The effects of p-chlorophenylalanine and ethanolamine-O-sulphate in an animal test of anxiety

SANDRA E. FILE* AND J. R. G. HYDE

Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, U.K.

p-Chlorophenylalanine, which produces a depletion of brain 5-HT concentration, had effects qualitatively similar to those previously found with chronic chlordiazepoxide and with acute ethanol in the social interaction test of anxiety. This result is compatible with the idea that a reduced turnover of 5-HT is important in anxiety reduction. On the same test, ethanolamine-O-sulphate, which raises brain γ-aminobutyric acid, was without effect, suggesting raised concentrations of this acid are not essential for anxiety reduction.

We have recently developed a new animal test of anxiety, in which social interaction between pairs of male rats is measured (File & Hyde, 1977). The time spent in active social interaction is greatest when the rats are tested at a low level of illumination in a box with which they are familiar. If the light level is high or if the test box is unfamiliar, there is a reduction in social interaction. We have shown that the decrease in interaction is not due to increased distraction or exploration of an unfamiliar environment, nor to changes in olfactory cues from the partner. As social interaction decreases there is an increased incidence of defaecation, freezing and displacement activity. The reduction in social interaction that normally occurs across test conditions can be prevented by acute administration of ethanol and by chronic administration of chlordiazepoxide (File, Hyde & Pool, 1976).

It has been reported that p-chlorophenylalanine (PCPA) has antianxiety effects (Robichaud & Sledge, 1969; Geller & Blum, 1970; Cook & Sepinwall, 1975). This is compatible with the proposal that a decreased turnover of 5-HT mediates anxiety reduction (Stein, Wise & Berger, 1973). One of the purposes of the present study was to obtain information about the effects of PCPA on our new animal test of anxiety. We used a dose of 400 mg kg⁻¹ which has been shown to be both behaviourally and biochemically effective (File, 1975) and 72 h after the injection of a 300 mg kg⁻¹ dose of PCPA the 5-HT concentrations are still only 33% of the control values (Miller, Cox & others, 1970).

More recently it has been suggested that the changes in 5-HT are secondary to the effects on γ-aminobutyric acid (GABA) of the benzodiazepines and that GABA may be the transmitter critically

* Correspondence.

involved in anxiety reduction (Stein, Wise & Belluzzi, 1975). One possible mechanism could be an increased release of GABA at 5-HT nerve endings causing a reduction, by presynaptic inhibition, of the release of 5-HT (Padjen & Bloom, 1975). To study the effect of raising GABA concentrations on our new test we used ethanolamine-O-sulphate (EOS), which is a specific GABA-T inhibitor (Fowler, 1973), in a behaviourally and biochemically effective dose of 0·5 mg kg⁻¹ (File, 1977).

METHODS

Animals

A total of 168 male hooded rats (*Rattus norvegicus*) 200–250 g were tested. They were housed singly for 5 days before the test and had free access to food and water. During this period they were weighed and handled daily and the position of the cages in the rack was changed so that all rats received equal experience of the different levels of illumination.

Drugs

p-Chloro-DL-phenylalanine (methyl ester HCl from Koch-light Laboratories) was dissolved in saline to give a concentration of 100 mg ml⁻¹. A dose of 400 mg kg⁻¹ was given intraperitoneally 3 days before the social interaction test. Control animals were injected with equal volumes of saline acidified with 0·1 N HCl to the same pH as the drug solution.

Ethanolamine-O-sulphate (courtesy of Dr L. J. Fowler) was dissolved in single strength Merlis solution to give a concentration of 10 mg ml⁻¹. Injections were given intracisternally in a volume of 15 μl (giving a dose of 0·5 mg kg⁻¹) to rats under halothane anaesthesia. Control animals were injected intracisternally with an equal volume of Merlis solution. The social interaction test was conducted 20 h after the intracisternal injections.

Procedure

(*a*) *PCPA.* (i) *Social interaction test.* Forty-eight control and 48 PCPA rats were randomly assigned to the four test conditions. Half the rats in each group were allocated to the 'familiar' test conditions. These were placed singly in the test box for 10 min on 2 consecutive days before injection with PCPA. The other half were allocated to the 'unfamiliar' conditions, and were placed in the test room for two 10 min sessions, but remained in their home cages. Half the rats in each of these groups were tested under high light and half under low. The familiarization sessions took place under the appropriate light level. The high and low light levels were 338 and 23·5 scotopic lux, respectively: scotopic units are appropriate since the rat has a predominantly rod retina.

Each rat was allocated an unknown test partner that did not differ by more than 10 g in weight. Both members of a pair had the same prior familiarization experience and the same drug treatment and six pairs of rats were tested in each condition. Pairs were tested in a random order between 0800 and 1100 h. The test box was 65 × 65 cm with walls 47 cm tall.

Pairs of rats were placed in this box for 10 min and their behaviour was observed on a television monitor in an adjacent room. The time they spent in active social contact was scored by two observers; this gave agreement to within 10 s. The following behaviours were scored: sniffing, nipping, grooming, following, mounting, kicking, boxing, wrestling, jumping on, crawling under or over the partner. Passive contact (sitting or lying with bodies in contact) was not included in this social interaction score. At the end of the session any boluses were removed and the floor and walls of the box wiped with detergent and dried.

(ii) *Motor activity.* Changes in social interaction might be secondary to changes in the level of motor activity. Therefore, following the social interaction test the level of motor activity was measured in 10 PCPA-treated rats and in 10 control rats. This was done by placing rats singly, for 10 min, in a black plastic cage (29 × 29 × 21 cm), which had nine touch sensitive plates in the floor. The output from these plates was fed to counters to give a measure of motor activity.

(iii) *Assay.* Four randomly selected PCPA-injected and four control animals were stunned with a blow to the head, the neck broken and the brain rapidly dissected out. 5-HT concentrations were assayed using the method of Curzon & Green (1970).

(*b*) *Ethanolamine*-O-*sulphate.* (i) *Social interaction test.* In this experiment, only three test conditions (low light, familiar; low light, unfamiliar; and high light, unfamiliar) were used and 36 EOS injected and 36 control rats were randomly allocated to these conditions. Familiarization took place before the intracisternal injections. In all other respects the test was carried out as above.

(*ii*) *Motor activity.* Ten EOS and 10 control animals were singly placed in the black activity box for 10 min, as above.

(iii) *Assay.* Twelve randomly selected EOS injected and six control animals were killed and the brains removed, as above, immediately after the social interaction test. The GABA concentrations were assayed using the method of Lowe, Robins & Eyerman (1958) with the modification of Sutton & Simmonds (1974).

RESULTS

A two-way analysis of variance was performed on the social interaction data, with one factor being the test conditions and the other the drug condition. The definition of an anxiolytic action is therefore a significant interaction between drug and test conditions. As can be seen (Fig. 1), the PCPA-treated rats showed no decline in social interaction
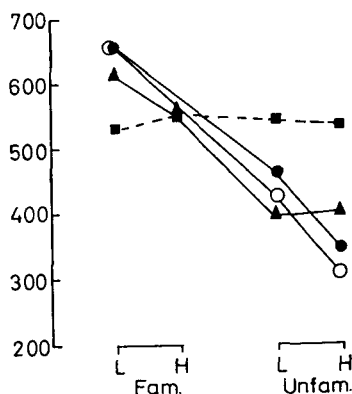

FIG. 1. Mean time spent in active social interaction for rats that were either familiar or unfamiliar with the test box and were tested under low or high light. ■ – – – ■ PCPA (400 mg kg⁻¹), ▲——▲ acidified saline, ●——● EOS (0·5 mg kg⁻¹), ○——○ intracisternal controls. Ordinate—Mean duration of active contact (s). Abscissa—Light level, L—low, H—High, Fam—familiar, Unfam—unfamiliar.

across the test conditions and this resulted in a significant drug × test interaction (F = 6·1, df = 3, 40, P <0·002).

The increased social interaction cannot be attributed to an increase in motor activity since this was slightly reduced in the PCPA-treated rats (t (18) = 2·44, P <0·05). Nor can the PCPA results be attributed solely to an increase in sexual activity, a common result of treatment with this drug (Zitrin, Dement & Barchas, 1973). In our experiment 2 out of 6 of the PCPA-injected pairs of rats tested in each condition showed persistent mounting. But the mean interaction scores from these rats did not differ from the means for the rats showing no hypersexuality.

The PCPA injections had resulted in a mean whole brain 5-HT concentration of 191 ± 7 ng g⁻¹, compared with the control brain concentration of 742·5 ± 7·5 ng g⁻¹, a 74% depletion. Known amounts of 5-HT were added to the homogenate and these internal standards were run to correct for recovery which averaged 60%.

Ethanolamine-O-sulphate

The effects of EOS on social interaction are shown in Fig. 1 and it is clear that this drug had no effect either on the level of social interaction or on its change across the test conditions. The level of motor activity was also unchanged by this dose of EOS, the controls having a mean score of 176·8 ± 29·3 and the EOS-injected rats a mean of 159·0 ± 16·7. It is unlikely that these negative results are due to 0·5 mg kg⁻¹ being a behaviourally ineffective dose since this same dose reduced exploration in the holeboard (File, 1977). Also the GABA concentrations in the whole brain in the control animals were 3·59 ± 0·49 μmol g⁻¹, compared with the treated level of 5·05 ± 0·35 μmol g⁻¹, an elevation of 41%.

DISCUSSION

Our results add further support to the evidence that PCPA has antianxiety actions, but because it reduces the concentrations of other putative neurotransmitters as well as of 5-HT, the anxiety reduction cannot necessarily be attributed to changes in the latter transmitter (Lane, Smith & others, 1976). However, it is unlikely that noradrenaline is important for anxiety reduction because concentrations of this amine were depeleted by only 16% compared with the much greater depletion of 5-HT. Secondly, we obtained anxiety reduction after chronic administration of chlordiazepoxide (File &

Hyde, 1977) and yet with chronic dosing there is tolerance to benzodiazepine-induced changes in noradrenaline (Stein, Wise & Berger, 1973). A third reason for suggesting that noradrenaline does not play a critical role in anxiety reduction comes from the behaviour of rats with 6-OHDA lesions of the locus coeruleus. These rats, like the controls, showed a decrease in social interaction if the light level was increased and the box was unfamiliar (Crow, Deakin & others, 1977).

The results of this experiment are certainly consistent with a role for 5-HT in anxiety reduction, but more specific manipulations of 5-HT are necessary to test this hypothesis further. However, the results obtained from enhancing GABA concentrations by injection of EOS provide no support for the involvement of GABA in anxiety reduction. Aminoxyacetic acid, which also produces raised GABA concentrations, did not have an anxiolytic effect when tested on the Geller-Seifter conflict test (Cook & Sepinwall, 1975). Moreover, the evidence on which Stein based his suggestion is open to alternative explanation. In support of the role of GABA, Stein & others (1975) found that picrotoxin, a GABA antagonist, reversed the anticonflict effect of oxazepam. This interpretation was based on the finding that picrotoxin reduced responding on the punished schedule (a CRF schedule) at a dose of 2 mg kg⁻¹, whereas responding on the unpunished schedule (VI = 2 min) was not reduced until a dose of 4 mg kg⁻¹. An alternative interpretation of these results is that the two types of reinforcement schedule (CRF vs VI), or the different response rates produced by those schedules, were not equally sensitive to the drug effects. Moreover, the results with picrotoxin may not have been specific to an action on GABA, since another convulsant, strychnine (a glycine antagonist), also reversed the antianxiety effects of oxazepam.

However, PCPA also produces changes in other amino acids (Lane & others, 1976) and although GABA does not appear to be a likely candidate, it is not yet known if the 20% reduction in alanine is of significance in the antianxiety effects of PCPA.

REFERENCES

COOK, L. & SEPINWALL, J. (1975). In: *Mechanism of Action of Benzodiazepines*, pp. 1–28. Editors: Costa, E. & Greengard, P. New York: Raven Press.

CROW, T. J., DEAKIN, J. F. W., FILE, S. E., LONGDEN, A. & WENDLANDT, S. (1977). *Brain Research*, in the press.

CURZON, G. & GREEN, A. R. (1970). *Br. J. Pharmac.*, 39, 653–654.

FILE, S. E. (1975). *Pharmac. Biochem. Behav.*, 3, 979–983.

FILE, S. E. (1977). *Brain Res.*, 131, 180–183.

FILE, S. E. & HYDE, J. R. G. (1977). *Br. J. Pharmac.*, in the press.

FILE, S. E., HYDE, J. R. G. & POOL, M. (1976). *Ibid.*, 58, 465P.

FOWLER, L. J. (1973). *J. Neurochem.*, 21, 437–440.

GELLER, I. & BLUM, K. (1970). *Eur. J. Pharmac.*, 9, 319–324.

LANE, J. D., SMITH, J. E., SHEA, P. A. & McBRIDE, W. J. (1976). *Life Sci.*, 19, 1663–1668.

LOWE, I. P., ROBINS, E. & EYERMAN, G. S. (1958). *J. Neurochem.*, 3, 8–18.

MILLER, F. P., COX, R. H., SNODGRASS, W. R. & MAICKEL, R. P. (1970). *Biochem. Pharmac.*, 19, 435–442.

PADJEN, A. & BLOOM, E. (1975). In: *Mechanisms of action of Benzodiazepines*, pp. 93–102. Editors: Costa, E. & Greengard, P. New York: Raven Press.

ROBICHAUD, R. C. & SLEDGE. K. L. (1969). *Life Sci.*, 8, 965–969.

STEIN, L., WISE, C. D. & BELLUZZI, J. D. (1975). In: *Mechanisms of action of Benzodiazepines*, pp. 29–44. Editors: Costa, E. & Greengard, P. New York: Raven Press.

STEIN, L., WISE, C. D. & BERGER, B. D. (1973). In: *The Benzodiazepines*, pp. 299–326. Editor: Garattini, S. New York: Raven Press.

SUTTON, I. & SIMMONDS, M. A. (1974). *Biochem. Pharmac.*, 23, 1801–1808.

ZITRIN, A., DEMENT, W. C. & BARCHAS, J. D. (1973). In: *Contemporary sexual behaviour: critical issues in the 1970s*, pp. 321–338. Editors: Zubin, J. & Money, J. Baltimore: John Hopkins Press.