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Interaction in the cerebral metabolism of the biogenic amines. Effect of phenelzine on this interaction

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Summary

1. Chronic administration of phenelzine to dogs caused the concentrations of homovanillic acid (HVA) in c.s.f. from both the lateral ventricle and cisterna magna to fall to new low levels at which they were maintained.

2. After 10–12 days treatment with phenelzine the caudate nucleus had elevated concentrations of dopamine and 3-methoxytyramine and lowered concentrations of 3,4-dihydroxyphenylacetic acid and HVA.

3. Intravenous administration of tryptophan to dogs pretreated with phenelzine caused in c.s.f. an increase in the concentrations of HVA and in the caudate nucleus a decrease in dopamine concentration and an increase in the concentrations of its metabolites, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid and HVA.

4. A model is proposed for the cerebral metabolism of dopamine and some of the points at which tryptophan and its metabolites may interact with dopamine metabolism are discussed.

Introduction

It has recently been shown (Moir, 1969 ; 1971a) that the intravenous administration of large amounts of L-tryptophan to dogs results not only in increases in the concentration of the 5-hydroxyindoles in brain and c.s.f. but also in even greater increases in the concentrations of homovanillic acid (HVA), the main acid metabolite of dopamine, in cerebrospinal fluid without altering the concentration of dopamine or its metabolites in caudate nucleus. As no point of interaction between dopamine and tryptophan metabolism in brain could be detected directly under normal conditions, it was thought that the interaction might become more apparent on re-examining the phenomena under altered conditions of metabolism. The original experiments have therefore been repeated with dogs chronically pretreated with the monoamine oxidase inhibitor phenelzine. It was anticipated that this drug treatment would produce a new steady state with elevated dopamine concentrations and a slower metabolic turnover in the dopamine pathway (Neff & Costa, 1968). This proved to be the case and the data in the present paper taken in conjunction with the results of the companion paper (Moir, 1972) clarify the interaction between tryptophan and dopamine in brain.

A preliminary communication concerned with some of the present data has already been given (Moir & Yates, 1970).

Methods

Animal methodology

Adult male beagles with implanted guide tubes directed towards the lateral ventricles and cisterna magna were used in these experiments. They were prepared as described previously (Ashcroft, Dow & Moir, 1968). The dietary schedule was rigidly standardized and experiments performed after an overnight fast. The dogs used in these experiments formed part of the group of animals used to investigate the effect of phenelzine on the metabolism of the 5-hydroxyindoles in brain (Moir, 1972).

Chronic phenelzine administration

After a control period of at least one week during which base line samples of c.s.f. from lateral ventricles and cisterna magna and venous blood were obtained as described (Moir, 1972), phenelzine (β-phenylethylhydrazine) hydrogen sulphate, 2 mg/kg was administered by daily subcutaneous injection at 10.00 hours for a period of ten to twelve days and further samples of blood and c.s.f. were obtained during this time at intervals of two to three days and just before the daily injection of phenelzine.

Acute experiments

The dogs used were pre-treated with phenelzine for ten to twelve days the last injection being given 24 h before the start of the acute experiment. The animals were maintained under light anaesthesia with sodium thiopentone while L-tryptophan (10 mg/ml of 0·9% (w/v) NaCl solution) solution was administered intravenously as an injection of 50 mg/kg followed by an infusion of (20 mg/kg)/h. In some animals, only an equivalent volume of 0·9% (w/v) NaCl solution was administered in the same manner. In each experiment samples of venous blood and c.s.f. were obtained before the onset and at hourly intervals for four hours after the start of the infusion. After the last samples were taken each dog was quickly exsanguinated via an arterial cannula. The brain was rapidly removed and dissected into regions which were stored at $-15°$ C until biochemical analyses could be performed.

Biochemical methodology

Estimations of tyrosine in plasma, erythrocytes, c.s.f. and brain were carried out as described previously (Moir, 1971b). The concentrations of HVA in c.s.f. were measured by the method of Ashcroft, Crawford, Dow & Guldberg (1968). All these results were corrected to 100% by comparison with internal standards added to duplicate portions of appropriate samples.

Analyses of the caudate nuclei for dopamine, methoxytyramine, 3,4-dihydroxyphenylacetic acid (DOPAC) and HVA were performed by the method of Crawford & Yates (1970).

Phenelzine and phenylacetic acid did not interfere with any of the above analyses.

Results

Chronic phenelzine treatment

Tyrosine concentration in body fluids during phenelzine administration

Table 1 gives the concentrations of tyrosine in erythrocytes, plasma and c.s.f. before and during chronic administration of phenelzine. Phenelzine administration produced no significant alteration in tyrosine concentrations in samples from any site (*t* test). There was no significant difference between the concentrations of tyrosine in c.s.f. from the lateral ventricle and cisterna magna (paired *t* test).

Homovanillic acid concentrations in cerebrospinal fluid during phenelzine administration

The analyses during the control period show a gradient for HVA from a concentration of 7·6 nmol/ml ±0·6 (9) (mean ± S.E. (number of estimates)) in c.s.f. from the lateral ventricle to a concentration of 0·35 ± 0·06 (8) in c.s.f. from the cisterna magna. These results show an even greater gradient for HVA than that initially reported by Guldberg, Ashcroft & Crawford (1966).

Following the daily administration of phenelzine, the HVA concentration in both lateral ventricular and cisternal c.s.f. was rapidly and significantly ($P<0·001$) reduced. In c.s.f. from the lateral ventricle the mean HVA concentration during the third to thirteenth day of phenelzine treatment was 0·66 ± 0·04 (17) nmoles/ml, less than a tenth of the previous control concentration, the further decline in concentration during this period being slight ((−0·04 nmol/ml)/day) in comparison with the initial fall from control concentrations. During the phenelzine administration period concentrations of HVA in c.s.f. from the cisterna magna fell from their control concentrations to levels that were either not detectable or just detectable (0·02 nmol/ml).

These results contrast with comparable data concerning 5-HIAA concentrations in c.s.f. in the same and similar experiments (Moir, 1972) where phenelzine produced a significant fall in the concentration of 5-HIAA in c.s.f. from the lateral ventricles to half its control values but the concentrations remained unaltered in c.s.f. from cisterna magna. It was found that phenelzine or its acid metabolite, phenylacetic acid, blocked the active transport system for the removal of HVA and 5-HIAA from c.s.f. The different effects of phenelzine on 5-HIAA concentration in c.s.f. from lateral ventricle and cisterna magna were thus explained since the

TABLE 1. *Tyrosine concentrations in erythrocytes, plasma and cerebrospinal fluid before and during chronic administration of phenelzine*

	Control	Phenelzine†
C.S.F.		
Lateral ventricle	20·6 ± 3·9 (11)*	22·0 ± 2·4 (22)
Cisterna magna	19·4 ± 3·7 (12)	18·4 ± 2·8 (21)
Plasma	45·5 ± 5·4 (13)	56·0 ± 4·9 (22)
Erythrocytes	200 ± 15 (13)	197 ± 16 (21)

* Mean ± S.D. (number of samples). Concentrations in nmol/ml.
† Phenelzine administered 2 mg/kg by subcutaneous injection. Values shown are obtained between the second and twelfth day of treatment.

active transport system concerned has been shown to be localized in the region of the fourth ventricle (Ashcroft, Dow & Moir, 1968).

Phenelzine produced a very much more marked reduction of HVA in ventricular c.s.f. than occurred with 5-HIAA and a significant reduction of HVA also occurred in c.s.f. from the cisterna magna. These results indicate that phenelzine inhibited the metabolic turnover of the dopamine pathway in brain to a much greater extent that the 5-hydroxyindole pathway.

Acute experiments

Tyrosine concentration in body fluids during the infusion period

Following the daily administration of phenelzine for a period of ten to twelve days the dogs were given intravenously either L-tryptophan or an equivalent volume of saline according to the schedule detailed in the **Methods** section. It has been demonstrated previously that with this schedule of administration in dogs not pre-treated with phenelzine, tryptophan administration does not alter tyrosine concentration in erythrocytes, plasma, c.s.f. or brain regions (Moir, 1971b). Similar results were found throughout the infusion period in the present experiments with dogs pre-treated with phenelzine. The slight alterations in tyrosine concentration in erythrocytes, plasma and c.s.f. were alike in both saline and tryptophan-infused dogs and were probably attributable to the administration of the large volume of fluid.

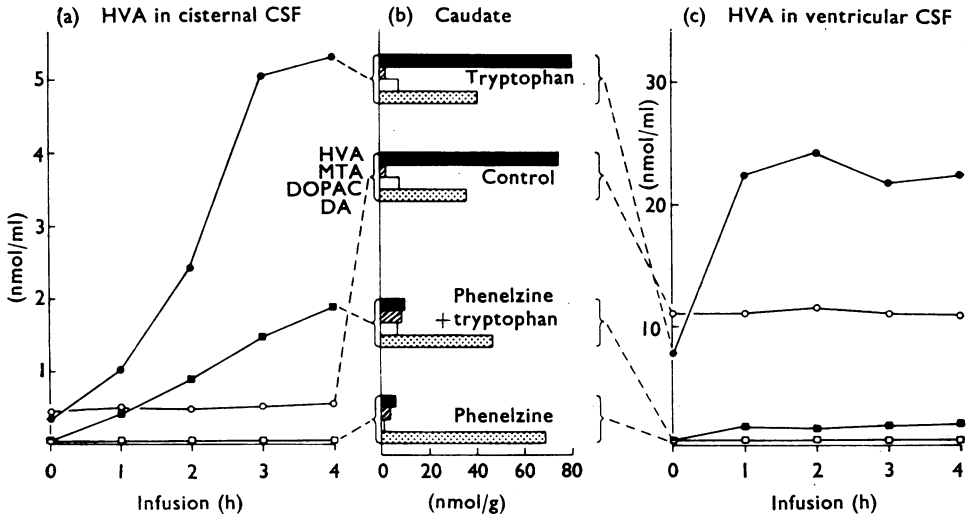

FIG. 1. (a) Concentrations of homovanillic acid (HVA) in serial samples of c.s.f. from cisterna magna: control dogs (○), tryptophan administration (●), chronic treatment with phenelzine followed by saline administration (□) and chronic treatment with phenelzine followed by tryptophan administration (■). Results are the means of at least three experiments. (b) Mean molal concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (MTA) and homovanillic acid (HVA) in caudate nucleus of dogs after treatment described in (a) (see text for details). Results derived from Table 2. (c) Concentration of homovanillic acid (HVA) in serial samples of c.s.f. from the lateral ventricle: control dogs (○) (data mean of 5 experiments from Ashcroft, Crawford, Dow & Guldberg (1968)), tryptophan administration (●), chronic phenelzine treatment followed by saline administration (□) and chronic treatment with phenelzine followed by tryptophan administration (■). Results other than controls are means of three experiments.

Homovanillic acid in cerebrospinal fluid during the infusion period

The mean concentrations of HVA in c.s.f. from the lateral ventricle are shown in Fig. 1c and those in c.s.f. from cisterna magna in Fig. 1a. The standard errors are omitted from the mean values because, as has been discussed in detail previously (Moir, 1972), when the data are expressed in terms of absolute values the highly significant ($P<0{\cdot}001$) variability between dogs obscures the consistency of the trend of change within each animal. Analysis of variance techniques were used for statistical comparison of data.

Serial sampling of c.s.f. caused no changes of HVA concentration in c.s.f. from the cisterna magna (Fig. 1a) or the lateral ventricle (Fig. 1c) either in control dogs or in those chronically pre-treated with phenelzine. Dogs which had been infused with tryptophan whether pre-treated with phenelzine or not, showed a highly significant increase ($P<0{\cdot}001$; analysis of variance) in HVA concentration in c.s.f. from both the cisterna magna (Fig. 1a) and the lateral ventricle (Fig. 1c). The increase occurred more slowly in the cisternal c.s.f. but at the end of the 4 h infusion period was proportionately greater (ten-fold) than in the lateral ventricular region (three-fold) (Fig. 1a, c).

Brain analyses after administration of tryptophan or saline

Tyrosine estimations were performed on portions of brain homogenate from caudate, hypothalamus, midbrain, thalamus, hippocampus, hindbrain, cerebral

TABLE 2. *Effect of phenelzine and tryptophan on the concentrations of dopamine and its metabolites in dog caudate nucleus*

Treatment	Dopamine	Methoxytyramine	Dihydroxyphenyl-acetic acid	Homovanillic acid
Controls	45·2	0·7	9·7	81·4
	24·9	3·3	8·5	66·8
	43·7	2·4	6·7	78·6
	33·6	3·1	6·1	72·9
Tryptophan*	42·8	4·0	7·9	83·1
	40·4	1·0	7·9	80·3
	38·4	1·0	7·9	77·4
Phenelzine†	72·8	3·7	1·5	7·9
	66·0	4·0	1·5	2·8
Phenelzine	52·2	1·5	7·3	12·4
+	35·2	18·4	6·7	10·0
Tryptophan	54·3	8·3	7·0	7·9

Concentrations in nmoles/gram of caudate

*Tryptophan—Intravenous injection of L-tryptophan 50 mg/kg followed by infusion of (20 mg/kg)/h for 4 h.

†Phenelzine—Subcutaneous injection of 2 mg/kg of phenelzine hydrogen sulphate daily for 10–12 days.

In both the phenelzine pre-treated and non-pre-treated group the analyses of the contrasts between control and tryptophan treated sub-groups were performed first by subtracting the appropriate mean control molal concentration of each substance from all the relevant data. For the null form of the proposed hypothesis that tryptophan causes displacement of brain dopamine to its metabolites, the coded dopamine values were all multiplied by —1. Analysis of variance of the coded data could demonstrate no difference between control and tryptophan treated animals in the absence of phenelzine treatment, but the data from animals chronically treated with phenelzine showed a significant difference ($P<0{\cdot}01$) between animals given saline subsequently, and those given tryptophan. Thus, the results of chemical analyses of the caudate nucleus offer significant support for the proposed hypothesis in the case of those animals which had been pre-treated with phenelzine.

cortex and cerebellum. The concentrations of tyrosine found in the various brain regions were unaltered by treatment of the animals with tryptophan (Moir, 1971b), phenelzine or tryptophan after phenelzine.

The concentrations of dopamine, 3-methoxytyramine (MTA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid HVA) in the caudate nuclei of dogs pre-treated with phenelzine and then given either tryptophan or an equivalent volume of saline are shown in Table 2 along with the corresponding results from similar experiments in dogs not pretreated with phenelzine. In animals not given phenelzine, the administration of tryptophan produced no signficant change in the concentration of dopamine and its metabolites, indeed, the mean concentration of 3-methoxytyramine and DOPAC had the same values as in controls and those for dopamine and HVA are within half a standard deviation of the respective mean control values. However, in dogs which had been pretreated with phenelzine it was found that the subsequent administration of tryptophan produced a significant ($P<0{\cdot}01$—footnote Table 2) reduction of dopamine in the caudate nuclei and a concomitant increase in all its metabolites (Fig. 1b, Table 2).

Discussion

The present experiments show that the normal specific pattern of distribution of tyrosine in erythrocytes, plasma, c.s.f. and different regions of brain (Moir, 1971b) was not altered by chronic phenelzine treatment with or without subsequent tryptophan administration. However, as tyrosine is normally present in amounts greater than those required to saturate tyrosine hydroxylase, minor changes of its effective concentration would not be likely to modify the rate of tyrosine hydroxylation.

The administration of tryptophan to animals untreated or pre-treated with phenelzine caused an increase in the HVA concentration in c.s.f. from both the cisterna magna and the lateral ventricles. However, only in the phenelzine pretreated dogs did tryptophan administration also cause a change in dopamine metabolites in the caudate nuclei with a significant fall in the concentration of dopamine and a rise in the concentration of its metabolites.

The cerebral metabolism of the 5-hydroxyindoles does not seem to be implicated in the alterations occurring in dopamine metabolism following tryptophan administration as it has been shown (Moir, 1972) that the administration of tryptophan to phenelzine pre-treated dogs, in spite of bringing about a sixfold increase in brain tryptophan concentration, produced no alterations in the concentrations of 5-hydroxytryptamine and 5-HIAA in brain or of 5-HIAA in c.s.f. additional to those induced by phenelzine alone. These findings indicate that with phenelzine pretreatment, normal tryptophan concentrations were already sufficient to saturate tryptophan 5-hydroxylase, the rate limiting enzyme in the cerebral metabolism of the 5-hydroxyindoles.

There is good evidence that noradrenaline (Sedvall, Weise & Kopin, 1968), 5-hydroxytryptamine (Macon, Sokoloff & Glowinski, 1971; Moir, 1972; Shields & Eccleston, 1972) and dopamine (Javoy & Glowinski, 1971) which are 'stored' in brain are in communication with smaller pools of newly synthesized amines which have more rapid turnover rates and are probably of greater functional significance. Also it would seem that the synthetic pathways of noradrenaline (Udenfriend, 1968)

and 5-hydroxytryptamine (Moir & Eccleston, 1968) require the localized coupling of the enzymes involved to the amine pools. A corresponding scheme of dopamine synthesis and storage which is compatible with the effects produced by tryptophan administration is shown in Figure 2.

The first point at which tryptophan could interact to cause the observed results would be by interfering with the transfer of 3-hydroxytyrosine (DOPA), newly formed by the tyrosine hydroxylase, to the appropriately localized DOPA decarboxylase (Fig. 2). *In vitro* the efflux of L-tryptophan from synaptosomes has been shown to be increased by the presence of related amino acids external to the synaptosomes (Grahame-Smith & Parfitt, 1970) thus it is likely that *in vivo* a high concentration of tryptophan external to the dopamine synthetic complex may divert an increased proportion of DOPA from the decarboxylase enzyme within this localized synthetic complex by the process of facilitated transport. This would reduce the amount of dopamine which would be formed for incorporation in the functional store and reduce feed back inhibition of tyrosine hydroxylase. In the absence of any metabolic inhibition the pathway could compensate to maintain the dopamine concentration and a normally functioning dopamine complex.

DOPA which had become diverted from the localized synthetic complex would be likely to be rapidly degraded by the cytoplasmic enzymes, decarboxylase and catechol-*O*-methyl transferase (COMT). The methoxytyramine so formed may be deaminated by monoamine oxidase (MAO) in regions of the neurone from which

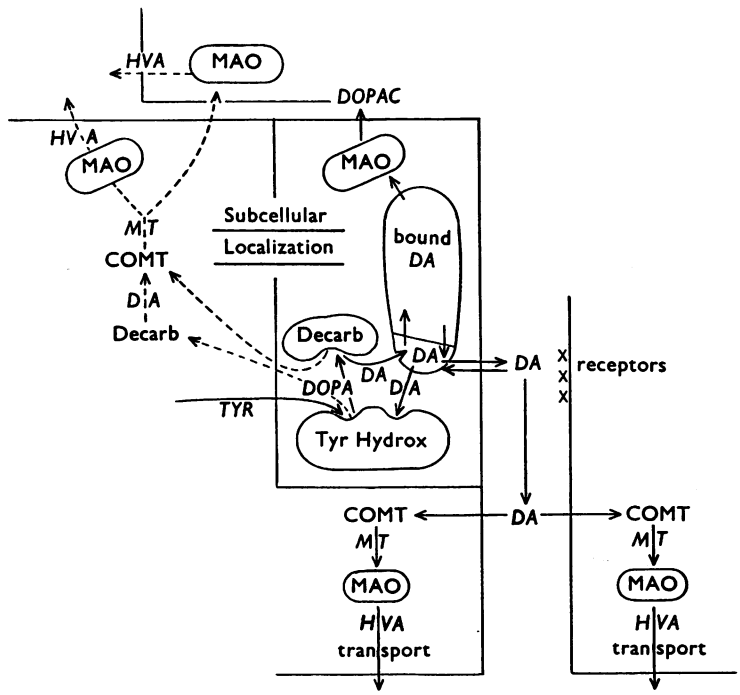

FIG. 2. Model of dopamine metabolism in brain. (——), Main routes of dopamine metabolism; (- - - -), diverted metabolism of DOPA and dopamine increased by tryptophan administration. Key: TYR HYDROX—Tyrosine hydroxylase; DECARB—L-aromatic amino acid decarboxylase; MAO—monoamine oxidase; COMT—catechol-*O*-methyl transferase; *TYR*—tyrosine; *DA*—dopamine; *MT*—3-methoxytyramine; *DOPAC*—3,4-dihydroxyphenylacetic acid; *HVA*—4-hydroxy-3-methoxyphenylacetic acid.

the HVA so formed may leave unimpeded. Alternatively the methoxytyramine itself may similarly leave the neurone from which due to the affinity of 3-*O*-methyl derivatives for the 'uptake 2' system (Iversen, 1967) it may be rapidly taken up and deaminated in sites from which the HVA so formed may have rapid access to c.s.f. The basic assumption is that DOPA diverted from its localized site of formation can be rapidly metabolized to HVA at sites in which neither it nor its intermediate metabolites will accumulate in brain.

It would seem likely in view of the present and previous (Moir, 1972) experiments that phenelzine inhibits (to different degrees) tyrosine hydroxylase, MAO and the transport of HVA from certain parts of brain. It has also been shown that phenelzine inhibits noradrenaline uptake processes (Iversen, 1967) and in addition that some inhibitors of COMT have a hydrazine moiety. In phenelzine pretreated animals there will be a larger proportion of dopamine in a functionally free state, thus changes in this pool will become more obvious when measuring total dopamine. The multiple inhibitory effects of phenelzine on dopamine catabolism and the initial low concentrations of the intermediary metabolites would allow an increase in dopamine synthesis rate to be more readily observed by an increase in these metabolites.

Despite failure to detect tryptamine, another explanation of the action of tryptophan on dopamine metabolism is that either tryptophan or one of its metabolites such as tryptamine acts as a 'false' dopamine transmitter. If the false transmitter has a low affinity for dopamine storage sites and for the inhibitory site of tyrosine hydroxylase and the association constant for the binding of dopamine in storage sites is relatively high then a false transmitter occupying a proportion of a free functional pool of dopamine may cause a significant increase in dopamine synthesis without overt alteration of total dopamine content. If the increased dopamine which is synthesized cannot be retained within the free pool then it will overflow on to metabolizing enzymes. As DOPAC does not increase in either c.s.f. (Moir, 1971a) or brain the route of metabolism is via COMT and MAO. The catabolism of dopamine 'overflowing' from the free pool of dopamine may take place at a site different from that dealing with functionally released dopamine and probably in a manner similar to that suggested for DOPAC which had been diverted from its localized metabolic pathway. It can be anticipated that the multiple inhibitory actions which have already been discussed for phenelzine would cause this type of effect of tryptophan on dopamine metabolism to be more clearly observed in terms of a reduction of dopamine and an elevation of its metabolites in brain.

It should be noted that the two hypotheses proposed are not mutually exclusive and it may be that both are playing some part in causing the effect of tryptophan administration on dopamine metabolism in brain.

That the increase in dopamine synthesis manifest by the rise in the HVA concentration in c.s.f. could be derived from increased turnover through the normal route of degradation would seem unlikely in view of the large changes which occur in c.s.f. without alteration of the intermediary metabolites in brain in the absence of phenelzine pre-treatment; particularly, as changes in c.s.f. concentrations of HVA and 5-HIAA normally reflect similar alterations in their concentrations in brain (Moir, Ashcroft, Crawford, Eccleston & Guldberg, 1970). The above scheme of dopamine catabolism modifies that suggested by Sharman (Sharman, 1969; Guldberg & Broch, 1971; Roffler-Tarlov, Sharman & Tegerdine, 1971). Sharman's

hypothesis suggests that DOPAC represents intraneuronal catabolism of dopamine and that HVA represents its extraneuronal metabolism. This hypothesis is well supported by data from the pharmacological actions of amphetamine derivatives and COMT inhibitors. The modification we would suggest is that DOPAC represents the degradation of dopamine within the dopamine synthesis-storage complex and that HVA may be derived from two sources; viz, from the catabolism of functionally released dopamine where the HVA so formed appears to depend on a specific transport system for its removal from brain, and from catabolism of DOPA or dopamine diverted from the synthesis-storage complex where the HVA formed may leave the brain more rapidly. The action of pharmacological agents such as probenecid, amphetamine and the tropolones may help to define the existence of these functionally distinct components of dopamine metabolism.

The marked species variation in the localization of brain enzymes, such as decarboxylase (McCaman, Rodriquez de Lores Arnais & de Robertis, 1965) and in specific brain transport systems for the acidic metabolites (Neff, Tozer & Brodie, 1964; Sharman, 1966; Werdinius, 1967; and Ahtee, Sharman & Vogt, 1970) suggests that interaction between tryptophan and dopamine metabolism such as have been described will vary in different species. The administration of tryptophan to rats pre-treated with tranylcypromine (Grahame-Smith, 1971) did not appear to alter brain dopamine concentration.

The practical importance of the interaction shown between the cerebral metabolism of tryptophan and dopamine is illustrated by the finding that the symptoms of Parkinsonism can be exacerbated by 5-hydroxytryptophan given together with a peripheral decarboxylase inhibitor (Chase, 1970b) and also by tryptophan particularly when given together with pyridoxine, which forms the co-factor for the decarboxylase enzyme (Hall, Weiss, Morris & Prange, 1971).

L-DOPA is already being shown to be of benefit in neurological diseases other than Parkinsonism (chronic manganese poisoning (Mena, Court, Fuenzalida, Papavasiliou & Cotzias, 1970); progressive nuclear palsy (Chase, 1970a); and certain dystonias (Coleman, 1970; Chase, 1970b)) and it is to be hoped that better understanding of the functional metabolism of dopamine in the brain may permit a rational approach to therapy in neurological diseases in which dopaminergic neurones are involved.

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