EFFECT OF P-CHLOROPHENYLALANINE ON BRAIN MONOAMINES AND BEHAVIOUR DURING ETHANOL WITHDRAWAL IN MICE

P.J. GRIFFITHS, J.M. LITTLETON & A. ORTIZ
Department of Pharmacology, King's College, London WC2R 2LS

The administration of *p*-chlorophenylalanine to mice prevents the rise in brain 5-hydroxytryptamine concentration associated with ethanol withdrawal but does not affect the increase in brain catecholamines which occurs at the same time. The locomotor excitement, piloerection, tremor and handling convulsions which occur during ethanol withdrawal were not affected. These results suggest that the increase in brain 5-hydroxytryptamine which occurs in ethanol withdrawal is a consequence of increased 5-hydroxytryptamine synthesis and that it is probably not involved in the above behavioural changes of ethanol withdrawal.

When ethanol is given to mice by inhalation or by the intragastric route, termination of ethanol administration is associated with locomotor excitation, increased susceptibility to seizures, tremor, piloerection and head twitches (Griffiths, Littleton & Ortiz, 1973a; Hammond & Schneider, 1973). These behavioural changes in mice may be related to different parts of the ethanol withdrawal syndrome in man.

Griffiths, Littleton & Ortiz (1973b) reported that chronic ethanol administration to mice was associated with changes in central monoamine concentrations. Chronic administration of ethanol by inhalation led to increases in noradrenaline, dopamine and 5-hydroxytryptamine and withdrawal of ethanol was associated with further transitory increases in monoamines. Administration of inhibitors of catecholamine biosynthesis before ethanol withdrawal reduced the brain catecholamine concentrations to below control values, prevented any increase in catecholamines on withdrawal and inhibited the initial locomotor excitation during withdrawal. However, these effects were accompanied by an increase in both the duration of increased susceptibility to convulsions and an increase in their severity (Griffiths, Littleton & Ortiz, 1974). Since 5-hydroxytryptamine concentrations showed changes qualitatively similar to those of the catecholamines during withdrawal, it was thought possible that modification of the metabolism of this neurotransmitter might also affect the behavioural changes of ethanol withdrawal. This communication describes the effect of pretreatment with the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (PCPA), on biochemical and behavioural changes taking place during withdrawal of ethanol from mice.

Methods Groups of 30 male white mice were exposed to ethanol vapour for 10 days in the way described by Griffiths *et al.* (1973a). Ethanol concentrations were increased slowly from about 10 mg/l to about 25 mg/l before withdrawal. For the four days preceding ethanol withdrawal half the mice in each group received PCPA methylester (125 mg/kg i.p. daily in 0.25 ml saline), the remaining mice received saline (0.25 ml i.p. daily) for the same period. The last injection of PCPA or saline was given 14 h before ethanol withdrawal. In some groups behavioural changes during ethanol withdrawal were assessed, whereas other groups were taken for estimation of brain monoamines (noradrenaline, dopamine, and 5-hydroxytryptamine) during ethanol withdrawal. The methods were those described previously (Griffiths *et al.*, 1973b).

All reagents were analar grade when these were available. Ethanol (A.R. grade 99.8% v/v) was obtained from James Burroughs Ltd., and *p*-chlorophenylalanine methylester hydrochloride was obtained from Sigma (London) Chemical Co. Ltd.

Results The administration of PCPA (4 x 125 g/kg i.p.) to otherwise untreated control mice resulted in a significant decrease in brain 5-hydroxytryptamine in the absence of a significant change in noradrenaline or dopamine concentrations (Table 1).

After ethanol administration for 10 days mice showed the previously reported elevation of brain monoamine concentrations. PCPA administration on the last four days of ethanol administration reduced brain 5-hydroxytryptamine concentrations to below those of untreated controls. Brain catecholamine concentrations were also reduced compared to values obtained in animals receiving ethanol alone (Table 1).

Table 1 The effect of *p*-chlorophenylalanine (PCPA; 4 x 125 mg/kg i.p.) on brain monoamine concentrations in mice during withdrawal from ethanol.

Pretreatment	Time after withdrawal (min)	n	Brain monoamine concentration (% of untreated controls) 5-HT	NA	DA
PCPA	–	12	68.75 ±8.6	90.79 ±8.3	83.82 ±6.3
Chronic ethanol + PCPA	0	10	87.52 ±4.5	118.3 ±8.4	89.26 ±5.4
	30	10	94.77 ±4.4	172.2 ±14.2	132.6 ±6.4
	60	8	95.56 ±4.3	118.9 ±19.2	142.8 ±18.21
Chronic ethanol	0	10	130.5 ±4.6	170.6 ±10.5	138.9 ±12.8
	30	10	156.6 ±7.2	228.7 ±15.4	196.5 ±20.2
	60	8	146.7 ±5.2	205.5 ±17.8	184.8 ±15.9

Brain monoamine concentrations are expressed as a mean (with s.e.) percentage of untreated control values. Absolute control concentrations were: 5-hydroxytryptamine (5-HT) 0.93 ± 0.03 μg/g; noradrenaline (NA) 0.67 ± 0.04 μg/g; dopamine (DA) 1.12 ± 0.04 μg/g. These are means with s.e. of at least ten determinations of concentrations per wet weight of brain tissue.

Withdrawal of ethanol was associated with a marked rise in brain catecholamine concentrations and a smaller rise in brain 5-hydroxytryptamine. Pretreatment with PCPA prevented the increase in brain 5-hydroxytryptamine concentration, but did not affect the magnitude of the increase in brain catecholamines (Table 1).

The administration of PCPA in this way did not affect the increase in locomotor activity which occurs early in ethanol withdrawal. Similarly, PCPA pretreatment had no significant effect on the severity or duration of ethanol withdrawal convulsions, piloerection, tail-lift, or tremor.

Discussion Inhibition of tryptophan hydroxylase by administration of PCPA has been shown to reduce the increase in brain monoamines associated with chronic ethanol administration to mice. However, PCPA does not prevent the increase in mouse brain catecholamines associated with ethanol withdrawal, although it does prevent the similar increase in 5-hydroxytryptamine concentration. This suggests that administration of PCPA in this way can be used to evaluate the relative importance of 5-hydroxytryptamine and catecholamines in the behavioural changes of ethanol withdrawal in mice. The results also indicate that the rise in brain 5-hydroxytryptamine concentration which occurs during ethanol withdrawal is probably a result of increased 5-hydroxytryptamine synthesis.

PCPA pretreatment did not produce any significant change in the locomotor activity, handling convulsions, piloerection, tail lift, or tremor associated with ethanol withdrawal, and so it seems unlikely that brain 5-hydroxytryptamine is directly involved in any of these changes. These results are supported by those of Goldstein (1973), but Collier, Hammond & Schneider (1974) have produced evidence that ethanol withdrawal head twitches in mice may be mediated in part by 5-hydroxytryptamine.

In conclusion, there is obviously a very complex relationship between monoamine neurotransmitters and behavioural aspects of ethanol withdrawal.

Dr Ortiz received a maintenance grant from the Medical Council on Alcoholism. This work has been financed in part by a grant from the Benescience Foundation.

References

COLLIER, H.O.J., HAMMOND, M.D. & SCHNEIDER, C. (1974). Biogenic amines and head twitches in mice during ethanol withdrawal. *Br. J. Pharmac.*, **51**, 310-311.

GOLDSTEIN, D.B. (1973). Alcohol withdrawal reactions

in mice: effects of drugs that modify neurotransmission. *J. Pharmac. exp. Ther.*, **186**, 1-9.

GRIFFITHS, P.J., LITTLETON, J.M. & ORTIZ, A. (1973a). A method for the induction of dependence to ethanol in mice. *Br. J. Pharmac.*, **47**, 669-670P.

GRIFFITHS, P.J., LITTLETON, J.M. & ORTIZ, A. (1973b). Evidence for a role of brain monoamines in ethanol dependence. *Br. J. Pharmac.*, **48**, 354P.

GRIFFITHS, P.J., LITTLETON, J.M. & ORTIZ, A. (1974). Changes in monoamine concentrations in mouse brain associated with ethanol dependence and withdrawal. *Br. J. Pharmac.* (in press).

HAMMOND, M.D. & SCHNEIDER, C. (1973). Behavioural changes induced in mice following termination of ethanol administration. *Br. J. Pharmac.*, **47**, 667P.

(Received April 19, 1974)