in the CNS [13]. FG 7142 specifically reduces punished drinking in a Vogel conflict test [1,17]; produces alertness, attentive behaviour and fearfulness in the cat [15] and causes subjective reports of intense anxiety in man [2], all of which have been interpreted as anxiogenic activity.

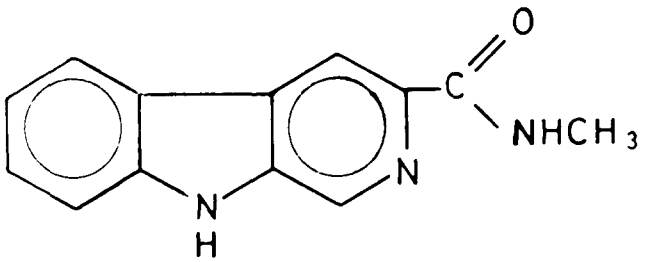
In the social interaction test an anxiogenic action is indicated by a decrease in the time spent in active social interaction by pairs of rats, without a concomitant decrease in motor activity. For a full description of the methodology and validation of the social interaction test see [3]. Anxiogenic profiles have been found in this test for drugs such as caffeine, which decreased social interaction, but increased locomotor activity [5] and has anxiogenic effects in man [12] and for drugs such as pentylenetetrazole which are anxiogenic in other animal tests [7,14] and in man [18].

Other β-carbolines, β-CCE (β-carboline-3-carboxylate ethyl ester) and β-CCP (β-carboline-3-carboxylate propyl ester), have exhibited anxiogenic profiles in the social interaction test [8,9]; we were therefore interested in investigating the effects of FG 7142 on social interaction. In order to compare further its profile with those of other β-carbolines, the effects of FG 7142 were also studied in the holeboard, to identify any action on exploratory head-dipping and locomotor activity. If FG 7142 produces a specific reduction in social interaction, without an accompanying decrease in locomotor activity, this will provide important validation for the use of this animal test, since it is the only drug acting at the benzodiazepine site that has been shown to cause anxiety in man. In man the anxiogenic action of FG 7142 was correlated with high levels of the drug in plasma [2]; we therefore measured plasma concentrations of the drug in rats to see if there was a correlation with the social interaction scores.

METHOD

Animals

Male Lister hooded rats (Olac Ltd., Bicester) were housed in a room with an 11 hr light: 13 hr dark cycle and allowed free access to food and water. Animals were singly housed for 5 days prior to the experiment and weighed 160–180 g. They were assigned to pairs on the basis of weight, but were randomly allocated to the drug groups.



FG 7142

FIG. 1. The structure of FG 7142.

Apparatus

The test arena was a wooden box, 60×60×35 cm. For the social interaction test, the floor was solid and infrared photocells 4.5 cm above the floor provided an automated measure of locomotor activity. A camera was mounted vertically over the box and the rats were observed on a monitor in an adjacent room. For the holeboard test, there were four holes equally spaced in the floor, each 3.8 cm in diameter. Head-dipping was measured by infra-red cells placed under the holes, and locomotor activity and rearing were measured by infrared cells in the walls of the box, 4.5 and 11 cm from the floor, respectively [11]. In both tests the level of illuminance was 35 scotopic lux.

Drugs

Micronised FG 7142 was suspended in distilled water with a drop of Tween 20, and injected intraperitoneally 20 min before testing in concentrations to give an injection volume of 2 ml/kg body weight. Control animals received distilled water with a drop of Tween 20. Both members of a pair always received the same drug treatment.

Procedure

Rat pairs were randomly allocated to the following drug groups, chosen on the basis of previous behavioural work [15,17]: control, FG 7142 5, 10 or 20 mg/kg (n=7 or 8 pairs per group). Animals were tested in an order randomised for drug treatment between 07.00 and 12.00 hr. Animals had previously been familiarised with the social interaction box for 7.5 min on 3 occasions prior to the test. During this familiarisation period one member of each pair was randomly selected and tail marked. On the test day, 20 min after injection, pairs of rats were placed in the centre of the test arena and their social behaviour scored for 7.5 min. The duration of the following behaviours were entered directly into a MINC-11 computer: sniffing, following, grooming, mounting, boxing, wrestling, kicking or pushing the partner. Passive body contact (when the rats were sitting or lying with their bodies in contact, but were not interacting with each other) was scored separately. Passive contact has been shown to be a sensitive measure of the sedative effects of benzodiazepines [3]. The two observers had no knowledge of the drug treatment that each pair of rats had received. At the end of the trial fecal boluses were removed and the box wiped clean.

After brief anaesthesia plasma was taken from the tail-marked rat of each pair by cardiac puncture, and the other rat was tested for 5 min in the holeboard, in a different room. The number of head-dips, time spent head-dipping, locomotor activity scores and number of rears were recorded. At the end of each trial, fecal boluses were removed and the box wiped clean. Plasma was then immediately obtained from this rat.

FG 7142 Analyses

Plasma aliquots of 300 μl were diluted to 500 μl with 0.9% saline and extracted with 4 ml diethyl ether. The ether phase was blown to dryness and the dry residue was dissolved in 100 μl 96% ethanol. Aliquots of 20 μl were applied on a 150×3.8 mm Nova Pack C HPLC column, and eluted with methanol:sodiumphosphate buffer, 13 mM, pH 7.0 (80:20 vol/vol) at a flow rate of 0.7 ml/min. Peak heights were compared to internal standard FG 7142. Detection was by fluorescence at an excitation wavelength of 270 nm, and emission at 386 nm.

Statistics

In order to assess the effect of FG 7142 on social interaction scores, independent of any change in motor activity, an analysis of covariance was carried out with social interaction as the dependent variable and motor activity as the covariate. A second analysis of covariance in which motor activity was the dependent variable and social interaction was the covariate allowed an assessment of drug effects on motor activity, independent of any change in social interaction. Figure 1 therefore shows the adjusted mean scores, calculated from the regression in each analysis of covariance, and for this reason there are no standard errors. The raw scores (±S.E.M.) are given in the figure legend. A Pearson product-moment correlation was calculated for the plasma levels of FG 7142 in each rat pair with (a) the social interaction score obtained for that pair and (b) the locomotor activity score for each pair in the social interaction test. The data from the holeboard experiment were analysed by one-way analyses of variance with the dose of FG 7142 as the independent factor. Post-hoc comparisons between individual drug groups and the controls were made with Dunnett's test.

RESULTS

FG 7142 (5–20 mg/kg) significantly decreased the time spent in active social interaction, $F(3,27)=4.29$, $p<0.01$. Dunnett's tests showed that each dose significantly reduced social interaction compared with controls ($p<0.01$, see Fig. 2). There was no significant effect on motor activity, $F(3,27)=0.44$, see Fig. 2.

FG 7142 (5–20 mg/kg) had no significant effect on the number of head-dips, $F(3,28)=1.76$, or the time spent head-dipping, $F(3,28)=1.16$ (see Fig. 3). However, there was a significant decrease in locomotor activity, $F(3,28)=4.29$, $p<0.05$, which reached significance at the 10 mg/kg ($p<0.05$) and 20 mg/kg ($p<0.01$) doses. There was also a significant effect on the number of rears, $F(3,28)=4.0$, $p<0.05$, and posthoc analysis showed a significant decrease at the 5, 10 mg/kg ($p<0.05$) and the 20 mg/kg ($p<0.01$) doses (see Fig. 4).

The plasma concentrations of FG 7142 are shown in Table 1. For FG 7142 5 mg/kg, the plasma concentrations were negatively correlated with the social interaction score ($r=-0.6$), but because of the small numbers of rats this did not reach significance. For FG 7142 10 mg/kg the correlation reached significance ($r=-0.74$, $p<0.05$). Thus those rats

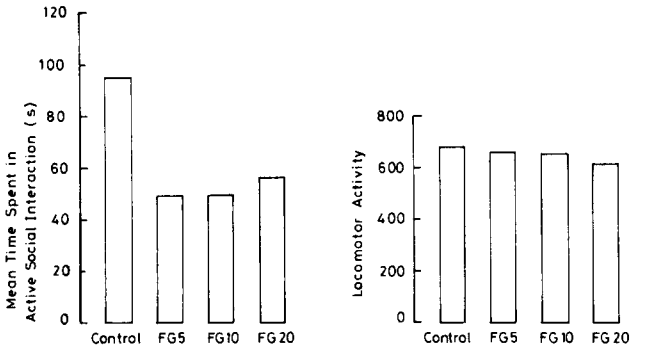


FIG. 2. Mean time (sec) spent in active social interaction and locomotor activity score for rats given a 7.5-min test, 20 min after injection of FG 7142 (5–20 mg/kg). Mean (±S.E.M.) raw scores for each group are: social—106.78±17.10; 47.00±4.32; 46.02±8.62; 48.72±6.84. Motor—759.75±40.30; 629.00±36.07; 620.37±46.33; 581.25±51.63.

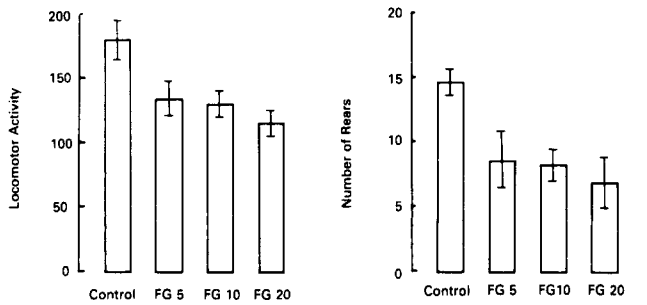


FIG. 4. Mean (±S.E.M.) locomotor activity scores and number of rears for rats given a 5-min test in the holeboard, 30 min after injection of FG 7142 (5–20 mg/kg).

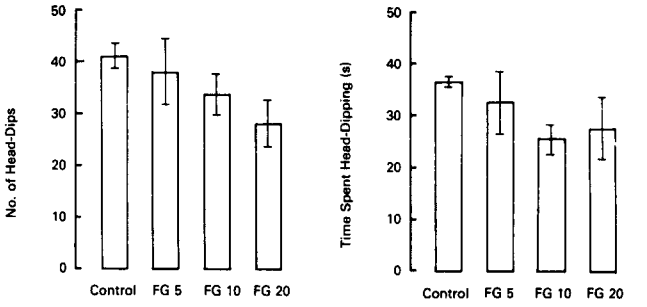


FIG. 3. Mean (±S.E.M.) number of head-dips and time spent head-dipping (sec) for rats given a 5-min test in the holeboard, 30 min after injection with FG 7142 (5–20 mg/kg).

TABLE 1

PLASMA CONCENTRATIONS OF FG 7142 (μg/ml PLASMA) IN RATS SAMPLED IMMEDIATELY AFTER THE SOCIAL INTERACTION TEST (A) AND IN THOSE SAMPLED 5 MIN LATER AFTER THE HOLEBOARD TEST (B)

Dose	A	B
5 mg/kg	1.86 ± 0.39	2.06 ± 0.39
10 mg/kg	5.84 ± 0.91	4.38 ± 1.46
20 mg/kg	6.03 ± 2.28	4.31 ± 1.95

with low scores in the social interaction test (indicating a strong anxiogenic effect) had high plasma concentrations of FG 7142. Correlations of plasma concentrations with the motor activity scores of the pairs of rats in the social interaction test did not reach significance for either the 5 or 10 mg/kg group and were no consistent in direction (+0.5 and −0.2, respectively). For FG 7142 20 mg/kg the plasma levels were no longer significantly correlated with the social interaction scores ($r=-0.2$) but the correlation with locomotor activity ($r=-0.61$) approached significance.

DISCUSSION

Like the other β-carbolines, β-CCE and β-CCP, FG 7142 displayed an anxiogenic profile in the social interaction test, i.e., a specific reduction in the time spent in active social interaction. That this effect was not secondary to any reduction in locomotor activity, was shown by a significant drug effect on analysis of covariance, which takes into account the extent to which locomotor activity scores may contribute to the observed effect on social interaction. At the doses tested, however, this effect was not dose-related. It seems that even the 5 mg/kg dose was high enough to achieve a maximal effect. In this respect, the social interaction test is more sensitive than the other tests used [17]. However, when the individual scores were examined the size of the anxiogenic effect was correlated with the plasma level of FG 7142 at least for the 5 and 10 mg/kg doses. At the dose of 20 mg/kg the correlation was hardly existent, due to high variation in plasma concentrations; it is most likely that this was due to absorption problems of the high drug concentration. Since FG 7142 has been reported to be anxiogenic in man [2] the results of the present experiment provide useful validation for the social interaction test.

In the holeboard, FG 7142 had no effect on exploratory head-dipping at the doses tested, but did reduce locomotor activity (10–20 mg/kg) and the number of rears (5–20 mg/kg). So, although motor activity was not reduced in the pairs of rats tested in the social interaction test (and therefore cannot account for the observed reduction in social interaction), some reductions could be detected when the rats were tested singly in the holeboard. Although we do not know the reasons for this difference, the two tests are quite different, and File and Pope [10] found that drug effects that are present when one rat is tested alone in the holeboard may not be present when two rats are tested together. The effects of FG 7142 in the holeboard differ from those of β-CCE and β-CCP which reduce head-dipping and rears, but not locomotor activity [6,9].

Thus, although FG 7142 has a profile in the social interaction test of anxiety similar to that of β-CCE and β-CCP, it can be distinguished from these other carbolines by its profile in the holeboard test. The classification of drugs on the basis of behavioural results in one test will not necessar-

ily apply to other test situations. Classifications based on anxiogenic properties do not always hold for drug effects on convulsions (see [16]) or for their effects on exploratory behaviour (see [4]).

ACKNOWLEDGEMENTS

We are grateful to Jane Freeman and Peter Mabbutt for expert technical assistance. SEF is a Wellcome Trust Senior Lecturer.

REFERENCES

1. Corda, M. G., W. D. Blaker, W. B. Mendelson, A. Guidotti and E. Costa. β-Carbolines enhance shock-induced suppression of drinking in rats. *Proc Natl Acad Sci USA* **80:** 2072–2076, 1983.
2. Dorow, R., R. Horowski, G. Paschelke, M. Amin and C. Braestrup. Severe anxiety induced by FG 7142, a β-carboline ligand for benzodiazepine receptors. *Lancet* **2:** 98–99, 1983.
3. File, S. E. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *J Neurosci Methods* **2:** 219–238, 1980.
4. File, S. E. What can be learned from the effects of benzodiazepines on exploratory behavior? *Neurosci Biobehav Rev* **9:** 45–54, 1985.
5. File, S. E. and J. R. G. Hyde. A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. *Pharmacol Biochem Behav* **11:** 65–69, 1979.
6. File, S. E. and R. G. Lister. β-CCE and chlordiazepoxide reduce exploratory head-dipping and rearing: no mutual antagonism. *Neuropharmacology* **21:** 1215–1218, 1983.
7. File, S. E. and R. G. Lister. Do the reductions in social interaction produced by picrotoxin and pentylenetetrazole indicate anxiogenic actions? *Neuropharmacology* **23:** 793–796, 1984.
8. File, S. E., R. G. Lister and D. J. Nutt. The anxiogenic actions of benzodiazepine antagonists. *Neuropharmacology* **21:** 1033–1037, 1982.
9. File, S. E., R. G. Lister, R. Maninov and J. C. Tucker. Intrinsic behavioural actions of propyl-β-carboline-3-carboxylate. *Neuropharmacology* **23:** 463–466, 1984.
10. File, S. E. and J. H. Pope. The action of chlorpromazine on exploration in pairs of rats. *Psychopharmacologia* **37:** 249–254, 1974.
11. File, S. E. and A. G. Wardill. Validity of head-dipping as a measure of exploration in a modified holeboard. *Psychopharmacologia* **44:** 53–59, 1975.
12. Greden, J. F. Anxiety or caffeinism: a diagnostic dilemma. *Am J Psychiatry* **131:** 1089–1092, 1974.
13. Jensen, L. H., E. N. Petersen and C. Braestrup. Audiogenic seizures in DBA/2 mice discriminate sensitively between low efficacy benzodiazepine receptor agonists and inverse agonists. *Life Sci* **33:** 393–399, 1983.
14. Lal, H. and G. Shearman. Attenuation of chemically induced anxiogenic stimuli as a novel method for evaluating anxiolytic drugs: a comparison of clobazam with other benzodiazepines. *Drug Dev Res (Suppl)* **1:** 127–134, 1983.
15. Ongini, E., C. Barzaghi and M. Marzanatti. Intrinsic and antagonistic effects of β-carboline FG 7142 on behavioural and EEG actions of benzodiazepines and pentobarbital in cats. *Eur J Pharmacol* **95:** 125–129, 1983.
16. Pellow, S. Can drug effects on anxiety and convulsions be separated? *Neurosci Biobehav Rev* **9:** 55–74, 1985.
17. Petersen, E. N., L. H. Jensen, T. Honore and C. Braestrup. Differential pharmacological effects of BZ receptor inverse agonists. In: *Benzodiazepine Recognition Site Ligands: Biochemistry and Pharmacology*, edited by G. Biggio and E. Costa. *Advances in Biochemical Psychopharmacology*, vol 38. New York: Raven Press, 1983, pp. 57–64.
18. Rodin, E., M. Rodin and L. Lavine. Electroclinical and ultrastructural changes associated with subconvulsant doses of pentylenetetrazol. *Exp Neurol* **64:** 386–400, 1979.