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Quinolines and Anxiety: Anxiogenic Effects of CGS 8216 and Partial Anxiolytic Profile of PK 9084

SANDRA E. FILE AND RICHARD G. LISTER

Department of Pharmacology, The School of Pharmacy, University of London Brunswick Square, London WC1N 1AX, England

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FILE, S. E. AND R. G. LISTER. *Quinolines and anxiety: Anxiogenic effects of CGS 8216 and partial anxiolytic profile of PK 9084.* PHARMACOL BIOCHEM BEHAV **18**(2) 185–188, 1983.—The effects of three quinoline derivatives, PK 8165 (7.5 mg/kg), PK 9084 (7.5 mg/kg) and CGS 8216 (1 and 10 mg/kg), alone and in combination with chlordiazepoxide (5 mg/kg), were investigated in the social interaction test of anxiety. PK 8165 had no effect on social interaction but both PK 8165 and PK 9084 in combination with chlordiazepoxide produced sedation. PK 9084 exhibited a partial anxiolytic profile reflected by a drug × light interaction in the familiar test conditions. CGS 8216 (1 mg/kg) showed no significant effects on social interaction, but did counteract the sedative effect of chlordiazepoxide. The 10 mg/kg dose of CGS 8216 reduced social interaction between pairs of animals in familiar test conditions which is indicative of an anxiogenic effect. These intrinsic anxiogenic properties of CGS 8216 demonstrate that it cannot be considered an inert benzodiazepine antagonist.

PK 8165 PK 9084 CGS 8216 Quinolines Benzodiazepines Rats Social interaction Anxiety Sedation

RECENTLY, several quinolines have been found to bind potently to the benzodiazepine receptors [3,15]. Two of them, PK 8165 and PK 9084, have been reported to have anti-conflict actions in the rat [15] and, like the benzodiazepines, their binding is enhanced in the presence of GABA [16]. However, they lack anti-convulsant activity [15] and it has been suggested that they might be partial agonists at benzodiazepine receptor sites [14]. In contrast, the binding of the pyrazoloquinoline, CGS 8216, is reduced by GABA [16] and it is able to antagonise the behavioural actions of the benzodiazepines [1].

We have recently reported [11] that two other benzodiazepine antagonists, ethyl β-carboline-3-carboxylate (β-CCE) and the imidazodiazepine RO 15-1788, have intrinsic anxiogenic actions, as well as being able to reverse the anxiolytic effects of the benzodiazepines [2, 13, 18]. The purpose of the present study was to investigate the effects of PK 8165, PK 9084 and CGS 8216 in an animal test of anxiety that is sensitive to both anxiogenic and anxiolytic effects [5]. As well as investigating the intrinsic effects of these compounds we also investigated their effects when combined with chlordiazepoxide, in order to see whether any intrinsic effects were enhanced or reduced by a benzodiazepine.

The social interaction test can distinguish between sedative, anxiolytic and anxiogenic drug effects. Sedation is typically seen following acute doses of benzodiazepines [4], and is reflected in a decrease in active social interaction in all the test conditions, accompanied by a decrease in motor activity. In contrast, after chronic treatment the benzodiazepines produce a typical anxiolytic profile in which the animals maintain a high level of social interaction, even when the test arena is unfamiliar and brightly lit, whereas control animals show decreased social interaction in these test conditions [5, 8, 9]. An anxiogenic action is shown by a decrease in social interaction, without a concomitant decrease in motor activity [11,12].

The doses of PK 8165 and PK 9084 were chosen on the basis of their effects in a conflict test [15] and in a holeboard in which possible sedative effects were investigated [7]. The doses of CGS 8216 were in the range of doses that antagonise the behavioural effects of diazepam [1]. The dose of chlordiazepoxide selected was the lowest one found to be anxiolytic with chronic administration [8] and sedative after acute doses [4].

METHOD

Animals

Male hooded Lister rats (Olac Ltd., Bicester), approximately 200 g, were housed singly and handled daily for 5 days prior to experimental testing. Food and water were freely available and the room lights were on from 0600 to 1700 hr. Rats were assigned test partners that did not differ in weight by more than 5 g.

Apparatus

The social interaction test arena was a wooden box 35 cm tall with a solid floor 60×60 cm. Infra-red photocells in the walls of the box (4.5 cm above floor level) provided an auto-

mated measure of total motor activity of the rats. A closed circuit TV camera with an automatic iris was mounted vertically over the test arena and the rats behaviour was observed on a monitor in the adjacent room. The frequency and duration of each category of behaviour was entered on-line into a MINC-11 computer and videotapes were kept of all trials. The illuminances in the test box were 30 and 300 scotopic lux for the low and high light test conditions, respectively.

Drugs

PK 8165 and PK 9084 (Pharmuka) were dissolved in distilled water to a concentration of 3.75 mg/ml. They were injected IP 60 min before test. CGS 8216 (Ciba-Geigy) was dispersed by ultrasound in water to which a drop of Tween-20 had been added and was injected IP 30 min before test. Chlordiazepoxide hydrochloride (CDP, Roche Products, Ltd.) was dissolved in distilled water to a concentration of 2.5 mg/ml and injected IP 30 min before test.

Statistics

The social interaction and motor activity scores were analysed separately for each drug by 3-way split-plot analyses of variance in which drug treatment and light level were independent factors and familiarity was a repeated measure. An anxiolytic action, as shown by chronically administered benzodiazepines, is manifested by significant drug × light and drug × familiarity interactions.

Procedure

Rats were randomly allocated, 14 or 16 pairs to each of the following drug groups: PK 8165 (7.5 mg/kg); PK 9084 (7.5 mg/kg); PK 8165 (7.5 mg/kg) plus CDP (5 mg/kg); PK 9084 (7.5 mg/kg) plus CDP (5 mg/kg); CGS 8216 (1 mg/kg); CGS 8216 (1 mg/kg) plus CDP (5 mg/kg); vehicle control (water).

Each pair of rats was tested twice, following the same drug treatment and under the same light level on each occasion. Both members of a test pair always received the same drug.

On the first day the pairs were unfamiliar with the test arena and in each drug group half the pairs were randomly allocated to the low light test condition, the other half were tested under high light. Each pair was placed in the centre of the arena for a 7.5 min trial and the following behaviours were scored by two independent observers who had no knowledge of the drug treatment or test condition: sniffing, following, grooming the partner, kicking, wrestling, boxing and mounting. Passive contact (when the rats were in body contact, but not interacting in any way) was scored separately.

At the end of the trial each rat was returned to its home cage. The following day each rat was placed alone in the test arena for a 7.5 min familiarisation trial, under the appropriate light level. The next day the pairs of rats were given a second social interaction test, in the by now familiar arena.

The rats were tested between 0700 and 1200 hr, in an order randomised for drug treatment and light level. The test order was the same for the two days.

At the end of the experiment 40 pairs of rats (from those previously tested with PK 8165, PK 9084 or control) were randomly reassigned to the following new drug groups: CGS 8216 (10 mg/kg); CGS 8216 (10 mg/kg) plus CDP (5 mg/kg); vehicle control (water-Tween). These rats were given a third social interaction test, with their original test partners and under the same light condition as previously, 2 days after the second test. The procedure for this test was as described above.

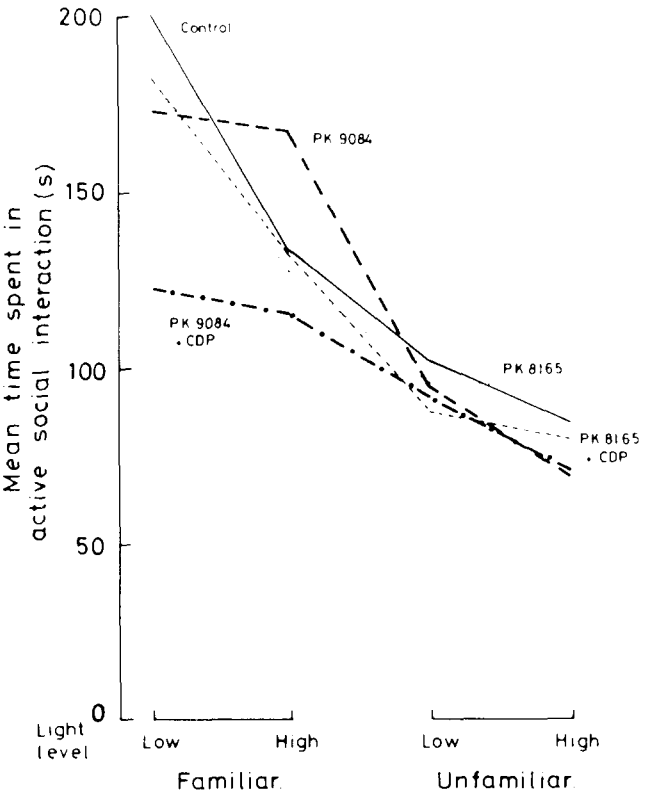

FIG. 1. Mean time spent in active social interaction by rats tested in four conditions after vehicle (—), PK 8165 (. . . .), PK 8165 + CDP (- - -), PK 9084 (— — —) or PK 9084 + CDP (—·—·—).

RESULTS

Figure 1 shows the mean time spent in active social interaction in the 4 test conditions by groups of rats treated with PK 8165 and PK 9084, alone and in combination with chlordiazepoxide. Overall, there was significantly less social interaction in the high than in the low light test condition, $F(1,42)=7.65$, $p<0.01$, and in the unfamiliar compared with the familiar test conditon, $F(1,42)=66.6$, $p<0.0001$.

PK 8165 was without significant effect alone or in combination with CDP ($F(2,42)=0.21$). PK 9084 had a significant overall effect on social interaction, $F(2,42)=3.9$, $p<0.03$, and resulted in a significant drug × familiarity interaction, $F(2,42)=4.2$, $p<0.03$. This is because the control rats showed a greater change in social interaction as the arena became familiar than did the rats injected with the drug. It can be seen from Fig. 1 that this drug × familiarity interaction is mainly the result of the effects of the combination of PK 9084 with CDP. PK 9084 when given alone resulted in a significant drug × light × familiarity interaction; this was because the drugged animals did not show lower scores in high light if the arena was familiar, whereas they did if the arena was unfamiliar. The control rats showed a decrease in high light in both the test conditions.

Although PK 8165 did not significantly alter social interaction, the pairs of rats showed significantly lower levels

TABLE 1

LOCOMOTOR ACTIVITY SCORES OF PAIRS OF RATS AFTER IP INJECTIONS OF PK 8165 (7.5 mg/kg) AND PK 9084 (7.5 mg/kg), ALONE AND IN COMBINATION WITH CDP (5 mg/kg)

	Low Unfamiliar	Low Familiar	High Unfamiliar	High Familiar
Control	710 ± 21	809 ± 35	681 ± 25	784 ± 45
PK 8165	690 ± 28	725 ± 38	809 ± 56	744 ± 66
PK 8165 + CDP	574 ± 39	670 ± 34	733 ± 57	614 ± 74
PK 9084	649 ± 6	731 ± 35	788 ± 52	718 ± 50
PK 9084 + CDP	602 ± 43	595 ± 64	729 ± 64	711 ± 52

Scores are means±SEM.

TABLE 2

TIME SPENT IN ACTIVE SOCIAL INTERACTION AND LOCOMOTOR ACTIVITY SCORES OF PAIRS OF RATS AFTER IP INJECTIONS OF CGS 8216 (1 mg/kg) ALONE AND IN COMBINATION WITH CDP (5 mg/kg)

	Control	CGS 8216	CGS 8216 + CDP
Time(s) in active social interaction			
Low Familiar	200 ± 25	172 ± 22	163 ± 22
High Familiar	133 ± 14	144 ± 8	117 ± 16
Low Unfamiliar	102 ± 10	84 ± 14	74 ± 15
High Unfamiliar	84 ± 10	80 ± 13	85 ± 18
Locomotor Activity			
Low Familiar	809 ± 35	808 ± 18	777 ± 29
High Familiar	784 ± 45	748 ± 37	715 ± 43
Low Unfamiliar	710 ± 21	685 ± 27	660 ± 21
High Unfamiliar	681 ± 25	660 ± 37	638 ± 48

Scores are means±SEM.

TABLE 3

TIME SPENT IN ACTIVE SOCIAL INTERACTION AND LOCOMOTOR ACTIVITY SCORES OF PAIRS OF ANIMALS AFTER IP INJECTION OF CGS 8216 (10 mg/kg) ALONE OR IN COMBINATION WITH CDP (5 mg/kg)

	Control	CGS 8216	CGS 8216 + CDP
Time(s) in active social interaction			
Low Familiar	148 ± 30	93 ± 19	117 ± 20
High Familiar	110 ± 7	83 ± 17	57 ± 11
Locomotor Activity			
Low Familiar	830 ± 34	786 ± 27	802 ± 32
High Familiar	908 ± 32	914 ± 31	863 ± 32

Scores are means±SEM.

of motor activity in this test, $F(2,42)=4.9$, $p<0.02$, and from Table 1 it can be seen that this was entirely due to the group given both PK 8165 and CDP. PK 9084 alone did not significantly reduce motor activity, but in the group also given CDP it was significantly reduced, $F(1,28)=5.4$, $p<0.03$.

CGS 8216 (1 mg/kg) did not significantly alter the overall level of social interaction, either alone or in combination with CDP, $F(2,40)=1.1$. The rats showed less social interaction in high than in low light, $F(1,40)=5.8$, $p<0.02$, and in the unfamiliar compared with the familiar test condition, $F(1,40)=70.7$, $p<0.0001$, but there were no significant drug × light or drug × familiarity interactions, $Fs<1.0$. The levels of motor activity were also unaffected by CGS 8216 (1 mg/kg) alone or in combination with CDP (Table 2).

In the experiment in which the rats were tested for a third time only the factor of light level was varied since all were by now very familiar with the test arena. Social interaction was significantly lower in the high light level, $F(1,34)=6.35$, $p<0.02$, and CGS 8216 (10 mg/kg) significantly reduced active social interaction, $F(2,34)=3.9$, $p<0.03$. CDP did not significantly alter this effect of CGS 8216, $F(1,22)=0.05$. This dose of CGS 8216 was without significant effect on motor activity, $F(2,34)=0.8$, alone or in combination with CDP.

DISCUSSION

The failure to find any evidence of an anxiolytic effect of PK 8165 may have been due to the selection of too low a dose. The dose selected was higher than the minimum effective dose in a conflict test and was equipotent with CDP (5 mg/kg) in this test [15]; whereas the social interaction test is sensitive to the anxiolytic effects of CDP (5 mg/kg) it may be less sensitive to the actions of quinolines. The selection of a higher dose of PK 8165 would have led to marked sedation [7].

PK 9084 was twice as effective as PK 8165 in the conflict test [15] and in the social interaction test it produced an anxiolytic profile as evidenced by a drug × light interaction in the familiar, but not in the unfamiliar test conditions. When combined with CDP an anxiolytic action was indicated by a significant drug × familiarity interaction, inspite of the sedative effects of this drug combination. However, the anxiolytic profile of PK 9084 is less clear than that seen with chronically administered benzodiazepines [8] and with low doses of CL 218,872 [6].

Only the combinations of CDP with PK 8165 or PK 9084 were significantly sedative (as reflected by a reduction in locomotor activity scores) and this is most likely to be due to

the sedative effects of acute CDP [4]. A reduction in locomotor activity was not seen in the present study when CDP was combined with either dose of CGS 8216 indicating that CGS 8216 can reverse this sedation. Antagonism of CDP induced sedation has also been found in a holeboard test [10].

CGS 8216, in contrast to the other quinolines, had a clear anxiogenic action at the higher dose and this resembles the effects found with other benzodiazepine antagonists, RO 15-1788 and β-CCE [11]. The anxiogenic effects of CGS 8216 (10 mg/kg) were not reversed by 5 mg/kg CDP, but this may have been too low a dose. Certainly the anxiogenic actions of β-CCE, RO 15-1788 and CGS 8216 suggest that these compounds are not just pharmacological antagonists of the benzodiazepines. One possibility is that the benzodiazepine receptor is unusual in having two groups of agonists that produce opposite behavioural effects [11,17].

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REFERENCES

1. Bernard, P., K. Bergen, R. Sobiski and R. D. Robson. An orally effective benzodiazepine antagonist. *Pharmacologist* **23:** 150, 1981.
2. Brown, C. L. and A. M. Johnson. Ethyl β-carboline-3-carboxylate reverses the effect of benzodiazepines in a test for detecting anxiolytic activity. *Br J Pharmacol* **75:** 43P, 1982.
3. Czernik, A. J., B. Petrack, H. J. Kalinsky, S. Psychoyos, W. D. Cash, C. Tsai, R. K. Rinehart, F. R. Granat, R. A. Lovell, D. E. Brundish and R. Wade. CGS 8216: Receptor binding characteristics of a potent benzodiazepine antagonist. *Life Sci* **30:** 363–372, 1982.
4. File, S. E. Anxiety, ACTH, and 5-HT. *Trends Neurosci* **1:** 9–11, 1978.
5. File, S. E. The use of social interactions as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *J Neurosci Methods* **2:** 219–238, 1980.
6. File, S. E. Animal anxiety and the effects of benzodiazepines. In: *The Pharmacology of Benzodiazepines*, edited by E. Usdin, S. M. Paul, P. Skolnick and D. J. Greenblatt. New York: Macmillan, 1982.
7. File, S. E. Sedative effects of PK 9084 and PK 8165 alone and in combination with chlordiazepoxide. Submitted.
8. File, S. E. and J. R. G. Hyde. Can social interaction be used to measure anxiety? *Br J Pharmac* **62:** 19–24, 1978.
9. File, S. E. and J. R. G. Hyde. A rest of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. *Pharmac Biochem Behav* **11:** 65–69, 1979.
10. File S. E. and R. G. Lister. RO 15-1788 and chlordiazepoxide each reverse the reduction in exploration caused by CGS 8216. *Br J Pharmac*, in press, 1983.
11. File, S. E., R. G. Lister and D. J. Nutt. The anxiogenic action of benzodiazepine antagonists. *Neuropharmacology* **21:** 1033–1037, 1982.
12. File, S. E. and S. V. Vellucci. Behavioural and biochemical measures of stress in hooded rats from different sources. *Physiol Behav* **22:** 31–36, 1979.
13. Hunkeler, W., H. Mohler, L. Pieri, P. Polc, E. P. Bonetti, R. Cumin, R. Schaffner and W. Haefely. Selective antagonists of benzodiazepines. *Nature* **290:** 514–516, 1981.
14. Karobath, M. Benzodiazepine receptor heterogeneity. In: *The Pharmacology of Benzodiazepines*, edited by E. Usdin, S. M. Paul, P. Skolnick and D. J. Greenblatt. New York: MacMillan, 1982.
15. Le Fur, G., J. Mizoule, M. C. Burgevin, O. Ferris, M. Haulme, A. Gauthier, C. Gueremy and A. Uzan. Mutilple benzodiazepine receptors: evidence of a dissociation between anticonflict and anticonvulsant properties by PK 8165 and PK 9084 (two quinoline derivatives). *Life Sci* **28:** 1439–1448, 1981.
16. Morelli, M., K. W. Gee and H. I. Yamamura. The effect of GABA on *in vitro* binding of two novel non-benzodiazepines, PK 8165 and CGS 8216 to benzodiazepine receptors in the rat brain. *Life Sci* **31:** 77–81, 1982.
17. Nutt, D. J., P. J. Cowen and H. J. Little. Unusual interactions of benzodiazepine receptor antagonists. *Nature* **295:** 436–438, 1982.
18. Rommelspacher, H. The β-carbolines (harmanes)—a new class of endogenous compounds: their relevance for the pathogenesis and treatment of psychiatric and neurological diseases. *Pharmacopsychiatry* **14:** 117–125, 1981.