Br. J. Pharmac. (1972), **45**, 249–264.

Interaction in the cerebral metabolism of the biogenic amines. Effects of phenelzine on the cerebral metabolism of the 5-hydroxyindoles in dog brain

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Summary

1. Chronic administration of phenelzine to dogs caused 5-hydroxyindol-3-ylacetic acid (5-HIAA) concentrations in c.s.f. from the lateral ventricle to be maintained at 50% of their normal values, but did not alter the concentrations of 5-HIAA in c.s.f. from the cisterna magna.

2. Following 10 days treatment with phenelzine, the active transport of 5-HIAA from c.s.f. which normally occurs in the region of the fourth ventricle, was inhibited. This transport system was also inhibited by the addition of phenylacetic acid, the acid metabolite of phenelzine, to the fluid perfusing the cerebral ventricles.

3. After 10–12 days treatment with phenelzine all regions of brain showed concentration increases to approximately 300% for 5-HT and 150% for 5-HIAA, but no alteration in the tryptophan concentration.

4. Intravenous administration of tryptophan to dogs pretreated with phenelzine caused large increases in the concentration of tryptophan in brain and body fluids but did not alter either the concentrations of 5-hydroxytryptamine and 5-HIAA in brain or of 5-HIAA in c.s.f.

5. A model of the cerebral metabolism of 5-hydroxytryptamine is proposed and the results are interpreted to mean that phenelzine has inhibitory actions, either directly or in some instances indirectly, on intracerebral tryptophan 5-hydroxylase, monoamine oxidase and the transport of 5-HIAA from both brain and c.s.f.

Introduction

The two hydrazine-type monoamine oxidase inhibitors, isocarboxazide and phenelzine have been shown to have an interesting difference in their effects on the cerebral metabolism of the 5-hydroxyindoles in man. Maclean, Nicholson, Pare & Stacey (1965) administered these two drugs to patients during the two to four week period preceding their deaths. Both drugs produced the anticipated marked rise in 5-hydroxytryptamine in the brain stem, but only isocarboxazide gave the expected fall in the concentration of the 5-hydroxytryptamine acid metabolite, 5-hydroxyindol-3-ylacetic acid (5-HIAA). After phenelzine treatment, 5-HIAA remained within normal limits. Maclean *et al.* (1965) offered no explanation for their observation on the action of phenelzine.

This paper attempts to assess the action of phenelzine at various points in the cerebral metabolism of the 5-hydroxyindoles and whether its main metabolite, phenylacetic acid (Dubnick, 1962) has actions on this metabolic pathway. The results demonstrate that the drug either directly or through its metabolites has an inhibitory action on tryptophan hydroxylase, monoamine oxidase and 5-HIAA transport from brain and cerebrospinal fluid (c.s.f.).

Preliminary communications concerned with parts of this work have already been published (Ashcroft, Crawford, Dow & Moir, 1969; Moir & Yates, 1970).

Methods

Animal methodology

The animals used in these experiments were adult male beagles, 10–14 kg which had had guide tubes directed towards the lateral ventricles implanted in the skull (Ashcroft, Dow & Moir, 1968b) at least two weeks before any subsequent experimental procedure.

To minimize the alterations of cerebral metabolism of the 5-hydroxyindoles and concentrations of amino acids, both of which can be influenced by dietary factors, each dog was given a constant amount of a carefully standardized balanced diet daily at approximately 15.00 hours. On the days prior to those on which experiments were performed, any food remaining was withdrawn at 17.00 hours; water was available overnight and experiments were started at 10.00 hours.

Chronic phenelzine administration

Two to three weeks after operation for the implantation of ventricular guide tubes there was a control period of one week during which samples of c.s.f. and blood were taken every two or three days at 10.00 hours. Following this, phenelzine (β-phenylethylhydrazine) was administered daily by subcutaneous injection as 2 mg phenelzine hydrogen sulphate/kg body weight. The dose of phenelzine in 1 ml of 0·9% NaCl solution was prepared immediately before injection and was given at 10.00 hours or directly following the collection of body fluid samples. Phenelzine administration was continued for a period of 10–12 days after which either acute experiments or ventricular perfusion experiments were carried out.

Sampling of blood and cerebrospinal fluid

The dog was anaesthetized with a minimal dose of sodium thiopentone. A 0·5 ml sample of c.s.f. was obtained from the lateral ventricle (Ashcroft, Crawford, Dow & Guldberg, 1968) and then a 2 ml sample of c.s.f. was taken from the cisterna magna (Eccleston, Ashcroft, Moir, Parker-Rhodes Lutz & O'Mahoney, 1968). Blood was then withdrawn from a cephalic vein into a polyethylene syringe and a 7 ml portion transferred to a polyethylene tube containing 0·2 ml heparin solution (1,000 i.u./ml). Plasma and erythrocyte fractions were separated as described (Moir, 1971b) previously. All samples were stored at $-15°$ C until biochemical analyses were undertaken.

Acute experiments

Twenty-four hours after the last dose of phenelzine, the dogs were anaesthetized with sodium thiopentone and supplementary doses were given to maintain very light

anaesthesia. It was found that smaller amounts of anaesthetic were required to maintain anaesthesia than is normally the case in dogs not pre-treated with phenelzine. Initial samples of blood and c.s.f. were obtained and then an intravenous injection of an L-tryptophan solution (10 mg/ml in 0·9% saline) was given in a dose of 50 mg/kg and followed by an intravenous infusion at the rate of (20 mg/kg)/h for a period of 4 hours. In some dogs an equivalent volume of 0·9% saline was administered instead of the tryptophan solution. Samples of venous blood and c.s.f. were obtained at hourly intervals throughout the infusion period and after the collection of the samples at 4 hours the dogs were rapidly exsanguinated via an arterial cannula. The brains were removed as quickly as possible and dissected into regions as described previously (Moir, 1971a). All samples were stored at −15° C until biochemical analyses were commenced.

Ventricular perfusion experiments

The dogs used in these experiments had an additional guide tube inserted in the skull which was directed towards the cisterna magna. Recirculatory perfusion of the ventricular space from lateral ventricle to cisterna magna was carried out with donor c.s.f. while the dogs were conscious and unrestrained as has been fully described previously (Ashcroft *et al.*, 1968b). Each recirculatory perfusion experiment was carried out in two parts. The first period of recirculation allowed equilibrium concentrations of endogenous 5-HIAA to be established. During the second part of the perfusion experiment a solution containing inulin and a low concentration of exogenous 5-HIAA was infused at a constant rate into the perfusion system until new equilibrium concentrations were established in the system. From the rate of infusion of exogenous 5-HIAA and the equilibrium concentrations of 5-HIAA in the perfusion system before and during this infusion, the rate at which exogenous 5-HIAA was removed from the c.s.f. system was calculated (Ashcroft *et al.*, 1968b). It was assumed that this was also the rate at which endogenous 5-HIAA was removed from the c.s.f. system and the rate at which endogenous 5-HIAA entered the c.s.f. was calculated from the equilibrium concentrations of endogenous 5-HIAA. An alteration in the amount of the metabolite released into the perfusion system will give an index of the change in the turnover rate of the metabolic pathway for any one animal given subsequent drug treatment, provided new steady state metabolic conditions are established and no change is produced in the relative proportion of the total metabolite from brain which is released into the perfusion fluid.

During the second part of two ventricular perfusion experiments in control dogs, a high concentration of phenylacetic acid was infused into the perfusion system instead of inulin and 5-HIAA.

Biochemical methodology

Estimations of tryptophan in plasma, erythrocytes, c.s.f. and brain were carried out as described previously (Moir, 1971b). 5-Hydroxyindoles in brain and plasma were measured by the method of Ashcroft, Eccleston & Crawford (1965). 5-HIAA concentrations in c.s.f. were measured by the method of Ashcroft *et al.* (1968a). Phenelzine and phenylacetic acid did not interfere with any of the above analyses. All results were corrected to 100% on the basis of recovery of internal standards added to duplicate portions of appropriate samples.

Phenelzine and phenylacetic acid concentrations were measured in plasma and brain using Dubnick's modification (Dubnick, 1962) of the method described by Hess, Weissbach, Redfield & Udenfriend (1958). Linear calibration was obtained for concentrations of 2–30 μg/ml with standard recoveries of both substances from plasma, but neither was detected in plasma nor in brain samples from the phenelzine-treated dogs.

Estimations of tryptamine concentrations in brain tissue were performed in two of the dogs which had received tryptophan after chronic treatment with phenelzine. One or two ml portions of acetic acid homogenate (Ashcroft *et al.*, 1965) of several regions of brain were taken to damp dryness under reduced pressure at an external temperature of 55° C. The residue was reconstituted in 9 ml water by vigorous mechanical shaking. Perchloric acid, 1 ml 4 N, was added at 4° C. The sample was mixed by inversion, allowed to stand for 10 min at 4° C and then centrifuged at 3,000 *g* for 5 minutes. The supernatant was then analysed for tryptamine by the method of Eccleston, Ashcroft, Crawford & Loose (1967) which ensures the complete separation of tryptamine from tryptophan before its estimation by the fluorimetric method of Hess & Udenfriend (1959).

Identification of the 5-hydroxyindoles

In one experiment, in which both phenelzine and tryptophan were administered, further evidence of the identity of '5-hydroxytryptamine' and '5-HIAA' estimated by chromatographic separation and specific 5-hydroxyindole fluorescence (Ashcroft *et al.*, 1965) was sought. Following the quantification of the 5-hydroxyindole fluorescence in 3 N HCl the samples of '5-hydroxytryptamine' and '5-HIAA' from all brain regions were saturated with sodium chloride and extracted with four volumes of peroxide-free, freshly distilled diethyl ether. The ether layer was shaken with 0·3 M phosphate buffer, pH 7, transferred to a tube containing 100 μg ascorbic acid in 0·1 ml 80% methanol and 0·1 ml 20% (v/v) acetic acid and reduced to damp dryness by a jet of nitrogen. The residue was dissolved in 1 ml of 0·1 N H_2SO_4. To these solutions and to portions of the phosphate buffer extracts 0·5 vol. of 10 N HCl was added. The resulting solutions and portions of the original extracted samples were then examined for 5-hydroxyindole fluorescence. In all the brain regions examined, the endogenous brain '5-hydroxytryptamine' and '5-HIAA' distributed between the different phases in the same manner as standards of authentic 5-hydroxytryptamine and 5-HIAA.

Results

Chronic phenelzine treatment

Behavioural effects during phenelzine administration

After three days treatment, the dogs became progressively more excitable and difficult to handle and by the time of the acute experiment, they appeared extremely agitated.

Tryptophan concentration in body fluids during phenelzine administration

The distribution of tryptophan in body fluids has been shown to be governed by complex factors (Moir, 1971b). As it was thought that these factors might be altered during drug administration, samples of plasma, erythrocytes and c.s.f. from

both cisterna magna and lateral ventricle were analysed for tryptophan during a control period and then at intervals of two to three days throughout the administration of phenelzine. However, chronic treatment with phenelzine did not significantly alter the concentration of endogenous tryptophan in the plasma, in the erythrocytes or in the c.s.f. from the lateral ventricle or cisterna magna (Table 1). The estimates of tryptophan in c.s.f. also confirm a previous finding (Geddes & Moir, 1969) that there is no gradient for the amino acid from lateral ventricle to cisterna magna unlike the very marked gradient that has been shown to exist for its carboxylic acid derivative, 5-HIAA (Guldberg, Ashcroft & Crawford, 1966).

5-Hydroxyindol-3-ylacetic acid concentration of cerebrospinal fluid during phenelzine administration

Figure 1 shows the concentration of 5-HIAA in c.s.f. samples taken during the control period and throughout treatment with phenelzine. The control samples

TABLE 1. *Tryptophan concentration (nmol/ml) in erythrocytes, plasma and cerebrospinal fluid before and during chronic administration of phenelzine*

	Control	Phenelzine†
Lateral ventricle	5·9± 2·9 (11)*	6·9± 2·0 (22)
Cisterna magna	5·9± 2·0 (12)	5·2± 1·9 (20)
Plasma	55 ±18.1 (13)	66·1±23·0 (21)
Erythrocytes	47 ± 7 (13)	46·8± 8 (21)

* Mean ±S.D. (number of samples).
† Phenelzine administered daily, 2 mg/kg by subcutaneous injection.
Values shown were obtained between the second and twelfth day of treatment.

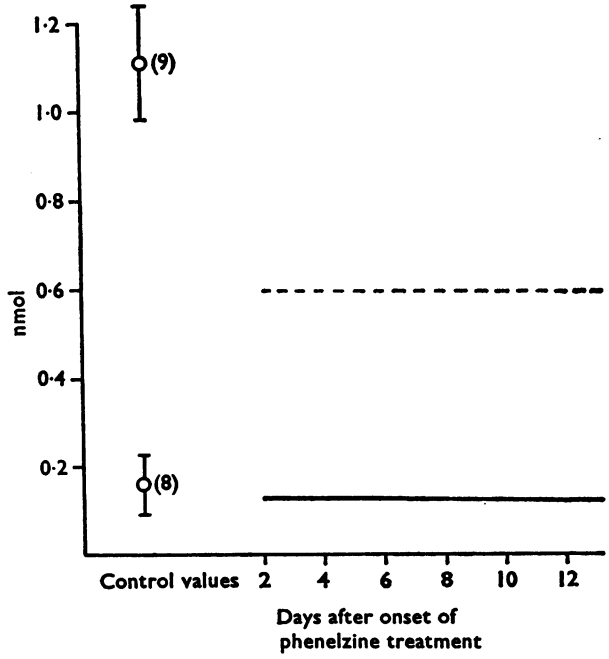

FIG. 1. Concentration of 5-HIAA in c.s.f. in five dogs before and during daily administration of phenelzine (2 mg/kg subcutaneously). Values for lateral ventricle (- - - -); values for cisterna magna (——). Control values, mean±S.E.M. (number of estimates); linear regression lines of 5-HIAA values for the 2–13 day period after the onset of phenelzine treatment. (Slopes of linear regression lines not significantly different from zero.)

demonstrate the marked concentration gradient of 5-HIAA which exists between lateral ventricle and cisterna magna. Following treatment with phenelzine there was no significant alteration in the concentration of 5-HIAA in c.s.f. from the cisterna magna. This is in agreement with a previous report (Roos, 1963) that phenelzine treatment did not alter 5-HIAA concentration in the lumbar c.s.f. of schizophrenic patients. The concentrations of 5-HIAA in c.s.f. from the lateral ventricle, however, showed a highly significant ($P<0{\cdot}001$) decrease from a mean control value of 1·13 nmol/ml $\pm$ 0·13 (9) (S.E. (number of estimates)) to a mean value of 0·59 $\pm$ 0·05 (27) following phenelzine treatment. After the first day of phenelzine administration the 5-HIAA concentrations were well stabilized (Fig. 1).

Acute experiments

Tryptophan concentrations in body fluids during the infusion period

After daily treatment with phenelzine for a period of 10 to 12 days the dogs were given a solution of L-tryptophan intravenously or an equivalent volume of 0·9% NaCl solution according to the schedule detailed in the **Methods** section. Administration of this type of tryptophan load or its equivalent volume of 0·9% NaCl solution to dogs not pre-treated with phenelzine has been shown to produce alterations in the concentration of tryptophan in erythrocytes, plasma and c.s.f.

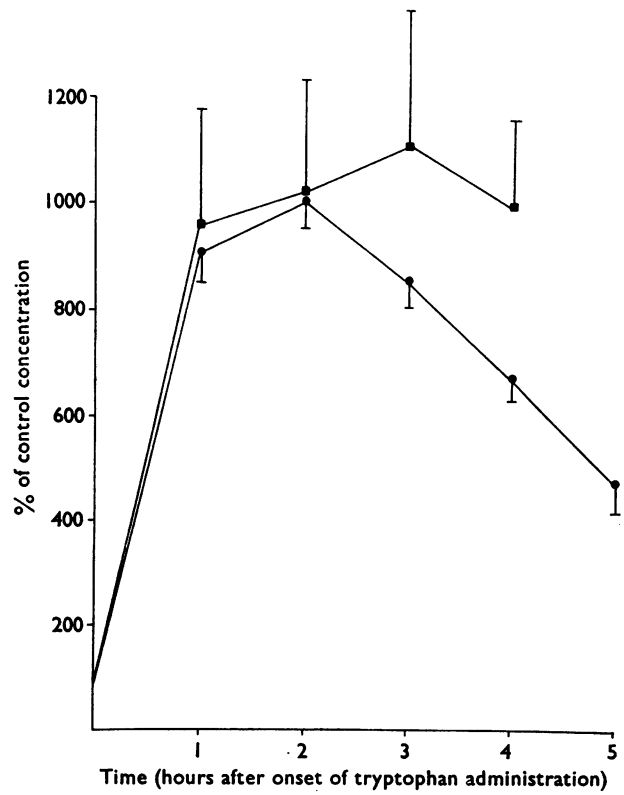

FIG. 2. Tryptophan concentrations in c.s.f. from the cisterna magna following intravenous administration of L-tryptophan (50 mg/kg then (20 mg/kg)/h) in control dogs (●) (means ± S.E.M. of 6 dogs) and in dogs which had been pretreated with daily phenelzine (2 mg/kg, subcutaneously) for 11–12 days (■) (means ± S.E.M. of 3 dogs).

which have a particular pattern (Moir, 1971b) due to factors affecting the distribution of tryptophan in the body. The pattern of change of tryptophan concentration in plasma and erythrocytes was exactly similar in dogs given phenelzine. However, as Fig. 2 shows, the concentrations of tryptophan found in cisternal c.s.f. after the administration of L-tryptophan were maintained at an elevated level for longer in animals which had been pre-treated with phenelzine. The combined values at 3 and 4 h were significantly different ($P<0.05$). On the other hand, there did not appear to be any significant difference between the concentrations of tryptophan found in brain (Fig. 4) in animals pre-treated with phenelzine and their comparable controls.

5-Hydroxyindol-3-ylacetic acid in cerebrospinal fluid during the infusion period

Although the concentration of 5-HIAA found in lateral ventricular c.s.f. varied considerably between dogs, serial samples of c.s.f. from the same dog had practically the same concentration. This finding has been discussed in detail (Ashcroft

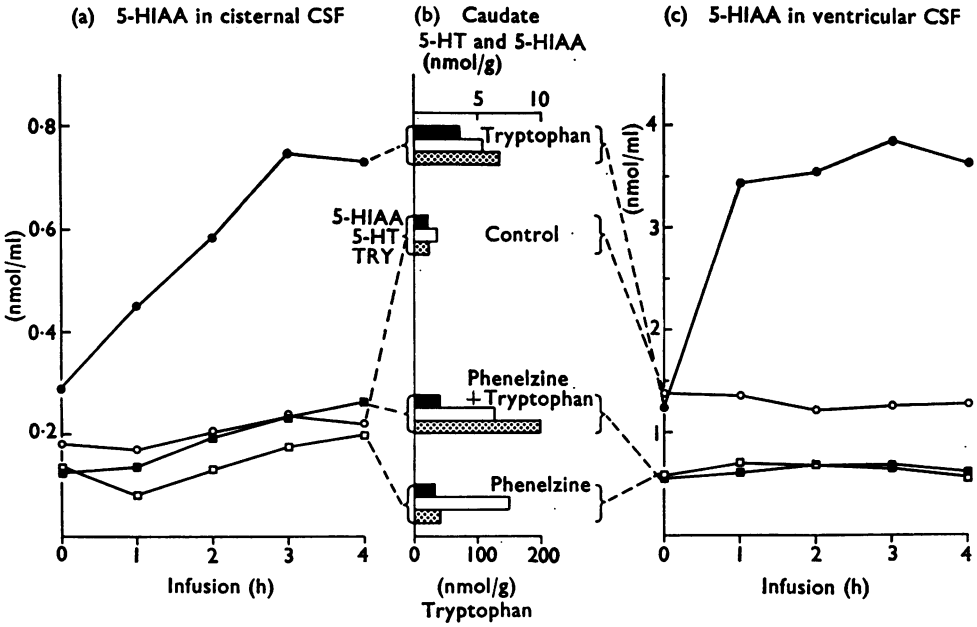

FIG. 3.* (a) Concentrations of 5-HIAA in serial samples of c.s.f. from cisterna magna of control dogs (○), following tryptophan administration (●), chronic treatment with phenelzine followed by saline administration (□) and chronic treatment with phenelzine followed by tryptophan administration (■). Results means of three to six experiments. (b) Mean molal concentrations of tryptophan (TRY), 5-hydroxytryptamine and 5-HIAA (note scale differences) in caudate nucleus of dogs after treatments as described in (a) (see text for details). Control results, mean of six experiments; other results, mean of three experiments. (c) Concentrations of 5-HIAA in serial samples of c.s.f. from the lateral ventricle of control dogs (○) (data mean of 5 experiments from Ashcroft, Crawford, Dow & Guldberg (1968)), following tryptophan administration (●), chronic phenelzine administration followed by saline administration (□) and chronic phenelzine treatment followed by tryptophan administration (■). Results other than controls are means of 3 experiments.

* S.E.M.S have been omitted from the mean values in Figs. 3a and 3c (see text) as they give a misleading impression of the variability of the data. The trend of change in concentration is similar in each dog within a group but the absolute concentration varies considerably from animal to animal. For example, the means of the coefficient of variation of 5-HIAA concentration in cisternal c.s.f. during tryptophan infusion are halved if they are expressed as percentage of control values, and this, despite the fact that such a manipulation should mathematically increase the variability of the data.

et al., 1968a) and has also been shown to apply to concentrations of 5-HIAA in c.s.f. from the cisterna magna (Moir, 1971a).

Previously it has been possible to plan experiments to allow the effects of drug treatment to be seen using each animal as its own control and comparing concentration changes in each dog with their initial value (Guldberg & Yates, 1968 ; Moir, 1971a). Such a design was obviously not possible in the experiments presented here where groups of dogs on different treatment schedules have to be compared. As the pattern of change in metabolite concentration against time was very reproducible within each dog in the different groups, and differences between dogs within treatments were highly significant ($P<0{\cdot}001$), standard errors have been deliberately omitted (footnote Fig. 3) from Figs. 3a and 3c which illustrate the comparative patterns of change found in 5-HIAA concentration in c.s.f. Where an accurate comparison of two groups was required, analysis of variance techniques was used.

The mean molal concentrations of 5-HIAA found in lateral ventricular c.s.f. during the various treatments are shown in Fig. 3a, and these in cisternal c.s.f. in Fig. 3c. They are shown in comparison with the mean molal concentrations of tryptophan, 5-hydroxytryptamine and 5-HIAA in a representative region of brain (caudate nucleus, Fig. 3b) from the comparable experiments at the end of the infusion period. The results show that in either control dogs or phenelzine pre-treated dogs, serial sampling of ventricular c.s.f. (Fig. 3c) produces no change from the initial concentration and that serial sampling of c.s.f. from the cisterna magna (Fig. 3a) produces only a very slight rise in 5-HIAA concentration.

Tryptophan administration to control dogs produced, as has been discussed previously (Moir, 1971a) a highly significant ($P<0{\cdot}001$) increase in the 5-HIAA concentration of c.s.f., which in ventricular c.s.f., reached 300% and, in cisternal c.s.f. 250% of its control values. It can also be seen from Fig. 3c that the rise in concentration of 5-HIAA occurred more rapidly in ventricular c.s.f. than in cisternal c.s.f.

In contrast, tryptophan administration to animals pre-treated with phenelzine produced no change in the concentration of 5-HIAA in ventricular c.s.f. and the slight rise seen in cisternal c.s.f. was comparable to that produced in animals given only a saline infusion following chronic phenelzine treatment. The lack of change in 5-HIAA concentration would seem to indicate that the precursor load in the phenelzine-treated animals did not increase the metabolism of 5-hydroxyindoles in brain.

Brain analyses following administration of tryptophan or saline

The chromatographic method of analysis used to estimate the 5-hydroxytryptamine and 5-HIAA in brain would have allowed the detection and measurement of 5-hydroxytryptophan in brain. None was detected in any of the brain regions ($<0{\cdot}2$–$0{\cdot}4$ nmol/g depending on region) or in plasma samples obtained after four hours of infusion ($<0{\cdot}2$ nmol/ml).

In Figure 4 the concentration of tryptophan, 5-hydroxytryptamine and 5-HIAA in various regions of brain in dogs pre-treated with phenelzine and subsequently given either tryptophan or an equivalent volume of saline are shown in comparison with their non-phenelzine treated controls. The distribution of the total molal concentrations of ([5-HT]+[5-HIAA]) in the brain regions of dogs given phenel-

zine or phenelzine plus tryptophan was similar to that found in control dogs and the major points of the results were not due to inter-regional differences, but to the contrasting treatments. Thus phenelzine treatment alone produced the expected increase in brain 5-hydroxytryptamine in all regions. However, it also caused increases in 5-HIAA concentration to approximately 150% of their control values, whereas one would have expected inhibition of monoamine oxidase alone to have produced a decrease in 5-HIAA concentration.

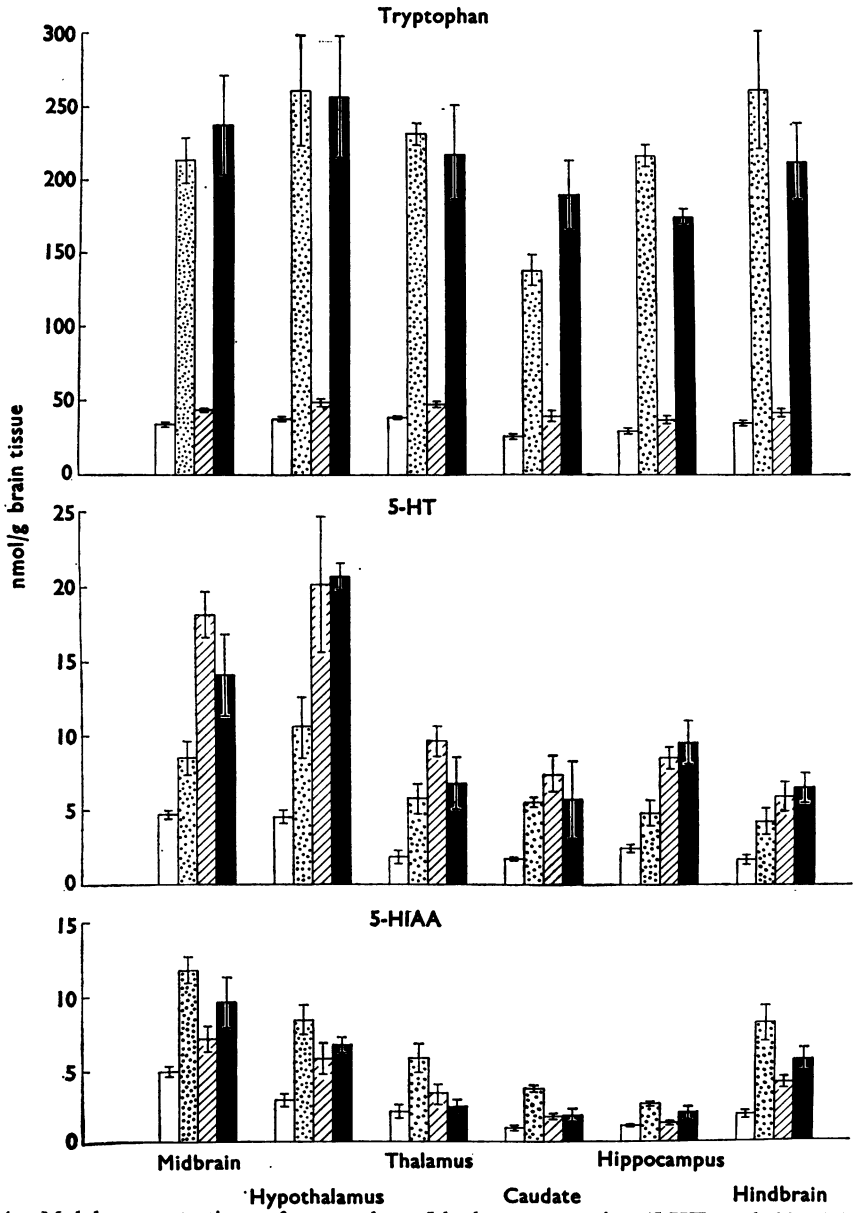

FIG. 4. Molal concentrations of tryptophan, 5-hydroxytryptamine (5-HT) and 5-HIAA (note scale differences) in various regions of dog brain in control dogs □, after tryptophan administration ⁝⁝, chronic treatment with phenelzine followed by saline administration ▨ and chronic treatment with phenelzine followed by tryptophan administration ■. Control results mean ± S.E.M. of 5 or 6 experiments; other results, mean ± S.E.M. of 3 experiments.

TABLE 2. *Effect of phenelzine pre-treatment on the influx and efflux of 5-hydroxyindol-3-ylacetic acid (5-HIAA) in cerebral ventricular perfusate*

Treatment		Clearance of inulin (μl of inflow perfusate/min)	Clearance of 5-HIAA (μl of inflow perfusate/min)	Mean endogenous 5-HIAA concentration (nmol/ml)	Rate of 5-HIAA release from brain (nmol/min)	5-HIAA Turnover rate %
Dog A	Control	49	146	0·58	0·094	100
	Phenelzine	63	93	0·68	0·073	79
Dog B	Control	51	168	0·71	0·131	100
	Phenelzine	54	89	0·74	0·079	60

Perfusion of the cerebral ventricles of dogs performed as described in the text. The data are from two control experiments and from two similar experiments performed on the same dogs after they had received phenelzine hydrogen sulphate 2 mg/kg subcutaneously daily for 10 days. The post drug experiments were started 24 h after the last dose of phenelzine.

Following the administration of tryptophan to phenelzine pre-treated animals, there was no further increase in the concentration of the 5-hydroxyindoles in brain despite the concentrations of tryptophan in brain rising to values similar to those found after the administration of tryptophan to control dogs; thus it would seem that tryptophan 5-hydroxylase, which is rate limiting in the cerebral metabolism of the 5-hydroxyindoles, was fully saturated at apparently normal control concentrations of tryptophan.

As Eccleston *et al.* (1967) were able to detect tryptamine in the brain of rats given tryptophan subsequent to a monoamine oxidase inhibitor, in two of the dogs which received both phenelzine and tryptophan brain regions which had sufficient homogenate available (hippocampus, hindbrain, cortex, cerebellum and midbrain) were analysed for tryptamine. None was detected; however, the limit of detection was rather high (1·5–2 nmol/g brain, depending on region) because of the small quantity of brain available and the losses incurred in the rigorous analytical technique required in order to estimate tryptamine in the presence of high concentrations of tryptophan.

At the time of the acute experiments neither phenelzine nor its acid metabolite phenylacetic acid were detectable in samples of brain and plasma from the phenelzine treated dogs.

Ventricular perfusion experiments

Turnover rate of 5-hydroxyindol-3-ylacetic acid in cerebrospinal fluid

Table 2 shows the concentrations of endogenous 5-HIAA found in perfusion fluid from a recirculatory perfusion of the ventricular spaces and also the rate at which inulin and a low concentration of 5-HIAA were 'cleared' from the system of the dog. Because steady state conditions were attained during the perfusion experiments, it was possible to calculate the rate at which 5-HIAA was released into the c.s.f. and this could be taken as an index of the turnover rate of the cerebral metabolism of the 5-hydroxyindoles. The results (Table 2) show, in both experiments, that phenelzine reduced the turnover through the 5-hydroxyindole pathway in brain.

It has been shown previously (Ashcroft *et al.*, 1968a) that inulin clearance represents the bulk removal of fluid from the c.s.f. system and that a high proportion of low concentrations of 5-HIAA and homovanillic acid are cleared from c.s.f. by a specific active transport system. It can be seen from the results in Table 2 that while phenelzine caused no significant change in the rate at which inulin was cleared, it produced a marked fall in the rate at which 5-HIAA was removed from the c.s.f.

The active transport mechanism for the removal of 5-HIAA and homovanillic acid from c.s.f. in the dog has been shown to be located in the region of the fourth ventricle (Ashcroft *et al.*, 1968a) and is presumably responsible for the marked gradient of 5-HIAA and homovanillic acid from lateral ventricle to cisterna magna. Thus the action of phenelzine which caused an inhibition of this localized transport mechanism explains why lowered concentrations of 5-HIAA were found in the c.s.f. from the lateral ventricles but not in the c.s.f. from cisterna magna (Fig. 1) where the reduced amount of 5-HIAA entering the c.s.f. was apparently compensated for by the inhibition of the mechanism for removing 5-HIAA.

Phenylacetic acid

The results shown in Table 3 are from experiments in conscious dogs in which, following a control period of recirculatory perfusion of the cerebrospinal fluid spaces, a solution of phenylacetic acid was infused into the system. The concentrations of 5-HIAA and homovanillic acid rose above control concentrations and almost reached values which have been found for endogenous concentrations of these acids in perfusion experiments with dogs treated with probenecid (Ashcroft *et al.*, 1968a) where the active transport mechanism has been shown to be inhibited. Despite the absence of detectable phenylacetic acid in brain, these results support the hypothesis that part of the action of phenelzine in blocking the egress of 5-HIAA from brain and c.s.f. may well be due to its acid metabolite, phenylacetic acid.

Discussion

The metabolic pathway to be considered is that from tryptophan through 5-hydroxytryptophan to 5-hydroxytryptamine and finally to 5-HIAA. The various regions of dog brain all showed similar changes in response to the action of the drug, the changes being slightly more marked in the midbrain and hypothalamus. Because of this the discussion will refer to ' brain ' concentrations without specifying any special regions. Chronic treatment with phenelzine caused no change in tryptophan concentration in the erythrocytes, plasma or c.s.f. No 5-hydroxytryptophan was detected in plasma or brain ; the 5-hydroxytryptamine concentration in brain was greatly increased and the 5-HIAA concentration, instead of being decreased as might be expected, was also increased. During chronic phenelzine administration the 5-HIAA concentration in c.s.f. from the cisterna magna remained unaltered from control levels, while in ventricular c.s.f. the concentration fell rapidly to about 50% of the control at which level it became stabilized. The results of experiments in which the cerebral ventricular system was perfused demonstrate that the metabolic turnover through the 5-hydroxyindole pathway in brain is reduced by phenelzine and also that the efflux of 5-HIAA from c.s.f. is impaired.

When the pathway was placed under a load by tryptophan administration and despite the fact that this produced the expected increases in the concentrations of tryptophan in erythrocytes, plasma, brain and c.s.f. there was no change in the concentrations found on treatment with phenelzine alone, of 5-hydroxytryptamine

TABLE 3. *Concentrations (nmol/ml) of 5-hydroxyindol-3-ylacetic acid (5-HIAA) and homovanillic acid (HVA) in fluid from a recirculatory perfusion of the cerebrospinal fluid spaces of conscious dog before and after infusion of phenylacetic acid*

		Control		Phenylacetic acid infusion		
Time of Sampling (h)		0·5	1	1·5	2	2·5
Dog C	5-HIAA	*0·6	0·6	0·9	1·2	1·3
	HVA	1·6	1·8	2·7	4·8	5·4
Dog D	5-HIAA	—	—	—	—	—
	HVA	1·9	1·7	2·5	6·3	

* Concentrations nmol/ml

and 5-HIAA in brain or of 5-HIAA in c.s.f. nor did 5-hydroxytryptophan become detectable in either plasma or brain

These results indicate that phenelzine was inhibiting the cerebral 5-hydroxyindole pathway to varying degrees at four separate points; the tryptophan 5-hydroxylase (no increase in turnover through the pathway after tryptophan administration), the monoamine oxidase (high concentrations of brain 5-hydroxytryptamine), the efflux of 5-HIAA from brain (the rise in brain 5-HIAA concentration instead of the expected fall), and the efflux of 5-HIAA from c.s.f. (the fall of 5-HIAA concentration in lateral ventricular c.s.f. but not in cisternal c.s.f. and the results from ventricular perfusion experiments).

The inhibitory effect of phenelzine on tryptophan 5-hydroxylase appeared to occur proportionally as the regional distribution of the total 5-hydroxyindoles in brain was unaltered. The inhibition might be a direct action of the drug or one of its metabolites or might be due to an inhibitory feedback from raised concentrations of 5-hydroxytryptamine.

It would seem likely that a small pool of free 5-hydroxytryptamine could cause an inhibitory feedback of tryptophan hydroxylase as administration of 5-hydroxytryptophan has been shown to inhibit the synthesis of the amine from tryptophan (Macon, Sokaloff & Glowinski, 1971). These findings and recent experiments concerning the relation of cerebral 5-hydroxytryptamine to a syndrome of hyperactivity and hyperpyrexia (Grahame-Smith, 1971) would suggest that the model previously proposed for cerebral 5-hydroxytryptamine metabolism (Moir & Eccleston, 1968) should be modified. The diagram shown in Fig. 5 illustrates two pools of 5-hydroxytryptamine in the subcellular synthetic complex, one in which the 5-hydroxytryptamine is stored, probably as a nucleotide complex of the type proposed by Pletscher and co-workers (Berneis, Da Prada & Pletscher, 1969; Goetz,

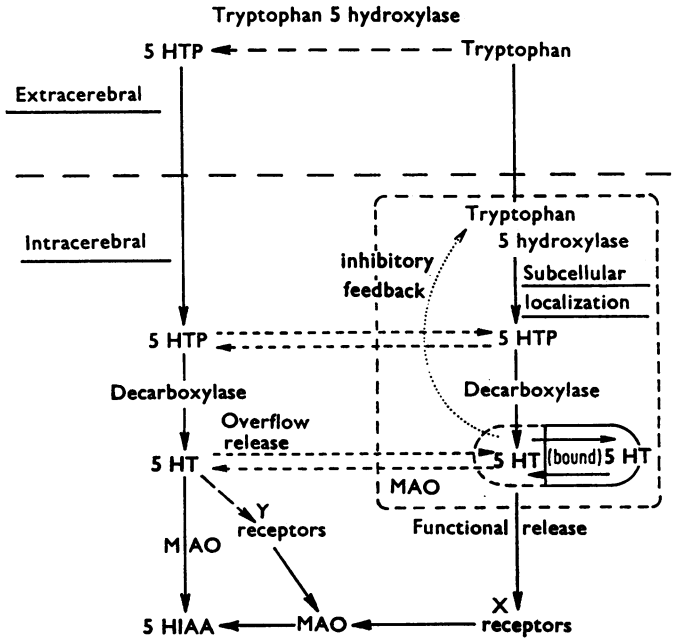

FIG. 5. Diagram of the functional relationships of the cerebral metabolism of 5-hydroxyindoles. (——), Routes of prime importance; (- - - -), routes of minor importance.

Da Prada & Pletscher, 1971) where turnover will be relatively slow and a second pool of newly synthesized 'free' 5-hydroxytryptamine which will turn over relatively rapidly and will give rise to the inhibitory feedback and functional release of 5-hydroxytryptamine and which may, in instances in which the catabolism of free 5-hydroxytryptamine is inhibited by monoamine oxidase inhibitors, overflow on to receptors which do not normally receive stimulation from 5-hydroxytryptamine.

Inhibition of 5-hydroxytryptophan decarboxylase which has been shown to occur in brain after phenelzine treatment (Dubnick, Leeson & Phillips, 1962) cannot be of major importance in producing the present biochemical changes, as 5-hydroxytryptophan was never detectable even after tryptophan administration.

The inhibitory action of phenelzine on the monoamine oxidases in brain, although still not well defined in terms of biochemical mechanisms, is not a controversial issue in terms of pharmacological actions and will not be discussed further here.

The hydrazine group of phenelzine is highly reactive and extensive review (Pletscher, Geoy & Burkard, 1966) of these types of drugs has reported them to inhibit very many enzymic mechanisms other than the monoamine oxidase. However, as the hydrazine type monoamine oxidase inhibitor, isocarboxazine does not appear to inhibit 5-HIAA egress from brain (MacLean *et al.*, 1965) it would appear that the effect of phenelzine on the transport of 5-HIAA from brain and c.s.f. is not due to its hydrazine group.

Phenylacetic acid, which occurs endogenously in man as one of the acid metabolites of phenylalanine (Ambrose, Power & Sherwin, 1933), has been shown to be the major metabolite of phenelzine in the mouse (Dubnick, 1962). The pharmacological actions of this acid metabolite are less widely known than those of its parent compound but like phenelzine it may act at more than a single point in the cerebral metabolism of the 5-hydroxyindoles. It was shown to have an inhibitory effect on 5-hydroxytryptophan decarboxylase *in vitro* (Davieson & Sandler, 1958) and *in vivo* (Dubnick *et al.*, 1962). Administered to carcinoid patients phenylacetic acid decreased the urinary 5-HIAA excretion, an observation interpreted by Sandler & Close (1959) and Sandler, Davis & Rimington (1959) on the basis of decreased 5-hydroxytryptamine formation by the inhibition of 5-hydroxytryptophan decarboxylase. As Milne (1959) pointed out there was insufficient evidence for this and he thought it was most likely that 5-HIAA, which had been shown by Despopoulos & Weissbach (1957) to be actively concentrated by renal cortex *in vitro*, would have its excretion by the kidney inhibited by phenylacetic acid. Milne, Crawford, Giraio & Loughridge (1960) showed that 5-HIAA was actively excreted by the kidney and that this could be inhibited by *p*-aminohippuric acid. However, the report most relevant to the present results was that by Neff & Tozer (1968) who demonstrated that the efflux of 5-HIAA from rat brain could be inhibited by massive doses of probenecid and certain other organic acids, including phenylacetic acid. Thus it would seem likely that phenylacetic acid derived from phenelzine might inhibit the efflux of 5-HIAA from brain and c.s.f.

This series of experiments, in addition to illustrating the power of using multiple pharmacological techniques and biochemical analyses in unravelling multiple points of action of a psychotropic drug and its metabolite, also shows that while metabolic manipulation of acid metabolite concentrations in c.s.f. can give valuable

insight into the state of cerebral metabolism (Moir, Ashcroft, Crawford, Eccleston & Guldberg, 1970) many complex mechanisms require to be taken into account in interpreting such data.

I am grateful to Drs. T. B. B. Crawford and G. W. Ashcroft for their valuable help and advice and to Miss Alice Urquhart, Mr. R. Strachan and Mr. I. Smith for expert technical assistance. Phenelzine was generously supplied by W. R. Warner & Co. Ltd.

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(*Received November* 8, 1971)