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EFFECTS OF DRUGS ACTING ON CEREBRAL 5-HYDROXYTRYPTAMINE MECHANISMS ON DOPAMINE-DEPENDENT TURNING BEHAVIOUR IN MICE

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1 The effects of drugs acting on cerebral 5-hydroxytryptaminergic mechanisms on drug-induced turning behaviour in mice with unilateral destruction of nigro-striatal dopaminergic nerve terminals have been studied.

2 Administration of L-tryptophan (400 mg/kg) or 5-hydroxytryptophan (200 mg/kg) increased brain 5-hydroxytryptamine and decreased the turning induced by both apomorphine (2 mg/kg) and amphetamine (5 mg/kg).

3 Parachlorophenylalanine (3 x 500 mg/kg) decreased brain 5-hydroxytryptamine and increased both apomorphine and amphetamine-induced circling behaviour.

4 Varying the protein content of dietary intake significantly altered brain 5-hydroxytryptamine and tryptophan levels, spontaneous locomotor activity and amphetamine-induced circling behaviour in these mice.

5 Systemic administration of methysergide (0.5–4 mg/kg), lysergic acid diethylamide (0.025–0.2 mg/kg), cyproheptadine (2.5–20 mg/kg) or clomipramine (0.6–20 mg/kg) produced no consistent effect on drug-induced turning behaviour.

6 The results suggest that circling behaviour due to striatal dopamine receptor stimulation is depressed by an elevation of brain 5-hydroxytryptamine and enhanced by a reduction in brain 5-hydroxytryptamine.

7 The possible physiological relationship between dopamine and 5-hydroxytryptamine neurones in the basal ganglia is discussed.

Introduction

Recent behavioural studies have stressed an interrelation between 5-hydroxytryptamine and the catecholamine transmitters in the brain (Hadžović & Ernst, 1969; Modigh, 1973a, 1974; Weiner, Goetz, Westheimer & Klawans, 1973; Jacobs, 1974; Jacobs, Eubanks & Wise, 1974). In rodents, an increase in dopamine receptor activity in one striatum as compared with the other causes the animal to turn in circles away from the side of higher activity (Andén, Dahlström, Fuxe & Larsson, 1966; Ungerstedt, 1971). In rats with unilateral nigro-striatal degeneration, but intact striatal dopamine receptors, directly acting dopamine agonists, such as apomorphine, induce circling away from the side of the lesion, because, it is suggested, the denervated receptors have become supersensitive (Ungerstedt, 1971). Indirectly acting dopamine agonists, such as amphetamine, cause release of dopamine from the nigro-striatal terminals of the intact side and cause the animal to circle towards the damaged side.

The basal ganglia is an area of the brain also rich in terminals of 5-hydroxytryptamine neurones (Fuxe, Hökfelt & Ungerstedt, 1968; Bak, Choi, Hassler, Usunoff & Wagner, 1975). The close anatomical relation between these two transmitter systems suggests a functional dependence; indeed 5-hydroxytryptamine has been shown to activate the release of dopamine in the rat striatum (Besson, Cheramy, Feltz & Glowinski, 1969) while dopamine receptor stimulation increases cerebral 5-hydroxytryptamine turnover (Grabowska, Antkiewicz, Maj & Michaluk, 1973).

In this study we have attempted to investigate the modulating role 5-hydroxytryptamine may exert on striatal dopamine function using the rotating mouse model of von Voigtlander & Moore (1973), a system

that directly involves the dopaminergic neurones of the striatum. The effect of administering precursors of 5-hydroxytryptamine such as tryptophan and 5-hydroxytryptophan, of *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase, and agents believed to block the 5-hydroxytryptamine receptor, has been studied on amphetamine and apomorphine-induced turning behaviour in mice with unilateral lesions of the nigro-striatal dopamine terminals.

Methods

Animals

Unilateral destruction of nigrostriatal dopamine terminals in male Swiss S strain mice was achieved by direct injection of 6-hydroxydopamine in a dose of 16 μg dissolved in 4 μl 0.9% w/v NaCl solution (saline) containing 0.2 mg/ml ascorbic acid, into the right striatum of a mouse under ether anaesthesia. The technique used was that described by von Voigtlander & Moore (1973), as modified by Pycock, Tarsy & Marsden (1976) for free hand injection. Mice were tested 2 days after operation with dexamphetamine sulphate (5 mg/kg) and those animals turning tightly towards the lesioned side were selected and tested at 10 days with apomorphine hydrochloride (2 mg/kg). Mice now turning away from the side of the lesion were selected for the following experiments.

Behavioural testing

Three groups of 20 mice were used to investigate the effect of systemic administration of 5-hydroxytryptophan (200 mg/kg), L-tryptophan (400 mg/kg) and *p*-chlorophenylalanine methyl ester (3 × 500 mg/kg) on amphetamine and apomorphine-induced circling behaviour. Ten mice from each group were randomly selected, marked and tested with apomorphine while the remainder were marked and tested with amphetamine. This enabled control turning rates of each group of mice to be determined. This procedure was repeated after 48 h when all control rates were found to be within 10% of those obtained previously. On the following day the 20-mouse groups were injected intraperitoneally with 5-hydroxytryptophan or L-tryptophan. The third group was injected three times at 24 hourly intervals with *p*-chlorophenylalanine. Amphetamine and apomorphine was administered to appropriate mice 30 min after 5-hydroxytryptophan or L-tryptophan and 16 h after the final injection of *p*-chlorophenylalanine. After injection of stimulant, all mice were placed in individual plastic boxes, measuring 12 × 12 × 8 cm. The number of complete revolutions made during a one-minute period was recorded 15 min after apomorphine (2 mg/kg) and 30 min after amphetamine (5 mg/kg). The number of counts in one minute was recorded for each group of mice and compared to the control values.

A further hour after counting, 10 mice from each of the three groups were randomly selected and killed, together with a group of 10 control animals, in order to determine brain 5-hydroxytryptamine levels.

For other pharmacological manipulations, groups of 20 mice were marked into subgroups of four for an incomplete Latin square design in order to randomize the distribution of drugs. Mice were tested on three separate days so that 12 observations were accumulated for each dose of drug. A saline-injected control group was run on each occasion. Methysergide, in a dose range 0.5–4 mg/kg and cyproheptadine, in a dose range 2.5–20 mg/kg, were administered 15 min before amphetamine and 30 min before apomorphine. Lysergic acid diethylamide (LSD) was administered in a dose range 25–200 μg/kg 5 min before amphetamine and 20 min before apomorphine.

In other experiments the effect of clomipramine in a dose range 0.6–20 mg/kg was investigated on submaximal turning in mice induced by 1.5 mg/kg amphetamine. As before an incomplete Latin square design was used, and animals were tested on three separate days. Clomipramine was administered intraperitoneally 30 min before amphetamine.

In a final series of experiments, the effect of a varying diet on the rate of turning induced by amphetamine was studied. Twenty mice were fed for two weeks on laboratory pellets (Dixons, Diet 41B). Throughout these experiments water was available *ad lib.* At the end of this period the mice were tested on three separate days with amphetamine (5 mg/kg) and divided into two equal groups so that the net turning rates of each group were the same. One group was fed high-protein (25%) pellets (Oxoid, breeding diet) while the other received a low protein diet composed of apple and potato. The groups were then tested weekly with amphetamine. Prior to amphetamine testing in the third week, spontaneous activity of the two groups was recorded in an Animex Activity recorder (LKB Farad Electronics). Following this third amphetamine testing the diet of the two groups was reversed, so that the low protein diet animals were now fed high protein pellets and *vice versa*. Animals were tested again at weekly intervals with amphetamine, and locomotor activity was recorded during the third week. At the end of this period mice from both diet groups were fed their normal diet of laboratory pellets (Diet 41B) and after one week the turning rate induced by 5 mg/kg amphetamine was observed. To complete the experiment, the 20 mice were randomly split into two groups again, fed the high and low protein diets for two weeks and then killed for brain biogenic amine and tryptophan estimation.

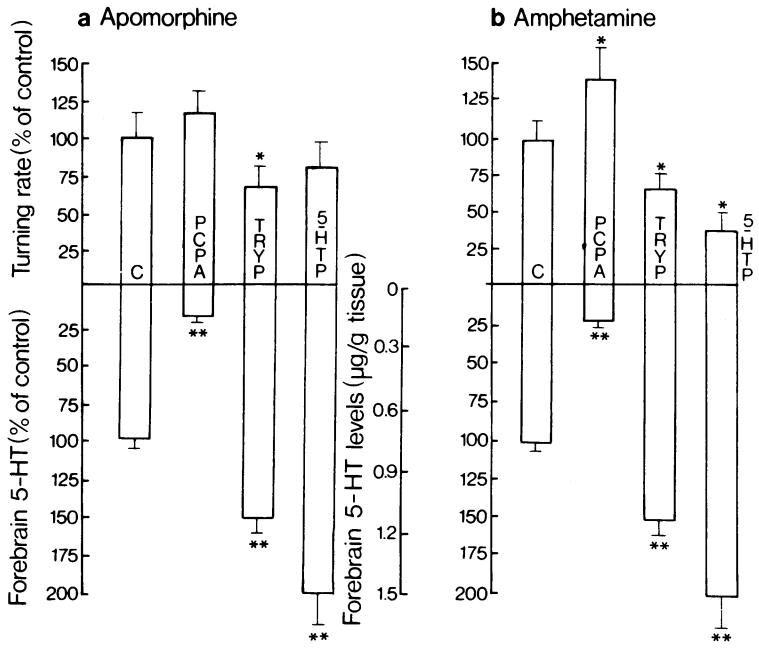

Figure 1 Effect of pretreatment of unilateral striatal lesioned mice with *p*-chlorophenylalanine (3 x 500 mg/kg) (PCPA), L-tryptophan (400 mg/kg) (Tryp), and 5-hydroxytryptophan (200 mg/kg) (5-HTP) on forebrain 5-hydroxytryptamine (5-HT) levels and on (a) apomorphine (2 mg/kg) and (b) amphetamine (5 mg/kg)-induced turning behaviour. C denotes control group of animals, and results are expressed as a percentage of control. Mean turning rates for control group were 7.6 ± 0.9, 30 min after amphetamine and 8.9 ± 0.7, 15 min after apomorphine. Percentage means are shown for 10 observations of each drug. Vertical lines show s.e. mean. * Denotes statistical significance at the level $P < 0.05$. ** Denotes high statistical significance at the level $P < 0.001$.

Biochemical analyses

Forebrains from mice were weighed, homogenized in cold acidified butanol and extracted into HCl. 5-Hydroxytryptamine was assayed fluorimetrically after condensation with *o*-phthaldialdehyde (Maickel, Cox, Saillant & Miller, 1968); tryptophan was assayed by the method of Denckla & Dewey (1967), modified for brain tissue; noradrenaline was assayed fluorimetrically (Chang, 1964); and dopamine, after purification with alumina, was assayed by the method of Laverty & Sharman (1965).

Drugs

All agents were dissolved in saline, unless otherwise stated, and administered intraperitoneally in volumes no greater than 0.5 ml. 5-Hydroxytryptophan (Sigma Chemical Co) and L-tryptophan (Koch Light Chemicals Ltd) were dissolved in a little 2 N HCl and diluted with saline. The following drugs were used: *p*-chlorophenylalanine methyl ester (Sigma); methysergide bimaleate (Sandoz); cyproheptadine hydrochloride (Merck, Sharp & Dohme); lysergic acid diethylamide tartrate (Sandoz); clomipramine hydrochloride (Geigy); dexamphetamine sulphate (Smith, Kline & French); apomorphine hydrochloride (Evans Medical). Weights of drugs are expressed in terms of the salt.

Statistical analyses

Changes in turning rates were statistically compared using Student's *t* test.

Results

5-Hydroxytryptophan, L-tryptophan and p-chlorophenylalanine pretreatments

The effect of 5-hydroxytryptophan, L-tryptophan or *p*-chlorophenylalanine treatments on forebrain 5-hydroxytryptamine levels and turning rates induced by either apomorphine or amphetamine is shown in Figure 1. All agents caused significant changes in

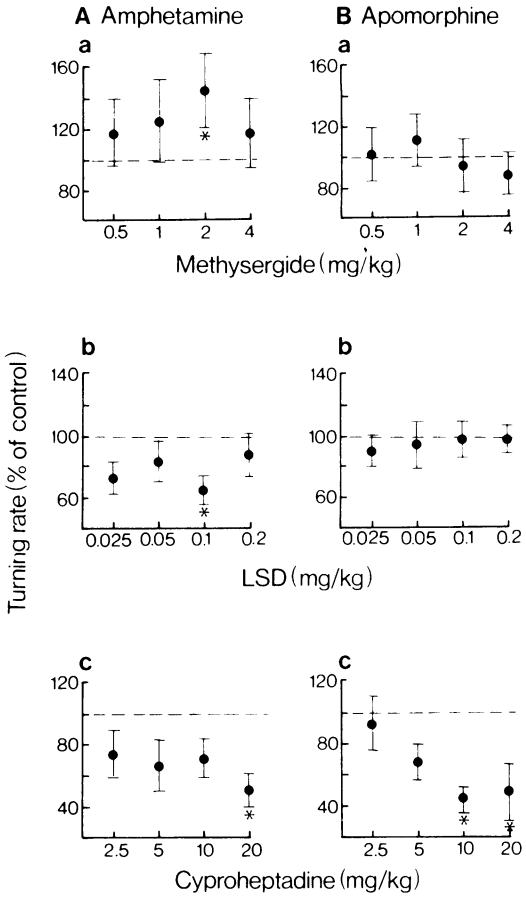

Figure 2 Effect of (a) methysergide (0.5–4 mg/kg), (b) Lysergic acid diethylamide (LSD, 0.025–0.2 mg/kg) and (c) cyproheptadine (2.5–20 mg/kg) on turning rates induced by amphetamine (5 mg/kg) (column A) and apomorphine (2 mg/kg) (column B). The mean number of turns per minute for each dose of drug is expressed as a percentage of the mean number of turns of the saline-treated control animals. The mean control turning rates were in the range 8.2–10.0 turns per min, 30 min after amphetamine and 8.3–10.7 turns per min, 15 min after apomorphine. Means for 12 observations at each dose level are shown. Vertical lines show s.e. mean. * Denotes statistical significance at the level $P < 0.05$.

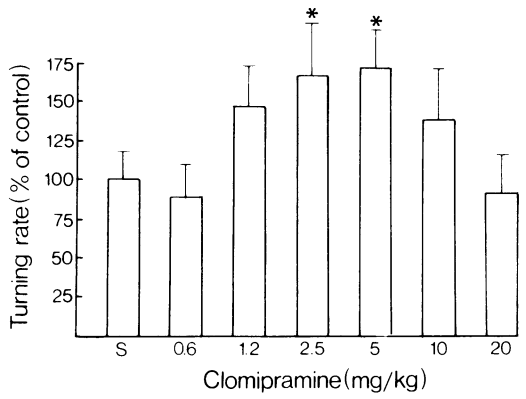

Figure 3 Effect of systematically administered clomipramine (0.6–20 mg/kg) on the rate of turning induced by amphetamine (1.5 mg/kg). The mean number of turns per min for each dose of clomipramine is expressed as a percentage of that of the saline-treated control group (S). The mean turning rate for the control group was 2.4 ± 0.4 turns/min after amphetamine. Means for 10 observations at each dose level are shown. Vertical lines show s.e. mean. * Denotes statistical significance at the level $P < 0.05$.

brain 5-hydroxytryptamine levels, but alone did not induce turning behaviour or any marked body postures.

5-Hydroxytryptophan, in a single dose of 200 mg/kg, produced a 200% rise in 5-hydroxytryptamine content and a small but insignificant decrease in the rate of turning to apomorphine (to 80% of control values), but a greater and significant reduction in turning to amphetamine (to 40% of control values) ($P < 0.01$). L-Tryptophan, in a single dose of 400 mg/kg, again elevated brain 5-hydroxytryptamine levels to 150% of control values and caused statistically significant depression of turning rates to about 70% of control counts for both apomorphine and amphetamine ($P < 0.025$ and 0.05 respectively). Parachlorophenylalanine methyl ester, in three daily doses of 500 mg/kg, depleted cerebral 5-hydroxytryptamine levels to 19% of control values. Parachlorophenylalanine pretreatment caused a small but insignificant increase in the rate of turning to apomorphine (117% of control), and a larger and significant increase of amphetamine-induced turning (142% of control) ($P < 0.05$).

Pharmacological agents

Methysergide (0.5–4 mg/kg) caused slight increases in amphetamine-induced turning rates, significant at the dose 2 mg/kg ($P < 0.05$), but had no effect on the rate of apomorphine-induced turning (Figure 2). Methysergide alone evoked no circling behaviour and caused no postural asymmetries.

Cyproheptadine (2.5–20 mg/kg) produced depression of both apomorphine and amphetamine-induced turning. The highest dose (20 mg/kg) caused a significant ($P < 0.05$) 50% reduction of turning behaviour to both drugs (Figure 2). Cyproheptadine

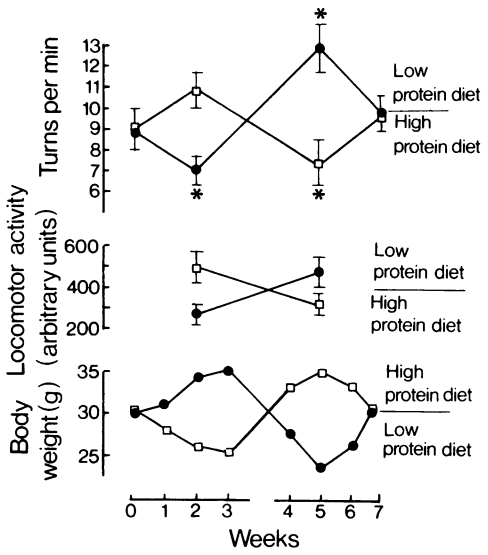

Figure 4 Effect of varying dietary protein intake on the rates of turning induced by amphetamine (5 mg/kg), on spontaneous locomotor activity and on body weight. Control turning rates were observed for two similar groups of mice. Group 1 mice (●) were then initially fed a high protein diet for 3 weeks, while Group 2 mice (□) received a low protein diet. The diet was reversed for a further 3 weeks. At the end of this period both groups were fed normal laboratory diet. Means for at least 10 observations at each time interval are shown. Vertical lines show s.e. mean. * Denotes a statistically significant difference from the control result at the level $P < 0.05$.

alone evoked no circling behaviour or postural asymmetries: the higher doses caused sedation.

LSD (0.025–0.2 mg/kg) caused a slight depression of amphetamine-induced turning activity signfiicant at the dose 0.1 mg/kg ($P < 0.05$) while having no effect on apomorphine-induced turning (Figure 2). LSD alone showed little behavioural effect in the majority of animals. In the remainder mild degrees of stereotypy and hyperactivity occurred, but no consistent postural deviations or turning behaviour were observed.

Clomipramine (0.6–20 mg/kg) did not evoke a turning response and postural asymmetries were not seen; higher doses had a sedative effect. The drug's effect on a submaximal dose of amphetamine (1.5 mg/kg) is depicted in Figure 3. The middle doses tested (1.25, 2.5 and 5 mg/kg) potentiated amphetamine-induced turning (significant at the level $P < 0.05$ for the 2.5 and 5 mg/kg doses) while the lower and higher doses depressed it, but not to a statistically significant extent.

Diet experiments

The rates of turning induced by amphetamine in the two groups of mice fed high and low protein diets are shown in Figure 4. Dosage of amphetamine was carefully controlled to allow for the significant weight changes of the animals in the two groups. In general, mice fed a high protein diet (25% protein) showed a lower circling rate to amphetamine than did the mice fed a low protein diet (mainly carbohydrate). Both groups when fed the normal laboratory diet (15.9% protein) had a mean turning rate of 9.1 ± 0.9 turns/min following 5 mg/kg amphetamine. When fed a high protein diet, the now heavier mice, had a mean rate of turning of 7.0 ± 0.7 turns/min, while the lighter, low protein diet animals showed a mean turning rate of 10.9 ± 0.8 turns/minute. Spontaneous locomotor activity changed in the same direction as the rate of turning. When the diet pattern was reversed after 3 weeks, the weight differences, spontaneous locomotor activity and rates of turning to amphetamine also reversed. When finally the two groups of mice were fed a normal diet both the weight and amphetamine-induced turning behaviour reverted to the same levels in the two groups.

The results of the biogenic amine and tryptophan levels in forebrains of the high and low protein diet animals are given in Table 1. The different diets caused no significant changes in noradrenaline or dopamine content. There was, however, a significant difference in the 5-hydroxytryptamine and tryptophan levels. The low protein diet caused a highly significant 20% decrease in the level of 5-hydroxytryptamine ($P < 0.001$) and a significant 30% decrease in the level of tryptophan ($P < 0.05$) when compared to the high protein diet mice.

Discussion

This study has used the turning mouse model of von Voigtlander & Moore (1973) to investigate the effect

Table 1 The effect of high and low protein diet on noradrenaline, dopamine, 5-hydroxytryptamine and tryptophan levels in mouse forebrain

Biogenic amine	*High protein diet*	*Low protein diet*	*% difference*	*Significance*
Dopamine	658 ± 47	666 ± 47	1	NS
Noradrenaline	207 ± 11	243 ± 26	15	NS
5-Hydroxytryptamine	728 ± 19	578 ± 12	21	$P < 0.001$
Tryptophan	832 ± 82	587 ± 96	30	$P < 0.05$

Biogenic amines expressed as ng/g wet weight of forebrain. All observations, mean ± s.e. mean, $n = 10$. NS denotes no statistical significance.

of drugs acting on 5-hydroxytryptamine upon amphetamine and apomorphine-induced circling behaviour. This circling phenomenon depends upon an imbalance in activity between the two nigro-striatal dopamine systems which in this instance results from the destruction of dopamine nerve terminals in one striatum by the direct injection of 6-hydroxydopamine. Amphetamine, an agent that releases dopamine from the intact nigro-striatal pathway, causes circling towards the lesioned side, while the dopamine agonist apomorphine causes turning away from the lesioned side presumably because the dopamine receptors on this side have become supersensitive (Ungerstedt, 1971). In this model striatal dopamine levels are reduced on the side of injection: forebrain 5-hydroxytryptamine concentrations are not grossly affected (Pycock *et al.*, 1976). Thus, modification of central 5-hydroxytryptaminergic mechanisms by systemic administration of various drugs would be expected to be effective on both sides of the brain, and the changes observed in circling behaviour probably reflect the action of bilateral changes in 5-hydroxytryptamine on an imbalanced dopamine system.

The rate at which these animals circle to either amphetamine or apomorphine has been shown to be influenced by gross changes in brain 5-hydroxytryptamine levels. Parachlorophenylalanine, an inhibitor of 5-hydroxytryptamine synthesis (Koe & Weissman, 1966), caused an 80% depletion of brain 5-hydroxytryptamine and potentiated the turning induced by amphetamine and, to a lesser extent, that caused by apomorphine. Parachlorophenylalanine has been reported to cause both an increase (Fibiger & Campbell, 1971a) and a decrease (Volicer, 1969) in spontaneous locomotor activity in rats; recently Breese, Cooper & Mueller (1974) have shown that *p*-chlorophenylalanine significantly potentiates amphetamine-induced locomotor activity in rats, although it has no apparent effect on the stereotypies produced by amphetamine. Both our results and those of Breese *et al.* (1974) may be explained by the fact that amphetamine itself may release 5-hydroxytryptamine as well as the catecholamines from nerve endings as suggested by Fuxe & Ungerstedt (1970). Grabowska (1974) also has shown that lesions of the midbrain raphé nuclei which reduce rat forebrain 5-hydroxytryptamine levels by some 38% potentiate apomorphine-induced locomotor activity, while Grabowska *et al.* (1973) have shown that *p*-chlorophenylalanine potentiates and 5-hydroxytryptophan inhibits the stimulating effect of apomorphine on motor behaviour.

Increase in brain 5-hydroxytryptamine levels produced by prior administration of L-tryptophan (Modigh, 1973b) or 5-hydroxytryptophan (Modigh, 1972) is associated with decreased spontaneous locomotor activity (Modigh, 1974). In our experiments these agents decreased the rates of circling induced by both apomorphine and amphetamine. L-Tryptophan increased brain 5-hydroxytryptamine levels by some 50% and decreased apomorphine and amphetamine-induced circling by about 30%. 5-Hydroxytryptophan increased brain 5-hydroxytryptamine levels by 100% and decreased apomorphine and amphetamine-induced circling by 20 and 60% respectively. The differences in the effects of L-tryptophan and 5-hydroxytryptophan on drug-induced circling in relation to their effects on brain 5-hydroxytryptamine are probably due to the relatively non-specific effect of 5-hydroxytryptophan. Whereas L-tryptophan is converted to 5-hydroxytryptamine only in cerebral 5-hydroxytryptaminergic neurones which possess tryptophan hydroxylase (Knapp & Mandell, 1973), 5-hydroxytryptophan is converted into 5-hydroxytryptamine in both 5-hydroxytryptaminergic and catecholaminergic neurones which possess the non-specific L-aromatic amino acid decarboxylase (Butcher, Engel & Fuxe, 1972; Ng, Chase, Colburn & Kopin, 1972), with consequent complex functional effects.

The results of the dietary experiments mimic those obtained with *p*-chlorophenylalanine, 5-hydroxytryptophan and tryptophan. It has been shown that changing dietary tryptophan content causes alterations in brain tryptophan and 5-hydroxytryptamine levels (e.g. Fernstrom & Wurtman, 1972; Biggio, Fadda, Fanni, Tagliamonte & Gessa, 1974). We have confirmed these observations in the present study, when mice fed a high protein diet had both higher brain tryptophan and 5-hydroxytryptamine levels than mice fed a low protein diet. Brain catecholamine levels were not affected. The high protein animals had lower spontaneous locomotor activity than the low protein mice, confirming the observations of Jacobs *et al.* (1974). The animals fed on a high protein diet also turned less vigorously to amphetamine than those fed on a low protein diet, an effect that was convincingly reversed by changing the diet. However perhaps the results obtained from the dietary experiments should be interpreted with some caution, as it has also been shown that food deprivation enhances amphetamine-induced locomotor activity in rats (Fibiger & Campbell, 1971b; Simpson, 1974).

The overall conclusion from these experiments in which brain 5-hydroxytryptamine levels were increased or decreased is that circling behaviour due to striatal dopamine receptor stimulation is depressed by an elevation of brain 5-hydroxytryptamine and enhanced by a reduction in brain 5-hydroxytryptamine. These results are in agreement with those of Baldessarini, Amatruda, Griffith & Gerson (1975) who have demonstrated similar effects on apomorphine-induced turning in rats with unilateral electrolytic lesions of the nigro-striatal pathway.

The results obtained with drugs believed to block or

stimulate central 5-hydroxytryptamine receptors were less clear. Methysergide, a drug claimed to block 5-hydroxytryptamine receptors (Bieger, Larochelle & Hornykiewicz, 1972), did not cause any consistent or dose-dependent effect on either apomorphine or amphetamine-induced circling behaviour. However there is doubt as to how well this drug penetrates into the brain (Doepfner, 1962), although it enhances apomorphine-induced locomotor activity (Grabowska & Michaluk, 1974) and stereotypy (Weiner, Goetz & Klawans, 1975) and apomorphine-induced turning in rats (Baldessarini *et al.*, 1975).

Similarly, no conclusive effect was demonstrated with LSD. Although LSD was originally described as an agent that blocks cerebral 5-hydroxytryptamine receptors (Boakes, Bradley, Briggs & Dray, 1970) and inhibits the release of this transmitter from central neurones (Chase, Breese & Kopin, 1967), later evidence suggests that it may stimulate 5-hydroxytryptamine receptors (Andén, Corrodi, Fuxe & Hökfelt, 1968; Aghajanian, 1972). Further, it has now been suggested that LSD is also a potent dopamine receptor agonist, for it causes strong contraversive turning in rats with unilateral lesions of the medial forebrain bundle (Pieri, Pieri & Haefely, 1974). However, no such effect was found in the present study, where only nigro-striatal terminals were damaged by the unilateral 6-hydroxydopamine lesion (Pycock & Anlezark, 1975). Pieri *et al.*'s (1974) result may have been due to the fact that their lesion affected pathways other than the nigro-striatal dopaminergic tract; particularly as Costall & Naylor (1974) have shown that a unilateral electrolytic lesion in the 5-hydroxytryptaminergic raphé nuclear system will result in a rat that circles in response to drugs. Furthermore, Grabowska *et al.* (1974) found that LSD had little effect on apomorphine-induced locomotor activity in rats, suggesting that it does not possess potent dopamine agonist action.

In contrast to methysergide and LSD, cyproheptadine, another anti-5-hydroxytryptamine agent (Stone, Wenger, Luddon, Stavorski & Ross, 1961), did cause dose-dependent inhibition of both amphetamine and apomorphine-induced circling. This result is the reverse of that expected on the basis of the earlier experiments. 5-Hydroxytryptamine receptor blockade should have mimicked the effects of brain 5-hydroxytryptamine depletion which caused an increase in drug-induced circling. We have no ready explanation for this finding, but it may be due to other unrecognized pharmacological actions of cyproheptadine, which is known, for example, to possess antihistaminic properties (Stone *et al.*, 1961).

The effect of the antidepressant drug, clomipramine, was investigated because it is a potent inhibitor of 5-hydroxytryptamine uptake into cerebral 5-hydroxytryptaminergic neurones (Carlsson, Corrodi, Fuxe & Hökfelt, 1969), with only a weak effect on the uptake of noradrenaline and dopamine into catecholamine neurones (Shaskan & Snyder, 1970, Tuck & Punell, 1973). Clomipramine might have been expected to enhance cerebral 5-hydroxytryptamine transmission, and therefore to depress circling induced by amphetamine. In fact, in doses of between 1.25 and 10 mg/kg, it caused a small increase in the rate of circling produced by a subthreshold dose of amphetamine. This may have been due to its albeit weak capacity to block reuptake of dopamine, for drugs such as nomifensine which are powerful dopamine reuptake blockers, do potentiate amphetamine-induced circling in this mouse model (Pycock, Milson, Tarsy & Marsden, unpublished observations). Also the inconsistency in the results may be explained by an inhibition of the rate at which amphetamine is metabolized similar to that seen after other tricyclic compounds as described by Lewander (1969).

The question arises as to the site at which 5-hydroxytryptamine impinges on the nigro-striatal dopamine system. Anatomical and biochemical evidence suggests that 5-hydroxytryptamine neurones enter both the substantia nigra and the striatum. Thus synaptosomes prepared from the substantia nigra contain large quantities of 5-hydroxytryptamine (Parizek, Hassler & Bak, 1971) which is consistent with the concept that there is a 5-hydroxytryptamine neurone projection to the substantia nigra (Andén, Dahlström, Fuxe, Larsson, Olson & Ungerstedt, 1966). Similarly, the large quantities of 5-hydroxytryptamine in the striatum probably lie mainly in the nerve terminals of 5-hydroxytryptamine neurones arising from the midbrain raphé nuclei (Andén *et al.*, 1966; Poirier, Singh, Boucher, Bouvier, Olivier & Larochelle, 1967; Kim, Hassler, Kurokawa & Bak, 1970). Stimulation of the raphé nuclei in cats results in release of 5-hydroxytryptamine from the caudate nucleus (Ashkenazi, Holman & Vogt, 1972; Holman & Vogt, 1972), while anatomical studies have identified a pathway from the anterior raphé nuclei to the basal ganglia in cats (Brodal, Taber & Walberg, 1960).

It is possible that such 5-hydroxytryptamine neurones influence the firing of nigro-striatal dopamine fibres themselves, or alternatively they could affect the activity of those nerve cells in the striatum controlled by incoming dopamine nigro-striatal fibres. The experiments undertaken in this study might have given some indication as to which of these two possibilities is the most likely. If 5-hydroxytryptamine manipulation affected only circling induced by amphetamine, an action directly on the nigro-striatal dopamine system would seem most plausible. On the other hand if in our model both apomorphine and amphetamine-induced circling was equally affected, then an action on striatal neurones would be most probable. In fact, the experiments did not give such a clear-cut answer. The exact electrophysiological inter-relation of basal

ganglia dopamine and 5-hydroxytryptamine pathways remains to be established by further experiment.

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