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Interaction in the cerebral metabolism of the biogenic amines:

Effect of intravenous infusion of L-tryptophan on the metabolism of dopamine and 5-hydroxyindoles in brain and cerebrospinal fluid

A. T. B. MOIR

M.R.C. Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ

Summary

1. In dogs, an intravenous injection of L-tryptophan followed by intravenous infusion of L-tryptophan, although unable to maintain stable concentrations of tryptophan in the plasma or cerebrospinal fluid, produced stable, raised concentrations of 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the cerebrospinal fluid (c.s.f.). This indicated that it was possible to raise the concentrations of the 5-hydroxyindoles in brain and to maintain the cerebral metabolism in a new steady state.

2. The regional distribution of the total molal concentration of the 5-hydroxyindoles in brain after the administration of tryptophan was similar to the distribution found in control animals, thus suggesting the normal rate limiting step of metabolism, the activity of the enzyme tryptophan 5-hydroxylase, was still the controlling factor.

3. Tryptophan administration caused a greater proportionate increase in the concentration of 5-HIAA than in that of 5-hydroxytryptamine (5-HT) in all regions of brain, perhaps indicating that the 'storage' capacity for 5-HT becomes filled under these conditions.

4. Administration of tryptophan caused a large rise in the concentration of homovanillic acid in c.s.f. demonstrating that there was an interaction between the cerebral metabolism of tryptophan and dopamine.

Introduction

Numerous studies have been performed investigating the role of the biogenic amines, dopamine and 5-hydroxytryptamine (5-HT) in brain and it has been shown that the metabolic pathways of both these amines include many similar steps, for example hydroxylation, decarboxylation, 'storage', oxidative deamination and the removal of acid metabolites. It has also been observed in many studies with psychotropic drugs that both dopamine and 5-HT metabolism may be affected in a similar manner though there is often a difference in the degree to which each amine is affected. This study shows that alteration of the metabolic load of one of these pathways (that of 5-HT) is itself sufficient to produce evidence of a profound alteration in the metabolism and, therefore, possibly the function of the other amine (dopamine). It is evident that this important interaction between the metabolism

of these two biologically active amines may be one of the factors which govern the normal modulation of neuronal activity.

Moir & Eccleston (1968) have shown that injections of either tryptophan or 5-hydroxytryptophan (5-HTP) resulted in a transient increase in the 5-hydroxyindole metabolites, 5-HT and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in brain. It was also shown in the dog that the pattern of the increased metabolites in the brain found after 5-HTP administration was not normal but reflected the distribution of the decarboxylase enzyme. However, the administration of L-tryptophan resulted in a normal pattern of distribution with similar percentage increases of the total 5-hydroxyindole metabolites in all the regions of the brain examined; the actual amount of the percentage increase depended only on the time between the administration of the tryptophan and the death of the animal. These studies showed that only tryptophan administration increased the concentrations of 5-hydroxyindoles in brain without disturbing the normal metabolism.

The changes produced in the 5-hydroxyindole metabolites in the brain were shown to be paralleled by similar changes in the 5-HIAA concentration in cerebrospinal fluid (c.s.f.) withdrawn from the cisterna magna, whether these changes were produced by L-tryptophan given alone or in addition to α-methyl dopa (Eccleston, Ashcroft, Moir, Parker-Rhodes, Lutz & O'Mahoney, 1968). As the above studies with a single injection of L-tryptophan posed problems in interpretation due to the transient nature of the changes in 5-hydroxyindole metabolism, the studies described here examined the possibilities of producing raised concentrations of 5-hydroxyindoles in a new steady state, and also allowed the investigation of their effect on cerebral dopamine metabolism.

There is good evidence that 5-HIAA (the acid metabolite of 5-HT) and HVA (the main acid metabolite of dopamine) in c.s.f. are derived only from the cerebral metabolism of their parent amines (Moir, Ashcroft, Crawford, Eccleston & Guldberg, 1970) and that alterations in their concentrations reflect alterations in amine metabolism in brain (Eccleston *et al.*, 1968; Guldberg & Yates, 1968; Moir *et al.*, 1970). There are considerable differences between dogs in the concentrations of these acids in c.s.f. (Ashcroft, Crawford, Dow & Guldberg, 1968a) and there are concentration gradients for both 5-HIAA and HVA between the lateral ventricle and the cisterna magna, which are due to the active transfer of these acids from c.s.f. by a specific mechanism situated in the region of the fourth ventricle (Ashcroft, Dow & Moir, 1968b).

In addition, alterations in the tryptophan concentration in cisternal c.s.f. have been shown to reflect the concentrations of tryptophan which occur in the brain after the intravenous administration of tryptophan.

Methods

Animal procedures

Male beagle dogs of approximately 10 kg were used. Food was withdrawn 12–16 h before the experiment, but water was available *ad libitum*. Each dog was lightly anaesthetized with sodium thiopentone, and supplementary doses were given throughout the experiment as required. In some animals a 0·5 ml sample of c.s.f. was withdrawn from the lateral ventricle by the method described by Ashcroft *et al.* (1968a). In all animals, a 2 ml sample of c.s.f. was withdrawn from the cisterna magna by the method of Eccleston *et al.* (1968).

After the initial samples had been obtained an L-tryptophan solution (10 mg/ml in 0·9% w/v NaCl solution) was administered intravenously in a dose of 50 mg/kg as an injection followed by a constant infusion at the rate of (20 mg/kg)/hour. Samples of c.s.f. were obtained at hourly intervals for 4 or 5 hours. In control experiments this schedule of serially withdrawing samples of c.s.f. at hourly intervals produces very little alteration in the concentrations of the metabolites measured, as was found with hourly sampling of ventricular c.s.f. alone (Ashcroft *et al.*, 1968a).

In some experiments, after the collection of the 4 h samples, the dog was heparinized and then exsanguinated through an arterial cannula. The brain was rapidly removed, bisected longitudinally and dissected in accordance with an atlas of dog brain (Lim, Liu & Moffitt, 1960). The brain samples were kept at −15° C for approximately 1 h until the biochemical analyses were commenced.

Biochemical procedures

One caudate nucleus from each dog brain was analysed for dopamine, 3-methoxytyramine, 4-hydroxy-3-methoxy-phenylacetic acid (HVA) and 3-4-dihydroxyphenylacetic acid (DOPAC) by the method of Crawford & Yates (1970). The other regions of the brain were weighed and then homogenized in acetic acid. Estimations of 5-HTP, 5-HT and 5-HIAA in the other brain regions and a venous plasma sample obtained after 4 h of intravenous infusion of L-tryptophan were carried out by the method of Ashcroft, Eccleston & Crawford (1965). Tryptophan in brain in c.s.f. was estimated as described by Moir (1971). Estimates of HVA, DOPAC and 5-HIAA in c.s.f. were performed by the method of Ashcroft *et al.* (1968a). Indolic and 5-OH indolic substances do not interfere with the estimation of HVA by this method.

All the biochemical results were corrected to 100% recovery by comparison with internal standards added to duplicate portions of appropriate samples.

In some experiments the authenticity of HVA estimated in samples of c.s.f. during the experiments was confirmed by extracting portions of the phosphate buffer used in the estimation of the concentration of HVA in c.s.f. obtained after 4 and 5 h of tryptophan infusion. These portions of phosphate buffer were brought to pH 1–2 with HCl saturated with NaCl and shaken with five volumes of ethyl acetate for 10 minutes. The water in the ethyl acetate extracts was removed with anhydrous sodium sulphate and after the addition of 0·1 ml 80% methanol (containing 50 mg ascorbic acid/100 ml) the extracts were taken to damp dryness under reduced pressure. Thin layer chromatography of the residue of the extracts was carried out in conjunction with standard amounts of authentic HVA as described by Ashcroft *et al.* (1968a) using a single 1 h development of silica gel H plates with the organic phase of a mixture of chloroform : acetic acid : water (2 : 2 : 1 by vol.) in an atmosphere of nitrogen. After the solvent had evaporated spraying with 0·1% ferricyanide in 5 N ammonium hydroxide the chromatogram of the c.s.f. extract showed a blue fluorescent spot isographic with authentic HVA. The intensity of fluorescence of the spot corresponded with the amount of HVA which had previously been estimated by the routine assay method to be in the sample.

Results

Cerebrospinal fluid

Table 1 shows the control concentrations of 5-HIAA and HVA in c.s.f. from the lateral ventricle and cisterna magna of the dogs used in this series of experiments. There were considerable differences between dogs in the concentrations of these acids in c.s.f. and concentration gradients for both 5-HIAA and HVA between lateral ventricle and cisterna magna.

TABLE 1. *Concentrations of 5-hydroxyindol-3-ylacetic acid (5-HIAA) and homovanillic acid (HVA) in dog cerebrospinal fluid*

	Lateral ventricle	Cisterna magna
5-HIAA	1·19±0·43 (15)*	0·20±0·15 (13)
HVA	7·95±1·82 (13)	0·39±0·21 (13)

* Mean concentration nmol/ml, ± standard deviation; number of estimates in parentheses.

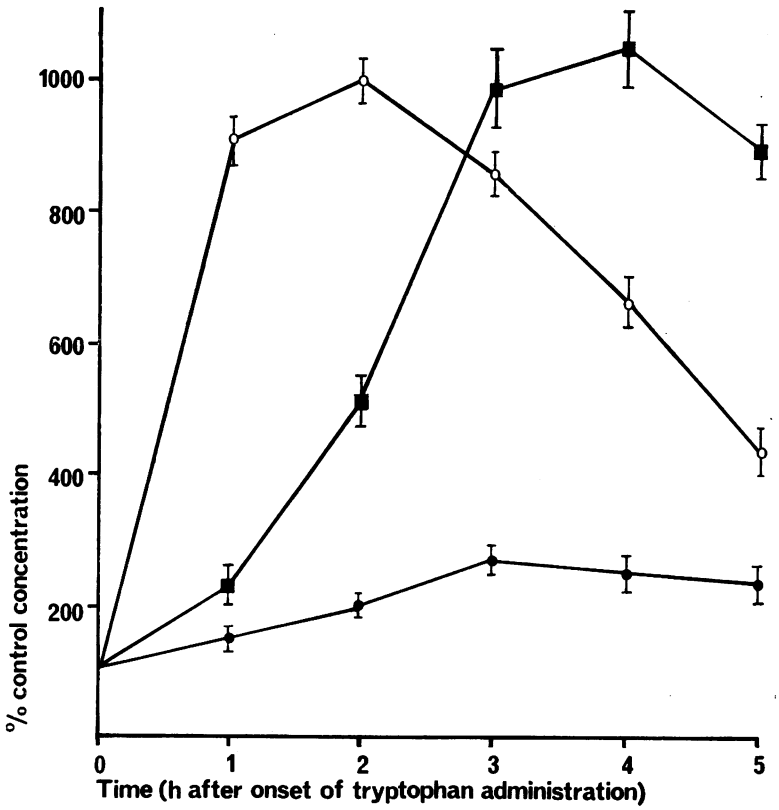

FIG. 1. Concentrations of tryptophan 5-hydroxyindol-3-ylacetic acid (5-HIAA) (●) and 4-hydroxy-3-methoxy-phenylacetic acid (HVA) (■) in c.s.f. from the cisterna magna of dogs after the intravenous administration of L-tryptophan (○). Results are expressed as percentages of the control values of each dog. Each point shows the mean and S.E.M. from five–six experiments unless otherwise indicated.

The plasma concentrations of tryptophan declined rapidly from their 1 h values despite the continuous infusion of tryptophan (Moir, 1971). The concentrations of tryptophan in cisternal c.s.f. (Fig. 1) reached a maximum 11 times control values at 2 h and then declined. The rate of decline of tryptophan concentrations was much slower in c.s.f. than in plasma (Moir, 1971). Despite the differences among the dogs in the actual values of the control concentrations of 5-HIAA and HVA in c.s.f. the alterations after tryptophan showed the same trend in each dog and when expressed in terms of their individual control values, each dog gave similar results. In cisternal c.s.f. (Fig. 1) the 5-HIAA concentration rose to a maximum 2·5 times the control value and this was maintained for 3–5 hours. Surprisingly the HVA in cisternal c.s.f. (Fig. 1) also showed a similar pattern of change ; however, the actual increase from control values was very much greater, being approximately 10-fold. The mean increment in HVA in cisternal c.s.f. is smaller than the 14-fold increase found in the initial experiments (Moir, 1969).

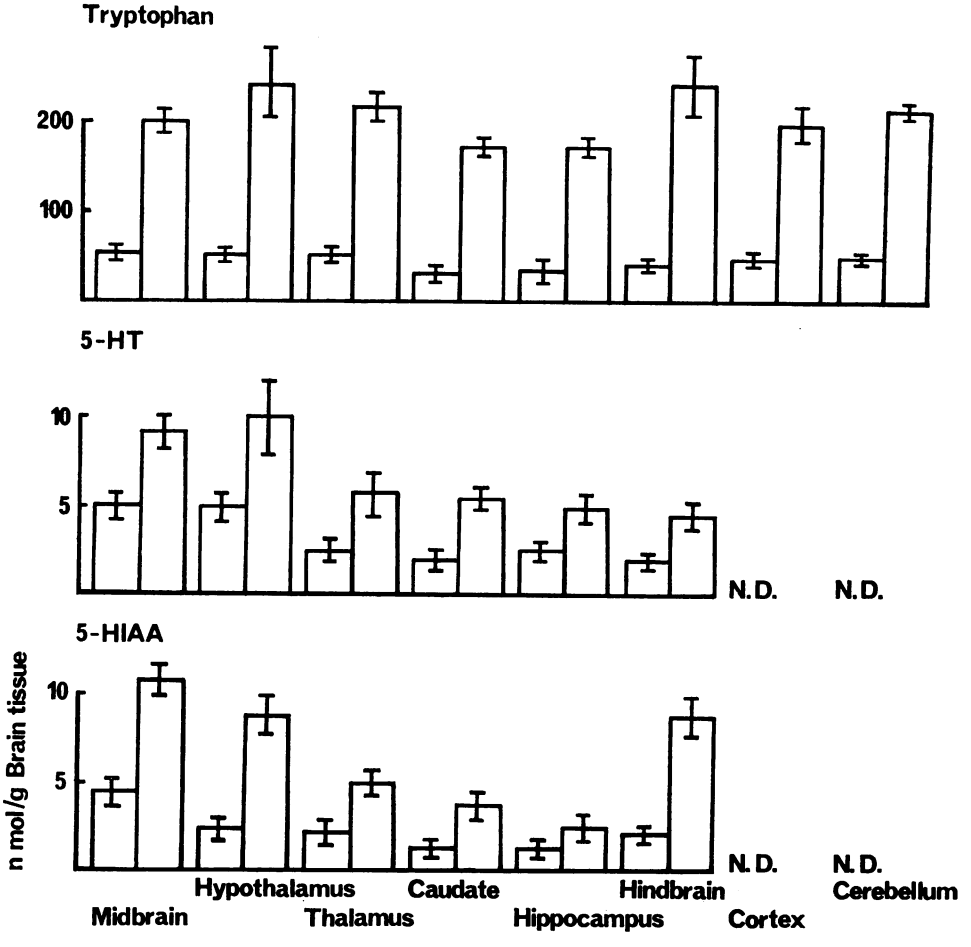

FIG. 2. Concentrations of tryptophan, 5-HT and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in various brain regions of dogs before and after intravenous administration of L-tryptophan. Concentrations in nmol/g brain tissue ± S.E.M. Control results from five–six dogs. Results after tryptophan administration from three dogs. N.D.—not detected.

In c.s.f. from the lateral ventricles, both 5-HIAA and HVA increased approximately 3-fold in the 2–5 h after starting the infusion of tryptophan.

In some experiments estimates were made of the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) in c.s.f. Small increases in concentration appeared to occur in both ventricular and cisternal c.s.f. after tryptophan administration, but the amounts assayed were too small to allow proper quantitation or characterization of the fluorophore ; however, the method would have been adequate to detect larger increases in DOPAC had they occurred.

Plasma

In plasma from control dogs neither 5-HTP nor 5-HIAA could be detected ($<0{\cdot}15$ nmol/ml). After 4 h of tryptophan infusion 5-HTP was still not detectable but 5-HIAA was present in low concentrations ($0{\cdot}43 \pm 0{\cdot}06$ nmol/ml (4)).

Brain

5-Hydroxyindoles

In animals killed after 4 h of tryptophan infusion 5-HTP could not be detected in any region of the brain (less than 0·2–0·4 nmol depending on region). The concentrations of 5-HT found in the different regions of dog brain after this form of tryptophan load were between 2 and 3 times those found in the control dogs (Fig. 2) and were greater than those concentrations found after a single intravenous injection of 50 mg/kg of tryptophan (Eccleston *et al.*, 1968). The concentrations of 5-HIAA were higher, relative to their control concentrations, than the 5-HT concentrations (Fig. 2). This disproportionate increase in 5-HIAA was particularly noticeable in the hindbrain region.

As the 3–5 h plateau in the 5-HIAA concentration in c.s.f. (Fig. 1) would appear to indicate that the higher concentrations of 5-hydroxyindoles in the brain were in a new steady state, the ratios of the molal concentrations of 5-HT/5-HIAA were

TABLE 2. *Ratio of the molal concentrations of 5-HT/5-HIAA in different regions of dog brain*

Region	Control	Tryptophan
Midbrain	$1{\cdot}12 \pm 0{\cdot}40$	$0{\cdot}80 \pm 0{\cdot}21$
Hypothalmus	$1{\cdot}82 \pm 0{\cdot}71$	$1{\cdot}21 \pm 0{\cdot}26$
Thalamus	$1{\cdot}06 \pm 0{\cdot}38$	$1{\cdot}08 \pm 0{\cdot}42$
Caudate	$2{\cdot}17 \pm 1{\cdot}02$	$1{\cdot}79 \pm 1{\cdot}07$
Hippocampus	$2{\cdot}70 \pm 0{\cdot}91$	$1{\cdot}84 \pm 0{\cdot}32$
Hindbrain	$1{\cdot}06 \pm 0{\cdot}41$	$0{\cdot}52 \pm 0{\cdot}21$
No. of dogs	6	3

Results are expressed as mean ± S.D. The results for the control and tryptophan treated dogs were contrasted by an analysis of variance. The difference between the two groups was significant ($P<0{\cdot}01$).

TABLE 3. *Concentrations of total 5-hydroxyindoles in different regions of dog brain*

Region	Control	Tryptophan
Midbrain	100%	100%
Hypothalamus	78 ± 20	88 ± 30
Thalamus	44 ± 24	55 ± 12
Caudate	30 ± 9	45 ± 6
Hippocampus	39 ± 13	36 ± 10
Hindbrain	39 ± 5	59 ± 13
No. of dogs	6	3

(5-HT)+(5-HIAA) expressed as a percentage of the concentration in the midbrain of each dog. Results are expressed as mean ± S.D.

examined. Table 2 shows these ratios in the tryptophan treated dogs compared with those in control dogs. The results have also been expressed as the concentrations of total 5-hydroxyindoles (5-HT and 5-HIAA) in Table 3 and are given as percentages of the concentration of 5-hydroxyindoles found in the midbrain region in order to make a comparison with the results obtained from control dogs.

The caudate nucleus did show some relative increase in its concentration of total 5-hydroxyindoles (Table 3). However, the most noticeable discrepancy was in the hindbrain region in the vicinity of which is a localized mechanism for the active transport of 5-HIAA from c.s.f. (Ashcroft *et al.*, 1968b). This region showed a relative increase in total 5-hydroxyindole content (Table 3) due almost entirely to a disproportionately high concentration of 5-HIAA (Fig. 2).

Dopamine and metabolites

One caudate nucleus from each dog was analysed for dopamine, methoxytyramine, 3,4-dihydroxyphenylacetic acid and HVA. Despite the very large changes produced in the HVA concentrations in c.s.f., the estimates obtained with tissues from tryptophan treated dogs were within the normal ranges for the caudate nuclei of control dogs.

Discussion

Despite the fact that the tryptophan concentrations in plasma and c.s.f. were not held constant throughout the experimental period, possibly due to the induction of tryptophan pyrrolase, the concentration of 5-HIAA in c.s.f. remained relatively uniform during the 3–5 h period. This would appear to indicate that the cerebral metabolism of 5-hydroxyindoles was taking place at a constant rate during this time. From previous studies (Moir & Eccleston, 1968 ; Eccleston *et al.*, 1968) it would seem probable that the turnover rate during these experiments was the maximum that could be achieved by the normal rate controlling step, intracerebral 5-hydroxylation of tryptophan. The raised concentrations of 5-hydroxyindoles found in the dog brain after 4 h of tryptophan infusion can, therefore, be regarded as being in a steady state.

The regional distribution in brain of the total 5-hydroxyindole metabolites in the new steady state (Table 3) was normal apart from the specific changes which might have been expected to occur in certain specialized brain regions, for example the hindbrain. Thus it would seem that in these experiments, as in previous ones (Moir & Eccleston, 1968 ; Eccleston et al., 1968) the localization of the 5-hydroxyindoles in brain was being controlled by the normal mechanism.

Examination of the relative concentrations of the amine and its acid metabolites in brain (Table 2) showed that the acid metabolite was increased more than the amine. With the present proposed scheme of cerebral 5-hydroxyindole metabolism (Moir & Eccleston, 1968) this finding is open to many interpretations. It has been suggested that in the steady state the ratio of the molal concentrations of 5-HT/5-HIAA (Table 2) is equal to the ratio of the 'efflux' constant for 5-HIAA to the 'efflux' constant for 5-HT (Tozer, Neff & Brodie, 1966). This ratio varies in different regions of the brain of control dogs, indicating that there are regional differences in the storage of 5-HT or in the pathway of its metabolism (Moir & Eccleston, 1968). The significant ($P<0{\cdot}01$) decrease in the ratio seen in tryptophan treated dogs may indi-

cate an increase in the proportion of 5-HT metabolized to that stored, a saturation of some component of the efflux of 5-HIAA from brain, or that part of the 5-HIAA has been derived from tryptophan which was hydroxylated to 5-HTP extracerebrally and would thus have contributed little (Moir & Eccleston, 1968) to the 5-HT concentration in the brain. The most likely explanation may be that the 5-HT 'storage' capacity becomes progressively filled giving rise to an increased relative concentration of 'free' 5-HT with a resultant relative increase in the 5-HIAA concentration relative to the total 5-HT concentration.

These results confirm a previous suggestion (Moir & Eccleston, 1968) that it should be possible to maintain the cerebral 5-hydroxyindole metabolism at its maximum rate by L-tryptophan administration. In this new maximally elevated steady state, the normal controlling factors of cerebral 5-hydroxyindole metabolism still seem to prevail and the steady concentration of 5-HIAA in the c.s.f. would seem to reflect the new state of the cerebral metabolism of the 5-hydroxyindoles. This technique would seem to allow the functional state of the controlling step in the cerebral metabolism of the 5-hydroxyindoles to be assessed from the concentration of metabolites in c.s.f., in animals after the administration of psychotropic drugs and perhaps in man in psychiatric disease states or after drug therapy.

Major changes in the concentration of tryptophan produced no alteration in the equilibrium concentration of tyrosine in brain and body fluids (Moir, 1971). Similarly, despite increases in tryptophan and 5-hydroxyindoles, the concentrations of dopamine and its main metabolites in the brain region primarily concerned with dopamine metabolism, the caudate nucleus, also appeared to be unaltered from the normal values. Thus, from an examination of brain tissue alone, even though all the main metabolite concentrations were determined, it would have seemed that an increased metabolic load of tryptophan had no influence on cerebral dopamine metabolism. However, the analysis of the main acid metabolite of dopamine (HVA) in c.s.f., particularly the c.s.f. from the cisterna magna, showed a very great rise after the administration of tryptophan (Fig. 1) thus revealing some interaction between the two metabolic pathways.

5-HIAA and HVA do appear to compete for the same active transport mechanism in the region of the fourth ventricle in the dog. This mechanism causes their active removal from c.s.f.; however, the affinity of HVA for transport appears higher than that of 5-HIAA and relatively large amounts of 5-HIAA given into the c.s.f. system produce comparatively small rises in HVA concentration (Ashcroft *et al.*, 1968b). Thus, it is not likely that this type of competition can explain more than a small part of the rise found in the HVA concentration in cisternal c.s.f. This idea was confirmed by the fact that the HVA concentration in c.s.f. from the lateral ventricle also showed considerable increases after the administration of tryptophan.

The fact that the analysis of the metabolites of the biogenic amines in c.s.f. reveal evidence of interaction between tryptophan and dopamine despite the absence of overt changes in the brain concentrations of dopamine or its metabolites might prove to be important in studying the metabolism of biogenic amines in the brain.

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