Pharmacology Biochemistry & Behavior, Vol. 14, pp. 259–262. Printed in the U.S.A.

Brain 5-Hydroxytryptamine Metabolism After Portocaval Anastomosis: Relationship with Ambulation

M. D. TRICKLEBANK,[1] D. L. BLOXAM,[2] B. D. KANTAMANENI AND G. CURZON

Department of Neurochemistry, Institute of Neurology, 33, John's Mews, London, WC1N 2NS

Received 1 July 1980

TRICKLEBANK, M. D., D. L. BLOXAM, B. D. KANTAMANENI AND G. CURZON. *Brain 5-hydroxytryptamine metabolism after portocaval anastomosis: Relationship with ambulation.* PHARMAC. BIOCHEM. BEHAV. 14(2) 259–262, 1981.—Chronic portocaval anastomosis (PCA) increased tryptophan and 5-hydroxyindoles in rat brain regions. PCA rats ambulated less than sham operated animals but showed positive correlations between ambulation and indices of brain 5-hydroxytryptamine (5-HT) metabolism, i.e., plasma free tryptophan, plasma non-esterified fatty acid, tryptophan in all brain regions, and 5-HT or 5-hydroxyindoleacetic acid in some regions. Results suggest a positive and non-causal association between motor activity and 5-HT metabolism in the PCA rats reflecting parallel responses of both variables to environmental stimulation. This may be superimposed on a normal inverse and causal relationship between motor activity and 5-HT metabolism.

Portocaval anastomosis 5-Hydroxytryptamine 5-Hydroxyindoleacetic acid Non-esterified fatty acid Ambulation

LIVER dysfunction has marked effects on tryptophan and 5-hydroxytryptamine (5-HT) metabolism. Thus, hepatectomy, hepatic devascularisation or carbon tetrachloride poisoning increase brain tryptophan concentration and 5-HT turnover [6, 13, 22]. Similar changes also occur after portocaval anastomosis (PCA) (the surgical diversion of the hepatic portal vein from the liver to the vena cava) and persist for many weeks [2, 3, 5].

The increase of brain tryptophan in acute liver failure is explicable by the increase in plasma free tryptophan [3, 6, 13, 16] due to displacement of the amino acid from albumin by non-esterified fatty acids (NEFA) but this only partly accounts for the brain changes in chronic PCA [3,5].

These disturbances, amongst others, may be involved in the development of hepatic encephalopathy [19]. Indeed, one behavioural consequence of anastomosis is decreased ambulation and this also occurred in sham-operated animals given tryptophan [23]. The present communication describes the relationships between values previously obtained [23] but not reported in detail for tryptophan, 5-HT and 5-hydroxyindole acetic acid (5-HIAA) concentrations and the motor activity of PCA and sham-operated rats on prolonged exposure to an open field. Results suggest that increases of plasma NEFA in anastomosed rats associated with changes of environment or behaviour may lead to an increase of brain tryptophan metabolism in addition to that resulting more directly from PCA.

METHOD

Full details of procedures have already been described [23]. PCA or sham-operation was performed [14] on 13 ten-week old male Sprague-Dawley rats from 6 litters. Mean body weight (±SD) was 338 ± 28 g. Animals were then caged in groups of 4 for seventeen days and subsequently caged singly. A white/red light cycle was used (deep red light between 1230 hr and 0030 hr). Five to six weeks later when mean body weights (±SD) of sham-operated and anastomosed rats were 489 ± 27 (n=7) and 380 ± 46 g (n=6), respectively, the animals were exposed singly to the open field between 1300 hr and 2000 hr for 95 min and behaviour observed remotely by television. The ambulation score was defined as the sum of the areas of the open field entered during six 5-min periods at 10 min intervals. The first period began 15 min after placing in the open field. This procedure is essentially that of Taylor [21] and differs from standard open field observations which are made in the first few minutes after placement. Rats were killed on removal from the open field and tryptophan, 5-HT and 5-HIAA determined in brain regions as described [23]. Plasma was assayed for free

[1]Send reprint requests to: Dr. M. D. Tricklebank, Department of Neurochemistry, Institute of Neurology, 33, John's Mews, London, WC1N 2NS (U.K.).

[2]Present address: Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London, W6 OXG (U.K.).

TABLE 1

CORRELATIONS BETWEEN 5HT METABOLISM AND AMBULATION SCORES AFTER PCA

	Correlation Coefficients			
	Sham-Operated (7)		PCA (6)	
	Ambulation Score	Regional Tryptophan Concentration	Ambulation Score	Regional Tryptophan Concentration
Plasma				
Total tryptophan	−0.31		−0.83*	
Free tryptophan	0.07		0.84*	
NEFA	−0.41		0.93†	
Mid-brain				
Tryptophan	0.32		0.87*	
5HT	−0.15	−0.09	0.26	0.59
5HIAA	−0.54	−0.36	0.59	0.87*
Hypothalamus				
Tryptophan	−0.30		0.84*	
5HT	−0.40	−0.65	0.75	0.52
5HIAA	−0.35	−0.34	0.47	0.71
Hippocampus				
Tryptophan	0.08		0.97‡	
5HT	−0.33	−0.43	−0.21	−0.03
5HIAA	−0.65	0.36	0.17	0.29
Striatum				
Tryptophan	−0.20		0.92‡	
5HT	0.10	−0.17	0.43	0.42
5HIAA	−0.10	0.08	0.95‡	0.92‡
Pons-medulla				
Tryptophan	0.12		0.95‡	
5HT	−0.80*	−0.47	0.81*	0.87*
5HIAA	−0.75	−0.46	0.91†	0.94‡
Rest of brain				
Tryptophan	−0.05		0.96‡	
5HT	−0.89‡	0.10	−0.14	−0.13
5HIAA	−0.47	0.53	0.85*	0.95‡

Number of rats in parentheses. *$p<0.05$, †$p<0.02$, ‡$p<0.01$.

and albumin bound tryptophan [1] and non-esterified fatty acid [4].

RESULTS

Tryptophan, 5-HT and 5-HIAA Concentrations in Plasma and Brain Regions after PCA

Results on rats killed after 95 min in the open field are not presented in detail as they were largely similar to previous findings on rats taken from their home cages [5]. Thus, PCA rats showed considerable and significant increases in tryptophan in all brain regions. 5-HIAA was significantly raised in all regions except pons-medulla and striatum. 5-HT showed smaller increases, significant only in the hippocampus, mid-brain and hypothalamus. Ratios of brain tryptophan (all regions) to plasma free tryptophan were approximately doubled.

Ambulation in the Open Field

Ambulation scores (±S.D.) were 73 ± 41 (n=7) and 20 ± 15 (n=6) for sham-operated and PCA rats, respectively ($p<0.01$, Mann-Whitney U test). The two groups showed strikingly different relationships of ambulation to indices of brain 5-HT metabolism (Table 1). Thus, the PCA group showed significant positive correlations between ambulation and the following: plasma free tryptophan; 5-HT in pons-medulla; 5-HIAA in striatum, pons-medulla and 'rest of brain'. Correlation coefficients of ambulation scores with regional 5-hydroxyindole concentrations largely paralleled those of regional tryptophan with 5-hydroxyindole concentrations. Plasma total tryptophan correlated significantly but negatively with ambulation.

None of the coefficients for the sham-operated rats were significant and of the same sign as those for the PCA

TABLE 2

PLASMA NEFA OF SHAM-OPERATED AND PCA RATS AFTER EXPOSURE TO DIFFERENT ENVIRONMENTS

Conditions	NEFA (meq/l)	
	Sham-operated	PCA
Home cage†	0.17 ± 0.07 (10)	0.31 ± 0.21 (10)
Home cage†	0.22 ± 0.11 (10)	0.28 ± 0.20 (12)
Open field 95 min	0.51 ± 0.25 (7)	0.83 ± 0.45 (6)
Intermittent foot-shock 35 min‡	0.32 ± 0.13 (5)	0.92 ± 0.02 (3)*

Values are means ± S.D. Numbers of rats in parentheses. Difference from sham-operated group, *$p<0.001$. Details of open field experiments are given under Method.

†For experimental details see [5].

‡For experimental details see [23].

animals. Thus the sham-operated group showed significant negative correlations between ambulation and 5-HT in pons-medulla and 'rest of brain'. Correlation coefficients between regional tryptophan and 5-hydroxyindole concentrations of the sham-operated rats were not significant ($p>0.1$ for all groups).

Plasma NEFA

Results in Table 2 show plasma NEFA concentrations of PCA and sham-operated rats killed after exposure to different environments. Mean NEFA values of PCA animals were invariably larger than those of controls. Differences were small when animals were taken from their home cages, somewhat larger after 95 min in the open field and still larger and significant after 35 min mild intermittent foot shock [23].

DISCUSSION

Rats with chronic PCA killed after 95 min in an open field had higher regional brain tryptophan concentrations than sham-operated animals. This agrees with results on rats taken from their home cages [2, 3, 5]. Three factors have been suggested to be involved: (a) decrease of plasma amino acids competing with tryptophan for uptake by brain [19]; (b) increase of plasma free tryptophan due to elevated plasma NEFA [13]; (c) increased activity of the carrier system transporting tryptophan to the brain [11].

Increases of plasma NEFA after PCA differ considerably [3,5] and may depend on environment (Table 2) as NEFA can rise due to stress [20]. The abnormally high plasma NEFA in stressed PCA rats could reflect both sympathetic abnormalities [7,17] and defective hepatic NEFA removal [18].

The positive correlation between ambulation and plasma NEFA of PCA but not sham-operated rats suggests that both variables are elevated by stress or environmental stimulation. As exercise decreases plasma NEFA [8] it is unlikely that the NEFA changes result from those of ambulation. The other correlations between ambulation and biochemical values and between brain tryptophan and 5-HIAA for the PCA rats are consistent with increased NEFA leading to increases of plasma free tryptophan and hence of brain tryptophan and 5-HT turnover. Thus, *within the PCA group* ambulation correlates positively with all brain regional tryptophan values and with 5-HT and/or 5-HIAA values in most regions even though PCA decreases ambulation and increases brain indoles.

Unlike PCA rats, the sham-operated animals did not show significant correlations between plasma NEFA or free tryptophan and ambulation. This possibly reflects the lower concentration and range of their NEFA values. The significant *negative* correlations between ambulation and 5-HT concentration in two of the larger regions (pons-medulla and 'rest of brain') is consistent with the effects of tryptophan [21,23], intraventricular 5-HT [24] and raphe lesions [10,15] on motor activity. The *positive* association between 5-HT metabolism and ambulation shown by the PCA rats only, may reflect parallel responses of both variables to environmental stimulation, superimposed on the above inverse relationship.

Results in general suggest that environmental stimuli may contribute to the increase of brain tryptophan and 5-HT synthesis in liver disease and may thus conceivably influence central symptoms.

ACKNOWLEDGEMENTS

We are grateful to the Medical Research Council and the Brain Research Trust for financial support, and to Professor J. Dobbing for use of his laboratories.

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