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THE EFFECT OF TRYPTOPHAN METABOLITES ON BRAIN 5-HYDROXYTRYPTAMINE METABOLISM

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Abstract—The effect on rat brain 5 hydroxytryptamine (5HT) metabolism of tryptophan metabolites formed subsequent to the action of tryptophan pyrrolase has been studied. Intraperitoneal injection of 5 mg./kg. L-kynurenine sulphate, L-3 hydroxykynurenine and 3 hydroxyanthranilic acid resulted in a fall of brain 5HT 2 hr after injection. Metabolites formed subsequent to 3 hydroxyanthranilic acid or formed on side branches of the pathway to it had no effect at this dose. None of the metabolites examined significantly affected the *in vitro* activity of either of the 5HT synthesising enzymes tryptophan 5-hydroxylase and 5-hydroxytryptophan decarboxylase. However, in common with other α-amino acids L-kynurenine or L-3 hydroxykynurenine at 1 mM concentration decreased uptake of tryptophan-^{14}C into brain slices. Tryptophan metabolites which were not α-amino acids had no effect.

Results are discussed in relation to the fall of brain 5HT associated with increased pyrrolase activity and in relation to the biochemistry of mental disorders.

A SINGLE intraperitoneal injection of hydrocortisone has been shown to cause a fall of both rat brain 5-hydroxytryptamine (5HT) and its metabolite 5-hydroxyindole acetic acid (5HIAA), which suggests decreased synthesis of brain 5HT.[1] The synthetic glucocorticosteroid betamethasone has recently been found to cause a similar 5HT change.[2] Decreased synthesis after hydrocortisone appears to be due to increased activity of the liver enzyme tryptophan pyrrolase, which is induced by hydrocortisone,[3] since Allopurinol, which decreases pyrrolase activity,[4–6] prevented the fall of brain 5HT.[5] The possibility that such a fall might result from diversion of tryptophan from the indole amine pathway by the action of pyrrolase upon it has often been suggested (see Ref. 7). This alone seems unlikely to be responsible for the effect of hydrocortisone on 5HT, as plasma tryptophan only fell transiently[8] while brain 5HT fell to a proportionately comparable extent, but much more prolongedly.[1] Therefore, the effect on brain 5HT metabolism of tryptophan metabolites formed subsequent to pyrrolase action was studied, since increased pyrrolase activity may well lead to increased formation of such metabolites. Their effects on rat brain 5HT level *in vivo*, on tryptophan 5-hydroxylase and 5-hydroxytryptophan decarboxylase activity *in vitro* and on uptake of L-tryptophan (methylene^{14}C) into brain slices, have been determined.

METHODS

Adult male Sprague–Dawley rats (180–230 g) were fed *ad lib.* diet of Oxo 41B pellets and tap water. Rats were kept in the laboratory in a housing which was acoustically lagged, with regular air changes and a controlled temperature of 25° ± 1°. Light

was automatically controlled on a 0600–1800 light–dark cycle and animals sacrificed at approximately the same time of day (14.30–16.30 hr) to minimise variations due to diurnal changes of 5HT[9] and of tryptophan pyrrolase activity.[10] The animals were removed from the housing only for injection or sacrifice which was by decapitation after stunning by pneumothorax.

Brain 5HT and 5HIAA

Brain 5HT was determined as Snyder, Axelrod and Zweig.[11] 5HIAA was determined by 0·4 N perchloric acid deproteinisation[12] followed by butyl acetate extraction, washing with 0·1 N hydrochloric acid saturated with sodium chloride and back extraction into 0·1 M phosphate buffer pH 7 by the method of Giacalone and Valzelli.[13] The gel formed in the buffer was broken by rapid shaking and centrifugation and the clear solution read on a Farrand spectrophotofluorometer (activation = 310 mμ, fluorescence = 550 mμ; both uncorrected) after bringing to 3 N with concentrated HCl. Ascorbic acid (0·01 %) was added to the phosphate buffer to stabilise the fluorescence, but not at earlier stages since this caused high blanks and was not necessary for adequate recovery of added 5HIAA (80–85 %).

Tryptophan pyrrolase activity

Tryptophan pyrrolase was measured essentially by the method of Knox and Auerbach,[3] with the addition of 2×10^{-6} M haematin to the reaction mixture.[14]

Tryptophan 5-hydroxylase

Activity was measured by determining conversion of L-tryptophan to 5-hydroxytryptophan (5HTP). One ml of an isotonic brain homogenate[15] prepared in 0·25 M sucrose + 0·001 M mercaptoethanol was incubated at 37° with 4 μg L-tryptophan [2 μg L-tryptophan + 2 μg L-tryptophan (methylene^{14}C) specific activity 54·5 mc/mM (Radiochemical Centre, Amersham)], NSD1015 (Final concn. 10^{-5} M) and potassium phosphate buffer pH 7·4 (Final concn. 0·05 M) to a total volume of 3 ml. After deproteinisation the percentage of ^{14}C-5HTP formed was determined by thin-layer chromatography and liquid scintillation spectrometry by the method of Häkanson and Hoffman.[16]

5-Hydroxytryptophan decarboxylase

Brain homogenates were incubated with DL-5-hydroxytryptophan by the method of Kuntzman *et al.*[17] except that the monoamine oxidase inhibitor Pargyline (as hydrochloride) was present at 10^{-5} M. After 30 min the 4 ml of mixture was added to 7·5 ml *n*-butanol + 2 ml 0·5 M-borate buffer pH 10 + 3 g sodium chloride and 5HT formed by decarboxylase action determined by the method of Snyder *et al.*[11]

Uptake of L-tryptophan (methylene^{14}C) by brain slices

Rat brain slices cut in the coronal plane (weight about 140 mg) were halved through the midline and each half after weighing was incubated at 37° with pargyline (final concentration 10^{-5} M), rat phosphate Ringer[18] and 4 μg L-tryptophan (2 μg L-tryptophan + 2 μg L-tryptophan [(methylene^{14}C) specific activity 54·5 mc/mM (Radiochemical Centre, Amersham)] in a total volume of 3 ml. After 50 min the slices were washed thoroughly with rat Ringer, homogenised in 3 ml acetone–0·1 N HCl

(95:5) containing 20 μg/ml each of L-tryptophan and 5HT and 0·05 ml supernatant after centrifugation spotted on to a 500 μ thick silica gel chromatoplate (Kieselgel G., Merck). Plates were developed in methyl acetate, 2-propanol, 25% ammonia (45:35:20)[19] and tryptophan and 5HT located with formaldehyde reagent.[19] Spots were transferred to vials containing 10 ml dioxane phosphor[18] and labelled tryptophan uptake and 5HT formed/mg measured by scintillation spectrometry.

In vivo *studies*

Tryptophan metabolites formed subsequent to pyrrolase action were prepared as 1 mg/l ml solutions or suspensions and injected intraperitoneally at 5 mg/kg except for 3-hydroxyanthranilic acid which was partly dissolved by addition of sodium hydroxide to pH 7 at the pH meter, with continual stirring under nitrogen. Animals were sacrificed 2 hr after injection and brain 5HT or 5HIAA determined. Allopurinol was suspended in saline with the addition of 0·01% methyl cellulose[4] and injected as above.

RESULTS AND DISCUSSION

In preliminary experiments, L-kynurenine caused a fall of brain 5HT which was maximal 2 hr after injection (Table 1) and thus occurred much more rapidly than the 5HT fall after injection of hydrocortisone which occurs via increased pyrrolase synthesis. As a maximal 5HT fall occurs 3 hr after the peak of hydrocortisone induced pyrrolase activity, the time of this effect of kynurenine is consistent with the effect of

TABLE 1. TIME COURSE OF CHANGES IN RAT BRAIN 5HT AFTER INJECTION OF L-KYNURENINE

	Time after injection (hr)				
Injected i.p.	0	1	1·5	2	3
Saline	0·64 ± 0·02 (4)	0·62; 0·62	0·67	0·64 ± 0·02 (4)	0·62
L-kynurenine sulphate (5 mg/kg)	—	0·58; 0·58	0·57; 0·52	0·48 ± 0·03 (4)	0·56; 0·58

Different from control $P < 0·001$.
No. of animals shown in parentheses.
Results expressed as μg 5HT/g brain (wet wt.)

hydrocortisone on 5HT being due to kynurenine formed by pyrrolase activity. Allopurinol, which inhibits pyrrolase, prevents the fall of brain 5HT which occurs after hydrocortisone injection,[5] but had no effect on the fall caused by kynurenine (Table 2).

The sequential kynurenine metabolites, 3-hydroxykynurenine and 3-hydroxyanthranilic acid also caused brain 5HT to fall (Table 2). However, metabolites formed on side branches of this pathway (Fig. 1) or subsequent to 3-hydroxyanthranilic acid had no effect. Nicotinic acid caused a 5HT fall but unlike nicotinamide this substance is proably not a significant product of tryptophan metabolism.[20] The effect of nicotinic acid may be related to its vasodilator properties as two other vasodilators, nicotinyl alcohol and glyceryl trinitrate, also caused a 5HT fall but nicotinamide which is not directly vasodilatory did not affect 5HT.

TABLE 2. EFFECT OF VARIOUS COMPOUNDS ON RAT BRAIN 5HT

Injected i.p.	μg 5HT/g brain (wet weight)
Saline	0·64 ± 0·02 (11)
L-kynurenine sulphate (5 mg/kg)	0·48 ± 0·03 (4)*
Kynurenic acid (5 mg/kg)	0·65 ± 0·02 (4)
Anthranilic acid (5 mg/kg)	0·65 ± 0·03 (4)
L-3 hydroxykynurenine (5 mg/kg)	0·49 ± 0·03 (4)*
Xanthurenic acid (5 mg/kg)	0·65 ± 0·02 (4)
3 hydroxyanthranilic acid (5 mg/kg)	0·50 ± 0·02 (4)*
Quinolinic acid (5 mg/kg)	0·63 ± 0·02 (4)
Picolinic acid (5 mg/kg)	0·63 ± 0·01 (4)
Nicotinic acid (5 mg/kg)	0·48 ± 0·02 (4)*
Nicotinamide (5 mg/kg)	0·66 ± 0·02 (4)
Nicotinyl alcohol (2·5 mg/kg)	0·48; 0·50 (2)
Glyceryl trinitrate (1 mg/kg)	0·46 ± 0·05 (5)*
Histamine (1 mg/kg)	0·67 ± 0·03 (4)
L-kynurenine (5 mg/kg) + Allopurinol (20 mg/kg)	0·50 ± 0·01 (4)*
Allopurinol (20 mg/kg)	0·66 ± 0·02 (4)

* Different from control $P < 0·001$.
No. of animals shown in parentheses.
Animals were sacrificed 2 hr after injection.

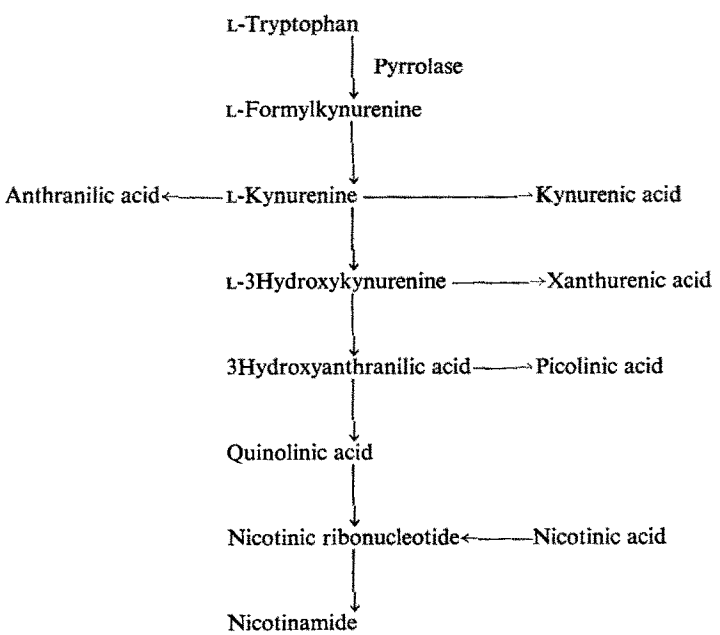

FIG. 1. The kynurenine pathway of tryptophan metabolism.

The fall of brain 5HT after kynurenine injection was similar whether 50 mg/kg or 1 mg/kg was injected (Table 3). This suggests that only a limited fraction of brain 5HT synthesis may be prevented by kynurenine. Similarly, it has been shown that the fall in brain 5HT after 20 mg/kg hydrocortisone injection is no greater than that after 5 mg/kg hydrocortisone[21] and the fall after α-methyltryptophan injection[5] is similar to that

TABLE 3. DECREASE OF RAT BRAIN 5HT AFTER DIFFERENT DOSES OF TRYPTOPHAN METABOLITES

Dose (μmoles/kg)	Injected i.p.	μg 5HT/g brain (wet wt.)
—	Saline	0·64 ± 0·02 (7)
165	L-Kynurenine	0·48 ± 0·04 (5)*
16·5	L-Kynurenine	0·48 ± 0·03 (4)*
3·3 (= 1 mg/kg Kynurenine SO_4)	L-Kynurenine	0·50 ± 0·02 (4)*
	3Hydroxyanthranilic acid	0·50 ± 0·02 (4)*
1·7	L-Kynurenine	0·63 ± 0·04 (4)*
	3Hydroxyanthranilic acid	0·57 ± 0·05 (4)†

* Different from saline control $P < 0·001$.
† Different from saline control $P < 0·01$.
No. of animals shown in parentheses.

after hydrocortisone even though the increase of pyrrolase activity is much greater after α-methyltryptophan.

Kynurenine caused a fall not only of brain 5HT but also of 5HIAA. Thus 2 hr after injection of 5 mg/kg kynurenine, brain 5HIAA was 0·28 ± 0·02 μg/g brain wet wt. (four animals) which is significantly less than 0·34 ± 0·02 (six animals) found for rats injected with saline ($P < 0·01$). Therefore, the 5HT fall after kynurenine, like that after hydrocortisone injection, appears to be associated with decreased 5HT synthesis. However, the 5HT change after injection of the tryptophan metabolites does not appear to be due to a direct effect on tryptophan-5-hydroxylase or on 5-hydroxytryptophan decarboxylase, the enzymes necessary for 5HT synthesis as these metabolites at a relatively high concentration had little effect on activities of the enzymes *in vitro* (Table 4). Marked inhibition of tryptophan-5-hydroxylase and 5-hydroxytryptophan

TABLE 4. EFFECT OF VARIOUS SUBSTANCES ON *IN VITRO* ACTIVITY OF 5HT SYNTHESISING ENZYMES AND ON TRYPTOPHAN UPTAKE AND 5HT FORMATION IN BRAIN SLICES

Substance added Final concn. 1mM	Inhibition of tryptophan 5-Hydroxylase activity (%)	Inhibition of 5HTP Decarboxylase (%)	Brain slices: Uptake of L-^{14}C-tryptophan (relative to control) (%)	Brain slices: Formation of ^{14}C-5HT (relative to control) (%)
L-Kynurenine	9 ± 1 (4)	2 (2)	66 ± 17 (13)*	60 ± 16 (6)†
L-3Hydroxykynurenine	13 ± 1 (4)	4 (2)	66 ± 15 (11)*	72 ± 11 (6)*
3Hydroxyanthranilic acid	− 4 ± 2 (4)	− 2 (2)	105 ± 13 (12)	84 ± 25 (4)
Anthranilic acid	n.d.	n.d.	105 ± 10 (4)	98 ± 6 (4)
Xanthurenic acid	n.d.	n.d.	107 ± 7 (4)	107 ± 7 (4)
Quinolinic acid	n.d.	n.d.	106 ± 7 (4)	104 ± 13 (4)
L-Alanine	n.d.	n.d.	78 ± 5 (4)†	65 ± 9 (4)†
L-Phenylalanine	73 ± 8 (4)*	n.d.	40 ± 11 (4)*	39 ± 9 (4)*
DL-α methyltryptophan	n.d.	n.d.	78 ± 10 (4)‡	66 ± 6 (4)†
DL-α methyl DOPA	n.d.	79 (2)	n.d.	n.d.
Allopurinol	n.d.	n.d.	104 ± 10 (4)	102 ± 8 (4)

* Different from control $P < 0·001$.
† Different from control $P < 0·01$.
‡ Different from control $P < 0·05$.
n.d. Not determined.
No. of animals shown in parentheses.

decarboxylase was shown by phenylalanine and α-methyl dopa respectively, which are known inhibitors of these enzymes.[22, 23] The lack of effect of the tryptophan metabolites on tryptophan-5-hydroxylase also points to a lack of effect upon transport of tryptophan into synaptosomes as an isotonic brain homogenate was used with a considerable fraction of the enzyme therefore presumably contained within intact synaptosomes[15] and thus defective transport could have led to decreased radioactivity of formed 5HTP, even in the absence of inhibition. Metabolism of kynurenine and 3-hydroxykynurenine requires pyridoxal dependent enzymes.[24] Therefore injection of kynurenine or 3-hydroxykynurenine by increasing demands for pyridoxal might lead to decreased 5HT synthesis due to low activity of 5-hydroxytryptophan decarboxylase which is also pyridoxal dependent.[25] This possibility is not excluded by the lack of direct effect of kynurenine and 3-hydroxykynurenine on the decarboxylase (Table 4) but it is made improbable by the finding that large amounts of pyridoxine or pyridoxal-5-phosphate did not prevent the fall of brain 5HT caused by kynurenine injection (Table 5).

TABLE 5. EFFECT OF PYRIDOXAL 5 PHOSPHATE OR PYRIDOXINE ON FALL OF RAT BRAIN 5HT AFTER L-KYNURENINE INJECTION

Injected i.p.	μg 5HT/g brain (wet wt.) 2 hr after final injection
Saline*	0·64 ± 0·02 (4)
Pyridoxal 5 phosphate (5 mg/kg)*	0·62; 0·64 (2)
Pyridoxal 5 phosphate (5 mg/kg) + L-Kynurenine sulphate (5 mg/kg)*	0·52 ± 0·02 (4)‡
Pyridoxine (25 mg/kg)*	0·65 ± 0·02 (4)
Pyridoxine (25 mg/kg) + L-Kynurenine sulphate (5 mg/kg)*	0·47 ± 0·01 (4)‡
Pyridoxine (25 mg/kg × 3)†	0·67; 0·64 (2)
Pyridoxine (25 mg/kg × 3) + L-Kynurenine sulphate (5 mg/kg)†	0·51 ± 0·03 (4)‡

* Injected 1300 hr. Sacrifice 1500 hr.
† Injected pyridoxine 1800 hr Day 1, 1000 hr Day 2, pyridoxine or pyridoxine + L-Kynurenine at 1300 hr with Sacrifice 1500 hr Day 2.
‡ Different from control $P < 0·001$.
No. of animals shown in parentheses.

Kynurenine and 3-hydroxykynurenine, in common with the other α-amino acids, alanine, phenylalanine, and α-methyl tryptophan, significantly inhibited uptake of ^{14}C-tryptophan into rat brain slices (Table 4). 3-hydroxyanthranilic acid which is not an α-amino acid and those tryptophan metabolites which did not alter brain 5HT *in vivo* did not affect uptake. Phenylalanine has been previously shown to inhibit transport of tryptophan into rabbit cortex slices.[15] These results are parallelled by the finding under different conditions to those of this study that several α-amino acids inhibit transport of tryptophan into rat brain synaptosomes.[26] In the present study a general quantitative agreement was found between the effects of substances tested on both ^{14}C-tryptophan uptake and formation of ^{14}C-5HT by brain slices. This indicates that the tryptophan changes are entirely responsible for the 5HT changes in brain slices and is consistent with a lack of effect of these substances on activities of enzymes necessary for 5HT synthesis. It is possible that inhibition of tryptophan transport into the brain may play a part in the fall of brain 5HT after peripheral injection of kynurenine, or 3-hydroxykynurenine. Whether such an effect of these substances is responsible for the 5HT fall due to hydrocortisone[5] or immobilisation[27] and provoked by

increased pyrrolase activity is more problematic partly because of the present lack of knowledge of the effect of pyrrolase changes on *in vivo* levels of tryptophan metabolites. The above findings give no indication of the mechanism by which 3-hydroxyanthranilic acid injection may influence brain 5HT as it had no significant effects *in vitro* on either 5HT synthesising enzymes or on tryptophan transport.

High levels of another amino acid, phenylalanine, cause decreased rat brain 5HT which may be due to the inhibition of tryptophan uptake by brain.[15, 28] There is evidence for defective 5HT synthesis by phenylketonurics[29] and for poor maze performance by rats fed phenylalanine which is reversed by feeding tryptophan.[30] Thus the mental disturbance in phenylketonuria may be related to the effect of excess phenylalanine on brain 5HT synthesis in infancy. Similarly, mental defect in infants with pyridoxine sensitive increased 3-hydroxykynurenine formation[31] could be due in part to an effect of this amino acid on brain 5HT formation, though deficiency of the pyridoxal co-factor of 5-hydroxytryptophan decarboxylase could also be involved. Also, in depressive illness, the decreased brain 5HT for which there is some evidence[7, 32] could perhaps be a consequence of increased formation of kynurenine and 3-hydroxykynurenine which is suggested by the results of tryptophan loading tests.[33–35]

TABLE 6. EFFECT OF ALLOPURINOL ON RAT LIVER PYRROLASE AND BRAIN 5HT CHANGES INDUCED BY DL-α-METHYL TRYPTOPHAN

Injected i.p.	Pyrrolase activity [μmoles Kynurenine/g liver (dry wt).]	μg 5HT/g brain (wet wt.)
Saline	7·86 ± 1·00 (4)*	0·63 ± 0·01 (6)
Allopurinol (20 mg/kg)	3·44 ± 1·28 (4)*	0·65 ± 0·05 (10)
DL-α-methyl tryptophan (25 mg/kg)	44·46 ± 1·74 (4)*	0·43 ± 0·01 (6)*
DL-α-methyl tryptophan (25 mg/kg) + Allopurinol (20 mg/kg)	16·80 ± 2·26 (4)*	0·59 ± 0·03 (4)*

No. of animals shown in parentheses.
* Different at level of significant $P < 0·001$.

Pyrrolase levels are raised not only by hydrocortisone, but also by α-methyltryptophan which also causes a fall of brain 5HT.[5, 36] This appears to be at least in part due to induction of pyrrolase as Allopurinol partly prevented the fall (Table 6). α-Methyltryptophan also inhibited tryptophan transport into brain slices (Table 4) and therefore this process may also be involved in its effect upon brain 5HT.

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