Effect of *p*-chlorophenylalanine on brain noradrenaline in mice

SIR,—*p*-Chlorophenylalanine is an effective inhibitor of the biosynthesis of 5-hydroxytryptamine (5-HT). Many investigators have viewed it as a relatively specific tool for studying the relation of 5-HT to behaviour and to various physiological functions (Koe & Weissman, 1966; Jéquier, Lovenberg & Sjoerdsma, 1967). However, we find that *p*-chlorophenylalanine slightly but consistently lowers brain noradrenaline at various dosages and time periods, in both sexes, and in several different strains of mice.

DL-*p*-Chlorophenylalanine was prepared as described by Koe & Weissman (1966) and administered intraperitoneally in 0·2 ml of a 0·9% saline suspension (facilitated by a drop of Tween 80), at pH 1·8 for Jackson Laboratory C_3H mice and at pH 7·0 for all other strains. Controls were injected with 0·9% saline at the same pH as their experimental congeners, and they were alternated with them in all experimental manipulations. Mice were killed by decapitation and their brains removed, weighed and frozen on dry ice within 2 min of death. The brains were homogenized in 0·01 N hydrochloric acid, extracted by the method of Shore & Olin (1958), and analysed for adrenaline by the method of Crout (1961). *p*-Chlorophenylalanine added to tissue homogenates offered no appreciable interfering fluorescence.

The data in Table 1 illustrate reductions in brain noradrenaline at times varying from 2 hr after a single dose to 24 hr after three consecutive daily doses of *p*-chlorophenylalanine in both males and females, and in five strains of mice.

Brain noradrenaline, in the whole brains of male Jackson BALB/C and C_3H mice, and in the telencephalon, diencephalon + mesencephalon, and pons + medulla of male Cumberland C_3H mice, was measured after the mice had been given 360 mg/kg of a neutral suspension of *p*-chlorophenylalanine either 10 min (Jackson) or 15 min (Cumberland) before being killed. There was a

TABLE 1. EFFECT OF *p*-CHLOROPHENYLALANINE ON BRAIN NORADRENALINE IN MICE

Strain dose, and time of death after dose of *p*-chlorophenylalanin	No of analysis	Noradrenaline† ng/g ± s.e.	P <
Dublin DUB/ICR ♂			
Control	32	501 ± 11	
360 mg/kg, 6 hr.	32	464 ± 9	0·001
Cumberland C_3H ♂			
Control	19	446 ± 18	
350 mg/kg, 4·5 hr.	20	413 ± 14	0·06
Jackson C_3H ♂			
Control	10	549 ± 30	
100 mg/kg, 24 hr. × 1	9	496 ± 20	n.s.
× 2	9	488 ± 8	n.s.
300 mg/kg, 24 hr. × 1	8	497 ± 18	n.s.
× 3	9	497 ± 18	n.s.*
Jackson C_3H ♀			
Control	9	543 ± 32	
300 mg/kg, 2 hr.	10	462 ± 20	n.s.
4 hr.	10	448 ± 12	0·05
18 hr.	10	429 ± 21	0·025
Jackson SWR/J ♀			
Control	12	330 ± 11	
115 mg/kg, 3 hr.	12	305 ± 10	0·05
Charles River HaM/ICR ♂			
Telencephalon			
Control	18	409 ± 9	
360 mg/kg	18	396 ± 8	n.s.
Brainstem			
Control	18	515 ± 16	
360 mg/kg	18	489 ± 18	n.s.

* When all dosages and time periods are pooled, Jackson C_3H mice receiving the drug differ significantly from their controls, $P < 0·025$.

† Whole brains of individual mice were analysed except where brain parts are indicated.

significant fall in noradrenaline in the whole brains of Jackson C_3H mice ($P < 0.05$; 15 mice control, 15 inhibitor) and in the pons + medulla of the Cumberland mice ($P < 0.025$; 30 mice control, 30 inhibitor; brain parts of 2 mice were pooled for each analysis). At this time, there were no significant changes in brain 5-HT or dopamine. This immediate effect of *p*-chlorophenylalanine upon brain noradrenaline was, however, paralleled by an effect upon behaviour. There was invariably a marked reduction of exploratory and motor activity, although the animals remained fully capable of coordinated motor activity if disturbed. The extent of this reduction in activity is illustrated by the fact that, in the 15 min experiment, all 30 saline controls, but not one of the 30 drug-injected mice, climbed out of a small (7 in × 3 in × 2½ in deep) refrigerator tray in which they were individually placed during the 15 min period between injection and death. Beyond this, however, the effect upon individual mice was highly variable. Some individuals evidenced mild hyperpnoea and were only moderately responsive to stimuli; others showed tachycardia, mild piloerection, assumed stereotype postures, and became super-sensitive to external stimuli such as blowing or snapping of the fingers. We were unable to predict in advance the response of particular individuals or of groups of mice. The initial behavioural effect usually passed within 30 min and the behaviour of the mice became grossly normal.

Koe & Weissman noted that *p*-chlorophenylalanine had an *in vitro* inhibitory effect against tyrosine hydroxylase about one-fortieth as great as its effect upon tryptophan hydroxylase, that it markedly inhibited phenylalanine hydroxylase, and that it caused a small reduction in brain catecholamines in mice, rats and dogs; however, they discounted this effect as being functionally unimportant compared with the quantitatively greater effect upon brain 5-HT.

We think that the effect which *p*-chlorophenylalanine has upon brain catecholamine levels may be functionally important, and must be considered in the interpretation of any studies made with this drug.

This contention is supported (1) by the small but consistent reduction of brain noradrenaline that is produced by *p*-chlorophenylalanine at various doses and time periods, in both sexes and in several strains of mice; (2) by the immediate reduction of brain noradrenaline, correlated with a behavioural effect, that occurs within 10 min after drug administration, at which time brain 5-HT is not detectably altered; and (3) by observations made in this laboratory that pretreatment of mice with *p*-chlorophenylalanine can prevent stress-induced increases in both brain dopamine and 5-HT and favour stress-induced reductions of brain noradrenaline and dopamine.

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