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EFFECTS ON PLASMA AND BRAIN TRYPTOPHAN IN THE RAT OF DRUGS AND HORMONES THAT INFLUENCE THE CONCENTRATION OF UNESTERIFIED FATTY ACID IN THE PLASMA

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1 The effects on tryptophan distribution and metabolism of drugs altering plasma unesterified fatty acid (UFA) concentration were investigated in the rat.

2 UFA and plasma free (i.e. ultrafilterable) tryptophan altered in the same direction.

3 Catecholamines and L-DOPA increased both plasma UFA and free tryptophan. L-DOPA also increased brain tryptophan and 5-hydroxyindoleacetic acid (5-HIAA) but decreased brain 5-hydroxytryptamine (5-HT).

4 Aminophylline increased plasma UFA and free tryptophan and also brain tryptophan, 5-HT and 5-HIAA. Food deprivation had qualitatively similar effects.

5 Insulin decreased plasma UFA and free tryptophan in both fed and food-deprived rats. However, while in fed rats these changes were associated with small decreases of brain indoles, in food-deprived animals small increases occurred.

6 Nicotinic acid had only small effects in fed rats but it opposed both the UFA and indole changes in food-deprived animals. Total plasma tryptophan increased in nicotinic acid treated, food-deprived rats.

7 There was a tendency towards inverse relations between changes of plasma free and total tryptophan.

8 The results suggest that drugs which influence plasma UFA through actions on cyclic AMP thereby alter the binding of tryptophan to plasma protein and that this leads to altered distribution and metabolism of tryptophan.

Introduction

Brain tryptophan hydroxylase is the rate limiting enzyme for 5-hydroxytryptamine (5-HT) synthesis. As this enzyme is normally unsaturated with its precursor tryptophan (Eccleston, Ashcroft & Crawford, 1965; Friedman, Kappelman & Kaufman, 1972) the mechanisms by which the concentration in brain of the latter is controlled can influence 5-HT turnover. It has been shown that food deprivation (Curzon, Joseph & Knott, 1972; Perez-Cruet, Tagliamonte, Tagliamonte & Gessa, 1972) or immobilization (Curzon, Joseph & Knott, 1972) both lead to increased brain tryptophan and increased turnover of brain 5-HT as indicated by raised concentrations of its metabolite 5-hydroxyindoleacetic acid (5-HIAA). The increase of brain tryptophan does not simply reflect non-specific brain amino acid changes (Knott, Joseph & Curzon, 1973) or plasma total tryptophan (Curzon *et al.*, 1972) but is associated with an increase of the small fraction of plasma tryptophan which is free, i.e. ultrafilterable (Knott & Curzon, 1972). Similarly a number of drugs which when given to rats increase their brain tryptophan also release protein bound (non-ultrafilterable) tryptophan from plasma *in vitro* (Tagliamonte, Biggio & Gessa, 1971).

During food deprivation, plasma unesterified fatty acid (UFA) increases together with free tryptophan, while heparin injection which increases plasma UFA concentration also increases free tryptophan (Knott & Curzon, 1972). Conversely, both UFA and free tryptophan decrease upon administration of an oral glucose load to human subjects (Lipsett, Madras, Wurtman & Munro, 1973). As the addition *in vitro* of UFA to rat and human plasma within the range of physiological concentration results in an increased concentration of free tryptophan (Curzon, Friedel &

Knott, 1973), it appears that plasma UFA changes can influence the availability of tryptophan to the brain and hence cerebral 5-HT turnover. Therefore it is possible that many drugs and hormones, known to alter lipolysis and UFA concentrations by changing cyclic AMP metabolism in fat cells (Robison, Butcher & Sutherland, 1971), may be able to alter the disposition of plasma tryptophan and thus also to alter brain tryptophan and 5-HT turnover. This paper describes studies with a number of such drugs and hormones and provides evidence consistent with the above mechanism. A brief account of part of the work has already been published (Curzon & Knott, 1973).

Methods

Adult male Sprague-Dawley rats (180-220 g; Carworth, Alconbury, Huntingdon, England) were kept at 25° C in acoustically lagged housing with a 06 h 00 min-18 h 00 min light-dark cycle. Animals were killed by guillotine at 16 h 00 min-17 h 00 min and blood collected into heparinized tubes, immediately centrifuged and the plasma frozen with solid CO_2 and stored at −20° C until required for chemical analysis. Brains were removed rapidly, frozen with CO_2 and stored at −20° C.

The following drugs were used: noradrenaline bitartrate injection (Bayer Products), isoprenaline sulphate injection (Macarthy Ltd), aminophylline injection (Evans Medical), theobromine, caffeine and nicotinic acid (BDH), L-DOPA (Hofmann-La Roche), glucagon free insulin (Wedell Pharmaceuticals). Drugs supplied as solids were dissolved in 0.9% sodium chloride solution. Injection volume was 2.5 ml/kg body wt. Control animals were injected with 0.9% w/v sodium chloride solution.

Analytical methods

Plasma tryptophan was determined by the method of Denkla & Dewey (1967) on whole plasma and also on plasma ultrafiltrate prepared by centrifuging 1.0 ml plasma in a CF50 Diaflo membrane cone (Amicon) at 800 *g* for 30 min at room temperature. L-DOPA administration interfered with the determination of plasma total and free tryptophan so that low values were obtained. These were avoided by the use of internal tryptophan standards added to plasma and ultrafiltrate samples. Plasma UFA was determined by the method of Laurell & Tibbling (1967). During all manipulations of plasma it was left unfrozen for the minimum time possible as in preliminary work when this precaution was not taken both UFA and free tryptophan concentrations gradually rose as a consequence of *in vitro* action of plasma lipase. Brain tryptophan, 5-HT amd 5-HIAA were determined as described by Curzon *et al.* (1972). Plasma glucose was determined by a glucose oxidase method (Biochemica Test Combination–Boehringer Gmbh).

Results

Effects of catecholamines and related substances

Infusion of noradrenaline or adrenaline is known to increase the concentration of plasma UFA (Havel & Goldfien, 1959). Preliminary experiments in which noradrenaline and adrenaline were injected subcutaneously gave results in agreement with these findings. Free tryptophan concentration in the plasma also increased (Table 1). Similarly 5 min after injection of 0.04 mg/kg i.v. of the agonist isoprenaline both these parameters were significantly increased.

Table 1 Effects of catecholamines on plasma unesterified fatty acid (UFA) and tryptophan

Injected	*Time after injection*	*UFA (mEq/l)*	*Tryptophan (μg/ml)*	
			Total	*Free*
0.9% Sodium chloride s.c. (2)	1 h	0.13	16.56	1.60
0.9% Sodium chloride s.c. (2)	2 h	0.04	16.58	1.38
Noradrenaline (1 mg/kg s.c.) (3)	1 h	0.38	10.34	2.16
Noradrenaline (1 mg/kg s.c.) (2)	2 h	0.52	12.53	3.35
0 9% Sodium chloride i.v. (5)	5 min	0.24 ± 0.09	14.88 ± 3.19	1.09 ± 0.12
Isoprenaline (0.04 mg/kg i.v.) (6)	5 min	0.45 ± 0.16*	16.36 ± 2.17	1.70 ± 0.25**

Results are expressed as means for the noradrenaline experiment and as mean ± one s.d. for the isoprenaline experiment. Numbers of determinations shown in parentheses.
Results compared by Student's *t* test; * $P < 0.05$; ** $P < 0.01$.

Table 2 Effect of L-DOPA on plasma unesterified fatty acid (UFA) and tryptophan metabolism.

	Plasma			*Brain (μg/g)*		
	Tryptophan (μg/ml)					
Injected	*UFA (mEq/l)*	*Total*	*Free*	*Tryptophan*	*5-HT*	*5-HIAA*
0.9% Sodium chloride (6)	0.28 ± 0.09	26.31 ± 6.05	2.01 ± 0.58	2.64 ± 0.43	0.57 ± 0.04	0.38 ± 0.03
L-DOPA 500 mg/kg (5)	0.64 ± 0.11***	16.88 ± 1.03*	4.91 ± 1.11***	4.52 ± 0.47***	0.49 ± 0.01**	0.56 ± 0.08**

Injections were made i.p. and rats killed 2 h later. Results are expressed as means ± one s.d. Nos. of determinations shown in parentheses. Results compared by Student's *t* test; $*P < 0.02$; $**P < 0.01$; $***P < 0.001$.

Injection of 500 mg/kg i.p. of the catecholamine precursor L-dihydroxyphenylalanine (L-DOPA) led 2 h later, to large and significant increases of both plasma UFA and free tryptophan concentrations and a moderately significant fall of plasma total tryptophan concentration (Table 2). Brain tryptophan and 5-HIAA concentrations both rose significantly and brain 5-HT concentration fell slightly but significantly.

Effects of methylxanthines. Methylxanthines inhibit enzymic destruction of cyclic AMP and increase UFA in isolated fat cell preparations (Butcher, Ho, Meng & Sutherland, 1965) in plasma and when administered to man (Bellet, Kershbaum & Finck, 1968). Thus injection of aminophylline (150 mg/kg i.p.) (Table 3) led 3 h later to highly significant increases in the concentrations of plasma UFA, brain tryptophan, 5-HT and 5-HIAA. The rise in the concentration of brain tryptophan was particularly large. Aminophylline or caffeine at lower dosage (50 mg/kg) did not lead to significant plasma changes at 3 h although a third methylxanthine, theobromine at this dosage caused a significant increase of UFA together with a smaller and non-significant increase of plasma free tryptophan. The apparent discrepancy between the effects of theobromine on these two parameters is in part at least related to a concomitant small fall of plasma total tryptophan. Rats given aminophylline (150 mg/kg) showed loss of muscle tone and decreased motor activity. Less severe toxic effects were noted in some animals given lower dosages.

Effect of insulin. Insulin decreases cyclic AMP in fat cells (Butcher, Baird & Sutherland, 1968) and has antilipolytic action. Thus 2 h after i.p. injection of insulin (2 iu/kg), the plasma concentration of UFA had fallen (Table 4). Plasma free tryptophan concentration also fell significantly. Although mean concentrations of brain indoles were below those of control rats the fall was significant only for 5-HT. These findings differ from those of Fernstrom & Wurtman (1971) who found that brain tryptophan and 5-HT increased 2 h after i.p. injection of insulin (2 iu/kg) to rats from which a largely carbohydrate diet had been withdrawn 15-18 h previously. An additional experiment was therefore performed with rats from which food was withdrawn 24 h before insulin injection. Plasma UFA and free tryptophan concentrations fell after injection as in the insulin treated rats, and total plasma tryptophan did not change significantly. Brain changes had some consistency with those found by Fernstrom & Wurtman (1971) as tryptophan concentration rose significantly, although 5-HT and 5-HIAA concentrations did not.

Table 3 Effect of methylxanthines on plasma unesterified fatty acid (UFA) and tryptophan metabolism.

	Plasma			*Brain (μg/g)*		
		Tryptophan (μg/ml)				
Injected	*UFA (mEq/l)*	*Total*	*Free*	*Tryptophan*	*5-HT*	*5-HIAA*
0.9% Sodium chloride (8)	0.41 ± 0.09	18.25 ± 2.66	1.59 ± 0.41	2.70 ± 0.26	0.42 ± 0.03	0.28 ± 0.03
Aminophylline 150 mg/kg (8)	0.78 ± 0.16**	20.55 ± 6.90	2.91 ± 0.68**	6.75 ± 0.97**	0.53 ± 0.04**	0.72 ± 0.06**
0.9% Sodium chloride (6)	0.39 ± 0.10	23.44 ± 3.00	1.54 ± 0.61			
Caffeine (50 mg/kg) (6)	0.35 ± 0.03	25.43 ± 2.93	1.57 ± 0.31			
Aminophylline (50 mg/kg) (6)	0.48 ± 0.15	23.00 ± 3.08	1.83 ± 0.86			
Theobromine 50 mg/kg (6)	0.67 ± 0.14*	19.89 ± 3.00	2.05 ± 0.59			

Injections were made i.p. and rats killed 3 h later. Results are expressed as means ± one s.d. Nos. of determinations shown in parentheses. Comparison with corresponding control value by Student's *t* test; * $P < 0.01$; ** $P < 0.001$.

Table 4 Effects of insulin on plasma unesterified fatty acid (UFA) and tryptophan metabolism of fed and food-deprived rats.

		Plasma			*Brain (μg/g)*		
	Glucose		*Tryptophan (μg/ml)*				
Injected	*(mg/100 ml)*	*UFA (mEq/l)*	*Total*	*Free*	*Tryptophan*	*5-HT*	*5-HIAA*
Fed rats							
0.9% Sodium chloride (6)	158 ± 9	0.51 ± 0.11	10.14 ± 0.21	1.70 ± 0.25	2.27 ± 0.24	0.55 ± 0.04	0.36 ± 0.07
Insulin 2 iu/kg (6)	92 ± 10***	0.32 ± 0.10*	10.35 ± 1.26	0.95 ± 0.10**	2.11 ± 0.23	0.47 ± 0.03**	0.31 ± 0.04
Food-deprived rats							
0.9% Sodium chloride (6)	74 ± 12	0.90 ± 0.16	8.74 ± 1.01	2.17 ± 0.37	2.28 ± 0.20	0.52 ± 0.04	0.48 ± 0.06
Insulin 2 iu/kg (6)	12 ± 10****	0.47 ± 0.11****	10.52 ± 2.23	1.19 ± 0.24****	3.05 ± 0.50**	0.54 ± 0.06	0.57 ± 0.07

Experiments on fed and food-deprived rats were done at different times. Injections were made i.p. and rats killed 2 h later. Food deprivation was for a total of 24 hours. Results are expressed as means ± one s.d. Nos. of determinations shown in parentheses. Results compared by Student's *t* test; * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

Table 5 Effects of nicotinic acid on plasma unesterified fatty acid (UFA) and tryptophan metabolism

	Plasma					
		Tryptophan (μg/ml)		*Brain (μg/g)*		
Injected	*UFA (mEq/l)*	*Total*	*Free*	*Tryptophan*	*5-HT*	*5-HIAA*
Fed rats						
0.9% Sodium chloride (6)	0.28 ± 0.06	16.21 ± 2.61	1.36 ± 0.19	2.72 ± 0.54	0.46 ± 0.02	0.35 ± 0.05
Nicotinic acid 50 mg/kg (6)	0.18 ± 0.03**	16.03 ± 1.77	1.46 ± 0.22	2.40 ± 0.35	0.47 ± 0.03	0.31 ± 0.03
Food-deprived rats						
0.9% Sodium chloride (6)	0.74 ± 0.18	16.41 ± 5.20	2.87 ± 0.46	5.22 ± 0.61	0.53 ± 0.02	0.48 ± 0.05
Nicotinic acid 50 mg/kg (6)	0.27 ± 0.10***	25.13 ± 1.45**	2.27 ± 0.23*	3.50 ± 0.55***	0.53 ± 0.02	0.41 ± 0.04*

All experiments were done concurrently. Injections were made i.p. and rats killed 1 h later. Food deprivation was for a total of 24 hours. Results are expressed as means ± one s.d. Nos. of determinations shown in parentheses. Results compared by Student's *t* test; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

A difference between the effect of insulin on fed and food-deprived rats was that no convulsions were noted in the former group but occurred in some of the latter. Also hypoglycaemia after insulin treatment was more striking in the food-deprived animals.

Effect of nicotinic acid. Nicotinic acid, like insulin, decreases cyclic AMP in fat cells (Butcher *et al.*, 1968) and has been extensively used to decrease plasma UFA concentration (Gey & Carlson, 1971). One hour after i.p. injection of nicotinic acid (50 mg/kg), plasma UFA concentration fell significantly (Table 5) but to a smaller extent than in the insulin treated rats. Concentrations of plasma free tryptophan and of the brain indoles were not significantly altered.

Nicotinic acid completely prevented the large increase of UFA after 24 h food deprivation if injected 1 h before killing the rats. The associated large increases of plasma free tryptophan, brain tryptophan and brain 5-HIAA following food deprivation were diminished but not prevented by nicotinic acid (Table 5). This limited effectiveness of nicotinic acid in opposing the effects of food deprivation is probably related to the significant increase of plasma total tryptophan in the group of rats which were both deprived of food and given nicotinic acid. Thus nicotinic acid decreased the percentage of plasma tryptophan which was free to a value similar to that obtained in fed rats.

Discussion

These results show that many drugs which alter rat plasma UFA also alter plasma free tryptophan in the same direction. Thus catecholamines, L-DOPA and aminophylline all increase the percentage of plasma tryptophan which is free, i.e. not bound to albumin, while insulin and nicotinic acid decrease both UFA and free tryptophan. The latter change probably results from the former as when UFAs are added to albumin (McMenamy, 1965) or to plasma (Curzon *et al.*, 1973) the binding of tryptophan to protein is decreased. That free rather than bound tryptophan is directly available for transport is indicated by the finding that changes of brain tryptophan upon food-deprivation (Knott & Curzon, 1972) or tryptophan administration (Tagliamonte, Biggio, Vargiu & Gessa, 1973) or in experimental hepatic coma (Curzon, Kantamaneni, Winch, Rojas-Bueno, Murray-Lyon & Williams, 1973) correlate well with change of plasma free but not total tryptophan.

The equivalence of percentage changes in UFA

and in free tryptophan after drug treatments which is usually found probably results from a fortuitous consequence of the conditions under which free tryptophan was determined (room temperature, pH 8.1) and therefore does not necessarily point to equivalence *in vivo* though approximate proportionality is indicated. Also, while percentage changes in different experiments may be similar, absolute changes may be very different (e.g. the UFA changes in the insulin and nicotinic acid experiments).

The results show a tendency towards an inverse relation between changes of plasma free and total tryptophan. Thus, after noradrenaline or L-DOPA injection, the increase of free tryptophan was associated with a decrease of total tryptophan which was significant in the L-DOPA treated animals. Conversely, when insulin or nicotinic acid were given to rats previously deprived of food the resultant decreases of free tryptophan were associated with an increase of total tryptophan which was significant in the nicotinic acid treated animals. These results are consistent with the hypothesis that only plasma free tryptophan is available for uptake by brain and other tissues and thus for intracellular metabolism in general. Therefore when free tryptophan increases these processes may also be enhanced so that equilibration results in release of more tryptophan from the bound form and eventual decrease of plasma total tryptophan. Conversely, total tryptophan might eventually increase if free tryptophan concentration decreases.

The apparently anomalous results with isoprenaline which increased free tryptophan without a fall of total tryptophan may reflect a time dependence of the latter change as determinations were made only 5 min after injection. The magnitude of the changes might also be expected to depend on the activity of tryptophan metabolizing enzymes, e.g. tryptophan pyrrolase. This enzyme increases upon starvation (Wu & Rosenthal, 1966) and it is therefore of interest that nicotinic acid increased plasma total tryptophan only in rats previously deprived of food. It is possible that a sufficiently prolonged elevation of activity of tryptophan catabolizing enzymes together with decreased binding of tryptophan to albumin could lead to such a depletion of total plasma tryptophan that finally a low free tryptophan concentration results.

A highly significant increase in concentration of brain tryptophan was found in all experiments in which plasma free tryptophan concentration rose and in which brain tryptophan was determined (L-DOPA and aminophylline injection, food deprivation). Conversely, brain tryptophan concentration fell when plasma free tryptophan fell upon giving nicotinic acid to rats previously deprived of food.

Brain tryptophan changes following insulin injection were less readily interpretable. Although plasma free tryptophan concentration decreased significantly, fed and insulin-treated rats only showed a small and non-significant reduction in the concentration of brain tryptophan while in corresponding food-deprived animals it increased significantly. Interpretation of the findings with insulin is not simple as insulin decreases plasma concentrations of amino acids which interfere with the transport of tryptophan to the brain (Fernstrom & Wurtman, 1972a,b). Another factor to be considered is that under our conditions insulin hypoglycaemia was greater in the food-deprived rats than in the fed rats and insulin convulsions were observed only in the food-deprived animals.

When the drugs or procedures altered brain tryptophan concentration then invariably brain 5-HIAA concentration changed in the same direction, but with the exception of aminophylline, to a proportionately smaller extent. The increase of 5-HIAA suggests that tryptophan hydroxylase, the rate limiting enzyme for 5-HT synthesis is unsaturated in brains of control rats. As the cerebral tryptophan concentration is approximately 10^{-5} M this is consistent with the *Km* value of 5×10^{-5} M for the purified rabbit hind-brain enzyme (Friedman *et al.*, 1972) and with an apparent *in vivo* *Km* of approximately 10^{-4} M for the rat brain enzyme calculable from the results of Grahame-Smith (1971).

In comparison with the above brain 5-HIAA changes those of 5-HT were usually small or negligible. This suggests that additional 5-HT, synthesized as a result of increased brain tryptophan, spills over from replete vesicles and is destroyed by monoamine oxidase. The small but significant fall in the concentration of 5-HT after L-DOPA injection even though brain tryptophan increased may be due to displacement of 5-HT stores by dopamine (Ng, Chase, Colburn & Kopin, 1970). The increase of 5-HT after aminophylline injection was noteworthy as though it was much smaller than that of 5-HIAA it was highly significant.

These changes after aminophylline are of specific interest as Berkowitz & Spector (1971) also report increased brain 5-HT and 5-HIAA concentrations after injection of another methylxanthine, caffeine (at higher dosage than that used in the present study). Increased brain tryptophan concentration might also be involved in the decreased depletion of brain 5-HT in rats treated with a tryptophan hydroxylase inhibitor when aminophylline or caffeine are also given (Corrodi, Fuxe & Jonsson, 1972).

The finding that catecholamines increase plasma free tryptophan suggests that in stress situations, enhanced sympathetic activity could lead to increased availability of tryptophan to the brain and therefore be responsible for at least part of the increased brain 5-HT turnover in stress reported by many workers. Thus, increased firing of 5-HT neurones in stress cannot necessarily be deduced from increased brain 5-HIAA. Indeed, as Aghajanian (1972) finds that tryptophan decreases the firing of these neurones, a coincidence between increased turnover and decreased firing is possible.

The effect on the UFA-tryptophan system of L-DOPA is consistent with the increases in the concentrations of brain tryptophan (Weiss, Munro & Wurtman, 1971) and plasma UFA (Rivera-Calimlim & Bianchine, 1972) reported to occur after injection of this drug in high dosage.

In general, the results obtained reinforce the finding of Tagliamonte, Tagliamonte, Perez-Cruet, Stern & Gessa (1971) that many drugs influence brain 5-HT metabolism by altering brain tryptophan concentration and indicate that the determination of the latter is required when investigating drugs or procedures affecting brain 5-HT metabolism. If brain tryptophan changes are found then investigation of the roles of plasma free tryptophan and UFA also become necessary.

Other factors as well as the above ones may also be determinants of brain tryptophan concentration. For example, pyrrolase can compete for tryptophan (Curzon & Green, 1971) and other amino acids can interfere with its uptake by the brain (Fernstrom & Wurtman, 1972b; Oldendorf, 1971). Finally, it is possible that drugs which influence cyclic AMP metabolism may affect brain tryptophan not only by altering lipolysis but also through cyclic AMP changes in brain membranes as Tagliamonte, Tagliamonte, Forn, Perez-Cruet, Krishna & Gessa (1971) report that cyclic AMP (albeit in pharmacological dosage) increases tryptophan uptake by brain slices *in vitro*.

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