Br. J. Pharmac. (1976), **58**, 533–545

EFFECT OF AMINOPHYLLINE ON TRYPTOPHAN AND OTHER AROMATIC AMINO ACIDS IN PLASMA, BRAIN AND OTHER TISSUES AND ON BRAIN 5-HYDROXYTRYPTAMINE METABOLISM

G. CURZON & J.C.R. FERNANDO
Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG

1 Aminophylline and other methylxanthines increase brain tryptophan and hence 5-hydroxytryptamine turnover. The mechanism of this effect of aminophylline was investigated.

2 At lower doses ($\geqslant$ 100 mg/kg i.p.) the brain tryptophan increase could be explained by the lipolytic action of the drug, i.e. increased plasma unesterified fatty acid freeing plasma tryptophan from protein binding so that it became available to the brain.

3 Plasma unesterified fatty acid did not increase when aminophylline (100 mg/kg i.p.) was given to nicotinamide-treated rats but as both plasma total and free tryptophan rose, a tryptophan increase in the brain still occurred.

4 The rise in brain tryptophan concentration following the injection of a higher dose of the drug (150 mg/kg i.p.) could no longer be explained by a rise of plasma free tryptophan as the ratio of brain tryptophan to plasma free tryptophan rose considerably. Plasma total tryptophan fell and the plasma insulin concentration rose.

5 The increase of brain tryptophan concentration after injection of 150 mg/kg aminophylline appeared specific for this amino acid as brain tyrosine and phenylalanine did not increase. However as their plasma concentrations fell the brain/plasma ratio for all three amino acids rose.

6 The higher dose of aminophylline increased the muscle concentration of tryptophan but that of tyrosine fell and that of phenylalanine remained unaltered. The liver concentrations were not affected.

7 The aminophylline-induced increases of the ratio of brain tryptophan to plasma free tryptophan no longer occurred when the drug was given to animals injected with the β-adrenoceptor blocking agent propranolol or the diabetogenic agent streptozotocin.

8 The changes in brain tryptophan upon aminophylline injection may be explained by (a) increased availability of plasma tryptophan to the brain due to increased lipolysis and (b) increased effectiveness of uptake of tryptophan by the brain due to increased insulin secretion.

Introduction

Much attention has recently been paid to mechanisms by which brain tryptophan concentration is controlled. This is principally because tryptophan hydroxylase, the rate limiting enzyme for brain 5-hydroxytryptamine (5-HT) synthesis is normally unsaturated with its precursor tryptophan (Eccleston, Ashcroft & Crawford, 1965; Friedman, Kappelman & Kaufman, 1972) so that alterations of brain tryptophan concentration can lead to changes of brain 5-HT turnover.

There is evidence that the following three factors can influence brain tryptophan concentration:

(a) Changes in the concentration of the relatively small fraction of unbound tryptophan in plasma (Knott & Curzon, 1972; Tagliamonte, Biggio, Vargiu & Gessa, 1973). This fraction can be increased in conditions in which the plasma concentration of unesterified fatty acids (UFA) increases as these substances decrease the binding of tryptophan to albumin (Knott & Curzon, 1972; Curzon, Friedel & Knott, 1973; Curzon, Kantamaneni, Winch, Rojas-Bueno, Murray-Lyon & Williams, 1973; Curzon & Knott, 1974; Knott & Curzon, 1975).

(b) Competition between tryptophan and a group of neutral amino acids for uptake by the brain This has been shown *in vitro* (Kiely & Sourkes, 1972) and *in vivo* (Oldendorf, 1971). The tryptophan concentrations used in these experiments were considerably greater than those of free tryptophan in plasma. However, this mechanism could be involved in the control of brain tryptophan concentration following food intake as brain tryptophan then no longer correlates with free plasma tryptophan (Madras, Cohen, Messing, Munro & Wurtman, 1974).

It has also been used to explain increased brain tryptophan in liver failure by Munro, Fernstrom & Wurtman (1975) who suggested that plasma insulin elevation in this disorder leads to a fall in the plasma concentrations of branched chain amino acids competing with tryptophan for transport to the brain. Although brain tryptophan changes in various kinds of acute liver failure have been explained largely in terms of plasma free tryptophan changes (Curzon, Knott, Murray-Lyon, Record & Williams, 1975) insulin injection does lead to an increase of brain tryptophan or of the ratio of its concentration to that of plasma free tryptophan (Fernstrom & Wurtman, 1971; Fernando, Knott & Curzon, 1976).

(c) Effectiveness of tryptophan uptake by the brain. This exhibits diurnal variations and may be affected by the activity of central catecholaminergic systems (Hery, Rouer, Kan & Glowinski, 1974) and cyclic adenosine-3′,5′-monophosphate (cyclic AMP) (Tagliamonte, Tagliamonte, Forn, Perez-Cruet, Krishna & Gessa, 1971).

One group of drugs which increase brain 5-HT turnover are the methylxanthines, e.g. aminophylline (Berkowitz & Spector, 1971). The increased 5-HT turnover due to aminophylline is associated with an increase in brain tryptophan concentration (Curzon & Knott, 1974) which appears to be related to inhibition of cyclic AMP breakdown in fat cells (Butcher, Ho, Meng & Sutherland, 1975) leading to an elevation of UFA and hence of free tryptophan in the plasma. However, the catecholamine releasing (Wooten, Thoa, Kopin & Axelrod, 1973), insulin releasing (Turtle, Littleton & Kipnis, 1967) or other actions of aminophylline could also be involved.

The present paper describes an investigation of the roles of plasma free tryptophan and insulin in the action of aminophylline on brain concentrations of tryptophan and on brain 5-HT metabolism. The effect of aminophylline on the disposition of other aromatic amino acids was also studied.

Methods

Male Sprague-Dawley rats (170–220 g, Anglia Laboratory Animals, Alconbury, Hunts.) were caged in groups of three or four and kept under a 06 h 00 min–18 h 00 min light-dark cycle in an acoustically lagged housing at 24 ± 1°C and fed with Oxoid 41B pellets and water *ad libitum*. The animals were killed by guillotine between 14 h 30 min and 16 h 30 min and blood collected into heparin-containing tubes except when required for insulin assay when citrate was used. It was immediately centrifuged and the plasma stored at −20°C. Separate samples were stored for insulin assay. The brains were removed rapidly, frozen with solid CO_2 and stored at −20°C.

The following drugs were used: aminophylline injection B.P. (Evans Medical), nicotinamide, propranolol (ICI), streptozotocin (Upjohn, Kalamazoo, Mich.,) dissolved in 0.07 M pH 4.5 sodium citrate buffer, caffeine citrate (BDH) and theobromine (BDH). Drugs supplied as solids were dissolved in 0.9% w/v Na Cl solution (saline) unless otherwise stated. Injection volume was 2.5 ml/kg body weight. Control animals were injected with the appropriate vehicles.

Analytical methods

Plasma total and ultrafilterable tryptophan, brain tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) and plasma and brain tyrosine were determined as previously described (Curzon, Joseph & Knott, 1972; Knott & Curzon, 1972). Phenylalanine was determined by the method of McCaman & Robins (1962) in plasma and in a 50 µl portion of the brain extract used for tyrosine determination (Curzon *et al.*, 1972). Tryptophan, tyrosine and phenylalanine were also determined in gastrocnemius muscle and liver by the above methods. Plasma UFA were determined fluorometrically (Curzon & Kantamaneni, unpublished) and plasma insulin by radioimmunoassay (Kit method, Lepetit, Maidenhead, Berks.).

Results

Behavioural effects of aminophylline

Rats given 50–150 mg/kg aminophylline intraperitoneally were more responsive than control ainmals to auditory, visual and tactile stimuli at about 45 min after injection. This soon disappeared and at 2 h the animals appeared sedated and flaccid and at 3 h were curled up and cool to the touch.

Effect of aminophylline on plasma unesterified fatty acids and tryptophan and on brain tryptophan and brain 5-hydroxytryptamine metabolism

Figure 1 shows that during the 3 h period after an injection of aminophylline (150 mg/kg i.p.) the total tryptophan concentration in the plasma gradually fell. The changes were significant at 2 and 3 hours. Plasma UFA and the percentage of plasma tryptophan which was unbound (i.e. ultrafilterable) did not alter significantly until the third hour when both rose to about twice their initial values. However, as total tryptophan fell the absolute increase in free tryptophan was not significant. The brain concentrations of tryptophan and 5-HIAA were significantly increased 1, 2 and 3 h after aminophylline injection (Figure 2) but not that of 5-HT. The brain changes did not simply reflect plasma free tryptophan changes as the ratio: brain tryptophan/plasma free tryptophan rose (significantly at 2 hours).

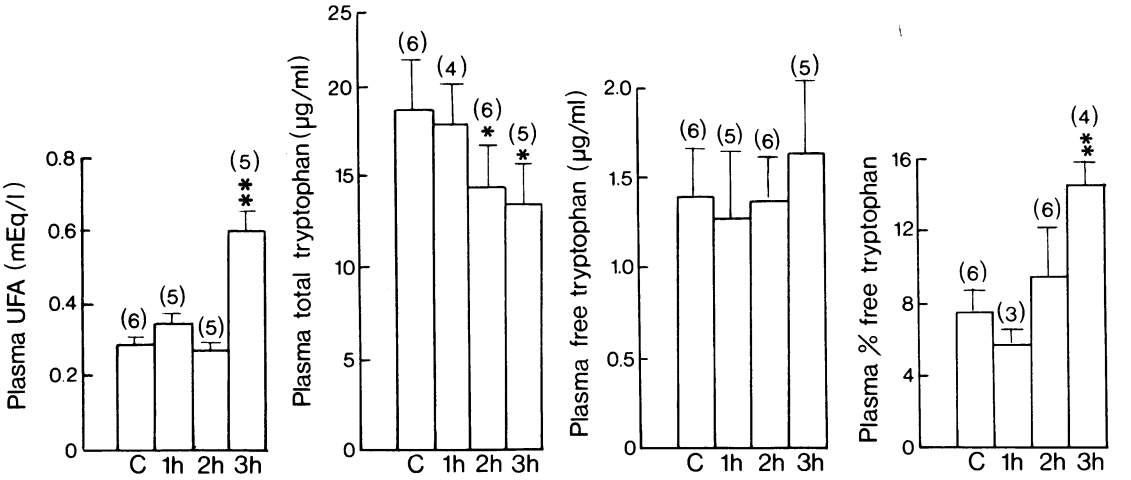

Figure 1 Time course of effect of aminophylline (150 mg/kg, i.p.) on the plasma concentrations of unesterified fatty acids (UFA) and tryptophan. Rats were killed 1, 2 or 3 h after the drug injection. Control animals were injected with saline. Control animals killed 1, 2 or 3 h after the injection did not show significantly different biochemical values and combined results are shown in column C. Results are expressed as means + one s.d. Numbers of determinations shown in parentheses. Significance of differences from controls (Student's *t* test): $*P<0.02$; $**P<0.001$.

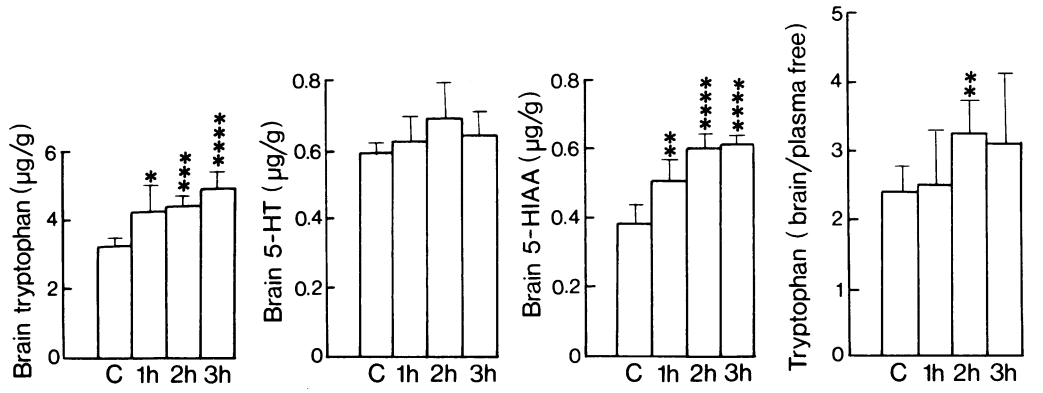

Figure 2 Time course of effect of aminophylline (150 mg/kg, i.p.) on brain indoles. Treatments as described under Figure 1. Results are expressed as means + one s.d. using 5–6 rats per group. Significance of differences from controls (Student's *t* test): $*P<0.05$; $**P<0.02$; $***P<0.01$; $****P<0.001$.

Table 1 shows the effects of 50, 100 and 150 mg/kg of aminophylline 3 h after intraperitoneal injection. Plasma total tryptophan fell significantly at the 150 mg/kg dose (as shown in Figure 1) At the lower dose it only altered slightly. UFA rose significantly at all doses but without apparent dose-dependence. There were associated rises in the percentage of free tryptophan in the plasma (not shown) and at the two lower doses the free tryptophan concentration in the plasma rose considerably and significantly. However, presumably because of the associated fall of total plasma tryptophan the concentration of free tryptophan did not rise at the highest drug dose. Nevertheless brain tryptophan and 5-HIAA concentrations rose significantly at all doses. The percentage increases of brain tryptophan and 5-HIAA were only slightly higher as the dose of aminophylline increased and were comparable for both substances. As in earlier work (Curzon & Knott, 1974) 5-HT rose slightly but significantly at all doses. The ratios of brain tryptophan to plasma free tryptophan were not significantly altered by the lower two doses of

Table 1 Effect of different doses of aminophylline on plasma unesterified fatty acid (UFA) and tryptophan metabolism

Injected	UFA (mEq/l)	Plasma Tryptophan (μg/ml) Total	Free	Brain (μg/g) Tryptophan	5-HT	5-HIAA	Brain tryptophan / Plasma free
Saline	0.30 ± 0.13 (6)	10.09 ± 1.39 (6)	1.03 ± 0.06 (6)	1.69 ± 0.15 (5)	0.33 ± 0.03 (6)	0.32 ± 0.07 (6)	1.62 ± 0.11 (4)
Aminophylline 50 mg/kg	0.64 ± 0.23* (6)	11.10 ± 0.98 (6)	1.80 ± 0.39** (6)	2.50 ± 0.31*** (6)	0.38 ± 0.02** (6)	0.52 ± 0.03*** (6)	1.42 ± 0.27 (6)
Saline	0.42 ± 0.23 (12)	13.07 ± 2.16 (13)	1.87 ± 0.45 (11)	2.64 ± 0.34 (13)	0.50 ± 0.04 (13)	0.41 ± 0.04 (13)	1.46 ± 0.33 (12)
Aminophylline 100 mg/kg	0.84 ± 0.28*** (13)	15.26 ± 2.47* (13)	2.51 ± 0.53** (13)	4.16 ± 0.92*** (13)	0.57 ± 0.05*** (13)	0.67 ± 0.09*** (13)	1.74 ± 0.62 (13)
Saline	0.29 ± 0.10 (6)	14.15 ± 1.71 (6)	0.91 ± 0.24 (6)	2.57 ± 0.10 (6)	0.36 ± 0.01 (6)	0.31 ± 0.03 (6)	2.63 ± 0.43 (5)
Aminophylline 150 mg/kg	0.56 ± 0.15*** (6)	9.13 ± 2.12** (6)	0.93 ± 0.17 (6)	4.55 ± 0.56*** (6)	0.47 ± 0.02*** (6)	0.55 ± 0.06*** (6)	4.27 ± 0.19** (4)

Experiments at different doses were not performed concurrently. Injections were made intraperitoneally and rats killed 3 h later. Results on two separate groups of rats given 100 mg/kg aminophylline are combined.

Results are expressed as means ± one s.d. Nos. of determinations are shown in parentheses. Results compared by Student's *t* test; Significance of difference from saline controls: $*P<0.05$; $**P<0.01$; $***P<0.001$.

aminophylline suggesting that the brain changes at these doses largely reflected those of the plasma concentrations of free tryptophan but at the 150 mg/kg dose this ratio was considerably increased.

Effect of aminophylline (100 mg/kg) on plasma unesterified fatty acids and tryptophan and brain tryptophan in nicotinamide-treated or fasted rats

The relationship between the effects of aminophylline on plasma UFA and on tryptophan and its metabolism was studied by the injection of aminophylline at a dose of 100 mg/kg. The effect of this dose on brain tryptophan can be explained by the increase in the plasma concentration of free tryptophan due to increased plasma UFA (Table 1). It was given either with nicotinamide (250 mg/kg) which prevents lipolysis (Dalton, Van Trabert & Dwyer, 1970) or after depriving rats of food for 24 h to promote lipolysis. Results are shown in Table 2. In nicotinamide-treated rats aminophylline increased neither plasma UFA nor the percentage of free tryptophan (not shown) 3 h later. However, as plasma total tryptophan concentration increased significantly the absolute concentration of plasma free tryptophan also increased and brain values rose comparably.

As previously found (Knott & Curzon, 1972), fasting increased both plasma free tryptophan and brain tryptophan comparably. Treatment of fasted rats with aminophylline led to a fall of plasma total tryptophan concentration and to a further increase of plasma free tryptophan concentration so that in contrast with the normal and the nicotinamide experiment the percentage of plasma tryptophan in the free state rose considerably (even though an increase of plasma UFA was not demonstrable). Brain tryptophan concentration increased by 55% and plasma free tryptophan concentration by 25%. Comparison with results in Table 1 did not suggest that fasted and fed rats responded differently to aminophylline.

Effect of aminophylline (150 mg/kg) on plasma unesterified fatty acids and insulin and on plasma and brain aromatic amino acids

The mechanism by which the higher dose of aminophylline increased brain tryptophan without apparently increasing the concentration of plasma free tryptophan (Table 1, Figures 1 and 2) was investigated further by studying the effects of aminophylline (150 mg/kg i.p.) not only on the concentrations of tryptophan but also on those of tyrosine and phenylalanine in plasma and brain 3 h after the injection in order to see whether the effect was specific to tryptophan. Plasma insulin was also determined to see whether a relation exists between the amino acid changes and the effect of aminophylline on insulin

Table 2 Effect of aminophylline on plasma unesterified fatty acid (UFA) and tryptophan disposition in nicotinamide treated and food-deprived rats

Treatment	Plasma UFA (mEq/l)	Plasma Tryptophan (μg/ml) Total	Plasma Tryptophan (μg/ml) Free	Brain tryptophan (μg/g)	Brain tryptophan / Plasma free tryptophan
Nicotinamide (250 mg/kg)	0.32 ± 0.33 (12)	14.47 ± 2.90 (13)	1.84 ± 0.51 (13)	2.76 ± 0.40 (13)	1.56 ± 0.26
Nicotinamide (250 mg/kg) + aminophylline (100 mg/kg)	0.24 ± 0.13 (13)	20.29 ± 3.97*** (13)	2.61 ± 0.33*** (11)	4.34 ± 0.53*** (13)	1.72 ± 0.34 (11)
Fed + saline (6)	0.53 ± 0.29	16.18 ± 2.71	2.27 ± 0.20	2.94 ± 0.25	1.30 ± 0.15
Fasted 24 h + saline (6)	1.09 ± 0.37†	15.83 ± 2.69	2.97 ± 0.37††	3.75 ± 0.39††	1.28 ± 0.23
Fasted 24 h + aminophylline (100 mg/kg) (5)	0.99 ± 0.22	11.71 ± 1.23*	3.71 ± 0.20**	5.80 ± 0.53***	1.58 ± 0.38

Injections were made intraperitoneally and rats killed 3 h later. Results on rats given nicotinamide are combined from two separate experiments.
Results are expressed as means ± one s.d. Nos. of determinations are shown in parentheses. Results compared by Student's *t* test. Significance of differences from corresponding nicotinamide or fasted and saline-injected controls: * $P<0.02$; ** $P<0.01$; *** $P<0.001$. Significance of difference from fed controls: † $P<0.02$; †† $P<0.01$.

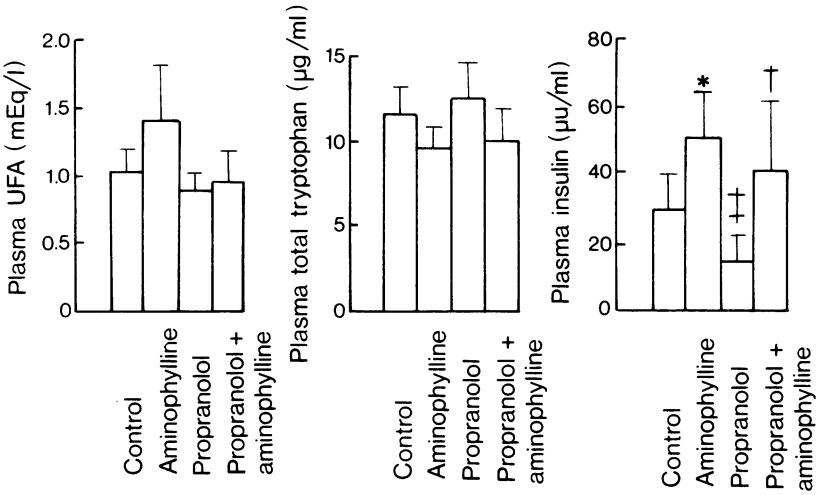

Figure 3 Effect of aminophylline (150 mg/kg i.p.) and propranolol (2 mg/kg i.p., ×2) on plasma concentrations of unesterified fatty acids (UFA), total tryptophan and insulin. Aminophylline was injected 3 h before killing and propranolol injected 3 h and 1 h before killing. All experiments were performed concurrently. Results are expressed as means + one s.d. using 6 rats per group. Significance of differences from controls (Student's *t* test): aminophylline *v.* saline-injected controls: $*P < 0.05$; aminophylline and propranolol *v.* propranolol-injected controls: $\dagger P < 0.05$; propranolol *v.* saline-injected controls: $\ddagger P < 0.05$.

secretion (Turtle *et al.*, 1967). Figure 3 shows the concentration of total plasma tryptophan together with that of UFA and insulin. Values for brain and plasma aromatic amino acids are shown in Figure 4.

In this experiment the decrease of plasma total tryptophan was proportionately less (19%) than that found before under the same conditions (Figure 1, 28%; Table 1, 36%); as before, plasma free tryptophan concentration was essentially unaffected. The plasma concentrations of tyrosine and phenylalanine fell significantly, indicating that the fall of the plasma concentration of total tryptophan was not specific for this amino acid. These changes occurred together with a significant increase of the plasma insulin concentration. The increased ratio of brain tryptophan to plasma tryptophan was not specific for this amino acid as the ratios for the other two amino acids were also raised, significantly so in the case of tyrosine. However, this was associated with decreases in the plasma concentrations of tyrosine and phenylalanine so that their concentrations in the brain (unlike that of tryptophan) did not rise. Brain tyrosine showed a small but significant decrease.

Effects of aminophylline in propranolol-treated rats (Figures 3 and 4)

The effect of β-adrenoceptor blockade on aminophylline-induced changes was investigated by giving propranolol. Propranolol alone did not alter the parameters measured except the plasma insulin concentration which fell by 50% (see Figure 3) and the ratio of brain tyrosine to plasma tyrosine which fell slightly but significantly.

Aminophylline (150 mg/kg) when given to propranolol-treated rats no longer increased plasma UFA. The plasma concentrations of total tryptophan and tyrosine fell, but that of phenylalanine was unaltered (Figure 3). The increase in the brain/plasma ratios for tryptophan and phenylalanine concentrations no longer occurred and the increase in the corresponding ratio for tyrosine was less pronounced than when aminophylline was given in the absence of propranolol. Thus propranolol largely prevented the effects of aminophylline on aromatic amino acid disposition between plasma and brain. It did not prevent the aminophylline-provoked rise in plasma insulin although the concentrations reached were somewhat lower. The insulin values were widely scattered (Figure 3). In a separate experiment propranolol decreased the raised plasma insulin concentration values 3 h after aminophylline injection from 119 ± 61 (s.d.) ($n=6$) to 53 ± 19 (s.d.) μu/ml ($n=6$). Therefore the relation between the amino acid changes and insulin remained unclear.

Effect of aminophylline (150 mg/kg) on aromatic amino acids in liver and muscle

As aminophylline (150 mg/kg) altered the disposition of aromatic amino acids between plasma and brain these amino acids were estimated in other tissues 3 h

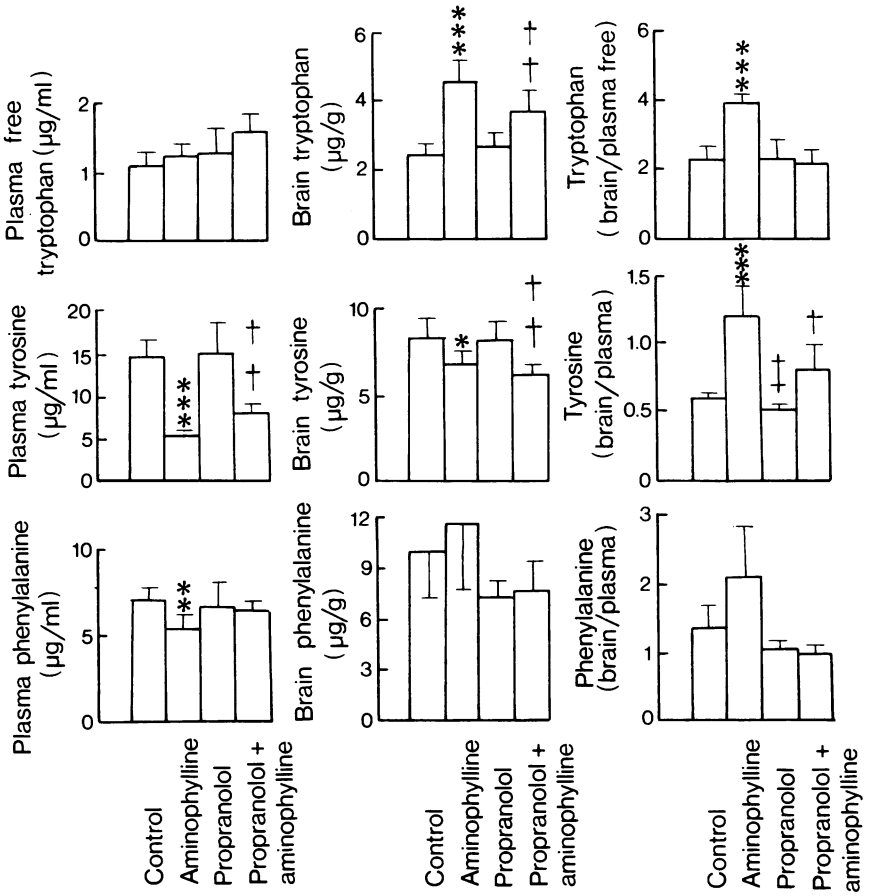

Figure 4 Effects of aminophylline (150 mg/kg i.p.) and propranolol (2 mg/kg i.p., ×2) on plasma and brain concentrations of aromatic amino acids. Details as in Figure 3. Results are expressed as means either + or − one s.d. using 5–6 rats per group. Significance of differences from controls (Student's *t* test): aminophylline *v.* saline-injected controls: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; aminophylline and propranolol *v.* propranolol: $\dagger P<0.05$, $\dagger\dagger P<0.01$; propranolol *v.* saline-injected controls: $\ddagger P<0.05$.

after aminophylline injection. The results in Figure 5 show that muscle tryptophan increased moderately but significantly in association with a comparable rise of plasma free tryptophan so that the muscle/plasma ratio remained unaltered. The concentrations in the liver of tryptophan, tyrosine and phenylalanine were not significantly changed. Muscle tyrosine fell significantly but as the plasma concentration fell even further the ratio, muscle tyrosine/plasma tyrosine rose significantly as did also the ratio: liver tyrosine/plasma tyrosine. These ratios were also increased for phenylalanine but not significantly.

Effect of aminophylline (150 mg/kg) in streptozotocin diabetic rats

In order to clarify the role of insulin in the action of aminophylline on the disposition of aromatic amino acids the parameters shown in Figures 3 and 4 were also measured in rats made diabetic with streptozotocin (Arison, Ciaccio, Glitzer, Cassaro & Pruss, 1967) before aminophylline injection. In a preliminary experiment (Experiment 2, Table 3) streptozotocin was injected into a tail vein (65 mg/kg) 72 h before aminophylline (150 mg/kg, i.p.); 3 h later streptozotocin-treated rats had significantly lower plasma insulin values than control rats (both injected with saline). Aminophylline did not significantly increase plasma insulin in the streptozotocin-treated rats. The data obtained on the brain were not easy to interpret. Although the brain/plasma ratio for tryptophan concentrations was no longer significantly increased by aminophylline the ratios for tyrosine and phenylalanine were still significantly raised (not shown). These increases could have been due to effects of aminophylline on residual pancreatic insulin stores

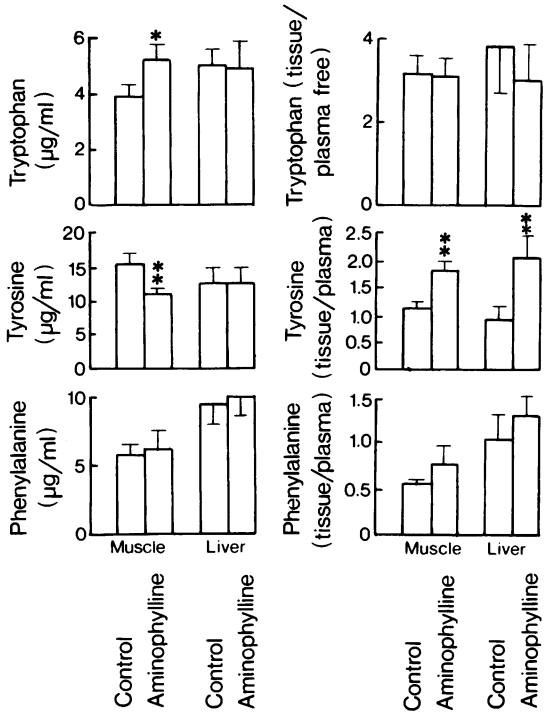

Figure 5 Effect of aminophylline (150 mg/kg i.p.) on aromatic amino acids in muscle and liver. Aminophylline was injected 3 h before killing. Results are expressed as means either + or − one s.d. using 6 rats per group. Significance of differences from controls (Student's *t* test): aminophylline *v.* saline-injected controls: $^{*}P<0.01$, $^{**}P<0.001$. Plasma and brain values are not shown being essentially the same as in Figures 3 and 4 except that brain phenylalanine fell by 21% ($P<0.001$) and the increase of the brain phenylalanine/plasma phenylalanine ratio was significant (+39%, $P<0.001$).

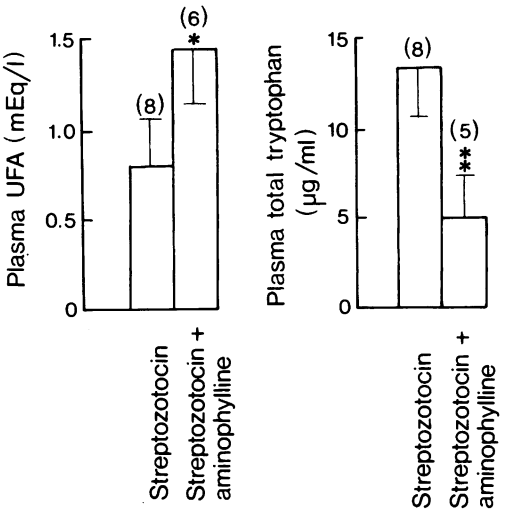

Figure 6 Effect of aminophylline (150 mg/kg i.p.) and streptozotocin (90 mg/kg i.p.) on plasma unesterified fatty acids (UFA) and total tryptophan. Streptozotocin was injected 48 h before aminophylline and rats killed 3 h later. Results are expressed as means+one s.d. Significance of differences from streptozotocin injected controls, (Student's *t* test): $^{*}P<0.01$, $^{**}P<0.001$. Insulin values are shown in Table 3, Experiment 3.

released during the first 2 h after aminophylline but no longer reflected by plasma insulin values 3 h later. This is possible because plasma insulin values of non-diabetic rats were much higher 1 h than at 3 h after aminophylline (Experiment 1, Table 3).

Therefore, in a subsequent experiment a larger dose of streptozotocin was given (90 mg/kg, i.p.). Principal

Table 3 Effect of aminophylline on plasma insulin in normal and streptozotocin-treated rats

	Treatment	*Plasma insulin (μu/ml)*	
		1 h[1]	*3 h*[1]
Experiment 1	Aminophylline	380 ± 32 (6)	119 ± 61 (6)
Experiment 2	Saline		65 ± 11 (6)
	Streptozotocin-treated rats (65 mg/kg, i.v. 72 h previously)		
	Saline		21 ± 11 (6)*
	Aminophylline		26 ± 9 (6)
Experiment 3	*Streptozotocin-treated rats (90 mg/kg, i.p. 48–72 h previously)*		
	Saline	10 ± 3 (6)	11 ± 6 (8)
	Aminophylline	7 ± 4 (8)	11 ± 6(5)

None of the three experiments was performed concurrently.
Results are expressed as means ± one s.d. Nos. of determinations are shown in parentheses. [1]Rats killed at these times after intraperitoneal injection of saline or aminophylline (150 mg/kg).
* Significance of difference from rats not given streptozotocin, $P<0.001$.

results are shown in Figures 6 and 7. Plasma insulin concentrations were not altered significantly 1 and 3 h after aminophylline (Experiment 3, Table 3) and both values were lower than in the previous experiment. The plasma concentrations of UFA and free tryptophan were doubled 3 h after aminophylline. This was accompanied by a large and significant fall of the concentration of plasma total tryptophan so that the percentage of free tryptophan was increased more than five-fold. Plasma tyrosine fell significantly but much less strikingly than total tryptophan. The brain concentrations of the three amino acids were essentially unaltered (not shown) and brain/plasma ratios were no longer increased by aminophylline and in the case of tryptophan the ratio fell significantly, i.e. the rise of plasma free tryptophan was not parallelled by a rise in the brain tryptophan concentration 3 h after aminophylline injection. However, brain tryptophan may have risen at an earlier time as both 5-HT and 5-HIAA were significantly increased (5-HT, +21%, $P<0.01$; 5-HIAA, +44%, $P<0.001$). Indeed, the experiment in Table 4 indicates that 1 h after aminophylline injection brain tryptophan did rise.

In this experiment aminophylline caused a large rise in the concentration of plasma UFA (in contrast with findings 1 h after giving the same dose of the drug to non-diabetic rats, see Figure 1) and percentage free tryptophan rose proportionately although as total tryptophan fell the mean free tryptophan concentration only rose to a lesser extent and not significantly. Nevertheless brain tryptophan did increase significantly and in proportion to the increase of plasma free tryptophan.

Effects of other methylxanthines on brain indoles

Table 5 shows that brain tryptophan and 5-HIAA were increased significantly by caffeine. The increase of 5-HIAA agrees with the findings of Berkowitz & Spector (1971). Theobromine at comparable doses had a similar but smaller effect and only the increased in 5-HIAA was significant.

Discussion

The results suggest that the increases in brain 5-HT metabolism following the injection of aminophylline or other methylxanthines are the consequence of increases of brain tryptophan. Previously (Curzon &

Table 4 Plasma unesterified fatty acid (UFA), insulin and tryptophan and brain tryptophan 1 h after injecting streptozotocin-treated rats with aminophylline

	Plasma				*Brain tryptophan*	*Brain tryptophan*
Treatment	*UFA (mEq/l)*	*Insulin (μu/ml)*	*Tryptophan (μg/ml) Total*	*Free*	*(μg/g)*	*Plasma free tryptophan*
Saline i.p. (4)	0.44 ± 0.23	12 ± 2	17.34 ± 2.19	2.55 ± 0.50	4.13 ± 0.63	1.68 ± 0.44
Aminophylline (150 mg/kg i.p.) (6)	1.19 ± 0.41*	8 ± 3	7.69 ± 1.05***	3.63 ± 0.91	5.65 ± 0.47**	1.64 ± 0.42

Streptozotocin (90 mg/kg i.p.) was given 72 h beforehand to rats which had been fasted overnight. Results are expressed as means ± one s.d. Nos. of determinations are shown in parentheses.
Significance of differences from saline-injected controls: *$P<0.02$; **$P<0.01$; ***$P<0.001$.

Table 5 Effects of caffeine and theobromine on brain indoles

	Brain (μg/g)		
Injected	*Tryptophan*	*5-HT*	*5-HIAA*
Saline (i.p.)	4.48 ± 0.84	0.54 ± 0.12	0.40 ± 0.05
Caffeine (64 mg/kg, i.p.)	5.80 ± 1.09*	0.65 ± 0.09	0.62 ± 0.11**
Theobromine (59 mg/kg, i.p.)	5.48 ± 0.80	0.59 ± 0.05	0.50 ± 0.04**

Doses were molar equivalents of 75 mg/kg aminophylline.
Rats were killed 3 h after injection of drug. Results are expressed as means ± one s.d. for 5–6 rats/group.
Significance of difference from saline-injected controls: *$P<0.05$; **$P<0.01$.

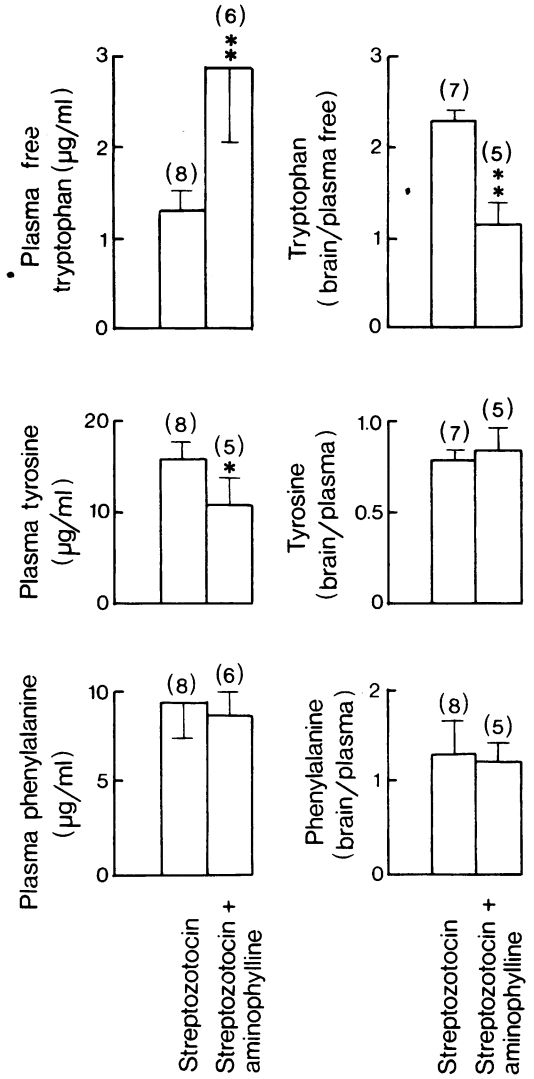

Figure 7 Effect of aminophylline (150 mg/kg i.p.) and streptozotocin (90 mg/kp i.p.) on aromatic amino acids in plasma and brain. Details as in Figure 5. Significance of differences from streptozotocin-injected controls (Student's *t* test): $*P<0.01$, $**P<0.001$.

Knott, 1974) the rise in brain tryptophan caused by aminophylline has been related to the lipolytic action of the drug increasing plasma UFA so that plasma tryptophan was freed from albumin binding and became more easily available to the brain. The present more detailed study suggests that the above mechanism can explain the increase of brain tryptophan after lower doses of aminophylline (▷100 mg/kg), in common with various other circumstances in which concentrations of both free plasma tryptophan and brain tryptophan alter together (Curzon *et al.*, 1973; Gessa & Tagliamonte, 1974; Knott & Curzon, 1975). However, the above mechanism does not adequately explain the increase of brain tryptophan when 150 mg/kg of aminophylline is injected.

This larger dose of aminophylline considerably increased brain tryptophan concentration whereas the plasma concentration of free tryptophan did not increase correspondingly. Thus the ratio of tryptophan concentration in brain to that in plasma was increased, suggesting increased effectiveness of tryptophan uptake by the brain. The rise of brain tryptophan concentration at the higher drug dose appeared to be specific for this amino acid as major changes in the brain concentrations of tyrosine and phenylalanine did not occur. However, the mechanism responsible for the rise of brain tryptophan is not indicated to be specific for this amino acid as brain/plasma ratios increased for all three amino acids. This agrees with the work of Petkov, Usunov & Kushev (1974) who found that theophylline injected intraperitoneally increased brain uptake of various amino acids. The fall of the plasma tyrosine concentration after aminophylline could involve increased muscle uptake of tyrosine due to the rise in insulin release (see below) as well as increased activity of tyrosine aminotransferase the synthesis of which is increased by aminophylline (Wicks, 1968) and by insulin (Gelehrter & Tomkins, 1970).

Possible mechanisms responsible for the chemical changes in the brain caused by aminophylline are suggested in Figure 8. Thus methylxanthines can increase cyclic AMP levels by releasing catecholamine (Wooten *et al.*, 1973) and thus stimulating cyclic AMP synthesis and/or by inhibiting cyclic 3',5'-nucleotide phosphodiesterase (Butcher *et al.*, 1965) and thus preventing cyclic AMP degradation. The cyclic AMP increases in fat cells and in the pancreas result in increased lipolysis (Butcher *et al.*, 1965) and insulin secretion (Turtle *et al.*, 1967) respectively. Thus in the present study aminophylline increased both plasma UFA and insulin.

These changes can exert mutual influences. Thus insulin has antilipolytic effects (Butcher, Baird & Sutherland, 1968) while UFAs can increase insulin secretion (Malaisse & Malaisse-Lagae, 1968; Balasse & Ooms, 1973). The antilipolytic action of insulin may explain why plasma UFA did not rise until 3 h after aminophylline injection (Figure 1) and also why the increase of UFA was not directly proportional to drug dosage (Table 1). Conversely, particularly large UFA increases occurred when aminophylline was given to animals with experimental diabetes following streptozotocin treatment. That insulin secretion as well as lipolysis could be responsible for the brain tryptophan changes following aminophylline injections is indicated by previous work in which

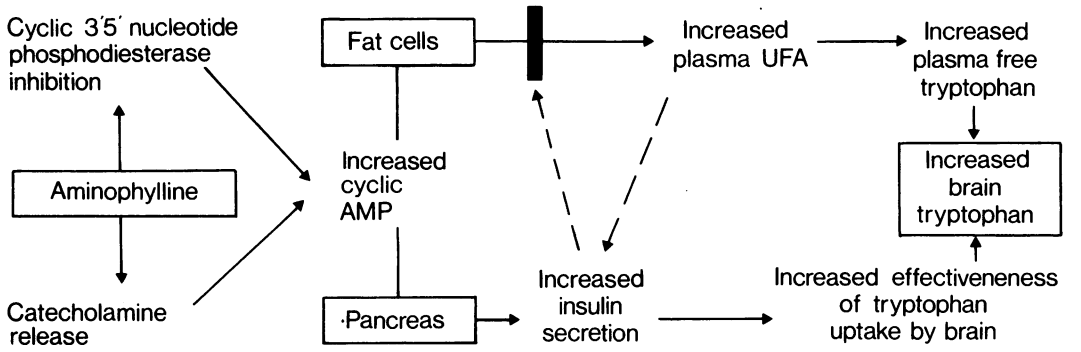

Figure 8 Relationships between aminophylline and brain tryptophan.

insulin was found to increase brain tryptophan in food-deprived rats (Fernstrom & Wurtman, 1971; Curzon & Knott, 1974) and also increased brain uptake of L-[G-^{3}H]-tryptophan (Dickerson & Pao, 1975). Furthermore, the effects of insulin and streptozotocin on the disposition of tryptophan between plasma and brain in fed rats have been explained in terms of concurrent insulin and UFA changes (Fernando *et al.*, 1976).

The increases in the plasma concentrations of insulin and UFA following aminophylline treatment are both mediated by β-adrenoceptors and such receptors seem also to be required for the effects of the drug on the disposition of tryptophan and other amino acids between plasma and brain (Figure 4) as these effects of aminophylline are prevented or decreased by propranolol. Insulin also seems to be involved in the above effect of aminophylline as it was opposed by previous streptozotocin treatment. However as aminophylline increased brain tryptophan concentration in these diabetic rats under at least some conditions (Table 4) the findings are consistent with the brain tryptophan changes not depending solely on insulin but also on plasma free tryptophan. The results in general suggest that both lipolytic and insulin secretory effects should also be considered when interpreting the effects of other drugs on brain tryptophan concentration. Whether such drugs increase lipolysis and/or insulin secretion a rise in the brain tryptophan concentration and 5-HT turnover will tend to occur. Similarly, increased lipolysis following food deprivation (Knott & Curzon, 1972) and increased insulin secretion following food intake (Fernstrom & Wurtman, 1971) can lead to increased brain tryptophan and 5-HT turnover. Thus, supplies of tryptophan to the brain for 5-HT synthesis and other purposes such as protein synthesis may be maintained in two contrasting situations.

The effectiveness of the supply of tryptophan to the brain depends on the responsiveness of β-adrenoceptors and may be opposed by stimulation of α-adrenoceptors which decrease both insulin secretion (Turtle *et al.*, 1967) and lipolysis (Boshart, Smith, Will, Perrine & Ringler, 1961; Burns, Mohs, Langley, Yawn & Chase, 1974). Another possible limitation on the availability of tryptophan to the brain from the plasma is its uptake by other tissues. This appears to be increased by aminophylline and also by carbon tetrachloride poisoning (Knott & Curzon, 1975). Both drugs led to an increase of plasma free tryptophan and the tryptophan concentration rose not only in the brain but also in muscle (which contains much larger amino acid pools). It is worth pointing out that the aminophylline-provoked increase in insulin secretion could enhance protein synthesis in muscle (Manchester, 1970) and therefore determinations of only the non-protein bound muscle tryptophan (or other amino acids) may give a falsely low index of increased flux into muscle.

Tryptophan catabolism in the liver on the pyrrolase pathway (Curzon, 1969; Green, Woods, Knott & Curzon, 1975) may also limit its availability to the brain although in the present study aminophylline may have an opposite effect as it inhibits pyrrolase *in vivo* (Young, Oravec & Sourkes, 1974).

Although the main purpose of this work was to use aminophylline to elucidate the mechanisms by which it influences brain tryptophan and 5-HT metabolism and which may also be relevant in other circumstances, the changes found may also be involved in the behavioural effects of the drug. Thus the increased sensitivity to external stimuli following aminophylline injection appears to be mediated via catecholaminergic mechanisms (Paalzow & Paalzow, 1974) and these may well be opposed by increased 5-HT synthesis as is suggested by work on the effect of altered 5-HT synthesis on the behavioural consequences of other drugs acting on catecholamine neurones (e.g. Mabry & Campbell, 1973).

We thank the MRC for a Programme Grant. Streptozotocin was generously supplied by the Upjohn Co.

References

ARISON, R.N., CIACCIO, E.I., GLITZER, M.S., CASSARO, J.A. & PRUSS, M.P. (1967). Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. *Diabetes*, **16**, 51–56.

BALASSE, E.O. & OOMS, H.A. (1973). Role of plasma free fatty acids in the control of insulin secretion in man. *Diabetologia*, **9**, 145–151.

BERKOWITZ, B.A. & SPECTOR, S. (1971). The effect of caffeine and theophylline on the disposition of brain serotonin in the rat. *Eur. J. Pharmac.*, **16**, 322–325.

BOSHART, C.R., SMITH, T.C., WILL, L., PERRINE, J. & RINGLER, I. (1961). Effect of phentolamine on body fat. *Nature, Lond.*, **189** 405–406.

BURNS, T.W., MOHS, J.M., LANGLEY, P.E., YAWN, R. & CHASE, G.R. (1974). Regulation of human lipolysis: *In vivo* observations on the role of adrenergic receptors. *J. clin. Invest.*, **53**, 338–341.

BUTCHER, R.W., BAIRD, C.E. & SUTHERLAND, E.W. (1968). Effects of lipolytic and antilipolytic substances on adenosine 3′,5′-monophosphate levels in isolated fat cells. *J. biol. Chem.*, **243**, 1705–1712.

BUTCHER, R.W., HO, R.J., MENG, H.C. & SUTHERLAND, E.W. (1965). Adenosine 3′,5′-monophosphate in biological materials. II. The measurement of adenosine 3′,5′-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. *J. biol. Chem.*, **240**, 4515–4523.

CURZON, G. (1969). Tryptophan pyrrolase — a biochemical factor in depressive illness? *Br. J. Psychiat.*, **115**, 1367–1374.

CURZON, G., FRIEDEL, J. & KNOTT, P.J. (1973). The effects of fatty acids on the binding of tryptophan to plasma protein. *Nature*, **242**, 198–200.

CURZON, G., JOSEPH, M.H. & KNOTT, P.J. (1972). Effects of immobilization and food deprivation on rat brain tryptophan metabolism. *J. Neurochem.*, **19**, 1967–1974.

CURZON, G., KANTAMANENI, B.D., WINCH, J., ROJAS-BUENO, A., MURRAY-LYON, I.M. & WILLIAMS, R. (1973). Plasma and brain tryptophan changes in experimental acute hepatic failure. *J. Neurochem.*, **21**, 137–146.

CURZON, G. & KNOTT., P.J. (1974). Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acid in the plasma. *Br. J. Pharmac.*, **50**, 197–204.

CURZON, G., KNOTT, P.J., MURRAY-LYON, I.M., RECORD, C.O. & WILLIAMS, R. (1975). Disturbed brain tryptophan metabolism in hepatic coma. *Lancet*, **i**, 1092–1093.

DALTON, C., VAN TRABERT, T.C. & DWYER, J.X. (1970). Hypolipidemic effects of nicotinamide in the rat arising from its transformation to nicotinic acid. In *Metabolic Effects of Nicotinic Acid and its Derivatives*, ed., Gey, K.F. & Carlson, L.A. pp. 65–75. Berne: Huber.

DICKERSON, J.W.T. & PAO, S.K. (1975). The effect of a low protein diet and exogenous insulin on brain tryptophan and its metabolites in the weanling rat. *J. Neurochem.*, **25**, 559–564.

ECCLESTON, D., ASHCROFT, G.W. & CRAWFORD, T.B.B. (1965). 5-Hydroxyindole metabolism in rat. A study of intermediate metabolism using the technique of tryptophan loading. *J. Neurochem.*, **12**, 493–503.

FERNANDO, J.C.R., KNOTT, P.J. & CURZON, G. (1976). The relevance of both plasma free tryptophan and insulin to rat brain tryptophan concentration. *J. Neurochem.* (in press).

FERNSTROM, J.D. & WURTMAN, R.J. (1971). Brain serotonin content: increase following ingestion of carbohydrate diet. *Science*, **174**, 1023–1025.

FRIEDMAN, P.A., KAPPELMAN, A.G. & KAUFMAN, S. (1972). Partial purification and characterisation of tryptophan hydroxylase from rabbit hindbrain. *J. biol. Chem.*, **247**, 4165–4173.

GEREHRTER, T.D. & TOMKINS, G.M. (1970). Postranscriptional control of tyrosine aminotransferase synthesis by insulin. *Proc. Nat. Acad. Sci.*, **66**, 390–397.

GESSA, G.L. & TAGLIAMONTE, A. (1974). Serum free tryptophan: control of brain concentrations of tryptophan and of synthesis of 5-hydroxytryptamine. In *Aromatic Amino Acids in the Brain. Ciba Foundation Symposium*, **22**, pp. 207–216. Amsterdam: Elsevier.

GREEN, A.R., WOODS, F., KNOTT, P.J. & CURZON, G. (1975). Factors influencing effect of hydrocortisone on rat brain tryptophan metabolism. *Nature, Lond.*, **255**, 170.

HERY, R., ROUER, E., KAN, J.P. & GLOWINSKI, J. (1974). The major role of the tryptophan active transport system in the diurnal variations of 5-hydroxytryptamine synthesis in the rat brain. *Adv. biochem. Psychopharmac.*, **11**, 163–167.

KIELY, M. & SOURKES, T.L. (1972). Transport of L-tryptophan into slices of rat cerebral cortex. *J. Neurochem.*, **19**, 2863–2872.

KNOTT, P.J. & CURZON, G. (1972). Free tryptophan in plasma and brain tryptophan metabolism. *Nature*, **239**, 452–453.

KNOTT, P.J. & CURZON, G. (1975). Tryptophan and tyrosine disposition and brain tryptophan metabolism in acute carbon tetrachloride poisoning. *Biochem. Pharmac.*, **24**, 963–966.

MABRY, P.D. & CAMPBELL, B.A. (1973). Serotonergic inhibition of catecholamine induced behavioural arousal. *Brain Res.*, **49**, 381–391.

MADRAS, B.K., COHEN, E.L., MESSING, R., MUNRO, H.N. & WURTMAN, R.J. (1974). Relevance of free tryptophan in serum to tissue tryptophan concentrations. *Metabolism*, **23**, 1107–1116.

MALAISSE, W.F. & MALAISSE-LAGAE. (1968). Stimulation of insulin secretion by non-carbohydrate metabolites. *J. lab. clin. Med.*, **72**, 438–448.

MANCHESTER, K.L. (1970). The control by insulin of amino acid accumulation in muscle. *Biochem. J.*, **117**, 457–465.

McCAMAN, M.W. & ROBINS, E. (1962). Fluorimetric method for the determination of phenylalanine in serum. *J. lab. clin. Med.*, **59**, 885–890.

MUNRO, H.N., FERNSTROM, J.D. & WURTMAN, R.J. (1975). Insulin, plasma amino acid imbalance, and hepatic coma. *Lancet*, **i**, 722–724.

OLDENDORF, W.H. (1971). Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection. *Amer. J. Physiol.*, **221**, 1629–1639.

PAALZOW, G. & PAALZOW, L. (1974). Theophylline increased sensitivity to nociceptive stimulation and regional turnover of rat brain 5-HT, noradrenaline and dopamine. *Acta Pharmac. tox.*, **34**, 157–173.

PETKOV, V., USUNOV, P. & KUSHEV, V. (1974). The role of 3′,5′-AMP system in amino acid transport across the blood-brain barrier. *Acta biol. med. germ.*, **33**, 371–383.

TAGLIAMONTE, A., BIGGIO, G., VARGIU, L. & GESSA, G.L. (1973). Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. *Life Sciences*, **12**, 277–287.

TAGLIAMONTE, A., TAGLIAMONTE, P., FORN, J., PEREZ-CRUET, J., KRISHNA, G. & GESSA, G.L. (1971). Stimulation of brain serotonin synthesis by dibutyryl-cyclic AMP in rats. *J. Neurochem.*, **18**, 1191–1196.

TURTLE, J.R., LITTLETON, G.K. & KIPNIS, D.M. (1967). Stimulation of insulin secretion by theophylline. *Nature, Lond.*, **213**, 727–728.

WICKS, W.D. (1968). Tyrosine-α-ketoglutarate transaminase: induction by epinephrine and adenosine-3′,5′-cyclic phosphate. *Science*, **160**, 997–998.

WOOTEN, G.F., THOA, N.B., KOPIN, I.J. & AXELROD, J. (1973). Enhanced release of dopamine- beta hydroxylase and norepinephrine from sympathetic nerves by dibutyryl cyclic adenosine 3′,5′-monophosphate and theophylline. *Molec. Pharmac.*, **9**, 178–183.

YOUNG, S.N., ORAVEC, M. & SOURKES, T.L. (1974). The effect of theophylline on tryptophan pyrrolase in the hypophysectomized rat and some observations on the validity of tryptophan pyrrolase assays. *J. biol. Chem.*, **249**, 3932–3936.

(Received March 18, 1976.
Revised May 19, 1976.)