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Interaction in the cerebral metabolism of the biogenic amines:

Effect of intravenous infusion of L-tryptophan on tryptophan and tyrosine in brain and body fluids

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Summary

1. The distribution of the amino-acids tryptophan and tyrosine has been determined in plasma ultrafiltrate, whole plasma, erythrocytes, cerebrospinal fluid (c.s.f.) and various regions of the brain in dogs.

2. The effect of tryptophan administration on the distribution of both these amino-acids showed that the alterations produced in tryptophan concentration did not appear to change the concentrations of tyrosine from their normal pattern.

3. The implications of these results with regard to amino-acid transport systems in man and dog are discussed.

Introduction

Interaction between the cerebral metabolism of tryptophan and dopamine has been demonstrated in dog brain (Moir, 1969; Moir & Yates, 1970; Moir, 1971). It was thought initially, in view of the experiments of Guroff & Udenfriend (1962) demonstrating that tryptophan inhibited the rate of uptake of radioactive tyrosine into rat brain, that the interaction demonstrated between the metabolism of the biogenic amines might be due to an interaction between their precursor amino-acids tryptophan and tyrosine. Because of this, concurrent estimations of these amino-acids were made in samples of various body fluids and brain regions during the experiments reported by Moir, 1971.

These results are reported separately, as although they demonstrate many interesting features of the behaviour of the amino-acids, tryptophan and tyrosine, in brain and body fluids, they provide no evidence of any interaction between them.

Methods

In some of the experiments reported by Moir (1971), estimates were made of tryptophan and tyrosine in plasma, plasma ultrafiltrates, erythrocytes, c.s.f. and various regions of brain.

Animal procedures

Dogs were lightly anaesthetized with intravenous sodium thiopentone. Venous blood was then withdrawn into a disposable syringe and a 7 ml portion transferred

to a 10 ml polyethylene tube containing 0·2 ml heparin (1,000 I.U./ml). After inverting the tube a few times, it was centrifuged at 2,500 *g* for 8 minutes. A 2 ml portion of plasma was transferred to Visking dialysis tubing-8/32 (Scientific Instrument Centre Ltd.) and an ultrafiltrate prepared by centrifuging at 3,000 *g* for 1 h at 37° C using an adaptation of the method of McMenamy, Lund, Van Mercke & Oncley (1961). This ultrafiltrate and portions of the plasma and red cell layer were transferred to separate polyethylene containers and stored at −15° C. A 2 ml sample of c.s.f. was withdrawn from the cisterna magna (Moir, 1971), transferred to a glass tube and stored at −15° C.

Once 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)/h and further samples of c.s.f., plasma, plasma ultrafiltrate and erythrocytes were obtained at hourly intervals for 4 or 5 hours. In some experiments after the collection of the 4 h samples, the dog was heparinized and rapidly exsanguinated through an arterial cannula. In these experiments the dog brain was then removed and dissected into its various regions (Moir, 1971). The brain samples were kept at −15° C for approximately 1 h until the biochemical analyses were commenced.

Plasma ultrafiltrate

McMenamy *et al.* (1961) showed that the small proportion of human plasma tryptophan which was dialysable could be reliably estimated by the concentration found in an ultrafiltrate of the plasma. The dog plasma ultrafiltrates analysed in these experiments were prepared in a manner similar to that described by McMenamy *et al.* (1961) and gave values similar to those found by Geddes (1969) for the proportion of dog plasma tryptophan which was dialysable.

Biochemical procedures

Samples of 0·1 ml of the plasma and erythrocytes and 0·5 ml of acetic acid homogenates of the various regions of brain (Ashcroft, Eccleston & Crawford, 1965) were diluted to 5·5 ml with distilled water and then the proteins were precipitated with 1 ml of 30% (w/v) trichloroacetic acid (TCA) which was allowed to act for exactly 10 minutes. After centrifugation at 2,500 *g* for 7 min, portions of these supernatants were used to estimate tryptophan and tyrosine by the fluorimetric methods of Hess & Udenfriend (1959) and Waalkes & Udenfriend (1957) respectively.

The estimates of tryptophan and tyrosine in c.s.f. and plasma ultrafiltrate were performed with 0·1 ml samples diluted to 0·85 ml with distilled water. The proteins were precipitated with 0·15 ml 30% (w/v) TCA which was allowed to act for 10 minutes. The fluorimetric assays were also scaled down and the fluorescence of the final solutions measured in a micro-cuvette using either a Farrand or Perkin Elmer spectrofluorimeter.

With some samples which were expected to contain large amounts of tryptophan, it was necessary to perform the tryptophan assays on dilutions which permitted the concentration of the resulting fluorophore to remain within the range of linearity of the assay method. All the biochemical results were corrected to 100% recovery by comparison with internal standards added to duplicate portions of appropriate samples.

Results

Concentrations in body fluids

Table 1 shows the control concentrations of tryptophan and tyrosine in plasma, plasma ultrafiltrate, erythrocytes and c.s.f. Seventy per cent of the plasma tyrosine, but only 20% of the tryptophan was ultrafiltrable. The control mean concentration gradients between ultrafiltrate and erythrocytes were 1:5·1 for tyrosine and 1:3·8 for tryptophan while the gradients between ultrafiltrate and c.s.f. were approxi-

TABLE 1. *Concentrations of tryptophan and tyrosine in plasma, plasma ultrafiltrate, cerebrospinal fluid and erythrocytes of control dogs*

	Tryptophan	Tyrosine
Cisternal c.s.f.	5·8± 1·8 (18)	18·0± 4·2 (18)
Plasma ultrafiltrate	11·3± 2·1 (6)	34·3± 8·2 (6)
Total plasma	55·8±15·1 (19)	48·5± 9·2 (19)
Erythrocytes	42·9± 7·9 (19)	174·0±15·8 (18)

Concentration in nmol/ml; mean ± S.D. (number of estimates).

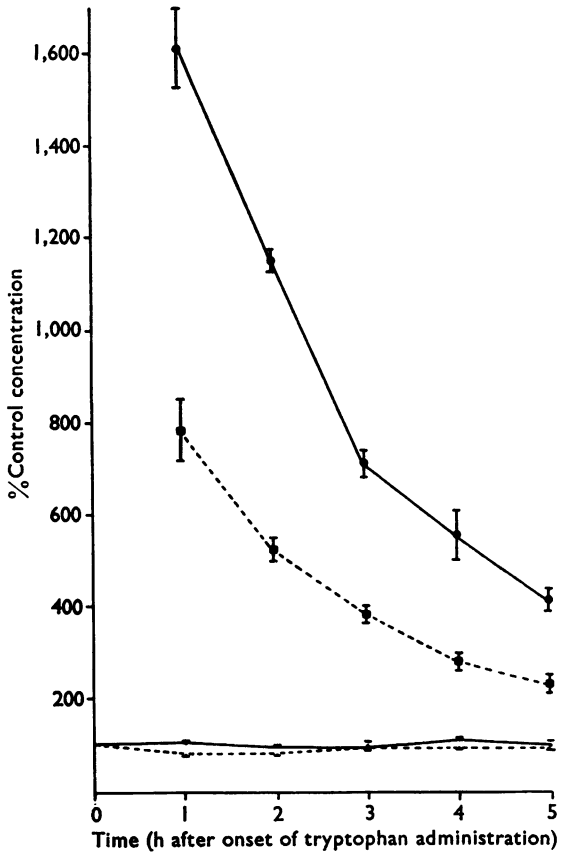

FIG. 1. Concentrations of tryptophan in plasma ultrafiltrate (●) and whole plasma (■) and concentrations of tyrosine in plasma ultrafiltrate (——) and whole plasma (– – –) after administration of L-tryptophan. Results expressed as percentage of control values of each dog. Ultrafiltrate results are means and S.E.M. from three experiments. Whole plasma results are means and S.E.M. from six experiments except 5 h values which are the means and S.E.M. from three experiments.

mately 1:0·5 for both amino-acids. Thus for tryptophan the gradient between plasma and c.s.f. was more apparent than real, and the apparently slight concentration gradient between plasma and erythrocytes became reversed and quite large when the 'free' tryptophan concentration in the plasma ultrafiltrate was considered. In the case of tyrosine, the apparent concentration gradients between plasma, erythrocytes and c.s.f. were little modified by considering the free tyrosine concentrations in the plasma ultrafiltrate.

Tryptophan concentrations after tryptophan administration

Plasma. Despite the constant infusion of tryptophan after the initial loading injection the concentration of tryptophan in plasma declined from its 1 h value towards its control value (Fig. 1).

Plasma ultrafiltrate. The tryptophan concentration in plasma ultrafiltrate reached relatively higher concentrations than in plasma and declined more rapidly (Fig. 1). Thus 1 h after tryptophan administration, 50% of the plasma tryptophan was filterable as opposed to 20% in control samples and 35% 5 h after tryptophan administration. The complex decline in tryptophan concentrations in plasma ultrafiltrate and whole plasma throughout the tryptophan infusion is probably the result of variable saturation of binding mechanisms and substrate induction of tryptophan pyrrolase.

Erythrocytes. The tryptophan concentration in the erythrocyte fraction was maintained throughout the infusion period (Fig. 2) at 350% of its control value, suggest-

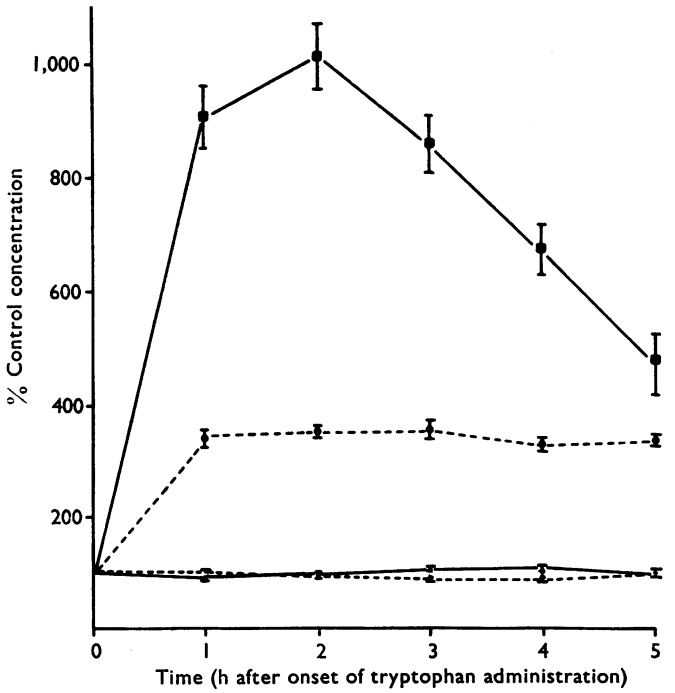

FIG. 2. Concentrations of tryptophan in cisternal c.s.f. (■) and erythrocytes (●) and concentrations of tyrosine in cisternal c.s.f. (——) and erythrocytes (– – –) after administration of L-tryptophan. Results expressed as percentage of control values of each dog. Results are the means and S.E.M. from six experiments except 5 h values which are the means and S.E.M. from three experiments.

ing that the capacity of the erythrocytes to contain tryptophan was fully saturated during the experimental period.

Cerebrospinal fluid. Tryptophan concentrations rose to maximal values at 1–2 h then showed a slow decline.

Tyrosine concentrations after tryptophan administration

There was little change in tyrosine concentrations in either plasma ultrafiltrate (Fig. 1) or c.s.f. (Fig. 2) throughout the infusion period. Concentrations of tyrosine in whole plasma (Fig. 1) showed a slight decline from control values particularly during the first two hours. Erythrocytes (Fig. 2) showed a slight decline in tyrosine concentration towards the end of the experimental period.

Brain

In control dogs, tyrosine concentrations in the different brain regions (Fig. 3) had a significant order ($P<0.05$) in which they could be ranked. However, this appeared to be due mainly to the contribution from three regions, hypothalamus, cerebellum and caudate which, respectively, were approximately 180, 130 and 115% of the average concentration in the other regions. Tryptophan infusion for 4 h produced no apparent alterations in the concentrations of tyrosine in brain.

In control dogs, tryptophan concentrations in the regions of brain analysed have a significant order ($P<0.05$) of ranking, but these regional differences in concentration could not be detected after an intravenous injection of L-tryptophan (Eccleston, Ashcroft, Moir, Parker-Rhodes, Lutz & O'Mahoney, 1968). In these experiments, after tryptophan infusion, all regions had tryptophan concentrations of approximately 200 nmol/g (Fig. 3) and there did not appear to be a significant rank order for the tryptophan concentrations. However, the concentrations in the caudate nucleus and hippocampus were consistently lowest and second lowest, which was also their concentration order in both the control dogs and tryptophan loaded dogs of previous experiments (Eccleston *et al.*, 1968). Thus it would appear that there

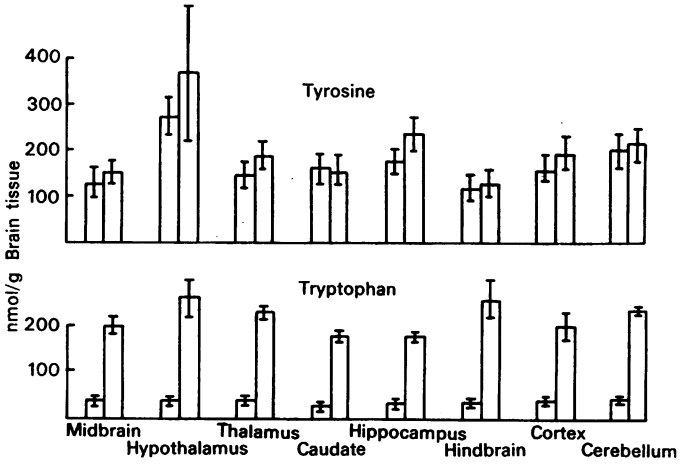

FIG. 3. Concentrations of tryptophan and tyrosine in various brain regions of dogs before and after intravenous tryptophan administered for 4 hours. Control results for tryptophan means and S.E.M. from five or six dogs. Control results for tyrosine and results after tryptophan administration are means and S.E.M. from three dogs.

is still some degree of regional variation of tryptophan concentration possibly due to regional differences in binding the amino-acid.

Discussion

McMenamy and his co-workers have investigated the distribution of tryptophan in human plasma and have shown that normally about 80% of the L-tryptophan is not dialysable but is bound to serum albumin in a specific manner (McMenamy & Oncley, 1958). The proportion of bound tryptophan is affected by the temperature, ionic strength and pH of the solution (McMenamy *et al.*, 1961). The portion of dialysable tryptophan in plasma can be reliably estimated in the plasma ultrafiltrate (McMenamy *et al.*, 1961). The above studies have demonstrated that total tryptophan can be quantitatively recovered from plasma after its precipitation with trichloroacetic acid (the method used in this study), although studies with radioactive L-tryptophan (Gal, Morgan, Chatterjee & Marshall, 1964) have suggested that there is yet another small fraction of tryptophan which remains in the protein precipitate, perhaps because of incorporation into the protein.

In contrast to plasma, however, McMenamy, Lund, Neville & Wallach (1960) found that tryptophan did not appear to be bound to the components of lysed human erythrocytes and that full quantitative recovery of added tryptophan could be made by dialysis. These workers also determined that in humans, the average ratio for dialysable tryptophan (mol/kg water) erythrocytes/plasma was 1 : 1·2.

In the dog, total plasma tryptophan cannot be estimated by dialysis. Geddes (1969) has shown with equilibrium dialysis studies that in the dog, as in man, approximately 80% of the plasma tryptophan is in a non-dialysable form. Thus the present studies confirm that estimations of tryptophan in a plasma ultrafiltrate give a reliable estimate of the free plasma tryptophan. If the normal concentrations found in dog erythrocytes, plasma and plasma ultrafiltrate (Table 1) are interpreted in the light of these previous studies, it would appear that either a proportion of the tryptophan in the animal erythrocytes is in a bound form which does not readily dissociate, or there is some mechanism concentrating diffusable tryptophan within the canine erythrocytes which is limited by a maximum absolute concentration that can be maintained within the cell.

The negative concentration gradient of tryptophan between plasma and c.s.f. (Table 1) is more apparent than real when the free concentration in the plasma ultrafiltrate is corrected, and indeed Geddes & Moir (1969) have shown that simple diffusion mechanisms would appear to be the most important factors controlling the interchange of tryptophan between these two body fluids in the dog. The concentration gradient between plasma ultrafiltrate and c.s.f. for tyrosine is almost identical with that for tryptophan suggesting perhaps similar passive mechanisms are involved in its maintenance.

McMenamy and his co-workers have shown that in humans, all the tyrosine in plasma (McMenamy, Lund & Oncley, 1957) and in erythrocytes (McMenamy *et al.*, 1960) is diffusable and that the ratio of the mean concentrations (mol/kg water) erythrocytes/plasma was 1 : 1·03, a value compatible with control of the tyrosine equilibrium being achieved by simple diffusion.

In dog plasma all the tyrosine would appear to be diffusable (Geddes, 1969) as measured by equilibrium dialysis ; however, these experiments show that not all

plasma tyrosine passes into the ultrafiltrate, suggesting that some of the plasma tyrosine may be bound. The concentrations of tyrosine found in canine erythrocytes were nearly 4 times higher than in plasma (Table 1) which would suggest that canine erythrocytes are capable of concentrating or binding tyrosine intracellularly. Similar results for tyrosine concentrations in canine erythrocytes and plasma have been obtained by Elwyn (1966).

While the effects of prolonged anaesthesia cannot be excluded, it would seem likely that the alterations of tyrosine concentrations in plasma and erythrocytes during the infusion of tryptophan are due to the large volume of saline in which the tryptophan was administered as similar changes were observed in control experiments where dogs received equivalent volumes of saline. The changes presumably reflect dilution of the ' bound ' plasma tyrosine and the erythrocyte tyrosine. That ultrafilterable tyrosine remains little altered during the experiment suggests that tyrosine concentration in body fluids equilibrate rapidly. This hypothesis is supported by the experiments of Elwyn (1966).

It has been suggested by Elwyn (1966) that the different patterns of amino-acid found concentrated in human and canine erythrocytes may be due to human erythrocytes having an active transport system with a leucine-preferring carrier and canine erythrocytes having an alanine-preferring carrier. This alanine-preferring carrier would appear to have similar characteristics to the uptake mechanism proposed for rat brain by Guroff & Udenfriend (1962).

In view of the fact that active amino-acid transport is usually a cation linked process, a major difference between human and canine erythrocytes which would seem relevant to their different patterns of amino-acid transport is that while human erythrocytes have a high intracellular potassium concentration, as do most body cells, canine erythrocytes have a high intracellular sodium concentration (Prankerd, 1961). Much evidence has suggested that neuroglial cells have relatively high intracellular concentrations of sodium compared with neurones and it might be expected that their amino-acid transport systems would also differ.

Different regions of the brain vary slightly in their tryptophan concentration (Eccleston *et al.*, 1968). Similarly, a few brain regions, for example the hypothalamus and cerebellum, have higher concentrations of tyrosine than the other regions of the brain. These regional variations in the concentration of the amino-acids may be important factors involved in the regulation of the various different functions of the separate brain regions. Alternatively, they may reflect a variation in the cellular population of the regions of brain with differences in the amino-acid uptake into cells or the binding capacity within cells.

These experiments appeared to show that in brain tissue, as in plasma, erythrocytes and c.s.f. a large elevation of the tryptophan concentration did not appear to alter factors concerned with the maintenance of normal equilibrium concentrations of tyrosine. Thus the interaction between tryptophan and dopamine in brain (Moir, 1969 ; Moir & Yates, 1970 ; Moir, 1971) is not due to interaction between the parent amino-acids. These experiments also illustrate particularly with reference to tryptophan, the dangers of regarding total plasma or brain concentrations as indicative of the quality of substance available for transport or metabolism. Thus it is naïve to relate such concentrations to the *in vitro* determined *Km* values of their metabolizing enzymes or to imply that alteration in total concentration of substrate

in whole brain must necessarily alter significantly the rate of subsequent neuronal metabolism (Tagliamonte, Tagliamonte, Perez-Cruet & Gessa, 1971).

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