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DRUGS ALTERING INSULIN SECRETION: EFFECTS ON PLASMA AND BRAIN CONCENTRATIONS OF AROMATIC AMINO ACIDS AND ON BRAIN 5-HYDROXYTRYPTAMINE TURNOVER

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

1 An investigation was made into the effects of drugs which alter insulin secretion on the concentrations of tryptophan and other aromatic amino acids in plasma and brain and on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in brain. Drugs used were streptozotocin, propranolol, tolbutamide and phentolamine.

2 Tolbutamide and phentolamine increased the plasma insulin concentrations by 100% and 300% respectively but with little effect on the brain/plasma ratios for the aromatic amino acids. Previously propranolol was found to decrease plasma insulin by 50% without altering the above ratios. The ratios were decreased by streptozotocin but only when plasma insulin fell by more than 50%.

3 Phentolamine and propranolol did not alter the brain/plasma ratios for the aromatic amino acids in streptozotocin-treated rats.

4 The results suggest that only large changes of insulin secretion e.g. those associated with food intake or aminophylline injection are likely to alter appreciably the brain/plasma ratios for the aromatic amino acids.

5 Tolbutamide displaced tryptophan from its binding to plasma albumin and increased brain 5-HIAA probably by inhibiting 5-HIAA efflux from brain. The other drugs did not alter brain 5-HT or 5-HIAA concentrations.

Introduction

When brain tryptophan concentration changes it can affect 5-hydroxytryptamine (5-HT) synthesis because tryptophan hydroxylase, the rate limiting enzyme for 5-HT synthesis is normally unsaturated with its substrate tryptophan (Eccleston, Ashcroft & Crawford, 1965; Friedman, Kappelman & Kaufman, 1972). Furthermore, recent work shows that quite moderate changes of brain tryptophan concentration can lead to altered behaviour (Lytle, Messing, Fisher & Phebus, 1975; Taylor, 1976; Bloxam, Curzon, Kantamaneni & Tricklebank, 1977). Therefore mechanisms by which brain tryptophan concentration is altered are of some importance. One determinant of brain tryptophan concentration is insulin secretion as this can result in decreased plasma concentrations of large neutral amino acids which compete with tryptophan for uptake by brain (Fernstrom & Wurtman, 1971; 1972; Madras, Cohen, Messing, Munro & Wurtman, 1974). Experiments with insulin and two drugs, aminophylline and streptozotocin, which alter its secretion suggest that the above mechanism and altered binding of plasma tryptophan to albumin can concurrently affect brain tryptophan concentration (Curzon & Fernando, 1976; Fernando, Knott & Curzon, 1976). Also the increase of plasma insulin following injection of the 5-HT receptor antagonist, methiothepin, has been suggested as a partial explanation of the increased brain 5-HT synthesis caused by this drug. (Jacoby, Shabshelowitz, Fernstrom & Wurtman, 1975).

It was of interest therefore to investigate whether drugs known to alter insulin secretion also alter the concentrations of tryptophan and other aromatic amino acids in plasma, brain and muscle and the concentrations of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in brain. Drugs used were streptozotocin and propranolol which decrease insulin secretion and tolbutamide and phentolamine which increase it.

Methods

Male Sprague-Dawley rats (ALA, Alconbury, Huntingdon, U.K.), 180–220 g were kept 3 or 4 to a cage in an acoustically lagged chamber at 24 ± 3°C on a 18 h 00 min–06 h 00 min light-dark cycle and fed with Oxoid 41B pellets and water *ad libitum*.

The following drugs were used: phentolamine (Ciba Ltd.) dissolved in 0.9% w/v NaCl solution (saline); probenecid (Sigma Ltd.) dissolved in the minimum

volume required of 1N NaOH, diluted with saline and pH adjusted to 9; (±)-propranolol (ICI Ltd.) dissolved in saline; streptozotocin (Upjohn, Kalamazoo, USA) dissolved in 0.07M sodium citrate buffer (pH 4.5) immediately before use, tolbutamide ('Rastinon' injection, Hoechst Ltd.). Drugs were injected intraperitoneally, 2.5 ml/kg body wt except for tolbutamide which was injected intraperitoneally, 5.0 ml/kg body wt. Control animals were injected with the appropriate vehicles.

At various intervals after injection as indicated in the Results section rats were killed by guillotine (between 15 h 00 min and 16 h 00 min) and the blood drained into tubes containing citrate. The plasma was separated by centrifugation and stored at −20°C. Brain and gastrocnemius muscle were removed, frozen immediately on solid CO_2 and stored at −20°C. Plasma unesterified fatty acids (UFA), insulin, tryptophan (total and free), tyrosine, phenylalanine, 5-HT and 5-HIAA and muscle tryptophan, tyrosine and phenylalanine were determined by methods described previously (Curzon & Fernando, 1976). The plasma was ultrafiltered for separation of free tryptophan at non-physiological pH (7.8–7.9) as pH rose during storage. Although the resultant *in vitro* decrease of free tryptophan concentration (McMenamy & Seder, 1963) would have apparently increased the ratios of tissue tryptophan to plasma free tryptophan concentrations, this effect would have been comparable in all experiments as plasma pH rose on storage independently of the drug treatment used.

Results

Effects on aromatic amino acid disposition of drugs decreasing plasma insulin concentration

Streptozotocin. Streptozotocin destroys pancreatic insulin secreting cells (Arison, Ciaccio, Glitzer, Cassaro & Pruss, 1967) and thus decreases plasma insulin concentration. Table 1 shows the results of two separate experiments in which streptozotocin was injected 72 h before rats were killed. Intravenous injection of 65 mg/kg of the drug led to a considerable fall of plasma insulin in association with a large and significant rise of free tryptophan. Despite this increased availability of tryptophan its concentration in the brain fell significantly and the ratio of brain tryptophan to plasma free tryptophan showed a large significant fall (−65%). These results were similar to previous findings (Fernando *et al.*, 1976) except for the fall of brain tryptophan concentration. It may be that this difference was due to the lower plasma insulin levels attained in the previous experiment.

Intraperitoneal injection appeared less effective as 90 mg/kg (i.p.) of streptozotocin led to a smaller (though significant) fall in plasma insulin. This was associated with small and non-significant changes in the brain tryptophan/plasma free tryptophan ratio (−20%) and in the corresponding ratios for the other aromatic amino acids (not shown).

Propranolol. Insulin secretion is increased by the action of catecholamines at pancreatic adrenoceptors (Turtle, Littleton & Kipnis, 1967). Previously the β-adrenoceptor blocking agent, propranolol was found to decrease plasma insulin significantly (Curzon & Fernando, 1976). The fall of insulin was similar to that found after streptozotocin injection (90 mg/kg i.p.) and brain tryptophan and its ratio to plasma free tryptophan were similarly unaffected. As it was conceivable that propranolol might have an action on tryptophan distribution which was not mediated by insulin and which might obscure the effects of insulin changes, propranolol was given to streptozotocin-treated rats (Table 2). Plasma insulin and both plasma and brain tryptophan concentrations were not significantly altered. Plasma and brain tyrosine or phenylalanine concentrations were also unaffected (results not shown).

Effects on aromatic amino acid disposition of drugs increasing plasma insulin concentration

Tolbutamide. Sulphonylureas such as tolbutamide increase pancreatic insulin secretion probably by increasing islet cell cyclic adenosine 3′,5′-monophosphate (Goldfine, Perlman & Roth, 1971). Table 3 shows the results of separate experiments in which rats were given tolbutamide and killed 1 h or 3 h later. Plasma insulin was considerably lower in the control group killed 1 h after injection of saline than in the corresponding control group killed 3 h after injection. The lower values in the former group may reflect a transient injection stress-provoked decrease of insulin secretion (Porte & Robertson, 1973). Plasma free tryptophan concentrations were unaltered but as total tryptophan fell significantly the percentage of free tryptophan rose. These tryptophan changes were most pronounced in rats killed 1 h after tolbutamide injection and occured without a significant UFA increase. The following observation suggests that they were probably due to the freeing by tolbutamide of tryptophan bound to plasma protein i.e. the addition of 4.5 mg tolbutamide dissolved in 0.2 ml saline to 1.0 ml plasma resulted in 81% of the plasma tryptophan becoming ultrafilterable. (The addition of 0.2 ml saline to 1.0 ml plasma resulted in only 11% of the tryptophan being ultrafilterable). The effect of tolbutamide was not due to the alteration of plasma pH as this was unchanged.

Brain tryptophan and its ratio to plasma free tryptophan increased slightly but except for the increase of brain tryptophan concentration 1 h after tolbutamide injection (Figure 1) the changes were not significant. Plasma values for tyrosine and phenylalanine (Table 4) were essentially unaltered while the

Table 1 Effect of streptozotocin on plasma concentrations of unesterified fatty acid (UFA), insulin and tryptophan and on brain concentrations of tryptophan

	Saline (8)	*Streptozotocin (90 mg/kg i.p.) (9)*	*Saline (4)*	*Streptozotocin (65 mg/kg i.v.) (4)*
Plasma UFA (mEq/l)	0.71 ± 0.40	0.79 ± 0.18	0.44 ± 0.16	0.83 ± 0.32
Plasma insulin (μu/ml)	28.4 ± 6.5	14.7 ± 6.4****	31.8 ± 11.6	6.8 ± 3.9**
Tryptophan				
Plasma total (μg/ml)	18.94 ± 2.54	18.15 ± 2.08	17.92 ± 1.09	13.51 ± 6.82
Plasma free (μg/ml)	1.94 ± 0.43	2.44 ± 0.39*	1.82 ± 0.29	3.59 ± 0.84***
Brain (μg/g wet wt)	4.94 ± 0.70	5.09 ± 0.49	4.20 ± 0.26	2.78 ± 0.62***
Brain/plasma free	2.67 ± 0.74	2.15 ± 0.46	2.36 ± 0.42	0.83 ± 0.33***

Rats were killed 72 h after injection of streptozotocin. Controls were injected with saline. Results are expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test. Differences from controls: *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$

Table 2 Effect of propranolol on plasma concentrations of unesterified fatty acid (UFA), insulin and tryptophan and on brain concentrations of tryptophan in streptozotocin treated rats

	Saline (7–8)	*Propranolol (7)*
Plasma UFA (mEq/l)	0.80 ± 0.30	0.87 ± 0.14
Plasma insulin (μu/ml)	12.4 ± 7.9	15.0 ± 7.7
Tryptophan		
Plasma total (μg/ml)	13.18 ± 2.65	11.54 ± 1.99
Plasma free (μg/ml)	1.39 ± 0.22	1.44 ± 0.37
Brain (ug/g wet wt)	3.15 ± 0.41	2.75 ± 0.45
Brain/plasma free	2.27 ± 0.19	2.09 ± 0.26

Propranolol (2 mg/kg i.p. × 2) was injected 3 h and 1 h before killing. Streptozotocin (90 mg/kg i.p.) was given 48 h earlier. Controls were injected with saline instead of propranolol. Results are expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test.
No significant differences.

Table 3 Effect of tolbutamide on plasma concentrations of unesterified fatty acid (UFA), insulin and tryptophan and on brain concentrations of tryptophan

	Experiment 1		*Experiment 2*	
	Saline (7–8)	*Tolbutamide (7–8)*	*Saline (5–6)*	*Tolbutamide (5–6)*
	Killed at 3 h		*Killed at 1 h*	
Plasma UFA (mEq/l)	0.31 ± 0.18	0.38 ± 0.15	0.71 ± 0.30	0.51 ± 0.22
Plasma insulin (μu/ml)	25.0 ± 8.0	41.0 ± 19.0*	9.5 ± 3.0	18.2 ± 8.7*
Tryptophan				
Plasma total (μg/ml)	16.29 ± 2.63	11.33 ± 4.06**	16.46 ± 3.61	5.73 ± 0.54***
Plasma free (μg/ml)	1.32 ± 0.35	1.29 ± 0.22	1.21 ± 0.27	1.25 ± 0.17
Brain (μg/g wet wt)	5.01 ± 0.59	5.51 ± 0.46	4.41 ± 0.39	4.95 ± 0.24
Brain/plasma free	4.01 ± 1.01	4.35 ± 0.56	3.77 ± 0.70	4.02 ± 0.49

Rats were killed either 1 or 3 h after injection of tolbutamide (230 mg/kg i.p.) as the Na salt. Controls were injected with saline. Results are expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test.
Differences from controls: *$P < 0.05$; **$P < 0.02$; ***$P < 0.001$

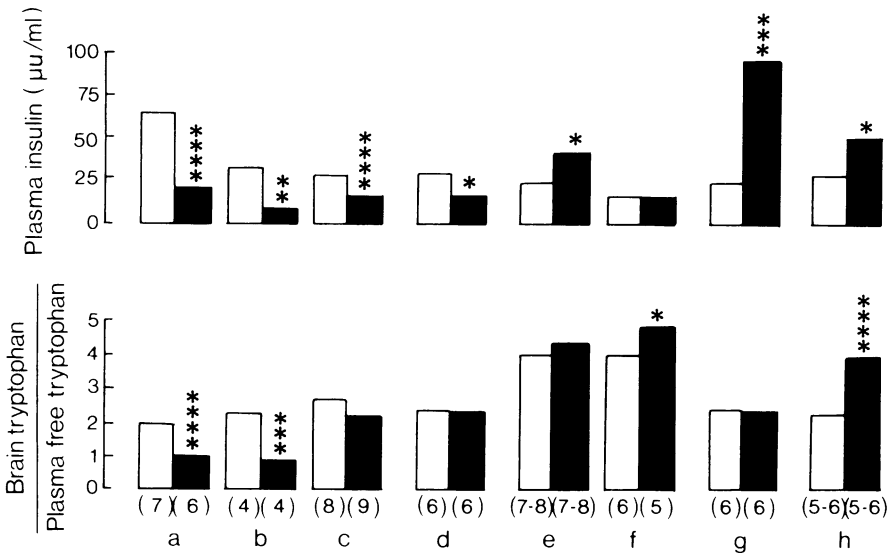

Figure 1 Effects of drugs on plasma insulin concentration and on the ratios of brain tryptophan to plasma free tryptophan concentrations. (a) Streptozotocin, 65 mg/kg i.v. (72 h); (b) streptozotocin, 65 mg/kg i.v. (72 h); (c) streptozotocin, 90 mg/kg i.p. (72 h); (d) propranolol, 2 mg/kg i.p. × 2 (1 h, 3 h); (e) tolbutamide, 230 mg/kg i.p. (3 h); (f) tolbutamide, 230 mg/kg i.p. (3 h) plus probenecid, 200 mg/kg i.p. × 2 (1 h, 3 h); (g) phentolamine, 20 mg/kg i.p. × 2 (1 h, 3 h); (h) aminophylline 150 mg/kg i.p. (3 h). Drugs were injected at times before the animals were killed as shown in parentheses. Results in (a) from Fernando *et al.* (1976). Significance of differences from controls: $*P < 0.05$; $**P < 0.02$; $***P < 0.01$; $****P < 0.001$

brain showed a slight increase so that brain/plasma ratios also increased slightly. The latter increase was significant for tyrosine only.

Phentolamine. Drugs blocking α-adrenoceptors increase plasma insulin (Turtle *et al.*, 1967). Thus phentolamine caused a very considerable increase of plasma insulin (Table 5). Changes of plasma UFA, plasma total and free tryptophan and brain tryptophan were negligible so that the ratio of the latter to plasma free tryptophan was not significantly altered. Muscle tryptophan concentration was also unaffected. When phentolamine was given to rats treated with streptozotocin in order to reveal any

Table 4 Effect of tolbutamide on plasma and brain concentrations of tyrosine and phenylalanine

	Saline (6)	Tolbutamide (5)
	Killed at 1 h	
Tyrosine		
Plasma (μg/ml)	11.45 ± 1.28	10.76 ± 1.02
Brain (μg/g wet wt)	9.04 ± 0.52	10.32 ± 1.00*
Brain/plasma	0.80 ± 0.09	0.97 ± 0.14*
Phenylalanine		
Plasma (μg/ml)	7.56 ± 1.08	7.29 ± 0.70
Brain (μg/g wet wt)	12.90 ± 0.56	14.55 ± 1.48*
Brain/plasma	1.72 ± 0.24	2.02 ± 0.35

Rats were killed either 1 or 3 h after injection of tolbutamide (230 mg/kg i.p.) as the Na salt Controls were injected with saline. Results are expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test.
Differences from controls: $*P < 0.05$.

Table 5 Effect of phentolamine on plasma concentrations of unesterified fatty acid (UFA) and insulin and on the disposition of tryptophan

			Streptozotocin treated	
	Saline (6)	*Phentolamine (6)*	*Saline (7–8)*	*Phentolamine (7–8)*
Plasma UFA (mEq/l)	0.57 ± 0.18	0.65 ± 0.36	0.80 ± 0.30	1.12 ± 0.13*
Plasma insulin (μu/ml)	24.2 ± 10.2	95.5 ± 45**	11.3 ± 5.70	15.0 ± 7.70
Tryptophan				
Plasma total (μg/ml)	12.19 ± 2.30	11.13 ± 2.69	13.18 ± 2.65	8.56 ± 1.42***
Plasma free(μg/ml)	1.34 ± 0.34	1.62 ± 0.44	1.39 ± 0.22	1.99 ± 0.87
Muscle (μg/g wet wt)	4.17 ± 0.76	3.96 ± 0.36	3.75 ± 0.79	3.85 ± 1.00
Brain (μg/g wet wt)	3.45 ± 0.45	3.42 ± 0.53	3.15 ± 0.41	3.35 ± 0.34
Brain/plasma free	2.39 ± 0.37	2.29 ± 0.67	2.27 ± 0.19	1.94 ± 0.75

Phentolamine (20 mg/kg i.p. × 2) was injected 3 h and 1 h before killing. Streptozotocin (90 mg/kg i.p.) was given 48 h earlier. Controls were injected with saline. Results expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test.
Differences from controls: *$P < 0.02$; **$P < 0.01$; ***$P < 0.001$

effects on amino acid distribution not mediated by insulin then plasma insulin concentrations were no longer significantly altered (Table 5), plasma UFA increased and total tryptophan fell significantly. Muscle tryptophan concentration and the ratio of brain tryptophan to plasma free tryptophan (as in the absence of streptozotocin) were not significantly altered.

Table 6 shows tyrosine and phenylalanine concentrations in the above groups of animals. Phentolamine injection led to significant decreases of tyrosine in plasma, muscle and brain. The ratio of brain to plasma concentrations was slightly increased. Comparable tyrosine changes occurred when phentolamine was given to streptozotocin-treated rats. Phentolamine did not significantly alter tissue phenylalanine concentrations in normal rats but caused small but significant decreases in both muscle and brain after streptozotocin treatment. The ratio of brain to plasma concentrations was not significantly changed.

Effects on 5-hydroxytryptamine metabolism of drugs altering plasma insulin concentration

Table 7 summarizes changes of brain tryptophan, 5-HT and 5-HIAA concentrations in the above experiments. Streptozotocin, propranolol or phentolamine had negligible effects on 5-HT or 5-HIAA. This was found even after 65 mg/kg streptozotocin intravenously which significantly decreased brain tryptophan concentrations.

Table 6 Effect of phentolamine on the disposition of tyrosine and phenylalanine

			Streptozotocin treated	
	Saline (6)	*Phentolamine (6)*	*Saline (7–8)*	*Phentolamine (7–8)*
Tyrosine				
Plasma (μg/ml)	13.87 ± 2.23	9.08 ± 1.57***	15.90 ± 2.29	10.76 ± 1.50****
Muscle (μg/g wet wt)	15.17 ± 1.51	11.50 ± 2.01***	12.47 ± 1.57	8.87 ± 1.81****
Brain (μg/g wet wt)	9.74 ± 0.62	7.90 ± 1.19***	12.64 ± 1.90	9.63 ± 1.07***
Brain/plasma	0.71 ± 0.08	0.88 ± 0.12**	0.77 ± 0.05	0.94 ± 0.23
Phenylalanine				
Plasma (μg/ml)	9.29 ± 0.95	9.36 ± 1.00	9.53 ± 2.28	8.97 ± 1.38
Muscle (μg/g wet wt)	5.75 ± 0.59	6.54 ± 1.20	12.47 ± 1.57	8.87 ± 1.81****
Brain (μg/g wet wt)	9.80 ± 0.79	10.88 ± 1.68	11.91 ± 1.30	10.67 ± 0.78*
Brain/plasma	1.06 ± 0.12	1.19 ± 0.30	1.32 ± 0.35	1.19 ± 0.12

Phentolamine (20 mg/kg i.p. × 2) was injected 3 h and 1 h before killing. Streptozotocin (90 mg/kg i.p.) was given 48 h earlier. Controls were injected with saline. Results expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test.
Differences from controls: *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$

Table 7 Effect of drugs on brain 5-hydroxytryptamine (5-HT) metabolism

	Brain concentrations (μg/g wet wt)		
Drugs administered	*Tryptophan*	*5-HT*	*5-HIAA*
Saline (8)	4.94 ± 0.70	0.63 ± 0.24	0.48 ± 0.09
Streptozotocin 90 mg/kg i.p. 72 h (9)	5.09 ± 0.49	0.72 ± 0.01	0.55 ± 0.06
Saline (4)	4.20 ± 0.26	0.61 ± 0.01	0.56 ± 0.08
Streptozotocin 65 mg/kg i.v. 72 h (4)	2.78 ± 0.62*	0.66 ± 0.07	0.56 ± 0.05
Saline (6)[1]	2.47 ± 0.27	0.52 ± 0.05	0.45 ± 0.08
Propranolol 2 mg/kg i.p. × 2; 1 h, 3 h (6)[1]	2.75 ± 0.33	0.56 ± 0.04	0.49 ± 0.03
Saline (8)	5.01 ± 0.59	0.62 ± 0.08	0.48 ± 0.04
Tolbutamide 230 mg/kg i.p.3 h (7)	5.51 ± 0.46	0.68 ± 0.04	0.80 ± 0.07**
Probenecid 200 mg/kg i.p. × 2; 1 h, 3 h (6)	4.71 ± 0.57	0.87 ± 0.07	0.93 ± 0.10
Probenecid 200 mg/kg i.p. × 2; 1 h, 3h Tolbutamide 230 mg/kg i.p. 3 h (5)	8.78 ± 1.09**	0.95 ± 0.08	1.20 ± 0.08**
Saline (6)	3.45 ± 0.45	0.54 ± 0.04	0.49 ± 0.05
Phentolamine 20 mg/kg i.p. × 2; 1 h, 3 h (6)	3.42 ± 0.53	0.57 ± 0.03	0.54 ± 0.07

Results expressed as means ± one s.d. Nos of determinations are shown in parentheses. Results compared by Student's *t* test.
Differences from controls: *$P < 0.01$; **$P < 0.001$
[1]Results from Curzon & Fernando (1976).

Tolbutamide caused a considerable and highly significant increase of brain 5-HIAA (+67%) 3 h later which was proportionately much greater than the increases of tryptophan (+12% at 1 h, +10% at 3 hours). When rats were also given probenecid to inhibit efflux of 5-HIAA from the brain, tolbutamide caused a relatively small increase of 5-HIAA (+29%) associated with a much larger increase of tryptophan (+86%).

Discussion

The results of drug treatments described here or elsewhere (Curzon & Fernando, 1976; Fernando *et al.*, 1976) showed that large changes of plasma insulin concentration can alter the ratios of the concentrations of aromatic amino acids in brain to their concentrations in plasma. The results are summarized in Figure 1.

Thus after streptozotocin injection (65 mg/kg i.v.) the plasma concentrations of insulin, the ratio for the concentrations of plasma free tryptophan to brain tryptophan (Figure 1a, b) and the corresponding ratios for tyrosine and phenylalanine (Fernando *et al.*, 1976) showed large falls. Conversely aminophylline increased these parameters (Curzon & Fernando, 1976 and Figure 1h). Also the absolute concentrations of tryptophan in the brain were decreased by streptozotocin (Table 1) and increased by aminophylline. The changes of brain concentration of tryptophan and of the other aromatic amino acids were less striking than the changes in the plasma-brain ratios (Fernando *et al.*, 1976) (Table 1); in some circumstances they were absent (Curzon & Fernando, 1976; Fernando *et al.*, 1976).

Other experiments with drugs indicated that although the above large changes of plasma insulin concentration were associated with major changes in the disposition of the aromatic amino acids between plasma and brain, quite appreciable but smaller insulin changes were not associated with alterations of amino acid disposition e.g. propranolol or streptozotocin (90 mg/kg i.p.) led to significant and proportionately comparable falls of plasma insulin but without significant or considerable changes of tryptophan disposition (Figure 1c, d). Furthermore, while tolbutamide did significantly increase both plasma insulin (Figure 1e) and aromatic amino acid concentrations in the brain (Tables 3, 4) the latter changes were relatively small and only in the case of tyrosine was the ratio of the brain to plasma concentrations significantly increased. The ratio of brain tryptophan to plasma free tryptophan concentration increased slightly but significantly when tolbutamide was given to probenecid-treated rats although interpretation of this result is difficult as plasma insulin concentration was not raised.

Phentolamine (Figure 1g) caused a much larger increase of plasma insulin concentration than tolbutamide but this had no effect on aromatic amino acid disposition: plasma, brain and muscle concentrations of tryptophan and phenylalanine were unaltered. The tissue tyrosine concentrations fell but as these changes were not prevented by streptozotocin

pretreatment they were probably unrelated to insulin secretion and may have been due to increased tyrosine catabolism as tyrosine aminotransferase is induced by phentolamine (Govier & Lovenberg, 1969).

It appears therefore that only a considerable plasma insulin increase such as might occur after food intake or a considerable insulin deficiency are likely to alter appreciably the disposition of tryptophan and other aromatic amino acids between plasma and brain. Thus decreases in plasma insulin of 50% (streptozotocin, Figure 1c; propranolol) or increases even up to 300% (phentolamine) had little effect. The results with aminophylline appear to contradict the above generalization as 3 h after its injection amino acid disposition was altered even though plasma insulin only increased by about 100%. However the amino acid changes may have resulted from the very much larger increases of plasma insulin found 1 h after aminophylline injection (Curzon & Fernando, 1976).

Other work supports the conclusion that considerable increases of insulin are needed to alter the tissue uptake of aromatic amino acids as plasma insulin had to be raised as high as 200–300 μu/ml in order to double the rate of entry of tryptophan into the rabbit brain (Daniel, Love, Moorhouse & Pratt, 1975). Plasma insulin may well rise comparably on food intake and thus lead to increased brain tryptophan (Fernstrom & Wurtman, 1971, 1972).

An apparent lack of effect of drugs on amino acid disposition even though they altered insulin secretion could occur if they had other effects on amino acid disposition which obscured those of insulin, e.g. effects dependent on the hypothermia due to phentolamine (Tsoucaris-Kupfer & Schmitt, 1972). Results indicated that propranolol or phentolamine-dependent mechanisms other than the effects of these drugs on insulin levels did not obscure the effects of the insulin changes on amino acid disposition i.e. changes of the latter were not revealed when propranolol or phentolamine were given to rats previously treated with the diabetogenic drug streptozotocin. The only exceptions were the small but significant decreases of muscle and brain phenylalanine when phentolamine was given to streptozotocin-treated rats.

In general, the drugs used did not alter brain tryptophan, 5-HT or 5-HIAA concentration. However, there were two exceptions. Firstly, 72 h after injection of streptozotocin (65 mg/kg i.v.) although brain tryptophan fell 5-HT and 5-HIAA remained unaltered which may suggest a concurrent increase of tryptophan hydroxylase activity. Secondly, tolbutamide caused a large increase of 5-HIAA. This appeared to result not from increased 5-HT turnover but from slower efflux of 5-HIAA from the brain. Thus when efflux was blocked by probenecid, tolbutamide caused only a small percentage increase of 5-HIAA which was probably due to a large increase of brain tryptophan that occurred only when both probenecid and tolbutamide were given.

Tolbutamide may well block 5-HIAA efflux from the brain as it is metabolized to *N-p*-carboxybenzene-sulphonyl N^1-n butylurea (Wittenhagen, Mohnike & Langenbock, 1959) which is structurally similar to probenecid (*p*-di-*n*-propylsulphamyl-benzoic acid). Furthermore another probenecid-like action of tolbutamide has been described as both drugs increase uric acid excretion (Iyer & Cyriac, 1970).

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