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BRAIN TRYPTOPHAN METABOLISM ON THE 5-HYDROXYTRYPTAMINE AND KYNURENINE PATHWAYS IN A STRAIN OF RATS WITH A DEFICIENCY IN PLATELET 5-HT

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1 Brain 5-hydroxytryptamine (5-HT) metabolism has been compared in albino (Sprague-Dawley; SD) and in Fawn-Hooded (FH) rats, which have an inherited platelet 5-HT deficiency.

2 It was confirmed that blood 5-HT levels in the FH rats were about one quarter of those in the SD rats.

3 Brain 5-HT and 5-HIAA were however higher in FH rats on a per gram basis; there was no difference between the strains on a per brain basis, because of the smaller brain weights of the FH rats.

4 Brain and plasma tryptophan were not significantly different in the two strains. Plasma kynurenine was higher in the FH rats, and brain kynurenine was also higher either on a per gram or on a per brain basis.

5 The reserpine-releasable brain 5-HT was the same proportion of total brain 5-HT in the two strains.

6 Experiments with pargyline suggested that the turnover of 5-HT was somewhat higher in the FH rats on a per gram basis, but not significantly so on a per brain basis.

7 It is concluded that although brain tryptophan metabolism may be somewhat accelerated along both the 5-HT and kynurenine pathways in the FH rats there is no gross deficiency in the binding of 5-HT in their brains analogous to that found in their platelets.

Introduction

The blood platelet is known to be able to take up and store 5-hydroxytryptamine (5-HT) (Pletscher, 1968) and it has been suggested that the platelet might provide a readily accessible model for aminergic nerve terminals in the brain both with respect to amines and their related enzymes (Sneddon, 1973; Stahl, 1977). Recently an inbred strain of rats has been described (Tschopp & Zucker, 1972) which have a prolonged bleeding time and a deficiency of platelet 5-HT, ADP and ATP in many ways similar to that found in platelets from humans with 'storage pool disease', a congenital disorder of haemostasis (Tschopp & Weiss, 1974; Weiss, Tschopp, Rogers & Brand, 1974). Since these 'Fawn-Hooded' (FH) rats are often described as having behavioural abnormalities (although no detailed description has appeared to my knowledge in the literature) it was of interest to compare 5-HT metabolism in these animals and in normal rats. If storage or metabolism of 5-HT were abnormal in the brains of these rats, then they might provide a model for the study of the hypotheses linking psychiatric and neurological disorders with alterations in brain 5-HT metabolism (Chase & Murphy, 1973; Green & Grahame-Smith, 1975).

Levels of 5-HT, its precursor, tryptophan and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were compared in the brains of FH and Sprague Dawley (SD) rats. Plasma kynurenine levels were also measured in order to study the kynurenine pathway of tryptophan metabolism, which can compete with the 5-HT pathway in some circumstances (Joseph, Young & Curzon, 1976). This pathway has recently been described in brain (Gal, 1974; Joseph, Baker & Lawson, 1978) and brain kynurenine levels were also compared in these rats. Monoamine oxidase (MAO) inhibitor administration was used to obtain a first index of brain 5-HT turnover in the two strains, and the 5-HT depleting effect of reserpine was also exam-

ined since this drug releases 5-HT from storage both in platelets and in brain and it is the storage pool in platelets that is deficient in the FH rats.

Methods

FH rats, (derived from stock generously supplied by Dr Th. B. Tschopp, Hoffmann-La Roche, Basle, Switzerland) and SD rats were bred in the Division of Comparative Medicine, Clinical Research Centre. Male rats were used for all experiments at 58 to 63 days of age, body weight 280 to 340 g (SD rats) and 63 to 68 days of age, body weight 245 to 305 g (FH rats). Drugs were administered intraperitoneally, pargyline hydrochloride (Abbott) at a dose of 75 mg/kg in 0.9% w/v NaCl solution (saline) and reserpine (Sigma) at a dose of 5 mg/kg in a 5% w/v solution of ascorbic acid. Two out of nine SD rats injected with reserpine showed neither the well-established behavioural changes, nor the expected changes in brain 5-HT and 5-HIAA and have been excluded from the analysis of results. Control animals were injected with appropriate vehicles and animals were decapitated between 15 h 00 min and 18 h 00 min. Trunk blood was collected into heparinized tubes and the brain rapidly dissected out and frozen on dry ice. Plasma was separated by centrifugation (for 10 min, 2000 *g* at 4°C) and stored, with the brains, at −40°C until analysed. For analysis, brains were deproteinised with five volumes, and plasma with ten volumes, of acid butanol as previously described (Joseph & Baker, 1976). The supernatant was back extracted with 0.1 N HCl and heptane, and aliquots of the aqueous phase were used for duplicate fluorimetric determinations of tryptophan (Denkla & Dewey, 1967, as modified by Bloxham & Warren, 1974) and 5-HT (Joseph & Baker, 1976) and for the determination of kynurenine by gas liquid chromatography (Joseph, 1978; Joseph *et al.*, 1978). The organic phase was re-extracted with 0.5 M phosphate buffer, pH 7 and the aqueous phase used for duplicate fluorimetric determinations of 5-HIAA (Joseph & Baker, 1976). Whole blood 5-HT levels were determined in heparinised trunk blood; 1 ml samples were added to ascorbate-disodium edetate (EDTA), snap frozen in liquid nitrogen, and analysed by fluorescence in strong acid (Geeraerts, Schimpfessel & Crockaert 1974). Some values were checked by the *o*-phthalaldehyde (OPT) procedure as previously described (Joseph & Baker, 1976) on the same deproteinised supernatants.

Results

The body and brain weights of the two strains of rats, and their whole blood 5-HT levels are shown in Table 1. The FH rats gain weight more slowly, and thus the mean body weights were lower, in spite of the use of somewhat younger SD animals as described in methods. The brain weights of the FH rats were lower in absolute terms, and also when expressed as a percentage of body weight. Whole blood 5-HT determinations showed that, as expected, the FH rats had a marked deficiency of 5-HT in platelets.

Table 2 shows the effects of reserpine and pargyline administration to SD rats. Reserpine depleted brain 5-HT (40% of control) and elevated 5-HIAA (190% of control) as expected, without significantly altering brain or plasma tryptophan. Conversely, pargyline elevated brain 5-HT and depleted 5-HIAA without significantly altering brain tryptophan. If a linear rate of accumulation of 5-HT is assumed (Tozer, Neff & Brodie, 1966) this corresponds to a 5-HT synthesis rate of 141 ng g^{-1} h^{-1} based on 5-HT accumulation and 127 ng g^{-1} h^{-1} based on 5-HIAA decline (Tozer *et al.*, 1966).

Table 3 shows the effects of the same agents on FH rats. Reserpine had similar effects, reducing 5-HT to 45% of control and raising 5-HIAA to 188% of control. Plasma tryptophan was also somewhat reduced. Pargyline elevated 5-HT and reduced 5-HIAA to an extent corresponding to a 5-HT synthesis rate of 190 ng g^{-1} h^{-1} based on 5-HT accumulation and 186 ng g^{-1} h^{-1} based on 5-HIAA decline. Brain tryptophan was somewhat increased in the pargyline-treated animals.

Since the values in the vehicle-treated rats of each strain were quite similar, with the possible exception

Table 1 Comparison of physical characteristics and blood 5-hydroxytryptamine (5-HT) levels in the two strains of rats

	Body weight (g)	*Brain weight (g)*	*Brain as % body weight*	*Whole blood 5-HT (ng/ml)*
Sprague-Dawley rats	305 ± 21 (28)	1.97 ± 0.08 (28)	0.64 ± 0.04 (28)	1333 ± 144 (10)
Fawn-Hooded rats	277 ± 17 (20)	1.62 ± 0.07 (20)	0.59 ± 0.03 (20)	357 ± 75 (9)
(FH as % SD)	(90.7)	(82.0)	(90.3)	(26.8)

Results are mean ± s.d. (no. of animals). Differences are significant ($P < 0.001$) in each case.

Table 2 Effects of reserpine and pargyline on concentrations of tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in brain and of tryptophan in plasma of Sprague-Dawley rats

			Brain			*Plasma*
Treatment	*Time (h) rats killed after treatment*	*No. of rats*	*Tryptophan (μg/g)*	*5-HT (ng/g)*	*5-HIAA (ng/g)*	*Tryptophan (μg/ml)*
Vehicle (5% ascorbic acid)	4	9	3.58 ± 0.76	410 ± 56	439 ± 36	16.2 ± 2.1
Reserpine (5 mg/kg)	4	7	3.67 ± 0.83	164 ± 22	836 ± 78	15.1 ± 1.2
% of control			102.5	40.0	190.3	93.2
P for drug vs control			NS	<0.001	<0.001	NS
Vehicle (saline)	2	5	3.39 ± 0.20	421 ± 49	398 ± 37	ND
Pargyline (75 mg/kg)	2	5	3.71 ± 0.48	702 ± 69	199 ± 45	ND
% of control			109.4	166.7	50.1	
P for drug vs control			NS	<0.001	<0.001	

Results are mean ± s.d. NS = not significant; ND = not done.

Table 3 Effects of reserpine and pargyline on concentrations of tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in brain and of tryptophan in plasma of Fawn-Hooded rats

			Brain			*Plasma*
Treatment	*Time (h) rats killed after treatment*	*No. of rats*	*Tryptophan (μg/g)*	*5-HT (ng/g)*	*5-HIAA (ng/g)*	*Tryptophan (μg/ml)*
Vehicle (5% ascorbic acid)	4	5	4.93 ± 1.35	509 ± 90	536 ± 86	15.2 ± 1.8
Reserpine (5 mg/kg)	4	5	4.64 ± 1.19	231 ± 26	1010 ± 128	11.7 ± 2.2
% of control			94.1	45.4	188.3	77.0
P for drug vs control			NS	<0.001	<0.001	<0.05
Vehicle (saline)	2	5	3.08 ± 0.30	551 ± 35	498 ± 30	ND
Pargyline (75 mg/kg)	2	5	3.54 ± 0.27	932 ± 66	221 ± 35	ND
% of control			114.9	169.1	44.4	
P for drug vs control			<0.05	<0.001	<0.001	

Results are mean ± s.d. NS = not significant; ND = not done.

of brain tryptophan in the FH rats, these groups have been combined in Table 4 to compare FH and SD rats not treated with drugs. In addition levels of brain and plasma kynurenine are shown as well as the mean of the two 5-HT turnover estimates previously quoted. Since the mean brain weight differed, the brain results are also presented on a per brain basis. Brain 5-HT and 5-HIAA are higher in the FH rats on a per gram basis, but these differences disappear on a per brain basis. Brain 5-HT turnover is higher in the FH rats on a per gram basis (2 way analysis of variance: strain × treatment interaction $P < 0.05$ for changes in 5-HIAA after pargyline) but the increase is not significant when expressed on a per brain basis. Plasma and brain kynurenine are also higher in FH rats; the brain difference is still observed on a per brain basis. Plasma and brain tryptophan levels are not significantly different.

Discussion

It was confirmed in this study that the FH rats had a marked deficiency of whole blood 5-HT (27% of that found in normal SD rats). Whole blood 5-HT is known to be mostly concentrated in platelets, and the deficiency found agrees well with values obtained in studies on isolated platelets (FH 30% and 26% of normal rat platelet 5-HT; Tschopp & Zucker, 1972; Tschopp & Weiss, 1974). However, greater deficiencies in whole blood 5-HT have been reported in mice (beige strain) with a similar prolonged bleeding time: 2.8% (Holland, 1976) and 1.0% (Meyers & Chen, 1976) of normal mice. The absolute values of whole blood 5-HT in SD rats in the present study seemed high in comparison with other reports (Lovenberg & Engelman, 1971) but variability is well-established. The results on some deproteinised whole blood supernatants from each strain were checked by fluorescence developed with OPT; the results were similar but about 10% higher in both groups. This corresponds to the difference reported previously for plasma determination of 5-HTP by OPT and by direct fluorescence (Joseph & Baker, 1976).

The brain content of 5-HT and 5-HIAA in vehicle-injected controls was not found to be reduced in FH rats, when compared with SD rats, indeed a small increase was seen. However, as shown in Table 4, when the results were expressed on a per brain basis the differences were virtually abolished, suggesting that a similar number of 5-HT neurones may be present, but in a smaller brain in the case of the FH rats. No difference between brain 5-HT levels in normal and beige mice was also reported (Meyers & Chen, 1976) during the course of this study. The differences in tryptophan levels in brain and plasma of the two rat strains are not significant and would anyway be unlikely to be important in view of the relatively wide physiological range encountered. A more marked elevation in kynurenine in plasma was seen, and that in brain was not entirely accounted for by weight differences.

The results with pargyline, an MAO inhibitor, enable a preliminary estimate of 5-HT turnover to be obtained. This again suggests (Table 4) that turnover is somewhat higher in the FH rats per unit brain

Table 4 Comparison of pooled results from control Sprague-Dawley and Fawn-Hooded rats

	Plasma		*Brain*				
	Tryptophan (μg/ml)	*Kynurenine (ng/ml)*	*Tryptophan (μg/g)*	*Kynurenine (ng/g)*	*5-HT (ng/g)*	*5-HIAA (ng/g)*	*5-HT turnover (ng g^{-1} h^{-1})*
Sprague-Dawley	16.2 ± 2.1 (9)	738 ± 90 (9)	3.51 ± 0.61 (14)	89.2 ± 19.9 (14)	415 ± 52 (14)	425 ± 40 (14)	133.9
Fawn-Hooded	15.2 ± 1.8 (5)	1033 ± 171 (5)	4.00 ± 1.34 (10)	141.1 ± 28.7 (10)	530 ± 68 (10)	517 ± 64 (10)	188.3
FH as % SD	93.8	140.0	113.9	158.2	128.0	121.6	140.6
P for FH vs SD	NS	<0.005	NS	<0.001	<0.001	<0.001	
			Brain results on a per brain basis				
SD			6.96 ± 1.21	177 ± 41	820 ± 93	842 ± 91	265.0*
FH			6.37 ± 1.98	225 ± 41	848 ± 109	825 ± 79	311.6*
FH as % SD			91.5	127.1	103.4	98.0	117.6
P for FH vs SD			NS	<0.02	NS	NS	

Results are mean ± s.d. (no. of animals); * based on mean brain weight for appropriate groups; NS = not significant.

weight but, as before, the elevation is not significant on a per brain basis. Thus the flux of tryptophan through both kynurenine and 5-HT pathways may be higher in FH rats. However, differences of this order are likely to be within the physiological range of flux rates within any one rat, and indeed greater differences in 5-HT turnover have been reported in rats nominally of the same strain from different suppliers (Miller, Cox & Maickel, 1968, Tonge & Leonard, 1971). It had been predicted by others that 5-HT might be catabolised more rapidly in the brain of FH rats, since their brain MAO activity towards 5-HT as substrate was higher (Bellin & Sorrentino, 1974). Finally the results with reserpine indicate that the relative size of the reserpine releasable pool is similar in the FH and SD rats.

The initial hypothesis, that there might be a deficiency in vesicular storage of 5-HT in the brain of FH rats analogous to that in their platelets, is therefore not supported. A small increase in turnover on the 5-HT and kynurenine pathways is suggested, but any relationship of this to the anecdotal behavioural differences (e.g. unusual docility—Bellin & Sorrentino, 1974) between FH and normal rats remain to be established.

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