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THE INFLUENCE OF CEREBRAL 5-HYDROXYTRYPTAMINE ON CATALEPSY INDUCED BY BRAIN-AMINE DEPLETING NEUROLEPTICS OR BY CHOLINOMIMETICS

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1 Catalepsy was produced in rats and mice by the subcutaneous injection of either tetrabenazine or the butyrophenone U-32,802A (4'-fluoro-4-{[4-(*p*-fluorophenyl)-3-cyclohexen-1-yl]amino} butyrophenone hydrochloride). Catalepsy was evaluated by the duration of total immobility on a vertical grid.

2 Pretreatment with *p*-chlorophenylalanine (PCPA) reduced the intensity of catalepsy by 50% or more, whereas its time course remained the same.

3 5-Hydroxytryptophan (5-HTP), 10 mg/kg, enhanced the catalepsy induced by U-32,802A or tetrabenazine, provided it was administered soon (45 min) after the neuroleptic; injections at 90 min had no effect. Otherwise untreated rats given this dose of 5-HTP behaved normally on the grid.

4 The anticataleptic effect of PCPA was reversed by 5-HTP.

5 Measurable changes in 5-hydroxytryptamine (5-HT) metabolism in the rat forebrain accompanied the modification of catalepsy by 5-HTP and PCPA.

6 Methysergide (5 mg/kg) given 30 min before the neuroleptics to either mice or rats reduced the catalepsy, assessed 2.5 h after the methysergide. It also prevented the increase in neuroleptic-induced catalepsy following 5-HTP, 10 mg/kg.

7 Tryptophan, like 5-HTP, increased the catalepsy seen in mice after U-32,802A and tetrabenazine, and increased the production of 5-hydroxyindol-3-ylacetic acid in the forebrain.

8 In the rat, intracerebroventricular injection of physostigmine produced catalepsy which was not modified by methysergide or PCPA but was abolished by atropine. Similarly, in the mouse, catalepsy induced by the subcutaneous injection of pilocarpine was abolished by atropine but not affected by either methysergide or 5-HTP.

9 Atropine greatly reduced the catalepsy induced by U-32,802A and tetrabenazine but lowered striatal homovanillic acid (HVA) only after U-32,802A. D,L-DOPA, 20 mg/kg, diminished the cataleptogenic effect of both neuroleptics and raised striatal HVA.

10 The results support the view that there is a facilitating or permissive action of 5-HT-containing neurones on neuroleptic-induced catalepsy.

Introduction

Although catalepsy induced by neuroleptics may be due to a blockade of dopaminergic neurotransmission in the striatum (Maj & Zebrowska, 1966; Hornykiewicz, 1973; Papeschi & Randrup, 1973), other putative neurotransmitters may be involved in the production of this syndrome. Cholinomimetic drugs are capable of producing catalepsy (Feldberg & Sherwood, 1954; Timsit, 1966; Zetler, 1968), and of increasing the cataleptic effect of both neuroleptics and raised striatal HVA.

The participation of 5-hydroxytryptamine (5-HT) in neuroleptic-induced catalepsy was shown by the finding that lesions involving the medial or dorsal raphe nuclei or pre-treatment with *p*-chlorophenylalanine (PCPA) reduced the intensity of catalepsy which follows haloperidol or chlorpromazine (Kostowski, Gumulka & Czlonkowski, 1972; Gumulka, Kostowski & Czlonkowski, 1973). These observations

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were extended by Costall, Fortune, Naylor, Marsden & Pycock (1975), and suggest that a functionally unimpaired 5-HT system is necessary for the cataleptogenic action of neuroleptic drugs. In agreement with this, Maj and co-workers (Maj, Mogilnicka & Przewlocka, 1975; Maj, Sowińska & Baran, 1976) found that administration of the 5-HT receptor blockers cyproheptadine and WA-335 antagonized the catalepsy induced by neuroleptics and also potentiated the anticataleptic effect of dihydroxyphenylalanine (DOPA) and amantadine.

On the other hand, Cerbo, Corchedi & Casacchia (1976) found that the administration of 5-hydroxytryptophan (5-HTP) reduced the cataleptic state produced by haloperidol or reserpine. They also reported that PCPA antagonized the cataleptic effect of haloperidol but not of reserpine and postulated that both increases and reductions in brain 5-HT reduce the cataleptogenic effect of neuroleptics.

These contradictory reports prompted a reinvestigation of the effect of the cerebral 5-HT system in catalepsy produced by two neuroleptic drugs, tetrabenazine and U-32,802A. The latter is the butyrophenone 4′-fluoro-4-{[4-(*p*-fluorophenyl)-3-cyclohexen-1-yl]amino} butyrophenone hydrochloride which has monoamine depleting properties unusual in butyrophenones (Lahti & Lednicer, 1974; Fuenmayor & Vogt, 1979). The compound is cataleptogenic (Fuenmayor & Vogt, 1977), and drugs which reduce the availability of cerebral 5-HT (PCPA and methysergide), as well as compounds which increase it (5-HTP and tryptophan) were administered to rats or mice made cataleptic by U-32,802A or tetrabenazine. Experiments were also carried out on the influence of the 5-HT system on catalepsy induced by intracerebroventricular injection of physostigmine in rats, or by subcutaneous administration of pilocarpine in mice. A few experiments were devoted to the anticataleptic effect of atropine and D,L-DOPA.

Methods

Animals

Adult male albino rats (180 to 260 g), obtained from the breeding colony of the Institute (Wistar-derived strain), and adult male albino mice (Tuck's No. 1) weighing 20 to 30 g were used. The animals were kept in groups of 8 to 15 with free access to food and water. In the rat room white light was on from 05h 00 min to 19 h 00 min and off from 19 h 00 min to 05 h 00 min. The mice were under reversed day-light with white light on from 22 h 00 min to 10 h 00 min and red light on from 10 h 00 min to 22 h 00 min. The experiments were never started before 2 h had elapsed after the change in illumination. In a few experiments, mice were fasted for 20 h before the assessment of catalepsy.

Evaluation of catalepsy and hypotonia

This has been described elsewhere (Fuenmayor & Vogt, 1979); briefly, animals were gently placed on a vertical grid and the time was measured during which the animal kept both head and limbs completely immobile. The intensity of catalepsy was usually assessed by measuring the duration of the longest immobile episode within a 2 min period. If catalepsy was pronounced, observation was continued till the first movement occurred. The longest period of complete immobility encountered was 10 min. Each experiment included animals treated in different ways and, at least one untreated or vehicle-treated animal. The catalepsy data were evaluated by the Mann & Whitney U test (Goldstein, 1964) as it was found that the cataleptic scores were not normally distributed. An animal was considered to be hypotonic if it slipped down the grid.

Chemical estimations

Animals were decapitated, the brains dissected on ice, the parts weighed, homogenized, and the amines 5-HT and dopamine and their metabolites 5-hydroxyindol-3-ylacetic acid (5-HIAA) and homovanillic acid (HVA) estimated fluorimetrically (see Fuenmayor & Vogt, 1979). 5-HT and 5-HIAA were estimated in the 'forebrain', and dopamine and HVA in the striatum. The 'forebrain' was obtained by cutting along the anterior border of the midbrain. The data were subjected to the two-tailed Student's *t* test for non-paired samples.

Drug treatment

U-32,802A (The Upjohn Co.), mol. wt. 361, was dissolved in 0.75% tartaric acid and the pH adjusted to 7 with $NaHCO_3$ immediately before subcutaneous administration. Rats received 2 or 5 mg/kg and mice 2 mg/kg. Tetrabenazine (Roche Products Ltd.) was dissolved in 0.05 N HCl to make a 0.1% solution. The pH was adjusted to 4 which $NaHCO_3$ before subcutaneous injection. The dose for rats was 4 mg/kg, and for mice 10 mg/kg or 15 mg/kg. Physostigmine sulphate (BDH Ltd.) was dissolved in a NaCl solution made up so that the total solute concentration was 0.15 N. Doses of 10, 15 or 20 μg (volume 2.5 μl) were administered into the left lateral ventricle of rats. A 0.15% solution of pilocarpine hydrochloride (BDH Ltd.) in water was injected subcutaneously to mice in a dose of 15 mg/kg. *p*-Chlorophenylalanine (Pfizer & Co., Inc.), 300 mg/kg, was suspended in 2 ml of a 0.5% solution of Tween 80

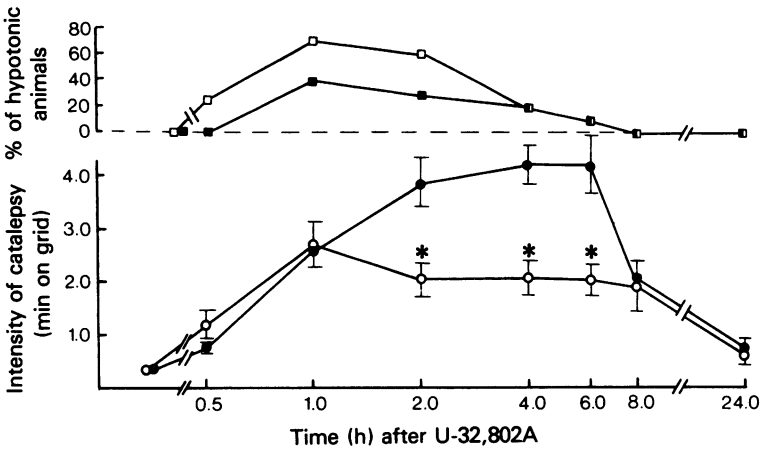

Figure 1 Effect of the administration of *p*-chlorophenylalanine (PCPA) on the catalepsy and hypotonia induced in rats by U-32,802A (5 mg/kg). Catalepsy is assessed by minutes of *total* immobility on the grid. PCPA, (300 mg/kp i.p) was injected 48 h before U-32,802A; vehicle-treated rats received 2 ml 0.5% solution of Tween 80. Open symbols: PCPA-treated; closed symbols: vehicle-treated animals. Symbols are means and vertical bars represent s.e. mean of at least 12 animals. $*P < 0.01$.

and given intraperitoneally to rats. Methysergide bimaleate (Sandoz Products Ltd.) was dissolved in distilled water and 5 mg/kg was injected subcutaneously. 5-Hydroxytryptophan (Roche Products Ltd.), 0.15% solution in water, was administered intraperitoneally to mice in a dose of 15 mg/kg. A 0.3% solution of L-tryptophan was made in distilled water acidified with HCl; the pH was adjusted to 4 before intraperitoneal injection into mice (dose 60 mg/kg). Atropine sulphate (BDH Ltd.) was dissolved in water and 10 mg/kg given subcutaneously. A 0.4% w/v solution of D,L-dihydroxyphenylalanine (Koch-Light Labs. Ltd.) in water acidified by a few drops of 2 N HCl was injected intraperitoneally into rats after adjusting the pH to 7. The dose was 20 mg/kg. Sodium pentobarbitone (May & Baker Ltd.) was dissolved in distilled water and 50 mg/kg injected intraperitoneally.

Implantation of a cannula in the rat lateral ventricle

Rats were anaesthetized with sodium pentobarbitone and the head fixed in a stereotaxic instrument (La Précision Cinématographique, France) in such a way that the upper incisor bar was 5 mm above the interaural line. If necessary, ether was given to maintain the anaesthesia. A hole of 1 mm diameter was made in the left parietal bone, 2 mm lateral to the mid-line and just behind bregma. A cannula, fitted with a stilette and similar to the one described by Myers, Casaday & Holman (1967), was introduced through the hole and its tip aimed at the coordinates 5.4 mm anterior, 2 mm lateral and 3 mm vertical (according to Pellegrino & Cushman, 1967), fixed to the skull with dental cement, and the skin sewn up. After the operation rats were housed individually, and used from the 10th post-operative day once or twice a week during four to five weeks. Once the experiments were completed the animals were killed, their brains fixed in 10% formalin and examined macroscopically. Results were discarded if the cannula tip was not found in the lateral ventricle.

Results

Effect of brain 5-hydroxytryptamine on catalepsy produced by U-32,802A and tetrabenazine

The administration of U-32,802A or tetrabenazine produced a cataleptic state in rats and mice, the duration and intensity of which were dose-dependent. In the rat, pretreatment with PCPA 48 h before U-32,802A, 5 mg/kg, greatly reduced the catalepsy seen 2, 4 and 6 h after the neuroleptic (Figure 1). The rats showed an approximately 50% reduction in the time of immobility on the vertical grid when compared with vehicle (Tween 80)-treated animals. The time of onset and total duration of catalepsy were not modified by PCPA. Figure 1 also shows that the catalepsy was accompanied by hypotonia in a proportion of both Tween 80 and PCPA-treated rats, and was more frequent after PCPA than after the vehicle. However, the difference between the percentage of hypotonic animals in the two groups was significant

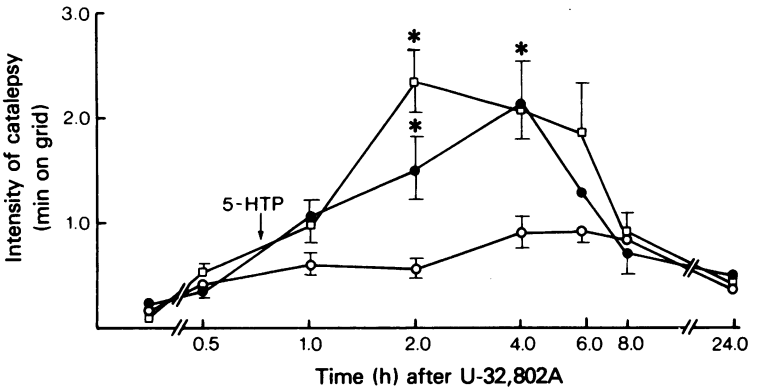

Figure 2 Effect of i.p. administration of 5-hydroxytryptophan (5-HTP) on the anticataleptic effect of *p*-chlorophenylalanine (PCPA) in rats. PCPA (300 mg/kg) was injected 48 h before U-32,802A (2 mg/kg), and 5-HTP (10 mg/kg) was given 45 min after that drug. (●) Vehicle-treated; (○) PCPA-treated; (□) PCPA plus 5-HTP treated animals. Symbols are means and vertical bars represent s.e. mean of at least 12 animals. $*P < 0.01$ vs PCPA-treated group.

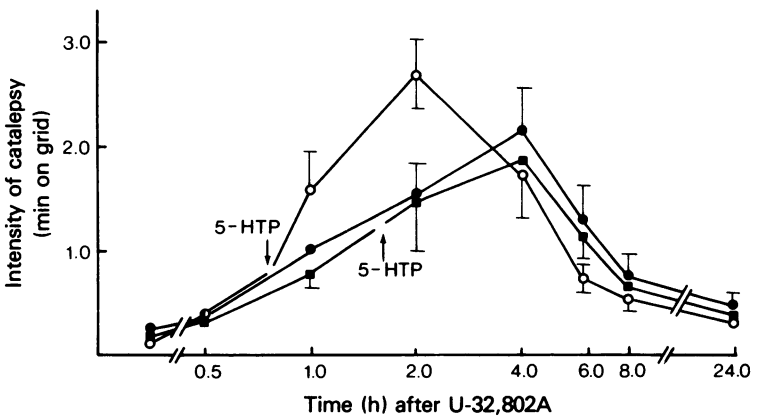

Figure 3 Effect of 5-hydroxytyrptophan (5-HTP) (10 mg/kg, i.p.,) on the catalepsy induced in rats by U-32,802A (2 mg/kg). 5-HTP enhanced the catalepsy when given 45 min, but not when given 90 min after U-32,802A. (●) Vehicle-treated rats; (○) 5-HTP at 45 min; (■) 5-HTP at 90 min. Symbols are means and vertical bars s.e. mean of at least 10 rats. $*P < 0.01$.

only at 0.5 h after the injection of U-32,802A. Hypotonia may interfere with the evaluation of catalepsy because it may reduce the time animals remain immobile on the vertical grid. Although this may have contributed to the reduction in catalepsy seen in rats treated with PCPA, the results cannot have been seriously affected by the hypotonia since the time course of its occurrence, did not coincide with the time course of the reduction in catalepsy by PCPA. Thus, at 4 and 6 h, the inhibition of catalepsy by pretreating the rats with PCPA was at its maximum, while the occurrence of hypotonia was not only infrequent but equal in the PCPA and the vehicle-treated group. Furthermore, at 0.5 and 1 h after U-32,802A, the catalepsy was of the same magnitude in both groups although the hypotonia was greater in the group treated with PCPA.

The catalepsy produced by 2 mg/kg of U-32,802A was also appreciably reduced in rats pretreated with PCPA (Figure 2). This was so in spite of the fact that hypotonia was almost completely absent after the lower dose. The onset and duration of the syndrome was again not modified by PCPA. PCPA on its own did not affect the normal behaviour of the rats on the vertical grid. When PCPA was injected only 24 h before the administration of U-32,802A, the intensity of catalepsy was not reduced although 64.3% of the rats showed hypotonia. Catalepsy after

tetrabenazine was also diminished in rats treated 48 h previously with PCPA; the effect was seen 1, 2 and 4 h after the tetrabenazine, while onset and duration of catalepsy were unaltered by PCPA.

The intraperitoneal administration of 5-HTP, 10 mg/kg, 45 min after the injection of U-32,802A, produced an increase in the intensity of catalepsy which reached a significant level 75 min later, at 2 h after the butyrophenone injection (Figure 3). The potentiation of catalepsy by this small dose of 5-HTP was transient and had completely disappeared 2 h later. When 5-HTP was injected 90 min after U-32,802A, there was no modification of the cataleptogenic effect of the drug (Figure 3). Thus, if its administration is carried out when the cataleptic effect is fully developed, no potentiation ensues. Similar results were obtained when 5-HTP was injected after tetrabenazine. The amino acid, given 45 min after the drug, potentiated catalepsy measured 75 min later, and the effect had subsided after another 2 h; when 5-HTP was given 90 min after tetrabenazine, there was no modification of the catelepsy. Rats treated with 5-HTP only did not differ from normal animals in their behaviour on the grid.

Table 1 Effects of methysergide and 5-hydroxytryptophan (5-HTP) on the intensity of catalepsy induced in mice by U-32,802A or tetrabenazine injected 2 h before the test

		Additional drug treatment		
	None	*Methysergide (5 mg/kg)*	*5-HTP (10 mg/kg)*	*Methysergide + 5-HTP*
U-32,802A	1.63 ± 0.23	0.98 ± 0.14*	2.93 ± 0.57*	1.30 ± 0.21**
2 mg/kg, 2 h	(13)	(14)	(16)	(15)
Tetrabenazine	0.93 ± 0.07	—	1.77 ± 0.53*	0.84 ± 0.13**
10 mg/kg, 2 h	(14)		(15)	(14)
Tetrabenazine	2.13 ± 0.37	1.17 ± 0.10*	—	—
15 mg/kg, 2 h	(18)	(16)		

Values for catalepsy are expressed as means ± s.e. mean of the duration (in min) of complete immobility on the grid. Number of animals indicated in parentheses. Methysergide was injected 30 min before the neuroleptic, and 5-HTP 45 min after U-32, 802A and 15 min after tetrabenazine. Mice were kept in reversed daylight for at least one week before the experiment. *$P < 0.025$ versus neuroleptic alone; **$P < 0.025$ versus neuroleptic + 5-HTP.

Table 2 Effects, in mice, of intraperitoneal injection of L-tryptophan on the intensity of catalepsy and the concentration of brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid (5-HIAA) following administration of U-32, 802A or tetrabenazine

	Catalepsy (min on the grid)	*5-HT (μg/g)*	*5-HIAA (μg/g)*
Control	0.06 ± 0.01	0.57 ± 0.03	0.35 ± 0.01
	(18)	(7)	(6)
U-32, 802A	2.15 ± 0.44	0.22 ± 0.01	0.49 ± 0.02
2 mg/kg, 2 h	(11)	(6)	(6)
U-32, 802A	3.25 ± 0.48*	0.24 ± 0.02	0.68 ± 0.03*
+ tryptophan	(12)	(6)	(6)
Tetrabenazine	0.91 ± 0.13	0.36 ± 0.03	0.49 ± 0.03
10 mg/kg, 2 h	(13)	(5)	(5)
Tetrabenazine	1.42 ± 0.20*	0.36 ± 0.01	0.86 ± 0.11*
+ tryptophan	(11)	(5)	(5)

Tryptophan, 60 mg/kg, was given (i.p.) 30 min after the neuroleptic. Grid test or killing for chemical analyses followed 90 min later.
Mice were kept in reversed daylight for at least one week and fasted for 20 h before the experiment.
Values for catalepsy are means ± s.e. mean of min spent on the grid in total immobility. Number of mice in parentheses. *$P < 0.02$ versus neuroleptic alone.

The injection of 5-HTP, 10 mg/kg, 45 min after either U-32,802A or tetrabenazine, reversed the reduction in catalepsy produced by PCPA. Figure 2 shows the effect for U-32,802A.

The 5-HT-receptor blocker methysergide, (5 mg/kg) when given 30 min before either U-32,802A or tetrabenazine, reduced by 43 and 68% the cataleptic effect of these drugs assessed 2 h after their administration.

The effect of methysergide, too, was reversed by 5-HTP, 10 mg/kg. The experiments (Table 1) were carried out on mice which, as also seen in Table 1, responded to the butyrophenone, tetrabenazine and their combination with methysergide in the same way as rats.

Since L-tryptophan is considered to be a more specific precursor of 5-HT than 5-HTP, another group of mice was given tryptophan 30 min after the butyrophenone or tetrabenazine (Table 2); this also caused an increase in catalepsy. All mice used in this experiment were fasted for 20 h before the experiment in order to avoid possible competition for entry into the brain between dietary amino acids and tryptophan. The catalepsy induced by both neuroleptics was of the same magnitude in fasted and fed mice.

Biochemical changes

Table 3 illustrates the effects in rats of U-32,802A and tetrabenazine on cerebral dopamine, 5-HT and their metabolites, and the changes produced by treatment with 5-HTP. It shows the fall in concentrations of dopamine and 5-HT and the rise in the metabolites caused by the neuroleptics, and the increases in 5-HIAA content of the forebrain 45 min after the injection of 5-HTP. Furthermore, it can be seen that there is a significantly greater fall in the cerebral 5-HT content when PCPA has been allowed to act for 2 days instead of one. Nevertheless, 48 h after PCPA, 5-HTP increased the turnover of 5-HT as seen by a four fold increase in the concentration of 5-HIAA.

The effect of tryptophan on the concentration of 5-HT and 5-HIAA in the forebrain of mice is shown in Table 2; the amino acid did not modify the depletion of 5-HT caused by either drug, but augmented the increase in 5-HIAA induced by the butyrophenone and tetrabenazine.

Effect of brain 5-hydroxytryptamine on catalepsy produced by cholinomimetics

Experiments were done on rats by injecting physostigmine into the cerebral ventricles, thus restricting its effect to the brain. A slight but dose-related cataleptic state was produced which was maximal 15 min after the injection (Table 4). Intraventricular administration of saline was without effect on the behaviour of the rats on the grid. Pretreatment with methysergide or with PCPA failed to modify the cataleptic effect of 15 or 20 μg of physostigmine, whereas atropine abolished it (Table 4). A characteristic effect of the intraventricular injection of physostigmine was the production within 3 min of compulsive drinking

Table 3 Brain amines and their metabolites in rats treated with neuroleptics or *p*-chlorophenylalanine (PCPA), and the effect of a combination of these drugs with 5-hydroxytryptophan (5-HTP) on the forebrain content of 5-hydroxyindol-3-ylacetic acid (5-HIAA)

	No 5-HTP				*5-HTP 10 mg/kg*
	DA (striatum) (μg/g)	*HVA (striatum) (μg/g)*	*5-HT (forebrain) (μg/g)*	*5-HIAA (forebrain) (μg/g)*	*5-HIAA (forebrain) (μg/g)*
Control	9.60 ± 0.55 (14)	0.74 ± 0.03 (16)	0.43 ± 0.02 (14)	0.44 ± 0.04 (14)	0.57 ± 0.02 (5)
U-32, 802A 2 mg/kg, 2 h	1.97 ± 0.18* (10)	2.05 ± 0.20* (8)	0.24 ± 0.01* (8)	0.54 ± 0.02* (8)	1.05 ± 0.20** (4)
Tetrabenazine 4 mg/kg, 2 h	1.27 ± 0.15* (7)	2.54 ± 0.17* (10)	0.21 ± 0.02* (6)	0.72 ± 0.03* (5)	1.34 ± 0.08** (4)
PCPA 300 mg/kg, 24 h	ND	ND	0.16 ± 0.01* (7)	0.12 ± 0.01* (7)	ND
PCPA 300 mg/kg, 48 h	9.78 ± 0.46 (10)	0.55 ± 0.03* (8)	0.07 ± 0.01*** (6)	0.07 ± 0.01*** (6)	0.28 ± 0.01** (6)

Values are means ± s.e. mean. Number of estimations indicated in parentheses. Time of killing after administration of first drug indicated in column 1. 5-HTP was injected 45 min before killing. *$P < 0.01$ versus control; **$P < 0.01$ versus drug without 5-HTP; ***$P < 0.01$ versus PCPA 24 h. ND = not determined.

which lasted for about 10 min. This response was present in 85% of the rats injected and was also abolished by atropine but not by PCPA or methysergide.

In the mouse, the catalepsy induced by subcutaneous injection of pilocarpine was not modified either by methysergide or by the administration of 5-HTP. In contrast, atropine, given 15 min before the cholinomimetic, prevented its cataleptic effect.

Effect of cholinolytics and DOPA on neuroleptic catalepsy

Atropine reduced by 72% the catalepsy induced in the rat by U-32,802A injected 15 min earlier, and greatly diminished the increase in striatal HVA produced by the butyrophenone (Table 5). Atropine was also a potent antagonist of the catalepsy induced by

Table 4 Effects of *p*-chlorophenylalanine (PCPA), methysergide or atropine on the intensity of catalepsy induced in rats by the injection of physostigmine into the left lateral ventricle

	15 min	*30 min*	*60 min*
Physostigmine (15 μg)	0.45 ± 0.06 (8)	0.35 ± 0.04 (8)	0.18 ± 0.02 (8)
Methysergide + physostigmine (15 μg)	0.40 ± 0.04 (8)	0.28 ± 0.03 (8)	0.18 ± 0.01 (8)
Physostigmine (20 μg)	0.62 ± 0.05 (9)	0.36 ± 0.04 (8)	0.20 ± 0.03 (9)
Methysergide + physostigmine (20 μg)	0.66 ± 0.12 (7)	0.43 ± 0.06 (7)	0.22 ± 0.04 (7)
PCPA + physostigmine (20 μg)	0.63 ± 0.15 (8)	0.36 ± 0.04 (8)	0.20 ± 0.03 (8)
Atropine + physostigmine (20 μg)	0.16 ± 0.02* (7)	0.12 ± 0.02* (7)	0.13 ± 0.04 (7)

PCPA, 300 mg/kg, was given 48 h, methysergide bimaleate, 5 mg/kg, 30 min, and atropine sulphate, 10 mg/kg, 15 min before physostigmine sulphate. The grid test was carried out at the times indicated and the values (means ± s.e. mean) are expressed in min of immobility. Number of animals in parentheses. $*P < 0.01$ versus physostigmine, 20 μg.

Table 5 Effects of D,L-DOPA or atropine on the intensity of catalepsy and the increase in striatal homovanillic acid (HVA) induced in the rat by U-32, 802A or tetrabenazine

	Catalepsy (min on the grid)	*HVA (μg/g)*	*HVA (μg/g) in the absence of a neuroleptic*	
U-32, 802A 2 mg/kg, 2 h	1.38 ± 0.20 (15)	2.05 ± 0.20 (8)	No drug	0.74 ± 0.04 (14)
U-32, 802A + DOPA	0.76 ± 0.09** (17)	2.87 ± 0.14* (6)	D,L-DOPA	1.04 ± 0.06 (5)
U-32, 802A + atropine	0.39 ± 0.08** (10)	1.26 ± 0.17**	Atropine	0.71 ± 0.05 (8)
Tetrabenazine 4 mg/kg, 2 h	2.86 ± 0.49 (9)	2.54 ± 0.17 (10)		
Tetrabenazine + DOPA	1.50 ± 0.34** (9)	3.75 ± 0.23* (8)		
Tetrabenazine + atropine	0.52 ± 0.08** (8)	2.39 ± 0.15 (8)		

Values are means ± s.e. mean. D,L-DOPA (20 mg/kg) was given i.p. 45 min after, or atropine sulphate (10 mg/kg) injected s.c. 15 min before the neuroleptic. Rats were tested or their brains analysed 2 h after injection of the neuroleptic. Number of animals in parentheses. $*P < 0.02$ and $**P < 0.01$ versus neuroleptic alone.

tetrabenazine, but did not appreciably modify the accompanying increase in striatal HVA (Table 5). Atropine alone did not change the content of HVA in the striatum.

The same table shows the effect of D,L-DOPA on the catalepsy and on the rise in striatal HVA concentration induced by the two neuroleptics. When DOPA was administered in a dose of 20 mg/kg 45 min after either drug, it reduced catalepsy by about 45% and further augmented the increase in HVA. DOPA, when given alone, produced a small increase in the striatal concentration of HVA (Table 5).

Discussion

In agreement with earlier observations on the anticataleptic effect of PCPA (Kostowsky *et al.*, 1972; Gumulka *et al.*, 1973), catalepsy after U-32,802A or tetrabenazine was reduced in rats treated with PCPA, provided they were tested at a time when the depletion of 5-HT was maximal. Since no reduction of catalepsy was obtained in rats tested only 24 h after PCPA, when loss of 5-HT had not yet reached its peak, it is most unlikely that the anticataleptic action of PCPA is related to an unspecific action of PCPA rather than the loss of 5-HT. This view is supported by the fact that the effect of PCPA was reversed by 5-HTP. All these observations suggest a participation of the 5-HT containing neurones in neuroleptic catalepsy. In the absence of PCPA, 5-HTP also increased catalepsy and this result is at variance with the results reported by Cerbo *et al.* (1976). These authors found a reduction, in rats pretreated with 5-HTP, of the catalepsy induced by haloperidol or reserpine. However, Cerbo *et al.* (1976), administered a high dose (100 mg/kg) of the amino acid and treated the animals with an inhibitor of the peripheral amino acid decarboxylase. Under their experimental conditions, the 5-HTP might have been taken up and decarboxylated to 5-HT in dopaminergic and noradrenergic neurones in addition to 5-HT neurones. The 5-HT so formed might have been releasing dopamine which in turn might have reduced catalepsy. Furthermore, any effect of the decarboxylase inhibitor on its own on the catalepsy by haloperidol or reserpine might have complicated the results.

The experiments in which the 5-HT receptor blocker, methysergide, was shown to reduce catalepsy agree with the findings of an anticataleptic action of other 5-HT receptor blockers, cyproheptadine (Maj *et al.*, 1975) and WA-335 (Maj *et al.*, 1976). Although the effect of these compounds was thought to be related to their 5-HT receptor blocking action, both drugs are known to have antihistaminic as well as anticholinoceptor activities, and the latter property might well contribute to their anticataleptic activity. Methysergide is probably the most specific blocker of 5-HT receptors currently available and its action can hardly be attributed to anticholinoceptor or antihistaminic properties. Recent work (Carter & Pycock, 1978) has localized at least some of the tryptaminergic neurones involved in the production of catalepsy by the neuroleptic fluphenazine. By injecting 5,7-dihydroxytryptamine the authors caused a localized loss of 5-HT in the nucleus accumbens or in the substantia nigra, and this was accompanied by a reduction in catalepsy not observed when the injections had been made in the tuberculum olfactorium or the striatum.

The fact that the administration of tryptophan enhances the catalepsy induced by neuroleptics is another finding suggesting a supporting role of the tryptaminergic system in this condition. Whereas the administration of 5-HTP might be followed by an increased synthesis of 5-HT not only in tryptaminergic neurones, but in any neurone containing dopa-decarboxylase, and the 5-HT so formed might act on nerve cells which are not normally under its influence, the administration of tryptophan increases the 5-HT content only in tryptaminergic neurones. Thus, the potentiation of catalepsy obtained by tryptophan is convincing evidence that the activity of tryptaminergic neurones facilitates the catalepsy induced by neuroleptics. That the dose of tryptophan used was effective in elevating the synthesis and release of brain 5-HT is shown by the increase in 5-HIAA found in the mouse forebrain after tryptophan administration. This finding also demonstrated that the synthesis of 5-HT was not blocked by either U-32, 802A or tetrabenazine. In a recent paper (Tachiki, Takagi, Tateischi, Kido, Nishiwaki, Nakamura, Nagayama & Takahashi, 1978) it was shown that the 'sedation', measured by the loss in locomotor activity of rats given tetrabenazine, was inhibited by PCPA, as here described for catalepsy.

Neither the anticataleptic effect of 5-HT antagonists nor the facilitating influence of 5-HTP were seen in the catalepsy induced by cholinomimetics. This was found for both the catalepsy following pilocarpine given systemically and physostigmine injected intraventricularly. These results agree with the report that cyproheptadine and WA-335 did not modify the catalepsy induced in rats by the systemic injection of physostigmine (Maj *et al.*, 1976). The lack of effect of the tryptaminergic system on the catalepsy following cholinomimetics excludes an interaction of brain 5-HT and cholinergic neurones as a cause of the influence of the 5-HT system on neuroleptic catalepsy. Furthermore, these results are also hardly compatible with the view that unspecific stimulatory or inhibitory actions of PCPA, methysergide or 5-HTP could be determining their actions in neuroleptic catalepsy.

The involvement of dopaminergic and cholinergic mechanisms in the cataleptogenic effect of U-32,802A

and of tetrabenazine is shown by the reduction in catalepsy obtained after treatment with D,L-DOPA or with atropine. The administration of D,L-DOPA has been shown to reduce catalepsy after other neuroleptics but only when doses of 200 mg/kg were given to rats or mice (Maj & Zebowska, 1966). A similar dose of L-DOPA was found to be anticataleptic against α-methyl-*p*-tyrosine (Papeschi & Randrup, 1973). In the present study a large reduction in catalepsy was obtained with a small fraction of that dose. It is possible that the difference is due to the method of assessing catalepsy: both groups of workers who used large doses limited their observation times of the cataleptic animals to periods of 1 min.

The well-known antagonism of antimuscarinic drugs against catalepsy induced by neuroleptics was confirmed for U-32, 802A. This is in contrast to the potentiation by atropine of the catalepsy produced by analgesics (Ahtee, 1976; 1978). Atropine also diminished the increase of striatal HVA caused by U-32, 802A, as it was shown (O'Keefe, Sharman & Vogt, 1970) to reduce the elevation in HVA elicited by chlorpromazine and haloperidol. On the other hand, atropine did not modify the HVA increase induced by tetrabenazine, thus indicating a difference in the mechanism of action between U-32, 802A and tetrabenazine.

Our results point to a facilitating, (permissive?) action of the cerebral 5-HT system on the catalepsy caused by malfunction of the nigrostriatal dopaminergic neurones. A modulatory action of brain 5-HT on other motor activities has been found for stereotype and rotatory behaviour. Thus, administration of 5-HTP inhibits, while administration of methysergide increases, the production of stereotypies by both amphetamine and apomorphine, the one acting pre-, the other postsynaptically. The circling behaviour produced by apomorphine in rats lesioned in one substantia nigra is increased by treatment with methysergide or PCPA, and diminished by the injection of 5-HTP or tryptophan (Baldessarini, Amatruda, Griffith & Gerson, 1975; Milson & Pycock, 1976). The findings on catalepsy, stereotyped behaviour and rotatory behaviour support the hypothesis that ascending 5-HT neurones modulate the output of the nigrostriatal dopaminergic neurones.

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