The effect of D- and L-*p*-chlorophenylalanine on the metabolism of 5-hydroxytryptamine in brain

The mechanism by which *p*-chlorophenylalanine (PCPA) inhibits 5-HT synthesis is not clear (Koe & Weissman, 1966). Jequier, Lovenberg & Sjoerdsma (1967) found it to inhibit cerebral tryptophan 5-hydroxylase irreversibly *in vivo*. The slow onset of depletion of 5-HT after PCPA has suggested that it may act through a metabolite. Accordingly, Gal, Roggeveen & Millard (1970) have shown that PCPA is incorporated into different proteins, including liver phenylalanine 4-hydroxylase and brain proteins with tryptophan 5-hydroxylase activity. This suggested that the selective inhibition of these two enzymes originates from the incorporation of PCPA at an enzymatic site essential for their activity.

L- and D-*p*-Chlorophenylalanine as ethyl ester hydrochlorides were synthesized by one of us (P.D.V., at Laboratory of Organic Chemistry of the Istituto Sieroterapico Milanese).

Male Wistar rats, 180–200 g, were injected intraperitoneally with D- or L-PCPA dissolved in distilled H_2O at pH 6–7 in a volume of 10 ml kg^{-1}. Doses of ethyl ester salts were calculated in terms of amino-acid.

Animals were killed with a guillotine, their brains were quickly removed, frozen in dry ice and stored at −30° until analysed.

Brain 5-HT and 5-HIAA were measured by the method of Curzon & Green (1970).

Groups of rats were injected intraperitoneally with 50, 100 and 200 mg kg^{-1} of D- or L-PCPA, respectively, and killed 24 h later.

Both isomers were equally potent in producing a dose-related depletion of brain 5-HT and 5-HIAA. Values for L-PCPA were for doses of 0, 50, 100 and 200 mg kg^{-1} i.p.: 5-HT—0·51 ± 0·01; 0·46 ± 0·02; 0·36 ± 0·02; 0·28 ± 0·03 and for 5-HIAA 0·61 ± 0·02; 0·44 ± 0·03; 0·40 ± 0·02; 0·25 ± 0·01 (n = 6). Values for D-PCPA were not significantly different.

Table 1 shows the time course of the effect of both isomers (100 ml kg^{-1}) on brain 5-HT and 5-HIAA levels. Brain 5-HT and 5-HIAA levels decreased maximally to about 75% of the control values 72 h after treatment and remained there for more than 144 h.

The increase in brain 5-HT level, normally observed after the MAO inhibitor pargyline, was equally blocked in rats treated with either D- or L-PCPA (100 ml kg^{-1}, i.p.) 72 h previously (control increase 0·4 ± 0·02 μg^{-1}; L-PCPA 0·03 ± 0·01, D-PCPA 0·04 ± 0·01 $\mu g\ g^{-1}$; n = 6).

The close similarity of the effects of the isomers on 5-HT metabolism and their equal effectiveness indicate that both act through a common mechanism in depleting

Table 1. *Brain 5-HT and 5-HIAA levels at different intervals after L- and D-PCPA (100 mg kg^{-1} i.p.).*

Time after treatment	L-PCPA 5-HT	D-PCPA 5-HT	L-PCPA 5-HIAA	D-PCPA 5-HIAA
h	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
0	0·51 ± 0·01		0·61 ± 0·02	
6	0·48 ± 0·01	0·45 ± 0·01	0·48 ± 0·02	0·51 ± 0·03
24	0·35 ± 0·02	0·32 ± 0·03	0·43 ± 0·04	0·40 ± 0·02
72	0·26 ± 0·03	0·22 ± 0·01	0·31 ± 0·02	0·27 ± 0·01
144	0·29 ± 0·02	0·25 ± 0·03	0·36 ± 0·01	0·34 ± 0·03

Each value is the mean ± s.e. of six determinations.

None of the values for L-PCPA is statistically different from the corresponding values for D-PCPA.

brain 5-HT and 5-HIAA and preventing 5-HT accumulation after pargyline. Thus D-PCPA, like its L-isomer, inhibits 5-HT synthesis. As previously shown for the racemic compound (Koe & Weissman, 1966), the depletion of brain 5-HT and 5-HIAA induced by D- or L-PCPA was maximal after a long latency and persisted for several days. These results suggest that the inhibition of 5-HT synthesis by either isomer is an irreversible process and depends on the slow formation of an active metabolite.

Since D-amino acids are not incorporated into proteins (Berg, 1959), the incorporation of D-PCPA into tryptophan hydroxylase is unlikely. A conversion of D-PCPA *in vivo* to L-PCPA is theoretically possible via its deamination by D-amino acid oxidase (Blaschko & Stiven, 1950) to *p*-chlorophenylpyruvic acid followed by transamination of the latter to L-PCPA (Spencer & Brock, 1962).

However, this is difficult to reconcile with the fact D-PCPA decreases the level of 5-HT and 5-HIAA at the same rate, to the same extent, and for the same time as the L-isomer.

These considerations lead to the conclusion that stereoisomerism is not essential for PCPA-induced inhibition of 5-HT synthesis.

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Reduction of food intake by apomorphine: a pimozide-sensitive effect

Some of the central effects of amphetamine are the result of an enhancement of dopaminergic nerve activity (Carlsson, 1970). Apomorphine, a selective stimulant of dopamine receptors (Ernst, 1967; Andén, Rubenson & others, 1967), like amphetamine, is capable of causing stereotyped (Ernst, 1965, 1969) or aggressive (McKenzie & Karpowicz, 1970) behaviour in rodents, hypo- or hyperthermia according to species (Kruk, 1972; Hill & Horita, 1972), and increased motor activity (Randrup & Munkvad, 1968).

We have investigated the reported similarities between dopamine and amphetamine, including the feeding behaviour. While the anorexic effect of amphetamine is well known (Ulrich, 1937; Nathanson, 1937), less is known about the involvement of dopamine in the eating response, although there is evidence of a stimulatory role for brain catecholamines (Booth, 1968; Slangen & Miller, 1969). The effect of direct stimulation of central dopamine receptors by apomorphine was examined on the