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EVIDENCE CONCERNING THE INVOLVEMENT OF 5-HYDROXYTRYPTAMINE IN THE LOCOMOTOR ACTIVITY PRODUCED BY AMPHETAMINE OR TRANYLCYPROMINE PLUS L-DOPA

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1 Pretreatment of rats with *p*-chlorophenylalanine (PCPA; 2 × 200 mg/kg) decreased the concentration of 5-hydroxytryptamine (5-HT) in the brain. It also decreased the locomotor activity produced by tranylcypromine plus L-DOPA administration 24 h after the second dose of PCPA.

2 Pretreatment with *p*-chloroamphetamine, which produced a similar decrease in brain 5-HT concentrations did not decrease the locomotor response to tranylcypromine and L-DOPA.

3 PCPA pretreatment decreased the rise in the concentration of DOPA and dopamine in the brain following tranylcypromine and L-DOPA, suggesting its effect on the dopamine-induced locomotor activity was the result of this drug diminishing dopamine formation in the brain, probably by inhibiting L-DOPA uptake.

4 The locomotor activity produced by tranylcypromine and L-DOPA was not decreased by pretreatment 6 h earlier with disulfiram (400 mg/kg). This argues against the locomotor activity being due to noradrenergic stimulation.

5 PCPA pretreatment did not alter amphetamine-induced stereotypy or the circling behaviour in unilateral nigro-striatal lesioned rats.

Introduction

When rats or mice have been pretreated with a monoamine oxidase (MAO) inhibitor, such as pargyline or tranylcypromine and are subsequently given a loading dose of L-DOPA they display various behavioural changes including enhanced locomotor activity. These effects have been termed the 'dopa potentiation test' (Everett, 1966). The test has been suggested as a useful model for the study of the action of some antidepressants (Everett, 1966).

Enhanced locomotor activity following monoamine oxidase inhibition plus L-DOPA administration is seen if the animals have been pretreated with either of the peptides thyrotropin releasing hormone (TRH) or melanocyte stimulating hormone release inhibitory factor (MIF) (Plotnikoff, Kastin, Anderson & Schally, 1971; Plotnikoff, Prange, Breese, Anderson & Wilson, 1972; Plotnikoff, Prange, Breese & Wilson, 1974; Green & Grahame-Smith, 1974a; Huidobro-Toro, Scotti de Carolis & Longo, 1974).

While it is likely that the behavioural changes seen after tranylcypromine and L-DOPA are partly due to stimulation of dopamine receptors in the brain, the fact that similar behavioural changes are also seen following tranylcypromine and L-tryptophan administration (Grahame-Smith, 1971) led Jacobs (1974) to suggest that some of these behavioural responses are due to increased functional activity of brain 5-hydroxytryptamine (5-HT), possibly as the result of DOPA being decarboxylated in 5-HT neurones. In support of this contention he reported that the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) partly inhibited the tranylcypromine/L-DOPA-induced behavioural changes.

To be able to interpret the findings on the action of peptides on the 'DOPA potentiation test' it is therefore necessary to know whether the behavioural changes are due solely to dopamine stimulation or whether 5-HT is also involved.

Methods

Adult male Sprague-Dawley rats weighing 150–200 g (Anglia Laboratory Animals, Alconbury, Huntingdon) were used in all experiments, except

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those in which animals were lesioned (see below) when rats weighing 300 g were used. All drugs except disulfiram were given by intraperitoneal injection dissolved in 0.9% w/v NaCl solution (saline) or suspended in saline containing 1.0% carboxymethyl cellulose. Control rats were given the vehicle only. Disulfiram was dissolved in the minimum volume of acetone and the control rats received the acetone only. Drugs were obtained from the following sources: tranylcypromine (Smith, Kline & French), L-DOPA, 6-hydroxydopamine, *p*-chlorophenylalanine, *p*-chloroamphetamine, methamphetamine and disulfiram (Sigma Chemical Co.), apomorphine (MacFarlan-Smith).

6-Hydroxydopamine lesions were made in rats anaesthetized with Equithesin (Jensen-Salsbery Labs). They were positioned in a stereotaxic apparatus and injected unilaterally with 6-hydroxydopamine hydrobromide (freshly dissolved in cold saline containing 1 mg/ml ascorbic acid) through a 30-gauge stainless steel needle aimed at the substantia nigra. The co-ordinates of the cannula tips were 2.8, 2.0, 8.0 according to the atlas of Pellegrino & Cushman (1967). Four µl of the 6-hydroxydopamine solution (2 mg base/ml) was injected at a rate of 1 µl/minute.

Brain 5-HT was measured by the method of Curzon & Green (1970) and noradrenaline and dopamine by the method of Chang (1964). L-DOPA was measured by the column chromatographic, fluorimetric technique described by Andén, Engel & Rubenson (1972).

Circling activity was measured in rotometer bowls of white translucent plastic supported on metal rings. Bowls measured 30 cm in diameter and 26 cm in height. Locomotor activity was measured on groups of three animals using LKB Animex activity meters (sensitivity and tuning: 30 µA) as described previously (Grahame-Smith, 1971; Green & Grahame-Smith, 1974b).

Results

Effect of p-*chlorophenylalanine on the locomotor activity following tranylcypromine and* L-*DOPA*

Rats were injected with *p*-chlorophenylalanine (PCPA; 200 mg/kg) or the vehicle on Day 1 and Day 2. Twenty-four hours after the second injection of PCPA both groups were given tranylcypromine (20 mg/kg); L-DOPA (100 mg/kg) was administered after a further 30 minutes.

Following tranylcypromine and L-DOPA the rats displayed irritability, reactivity, squeaking, aggression, forepaw padding, headweaving, tremor, salivation, piloerection, locomotor activity and rearing to other animals.

The animals that had been pretreated with PCPA no longer showed rigidity, forepaw padding or tremor and headweaving and salivation were much decreased. Aggression and rearing to other animals was still sometimes seen. The locomotor activity now more closely resembled that seen after low doses of methamphetamine (1–2 mg/kg) and was much reduced in comparison to control animals (Figure 1).

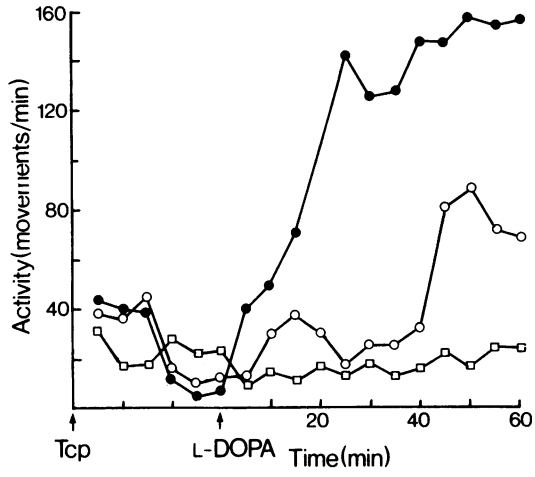

Figure 1 Effect of *p*-chlorophenylalanine (PCPA) on the locomotor activity produced by tranylcypromine and L-DOPA administration. Rats were injected with PCPA (200 mg/kg) or vehicle on Day 1 and Day 2. On Day 3, 24 h after the second injection both groups were injected with tranylcypromine (Tcp; 20 mg/kg) and after a further 30 min with L-DOPA (100 mg/kg). Activity of vehicle-injected group (●), PCPA-treated (○) group and control group given tranylcypromine only (□).

Biochemical measurements showed that at the start of the experiment the brain 5-HT concentration in the PCPA-treated rats was decreased by 75% (Table 1). Noradrenaline concentrations were unaltered but there was a small decrease in dopamine following PCPA.

In rats given tranylcypromine and L-DOPA it was found that 90 min after L-DOPA administration, the PCPA-treated rats had a lower brain dopamine concentration than controls. This was presumably because the L-DOPA concentration in the brain following tranylcypromine and L-DOPA is decreased by pretreatment with PCPA (Table 2).

Effect of p-*chloroamphetamine on the locomotor activity following tranylcypromine and* L-*DOPA*

p-Chloroamphetamine (PCA) inhibits 5-HT synthesis leading to prolonged brain 5-HT depletion (Sanders-Bush, Gallager & Sulser, 1974) although it also has

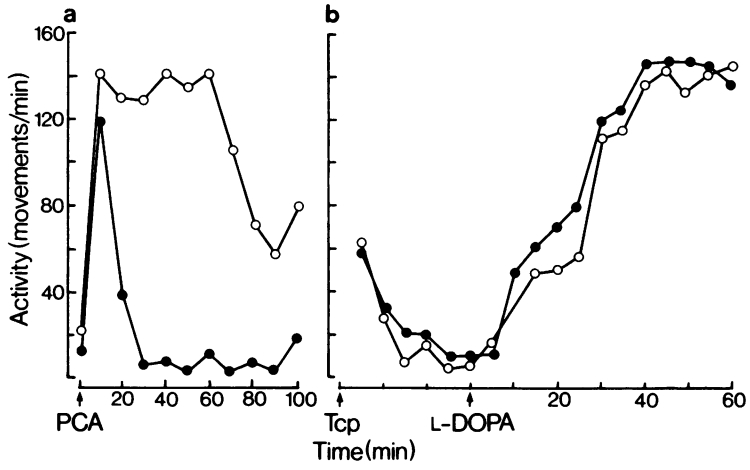

Figure 2 Effect of *p*-chloroamphetamine (PCA) on locomotor activity and activity following tranylcypromine and L-DOPA administration. Rats were injected with saline (●) or PCA, 10 mg/kg (○). (a) Shows effect of these treatments on locomotor activity immediately after injection. (b) Shows locomotor activity when both groups were injected with tranylcypromine (Tcp; 20 mg/kg) followed by L-DOPA (100 mg/kg), 24 h after the PCA injection.

several other actions on brain amine metabolism (see Fuller & Molloy, 1974). It was used in the present study to decrease brain 5-HT and investigate whether this resulted in decreased locomotor activity following tranylcypromine and L-DOPA administration.

Rats were injected with saline or PCA (10 mg/kg). In agreement with previous reports (Frey & Magnussen, 1968; Buus Lassen, 1974) the PCA-treated animals rapidly became hyperactive and behaviourally were identical to rats given tranylcypromine and L-tryptophan (Figure 2a). This effect lasted for over 2 h and will be discussed later. Twenty-four hours after the PCA injection brain 5-HT was greatly decreased, while noradrenaline and dopamine concentrations were unaltered (Table 1). The PCA and saline-injected animals were then given tranylcypromine (20 mg/kg) and after a further 30 min L-DOPA (100 mg/kg). PCA-pretreatment had no effect on the locomotor activity (Figure 2b). Behaviourally they were also very similar to the control animals except that they seemed to be more reactive both to the other rats in the cage, jumping violently when they touched each other, and to outside stimuli such as noise. These features appeared much later after L-DOPA in the control rats. PCA-pretreatment did not alter the increase in dopamine and noradrenaline concentrations, seen after tranylcypromine and L-DOPA administration (Table 2).

Table 1 Effect of *p*-chlorophenylalanine (PCPA) and *p*-chloroamphetamine (PCA) on rat brain 5-hydroxytryptamine (5-HT), dopamine and noradrenaline

	Brain amine concentrations (μg amine/g brain (wet wt.))		
Injected	*5-HT*	*Dopamine*	*Noradrenaline*
Saline	0.51 ± 0.01 (4)	1.46 ± 0.08 (8)	0.24 ± 0.03 (6)
p-Chlorophenylalanine (200 mg/kg × 2)	0.14 ± 0.02 (4)*	1.14 ± 0.11 (4)	0.27 ± 0.02 (6)
p-Chloroamphetamine (10 mg/kg)	0.18 ± 0.02 (6)*	1.27 ± 0.04 (4)	0.28 ± 0.02 (4)

Rats were injected with PCPA 200 mg/kg on Day 1 and 2; 24 h later they were killed for brain amine determinations. PCA (10 mg/kg) was injected 24 h before rats were killed and brain amines determined. Results show mean ± s.e. mean with number of determinations in brackets. *Different from control ($P < 0.001$).

Effect of p-*chlorophenylalanine on activity following methamphetamine*

These results suggested that PCPA altered the tranylcypromine/L-DOPA activity by inhibiting L-DOPA uptake into the brain and not because of alterations in brain 5-HT concentrations. We therefore next examined the effect of PCPA on locomotor activity or circling behaviour. These effects appear to depend on amphetamine-induced release of dopamine (Ungerstedt, 1971; Thornburg & Moore, 1973; Kelly, Seviour & Iversen, 1975; Kelly, 1975) and are independent of L-DOPA uptake.

Rats were injected with vehicle or PCPA (200 mg/kg) on Day 1 and Day 2. Twenty-four hours after the second injection both groups were given methamphetamine (2 mg/kg or 5 mg/kg). The lower dose of methamphetamine produces locomotor stimulation whereas the higher dose results in stereotypy.

PCPA-pretreatment did not alter the locomotor activity produced by 2 mg/kg methamphetamine (total movements over 90 min: controls; 9440 ± 150 (3), experimental; 9500 ± 220 (3)) nor did PCPA alter the degree of stereotypy observed when methamphetamine (5 mg/kg) was given.

The effect of PCPA-pretreatment on the methamphetamine-induced rotational behaviour of unilaterally nigro-striatal lesioned rats was also observed. The lesioned rats were tested at least twice with methamphetamine (5 mg/kg) in control experiments and the number of contralateral turns did not alter significantly. They were then injected with PCPA (200 mg/kg) on two successive days. On the third day, 24 h after the final injection they were again given methamphetamine (5 mg/kg) and circling

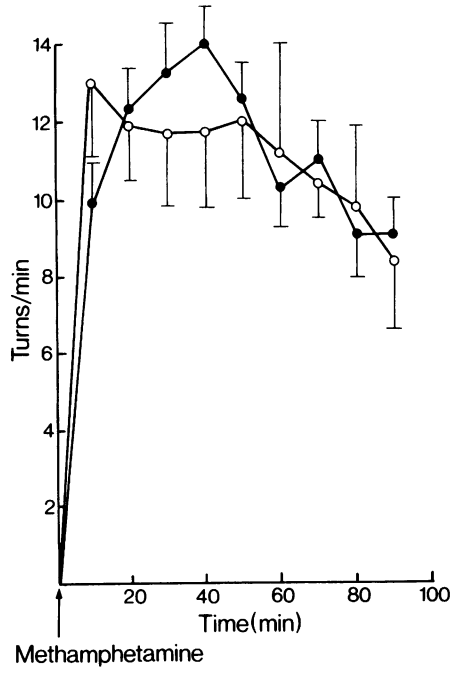

Figure 3 Effect of *p*-chlorophenylalanine (PCPA) on circling behaviour of unilateral nigro-striatal lesioned rats. Lesioned rats (see methods section) were tested at least twice with methamphetamine (5 mg/kg) in control experiments and the number of contralateral turns (which did not alter significantly from test to test) measured (●). They were then injected with PCPA (200 mg/kg) on two successive days. On Day 3 (24 h after the last PCPA injection) they were given methamphetamine (5 mg/kg) and number of turns measured (○). Vertical lines show s.e. mean.

Table 2 Effect of *p*-chlorophenylalanine (PCPA) and *p*-chloroamphetamine (PCA) on rat brain L-DOPA, dopamine and noradrenaline concentrations following subsequent tranylcypromine (Tcp) and L-DOPA administration

	Brain catechol concentrations (μg catechol/g brain (wet wt.))		
Injected	*L-DOPA*	*Dopamine*	*Noradrenaline*
Saline	0.78 ± 0.29 (4)	1.46 ± 0.08 (8)	0.24 ± 0.03 (6)
Tranylcypromine (20 mg/kg) + L-DOPA (100 mg/kg)	55.1 ± 7.4 (4)	11.74 ± 1.29 (7)	0.57 ± 0.05 (7)
PCPA (200 mg/kg × 2) + Tcp (20 mg/kg) + L-DOPA (100 mg/kg)	11.5 ± 4.0 (4)*	4.71 ± 0.61 (8)*	0.49 ± 0.04 (6)
PCA (10 mg/kg) + Tcp (20 mg/kg) + L-DOPA (100 mg/kg)	N.D.	9.10 ± 0.88 (6)	0.53 ± 0.03 (6)

Rats were injected with PCPA or PCA as described in footnote to Table 1. Twenty-four hours after the PCA or second PCPA injection they were injected with tranylcypromine (20 mg/kg) and after a further 30 min with L-DOPA (100 mg/kg). Rats were killed for brain noradrenaline and dopamine determinations 90 min after L-DOPA and after 60 min for L-DOPA determinations. Results show mean ± s.e. mean with number of determinations in brackets. N.D.—not determined.

* Different from tranylcypromine/L-DOPA values $P < 0.001$.

behaviour measured. No difference in the degree of turning was observed following PCPA-pretreatment (Figure 3).

Effect of disulfiram on tranylcypromine/L-DOPA locomotor activity

Because administration of tranylcypromine and L-DOPA results in a significant rise not only of dopamine but also of noradrenaline (Table 2) it seemed possible that some of the activity observed was due to noradrenergic stimulation. This was investigated by inhibiting the conversion of dopamine to noradrenaline with the dopamine β-hydroxylase inhibitor, disulfiram. The dose used has been shown to produce a 70% reduction in brain noradrenaline concentrations (Green & Grahame-Smith, 1974b).

Rats were injected with vehicle or disulfiram (400 mg/kg). Six hours later both groups were given tranylcypromine (20 mg/kg) with L-DOPA (100 mg/kg) after a further 30 minutes. No difference was observed between the activity of the two groups.

Discussion

In agreement with Jacobs (1974) we found that PCPA altered the behaviour following monoamine oxidase inhibition and L-DOPA administration. However, the biochemical results suggest that this effect may not be due to involvement of 5-HT in the behavioural syndrome, but may be an effect of PCPA-pretreatment on L-DOPA uptake and subsequent dopamine formation. The PCPA-treated rats displayed about 40% less activity than the controls and PCPA decreased dopamine concentrations in the brain by a similar value. Furthermore, PCA administration produced a similar decrease in brain 5-HT concentration but did not alter the increased rate of dopamine formation following tranylcypromine/L-DOPA administration and did not inhibit the locomotor activity.

While in the current experiments the last PCPA injection was given 24 h before L-DOPA administration and in the experiments of Jacobs (1974) it was given 72 h before the DOPA, there is evidence that PCPA would still be present in significant amounts in both brain and plasma at this time (Gal, Roggeveen & Millard, 1970). The decreased L-DOPA concentration in the brain may be due to the PCPA competing with this amino acid for uptake. PCPA and tryptophan compete for uptake into synaptosomes (Grahame-Smith & Parfitt, 1970) and the tryptophan concentration in the brain following a load is decreased in PCPA-treated rats (Grahame-Smith, 1971). However, the finding that tryptophan uptake into synaptosomes was decreased if the animals had been injected with PCPA 24 h earlier (Parfitt, 1972) raises the possibility that PCPA in the brain might block subsequent entry of other amino acids.

When dopamine was released from dopaminergic neurones by methamphetamine injection, PCPA-pretreatment failed to alter either the stereotypy in normal rats or circling behaviour in nigro-striatal lesioned animals, in agreement with other reports (Marsden & Guldberg, 1973; Breese, Cooper & Mueller, 1974).

Unlike Breese *et al.* (1974) we were unable to show enhanced locomotor activity following amphetamine in rats pretreated with PCPA. It is possible that this is the result of the different dose of amphetamine used (we used 2 mg/kg while they used 3 mg/kg). However, it should be pointed out that they found potentiation of marginal significance at a dose of 1 mg/kg. Jacobs, Wise & Taylor (1975) concluded on the basis of their studies that while PCPA-pretreatment may result in amphetamine-treated rats displaying higher activity than control animals, the significance is doubtful as the PCPA-injected rats also have greater spontaneous locomotor activity (Fibiger & Campbell, 1971). We feel that our results together with those of Jacobs *et al.* (1975) seriously weaken the concept of an amphetamine-5-HT interaction or a 5-HT involvement in dopamine-induced locomotor activity produced by tranylcypromine/L-DOPA administration. Quock & Horita (1974) observed no alteration in behavioural responses of rabbits given the dopamine agonist apomorphine after depletion of 5-HT by PCPA. It should be noted, however, that raphé lesions have been reported to alter responses to agonists acting on dopamine receptors (Costall & Naylor, 1974).

While Dolphin, Jenner & Marsden (1975) have shown involvement of noradrenaline in L-DOPA reversal of reserpine-induced akinesia, a noradrenergic component of the tranylcypromine/L-DOPA hyperactivity was not demonstrated.

Decarboxylation of 5-hydroxytryptophan (5-HTP) and L-DOPA is performed by the same enzyme (Kuntzman, Shore, Bogdanski & Brodie, 1961). Loading doses of L-DOPA result in catecholamine fluorescence in 5-HT neurones and 5-hydroxytryptophan (5-HTP) loading produces 5-HT fluorescence in catecholamine neurones (Fuxe, Butcher & Engel, 1971; Butcher, Engel & Fuxe, 1972). It is often suggested, therefore, that administration of either of these precursors results in non-specific stimulation. However, the failure of PCA to decrease tranylcypromine/L-DOPA activity and α-methyl *m*-tyrosine to decrease 5-HTP-induced activity (Modigh, 1971) argues against this non-specific decarboxylation necessarily having functional significance.

Finally, we wish to suggest that the hyperactivity following PCA injection (Figure 2a) is not due to an amphetamine-like action of this drug in releasing dopamine as previously suggested (Strada, Sanders-Bush & Sulser, 1970; Strada & Sulser, 1971; Costa, Naimzada & Revuelta, 1971) but the result of 5-HT

receptor stimulation. PCA releases 5-HT (Wong, Horng & Fuller, 1973), is a potent monoamine oxidase inhibitor (Fuller, 1966; Fuller & Hines, 1970) and inhibits 5-HT re-uptake (Carlsson, 1970; Wong *et at.*, 1973). Therefore, when 5-HT is released it is not metabolized intraneuronally and 'spills-over' into the synaptic cleft producing stimulation of 5-HT receptors, explaining why the hyperactivity following PCA is identical to that seen after tranylcypromine/L-tryptophan administration; an observation also made by Jacobs, Mosko & Trulson (1976). It also explains why PCPA abolishes PCA-induced hyperactivity (Buus Lassen, 1974; Jacobs *et at.*, 1976). α-Methyl *p*-tyrosine blocks PCA-induced hyperactivity (Buus Lassen, 1974) and 5-HT-induced hyperactivity is also inhibited by this drug (Green & Grahame-Smith, 1974b).

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