Br. J. Pharmac. (1976), **58**, 3–7

A COMPARISON OF D AND L-TRYPTOPHAN ON THE CEREBRAL METABOLISM OF 5-HYDROXYTRYPTAMINE AND DOPAMINE IN THE DOG

J. BUDNY
The Proctor and Gamble Co., Miami Valley Laboratories, P.O. Box 39175, Cincinatti, Ohio 45239, U.S.A.

R.C.DOW, D. ECCLESTON, A.G. HILL & ISOBEL M. RITCHIE
MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ

1 After D and L-tryptophan (50 mg/kg) were given intravenously in the dog, the concentration of the amino acid was increased in ventricular cerebrospinal fluid (CSF) during the subsequent 4 h of sampling, although the concentrations were significantly lower following the administration of the D-isomer.

2 There was no evidence that D-tryptophan increased the synthesis of 5-hydroxytryptamine (5-HT) in dog brain as judged by the failure to cause a change in 5-hydroxyindoleacetic acid (5-HIAA) concentrations in ventricular CSF different from that seen with controls.

3 There was no appreciable conversion of D-tryptophan to L-tryptophan in the dog.

4 D-tryptophan was cleared more rapidly from plasma than L-tryptophan.

5 No difference in plasma binding between D and L-tryptophan was detected.

Introduction

In exploring the effects of D-tryptophan on brain indoles in rats, Yuwiler (1973) observed that there was an increase in the cerebral 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations. Since he also observed that D-tryptophan only sparingly crossed the blood brain barrier, he attributed the rise in 5-hydroxyindole concentrations to extra-cerebral conversion of D-tryptophan to L-tryptophan via indole pyruvate. Numerous studies (du Vigneaud, Sealock & Van Etten, 1932; Berg, 1934; Oesterling & Rose, 1952) have shown that the rat is able to use D-tryptophan almost as well as L-tryptophan for growth, which is consistent with Yuwiler's explanation of increased formation of 5-hydroxyindoles from D-tryptophan. There is a likelihood of human exposure to D-tryptophan because it is sweet (Berg, 1942; Berg, 1953). It could then be considered as a substitute for the currently used artificial sweetener Saccharin, cyclamate already having been withdrawn for toxicological reasons. It is also a constituent of oral and intravenous amino acid supplements. If one then wishes to extrapolate animal studies on D-tryptophan to man, it seems likely that the rat is an inappropriate model since man is probably unable to use D-tryptophan (Baldwin & Berg, 1949; Rose, 1949). The dog on the other hand appears to be unable to convert D-tryptophan to kynurenic acid (Correll, Berg & Cowan, 1938). Furthermore, the urinary metabolite profile from dogs given D-tryptophan (L. M. Henderson and K.C. Triebwasser, personal communication) seems to be reasonably similar to the urinary metabolites of D-tryptophan from man (Hankes, Brown, Leklem, Schmaeler & Jesseph, 1972). Consequently the dog is probably a better model than the rat for examining the effect of D-tryptophan on the 5-HT pathway in animals if it is intended to extrapolate the findings to man. Therefore, we initiated these studies to determine what effect, if any, D-tryptophan had on the 5-HT pathway in the brains of dogs, and indirectly on that of dopamine.

Methods

Four male Beagle dogs with indwelling ventricular cannulae (Ashcroft, Dow & Moir, 1968) had 0.5 ml of CSF withdrawn on 3 occasions, at 0, 30 and 60 min

before an intravenous loading dose of equal volumes of 0.9% w/v NaCl solution (saline), or D or L-tryptophan (50 mg/kg made up in saline at a concentration of 20 mg/ml). This dose was chosen as that producing a change in 5-HT synthesis when cisternal CSF was used as an index of cerebral turnover (Eccleston, Ashcroft, Moir, Parker-Rhodes, Lutz & O'Mahoney, 1968). Further samples of CSF were withdrawn at 30 min, 1, 2, 3 and 4 h after the intravenous injection. Five ml of blood was removed by venepuncture at the same time as the CSF samples. The experiments were always performed at the same time of day to obviate possible errors due to circadian variation.

Biochemical estimations

Free and total tryptophan. The 5 ml of blood was centrifuged immediately at room temperature for 5 min at 1000 *g*. The plasma was separated and an aliquot deep frozen until the total tryptophan was estimated; 1 ml of plasma was centrifuged at 1000 *g* for 1 min at room temperature through an Amicon membrane (Centriflo 224-CF-50). The ultrafiltrate was deep frozen until required for estimation (not more than 2 days). Tryptophan was then measured on 0.1 ml of plasma ultrafiltrate and 0.1 ml of CSF by a micro method based on that of Guroff & Udenfriend (1962) from the original method of Hess & Udenfriend (1959).

Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid in CSF. These were estimated according to the spectrophotofluorescence method of Yates & Guldberg (1968) modified for small CSF volumes by Pullar, I.A. (unpublished).

Statistical treatment of the data

Because the variation in the data between animals and between experiments was large, it was decided to use all the CSF data in the analysis rather than that obtained at particular time points and to treat each set as a regression problem (Kendall & Stuart, 1967). The best fitting polynomial was ascertained by computer programme and this gave information as to whether metabolites rose significantly, if they rose in a linear fashion or if they rose and fell. Such treatment of data in similar previous experiments in cisternal fluid had indicated a rise and fall of 5-HIAA after treatment with L-tryptophan (Eccleston *et al.*, 1968).

Results

Tryptophan

The administration of D and L-tryptophan intravenously led to an elevation of the amino acid

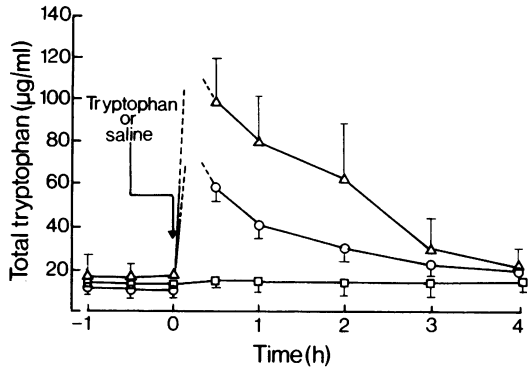

Figure 1 Total concentration of tryptophan (free and bound) in plasma of dog both before and after an intravenous dose of D (O) or L-tryptophan (△) (50 mg/kg) or saline (□). Values are mean of 4 experiments; vertical lines show s.d.

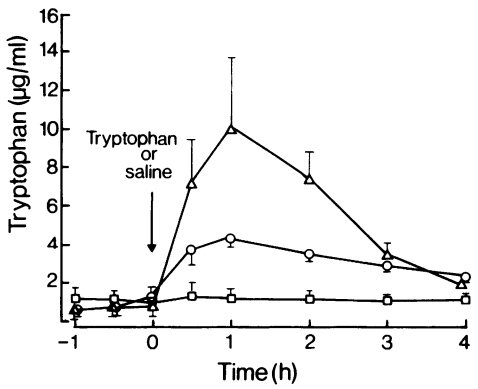

Figure 2 Concentration of tryptophan in ventricular CSF of dog before and after an intravenous injection of D (O) or L-tryptophan (△) (50 mg/kg) or saline (□). Values are mean of 4 experiments; vertical lines show s.d.

both in plasma and CSF (Figures 1 and 2). Even though equal quantities of the isomers were given, after 30 min and 1 h the total concentration of D-tryptophan in plasma was significantly ($P < 0.02$ and $P < 0.025$ respectively, Student's t test) lower than that for L-tryptophan and at 1 h the concentration of free tryptophan was lower for the D-isomer than the L-isomer ($P < 0.05$, Student's t test). There was no difference in the percentage of amino acid bound to the plasma protein between the D and L forms. Taking the best fit cubic equations for the results with CSF (significance of fit $P < 0.001$) the concentration of D-tryptophan peaks at 1.3 h with a value of 4.3 ± 0.3 µg/ml and the concentration of the L-isomer peaks (significance of fit cubic equation $P < 0.01$) at a similar time, 1.2 h reaching 9.9 ± 1.1 µg/ml which is

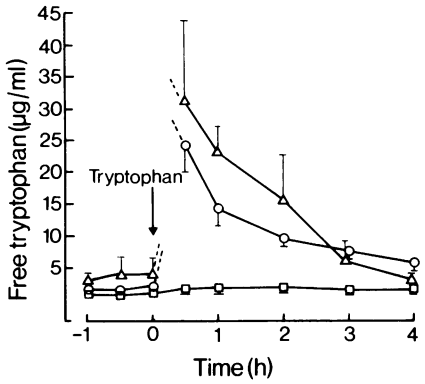

Figure 3 Concentration of free tryptophan in plasma of dog after an intravenous dose of D (○) or L-tryptophan (△) (50 mg/kg) or saline (□). Values are mean of 4 experiments; vertical lines show s.d.

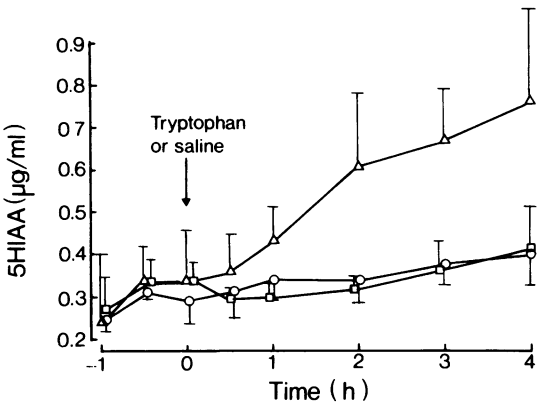

Figure 4 Concentration of 5-hydroxyindoleacetic acid (5-HIAA) in ventricular CSF of dog after an intravenous injection of D (○) or L-tryptophan (△) (50 mg/kg) or saline (□). Values are mean of 4 experiments; vertical lines show s.d.

significantly different from the maximum concentration of D-tryptophan at the 0.001 level (Student's *t* test). This suggests that there is no delay in entry of D-tryptophan into CSF compared with L and that the lower concentrations are a reflection of the lower concentrations of the amino acid in plasma. In the experiments in which saline was injected there was neither a change in free or total tryptophan in blood or plasma with time (Figures 1 and 3) nor in CSF (Figure 2).

5-Hydroxyindoleacetic acid and homovanillic acid

The data for 5-HIAA in CSF (Figure 4) after both L-tryptophan and saline significantly fits a linear

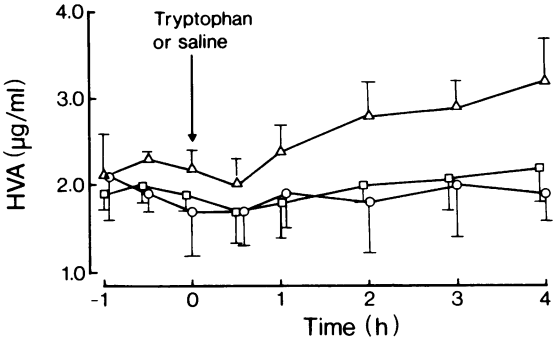

Figure 5 Concentration of homovanillic acid (HVA) in ventricular CSF of dog after an intravenous injection of D (○) or L-tryptophan (△) (50 mg/kg) or saline (□). Values are mean of 4 experiments; vertical lines show s.d.

equation ($P < 0.005$ and < 0.02 respectively) whilst for D-tryptophan there is a linear trend which did not reach statistical significance ($0.1 > P > 0.05$). Inspection of the data (Figure 4) shows the results with D-tryptophan and saline to be initially superimposible and certainly not significantly different from each other. The rate of rise was, however, higher for L-tryptophan than saline ($P < 0.001$). In the case of HVA in CSF (Figure 5), only L-tryptophan produced a linear rise ($P < 0.001$), saline just failing to reach significance ($0.1 > P > 0.05$).

Discussion

The aim of the present experiments was to determine whether or not there is an increase in 5-HT synthesis in the dog after the administration of D-tryptophan as has been shown in the rat (Yuwiler, 1973). It was hoped to use the technique of tryptophan loading with cisternal CSF sampling as used in previous experiments (Eccleston *et al.*, 1968). Unfortunately in preliminary experiments the data showed wide variation and ventricular CSF studies were initiated. It was found that after L-tryptophan there was a sustained rise in 5-HIAA concentration in ventricular CSF (Figure 4) and this continued throughout the 4 h of the experiment, the data fitting linear rather than quadratic equations. The previous experiments examined the concentration of 5-HIAA in both brain and cisternal CSF and showed peaks in both concentrations at about 2.3 h following the administration of L-tryptophan with a return to normal at 4 hours. The difference between the CSF from the two sites, ventricle and cisterna, may lie in the proximity of the cisterna to the choroid plexus of the 4th ventricle, the site at which acid metabolites are actively removed from the CSF (Ashcroft *et al.*, 1968). The results

suggest that ventricular CSF levels of metabolites remain high after brain concentrations have fallen.

In the rat Yuwiler (1973) found that D-tryptophan penetrated brain only slowly compared with L-tryptophan and although concentrations of tryptophan in brain were almost as high after peripheral injection of the D-isomer as after the L-isomer, an analysis of the amino acid present in the brain showed it to be almost entirely L, suggesting a rapid peripheral conversion of D-tryptophan to L-tryptophan with subsequent uptake into brain. Under these circumstances a rise in cerebral 5-HT synthesis was anticipated and, in the case of the rat, found.

Our results in the dog are clearly different from the rat since there is no rise in the synthesis of 5-HT after D-tryptophan administration. After injection of the D-isomer, the concentration of tryptophan is subsequently increased in the ventricles but not after injection of saline. After both these treatments there was no rise in 5-HIAA. When L-tryptophan was given there was a rise in the concentration of both tryptophan and 5-HIAA in the ventricular CSF. This suggests that the tryptophan isomer found in the CSF after infusion of tryptophan is the D-form and supports the view that the peripheral conversion of D-tryptophan to L-tryptophan is small. A further point in the failure of D-tryptophan to raise 5-HT synthesis in these experiments is the stereo-specific nature of the hydroxylase enzyme being greatly in favour of the L-form (Gal, Armstrong & Ginsberg, 1966).

Clearance from the periphery of the two isomers was also different. The plasma concentration of D-tryptophan, both total and free at the earliest time of sampling, 30 min after administration, is lower than the plasma concentration of tryptophan measured after administration of the L-isomer and is presumably due to the more rapid renal clearance of D-tryptophan, perhaps because of failure to reabsorb the D-isomer from the glomerular filtrate. Differences in the binding of D-tryptophan compared with L-tryptophan to plasma proteins were not apparent in this study. This is in conflict with the findings of McMenamy & Oncly (1958) who demonstrated that D-tryptophan had essentially no affinity for human serum albumin. However, these experiments were *in vitro* measurements done at equilibrium (5 h and 2°C) experimental conditions which differed substantially from our own.

After L-tryptophan there was also a massive rise in the concentration of HVA in ventricular CSF. This phenomenon is probably a result of competition for active transport sites out of CSF between HVA and the increased quantities of 5-HIAA which has a high affinity for these sites (Moir & Yates, 1970). The concentration of 5-HIAA also rose slightly after saline and also after D-tryptophan but there was no significant difference between these two treatments. This rise was significantly lower ($P < 0.001$) than the rise seen after L-tryptophan. These results suggest that a rise in 5-HT synthesis occurs only after L-tryptophan. The small rise after saline or D-tryptophan is enigmatic but could be due either to non-specific stress (Thierry, Javoy, Glowinski & Kety, 1968) or to some impairment of the removal of 5-HIAA due to leakage of blood into the ventricular system when the blood proteins might interfere with transport mechanisms.

Our findings are clearly different from those of Yuwiler (1973). They suggest that little peripheral conversion from D-tryptophan to L-tryptophan occurs in the dog compared with the rat. The data also suggest that because of the similarities between the peripheral metabolism of D-tryptophan in man and dog that in looking at the cerebral metabolism of the D-isomer the dog is the more appropriate animal model than the rat.

Reprints from D.E. (MRC Brain Metabolism Unit).

References

ASHCROFT, G.W., DOW, R.C. & MOIR, A.T.B. (1968). The active transport of 5-hydroxyindol-3-ylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid from a recirculatory perfusion system of the cerebral ventricles of the unanaesthetised dog. *J. Physiol., Lond.*, **199**, 397–425.

BALDWIN, H.R. & BERG, C.P. (1949). The influence of optical isomerism and acetylation upon the availability of tryptophan for maintenance in man. *J. Nutr.*, **39**, 203–218.

BERG, C.P. (1934). Tryptophane metabolism. IV the influence of optical activity on the utilization of tryptophane for growth and for kynurenic acid production. *J. biol. Chem.*, **104**, 373–384.

BERG, C.P. (1942). The metabolism of the d-amino acids. *Fedn Proc.*, **1**, 281

BERG, C.P. (1953). Physiology of the D-amino acids. *Physiol. Rev.*, **33**, 145–189.

CORRELL, J.T., BERG, C.P. & COWAN, D.W. (1938). Tryptophane metabolism. IX the excretion of kynurenic acid in the bile and urine of the dog after the administration of kynurenic acid and L- and D,L-tryptophane. *J. biol. Chem.*, **123**, 151–159.

ECCLESTON, D., ASHCROFT, G.W., MOIR, A.T.B., PARKER-RHODES, A., LUTZ, W. & O'MAHONEY, D.P. (1968). A comparison of 5-hydroxyindoles in various regions of dog brain and cerebrospinal fluid. *J. Neurochem.*, **15**, 947–957.

GAL, E.M., ARMSTRONG, J.C. & GINSBERG, B. (1966). The nature of *in vitro* hydroxylation of L-tryptophan by brain tissue. *J. Neurochem.*, **13**, 643–654.

GUROFF, G. & UDENFRIEND, S. (1962). Studies of aromatic amino acid uptake by rat brain *in vivo*. *J. biol. Chem.*, **237**, 803–806.

HANKES, L.V., BROWN, R.R., LEKLEM, J., SCHMAELER, M. & JESSEPH, J. (1972). Metabolism of C^{14} labelled enantiomers of tryptophan kynurenine and hydroxykynurenine in humans with scleroderma. *J. Invest. Dermatol.*, **58**, 85–95.

HESS, S.M. & UDENFRIEND, S. (1959). A fluorometric procedure for the measurement of tryptamine in tissue. *J. Pharmac. exp. Ther.*, **127**, 175–177.

KENDALL, M.G. & STUART, A. (1967). *The Advanced Theory of Statistics*, Vol. II, pp. 346–373. London: Griffin.

McMENAMY, R.H. & ONCLY, J.L. (1958). The specific binding of L-tryptophan to serum albumin. *J. biol. Chem.*, **233**, 1436–1447.

MOIR, A.T.B. & YATES, C.M. (1970). Actions of phenelzine on the interactions of the metabolism of tryptophan and dopamine in brain. *Br. J. Pharmac.*, **40**, 563P.

OESTERLING, M.J. & ROSE, W.C. (1952). Tryptophan requirement for growth and utilization of its optical isomers. *J. biol. Chem.*, **196**, 33–43.

ROSE, W.C. (1949). Amino acid requirements of man. *Fedn. Proc.*, **8**, 546–552.

THIERRY, A.M., JAVOY, F., GLOWINSKI, J. & KETY, S.S. (1968). Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. *J. Pharmac. exp. Ther.*, **163**, 163–171.

VIGNEAUD, V. DU, SEALOCK, R.R. & VAN ETTEN, C. (1932). The availability of D-tryptophan and its acetyl derivative to the animal body. *J. biol. Chem.*, **98**, 565–575.

YATES, C.M. & GULDBERG, H.C. (1968). Some studies of the effects of chlorpromazine, reserpine and dihydroxyphenylalanine on the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindol-3-ylacetic acid in ventricular cerebrospinal fluid of the dog using the technique of serial sampling of the cerebrospinal fluid. *Br. J. Pharmac. Chemother.*, **33**, 457–471.

YUWILER, A. (1973). Conversion of D- and L-tryptophan to brain serotonin and 5-hydroxyindoleacetic acid and to blood serotonin. *J. Neurochem.*, **20**, 1099–1109.

(Received March 5, 1976)