Dopamine and Serotonin Metabolism in Hepatic Encephalopathy

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Summary

Patients with stupor or coma from fulminant hepatic failure were found to have high cerebrospinal fluid concentrations of homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA), metabolites of dopamine and serotonin respectively. Excessive amounts of their precursors—phenylalanine and tyrosine and free tryptophan—were found in the patients' plasma. Methionine, which participates in dopamine degradation, was also much increased. Similar disturbances were found in patients suffering an acute exacerbation of chronic encephalopathy. These abnormalities would be consistent with other evidence of an increased turnover of serotonin and possibly dopamine in the brain during hepatic encephalopathy.

Introduction

Our interest in the cerebral metabolism of dopamine in hepatic encephalopathy was stimulated by two clinical observations. The first was the arousal effect of levodopa noted in some patients (Parkes *et al.*, 1970). The second was the frequent occurrence of physical signs attributable to dysfunction of the extrapyramidal system, parts of which contain substantial amounts of dopamine. Thus loss of facial expression and more general bradykinesia are early features of chronic encephalopathy (Summerskill *et al.*, 1956), while in acute coma a normal plantar response often accompanies increased muscle tone, ankle clonus, and brisk tendon reflexes (Walshe, 1951; Summerskill *et al.*, 1956). Other evidence suggests that changes in the neurotransmitter—serotonin—might also be involved. Thus tryptophan, the precursor of serotonin, has little toxicity if liver function is normal (Wyatt *et al.*, 1970) but when given by mouth to patients with liver disease it can precipitate or exacerbate encephalopathy (Sherlock, 1968). This also leads to neurological signs in the dog with a portacaval shunt (Oginara *et al.*, 1966).

We describe investigations into the possible changes in cerebral metabolism of these two neurotransmitters in hepatic encephalopathy. The degradation product of dopamine, homovanillic acid (HVA), was measured in the cerebrospinal fluid as well as the plasma concentrations of the amino-acids needed for its synthesis and breakdown—that is, phenylalanine, tyrosine, and methionine. Serotonin metabolism was investigated by measuring its degradation product, 5-hydroxyindole acetic acid (5-HIAA), in the cerebrospinal fund and of its precursor amino-acid in plasma.

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Patients and Methods

Of the 32 patients with hepatic encephalopathy investigated 20 had fulminant hepatic failure (10 after a paracetamol overdose, seven with acute viral hepatitis, and three with halothane-associated hepatitis). The other 12 patients had encephalopathy complicating chronic liver disease. They were all either stuporose or in coma and were considered to have grade III or grade IV encephalopathy, according to the classification of Trey *et al.* (1966). The cerebrospinal fluid samples were obtained while investigating the course of the cerebral oedema which can develop in fulminant hepatic failure, though the cerebrospinal fluid pressure was not raised in any of the patients. Cerebrospinal fluid from four other patients with liver disease but no clinical evidence of encephalopathy were also investigated. Results in a further 23 patients with miscellaneous neurological diseases and no liver damage were available for comparison.

The samples of cerebrospinal fluid obtained by lumbar puncture were stored at −20°C until analysed. HVA was measured as previously described (Curzon *et al.*, 1970), except that the modification of Pullar *et al.* (1970) was used. 5-HIAA was measured by the cysteine-phthalaldehyde method (Korf and Valkenburgh-Sikkemn, 1969). A Sephadex column method (Korf *et al.*, 1971) was used on a few samples to check HVA and 5-HIAA values. Plasma levodopa was measured by the method of Curzon *et al.* (1972). Phenylalanine, tyrosine, and methionine concentrations were measured by automated ion-exchange chromatography, using either a Bio-Cal BC-200 or BC-100 machine. Sulphosalicyclic or picric acid procedures (Tallan *et al.*, 1954) were used to remove protein from plasma samples within 10 minutes of venepuncture and the treated extracts were stored at −20°C until analysed. Elution buffers were usually made up with sodium citrate but sometimes a lithium citrate sequence (Atkin and Ferdinand, 1970) was used. Peak areas were compared with those of a standard amino-acid mixture to calculate plasma concentrations. The normal ranges reported by Soupart (1962) were checked and excellent agreement found. Total plasma tryptophan was estimated by the method of Denkla and Dewey (1967). This was also used to measure free tryptophan in an ultrafiltrate from a 1.7-ml aliquot of each plasma sample, prepared by centrifuging at 800 g in a Diaflo cone for 30 minutes at room temperature. Unesterified fatty acids in whole plasma were measured by the technique of Laurell and Tibbling (1967).

Results

Concentrations of HVA and 5-HIAA were substantially increased in the cerebrospinal fluid of patients with hepatic encephalopathy (fig. 1). The levels in patients with liver disease but no encephalopathy and in patients with primary neurological disorders were similar (table I). There was no relationship between the concentrations in those with hepatic encephalopathy and the aetiology of the underlying liver damage. The difference of HVA and 5-HIAA concentrations in patients with encephalopathy and in controls had statistical significance at confidence levels of 99% or greater (table II).

Increased plasma concentrations of phenylalanine, tyrosine, and methionine (fig. 2) were found in the patients with encephalopathy. Methionine showed the greatest relative increase and the abnormalities tended to be most severe in those with

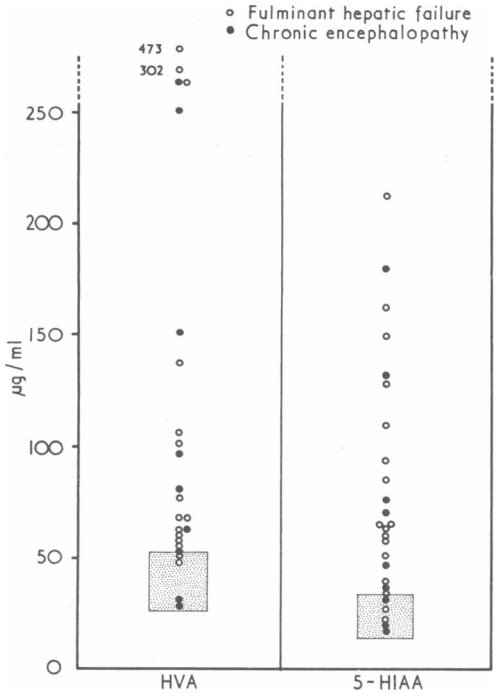

FIG. 1—Cerebrospinal fluid concentrations of homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA). Shaded areas show normal range.

TABLE I—*Concentrations of Homovanillic Acid (HVA) and 5-Hydroxyindole Acetic Acid (5-HIAA) in Groups Studied. Figures are Means ± S.D. Numbers of Patients are given in Parentheses.*

	Age (Years)	HVA Concentration (ng/ml)	5-HIAA Concentration (ng/ml)
Encephalopathy from fulminant hepatic failure	36 ± 13 (20)	123 ± 120* (16)	86 ± 81* (18)
Encephalopathy complicating chronic liver disease	52 ± 19 (12)	147 ± 113* (11)	78 ± 63* (10)
All patients with hepatic encephalopathy	39 ± 15 (32)	129 ± 112* (27)	80 ± 53* (28)
Liver disease without encephalopathy	44 ± 24 (4)	23 ± 13 (4)	27 ± 4 (3)
Primary neurological disorders	42 ± 17 (23)	38 ± 13 (23)	22 ± 10 (23)

*Significantly greater than in patients with miscellaneous neurological disorders; P = < 0.001.

TABLE II—*Plasma Tryptophan and Fatty Acid Concentrations in nine Patients with Fulminant Hepatic Failure*

	Age (Years)	Sex	Plasm Tryptophan (µg/ml) Total	Plasm Tryptophan (µg/ml) Free	Plasma F.F.A. (mEq/l.)
Paracetamol overdose	19	F.	13·15	10·48	3·41
	26	F.	14·36	7·86	0·66
	52	F.	12·26	8·02	1·88
Halothane anaesthesia	32	M.	8·33	5·99	1·72
	66	F.	5·10	1·80	—
	53	M.	9·81	4·32	—
Viral hepatitis	58	F.	11·20	3·10	0·77
	70	M.	18·87	14·15	0·25
	46	M.	14·20	—	1·54
Mean ± S.D.	Patients		11·92 ± 3·96	6·96 ± 4·06	1·46 ± 1·06
	Controls		10·7 ± 1·07	0·8 ± 0·2	0·40 ± 0·16
	Significance		N.S.	P = <0·001	P = 0·02

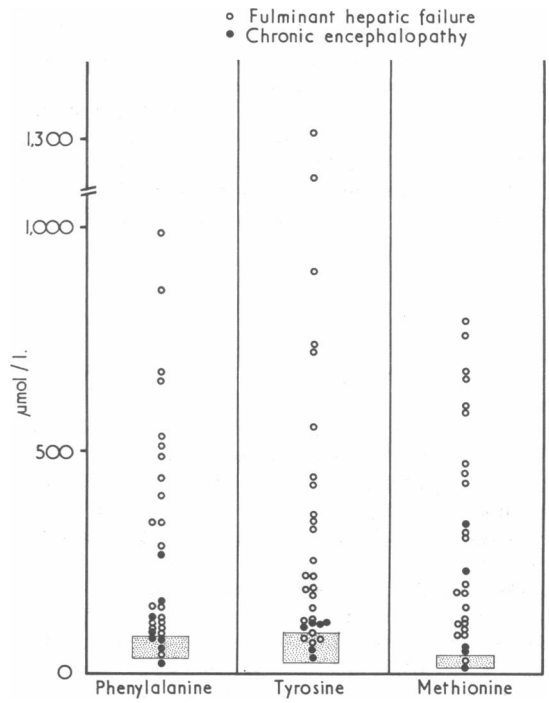

FIG. 2—Plasma concentrations of phenylalanine, tyrosine, and methionine. Shaded areas show normal range.

the deepest coma (grade IV encephalopathy). Measurements in two of these patients showed negligible quantities of dopa. In the nine patients with hepatic encephalopathy the mean total plasma tryptophan concentration was normal but the free tryptophan fraction was increased. This increase was significant at the 99.9% confidence level. Plasma unesterified fatty acid levels were also significantly increased. (table II).

Discussion

The most obvious explanation for the high concentrations of HVA and 5-HIAA found in the cerebrospinal fluid would be an increased brain turnover of dopamine and 5-hydroxytryptamine respectively due to increased availability of the precursor amino-acids phenylalanine, tyrosine, and tryptophan. When tryptophan is given by mouth to human subjects 5-HIAA increases in the lumbar cerebrospinal fluid with a time course which suggests derivation from an increase in brain 5-hydroxytryptamine turnover (Eccleston *et al.*, 1970). Though the total plasma tryptophan concentration in the present patients was not increased there is evidence to suggest that only the fraction which is free—that is, not bound to protein—is directly available to the brain (Knott and Curzon, 1972; Curzon *et al.*, 1973; Tagliamonte *et al.*, 1973). The free tryptophan fraction was strikingly high, presumably because of increases in plasma unesterified fatty acid concentration. The addition of such fatty acids to plasma is followed by a rise in the free tryptophan fraction (Curzon *et al.*, 1973 a). Furthermore, increases in plasma unesterified fatty acid and free tryptophan in association with increased brain tryptophan and 5-HIAA have been reported in pigs with hepatic encephalopathy resulting from experimentally-induced necrosis of the liver (Curzon *et al.*, 1973 b).

A similar mechanism cannot be invoked to explain the high HVA concentrations. Though high plasma concentrations of the dopamine precursors phenylalanine and tyrosine are found the rate-limiting enzyme for dopamine synthesis—that is, tyrosine hydroxylase—is thought to be normally saturated with substrate (McGeer *et al.*, 1971). It may be relevant that in dogs an intravenous infusion of tryptophan leads to increased HVA as well as 5-HIAA in the cerebrospinal fluid, even though brain dopamine or HVA was unchanged (Moir, 1971). The present findings in hepatic encephalopathy could

therefore result from defective transport of HVA owing to the raised free tryptophan levels rather than to abnormal brain dopamine metabolism. Investigations on brain tissue are required to decide this question.

We had hoped that this study would elucidate the arousal effect of levodopa seen in hepatic coma. The results have shown that this cannot be attributed to defective brain dopamine synthesis, but whether it is due to the flushing away of false neurotransmitters accumulating in the brain in liver failure requires further study (Fischer and Baldessarini, 1971). Conceivably the raised plasma free tryptophan levels are important in the mechanism of the cerebral impairment in hepatic encephalopathy in view of the reported toxic effects of tryptophan in the presence of defective liver function.

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References

Atkin, G. E., and Ferdinand, W. (1970). *Analytical Biochemistry*, **38**, 313.
Curzon, G., Friedel, J., and Knott, P. J. (1973 a). *Nature*, **242**, 198.
Curzon, G., Godwin-Austen, R. B., Tomlinson, E. B., and Kantameneni, B. D. (1970). *Journal of Neurology, Neurosurgery and Psychiatry*, **33**, 1.
Curzon, G., Kantameneni, B. D., and Trigwell, J. (1972). *Clinica Chimica Acta*, **37**, 335.
Curzon, G., *et al.*, (1973 b). *Journal of Neurochemistry*, **21**, 137.
Denkla, W. D., and Dewey, H. K. (1967). *Journal of Laboratory and Clinical Medicine*, **69**, 160.
Eccleston, D., *et al.* (1970). *Journal of Neurology, Neurosurgery and Psychiatry*, **33**, 269.
Fischer, J. E., and Baldessarini, R. J. (1971). *Lancet*, **2**, 75.
Knott, P. J. and Curzon, G. (1972). Nature (London), **239**, 452.
Korf, J., and Valkenburgh-Sikkema, T. (1969). *Clinica Chimica Acta*, **26**, 301.
Korf, J., Van Praag, H. M., and Sebens, J. B. (1971). *Biochemical Pharmacology*, **20**, 659.
Laurell, S., and Tibbling, G. (1967). *Clinica Chimica Acta*, **16**, 57.
McGeer, E. G., McGeer, P. L., and Wada, J. A. (1971). *Journal of Neurochemistry*, **18**, 1647.
Moir, A. T. B. (1971). *British Journal of Pharmacology*, **43**, 715.
Oginara, K., Mozai, T., and Hirai, S. (1966). *New England Journal of Medicine*, **275**, 1251.
Parkes, J. D., Sharpstone, P., and Williams, R. (1970). *Lancet*, **2**, 842.
Pullar, I. A., Weddell, J. M., Ahmed, R., and Gillingham, F. J. (1970). *Journal of Neurology, Neurosurgery and Psychiatry*, **33**, 851.
Sherlock, S. (1968). *Diseases of the Liver and Biliary System*, 4th edn., p. 113. Oxford, Blackwell.
Soupart, P. (1962). *Amino-acid Pools*, Ed. J. T. Holden, p. 220. Amsterdam, Elsevier.
Summerskill, W. H. J., Davidson, E. A., Sherlock, S., and Steiner, R. E. (1956). *Quarterly Journal of Medicine*, **25**, 245.
Tagliamonte, A., Biggio, G., Vargiu, L., and Gessa, L. G. (1973). *Life Sciences*, **12**, 277.
Tallan, H. H., Moore, S., and Stein, W. H. (1954). *Journal of Biological Chemistry*, **211**, 927.
Trey, C., Burns, D. G., and Saunders, S. J. (1966). *New England Journal of Medicine*, **274**, 473.
Walshe, J. M. (1951). *Quarterly Journal of Medicine*, **20**, 421.
Wyatt, R. J., *et al.* (1970). *Lancet*, **2**, 842.

MEDICAL MEMORANDA

Osteomalacia due to Phosphate Depletion from Excessive Aluminium Hydroxide Ingestion

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The theoretical dangers of severe phosphate depletion have been known for a long time. Minor degrees might be expected first to affect bone mineralization and then to produce a disease similar to rickets (or osteomalacia) while severe depletion would lead to death. Aluminium hydroxide is nearly always given as an antacid. Rarely, as in certain patients with renal failure, it is given in larger doses to produce a slight phosphate depletion, for the hydroxide not neutralized by the gastric acidity (and probably most that is) is likely to bind with the phosphate in the diet and intestinal secretions and be excreted unabsorbed as aluminium phosphate. With the usual doses given severe phosphate depletion should not occur but with larger doses it would seem to be inevitable. We describe such a case here.

Case Report

A housewife aged 49 years was admitted in April 1973 complaining of pain in the left hip and of weakness and difficulty in walking over a period of five months. The pain had begun suddenly for no apparent reason, had later become severe, and was made worse by rising from a sitting position and climbing stairs. More recently she had felt pain in both upper arms and in the lower lumbar region. Weakness was most noticeable when she tried to rise from a sitting position. Her gait had been described by friends as waddling, and on admission she was able to walk a few paces only with great difficulty. The results of previous investigations had been normal, but later abnormalities in plasma calcium, phosphorus, and alkaline phosphatase led to her referral to this unit.

She had a history of severe heartburn, associated with reflux oesophagitis and hiatus hernia, which antacids relieved. Her appetite was normal but she had eaten sparingly for two years, avoiding all starchy foods and alcohol, and had lost 12 kg in weight. There were no other symptoms. She had had a hysterectomy for menorrhagia in 1971. Since January 1971 her antacid intake had consisted of 500-600 ml Aludrox a week, equivalent to about 4·7 g aluminium hydroxide daily. This was discontinued after nine months in favour of Mucaine at least 10 ml hourly, equivalent to about 11·4 g aluminium hydroxide, 4·0 g magnesium hydroxide, and 400 mg oxathazaine daily. In addition she had taken 15-20 Asilone tablets daily for the last 20 months, equivalent to about 8·5 g aluminium hydroxide and 4·2 g polymethylsiloxane daily. Thus for some months before admission she had taken about 20 g of aluminium hydroxide each day—far in excess of the normal therapeutic dose. She had also taken nightly nitrazepam 5 mg, levorphanol tartrate 3 mg, and two tablets each of Tuinal and Equagesic.

The patient was small and thin (weight 51·0 kg, height 152·2 cm), but looked in good general health. She had a pronounced waddling gait and weakness of the hip and knee flexors. Passive joint movement was normal. There was tenderness around both humeri and the left hip but no generalized bone tenderness and no weakness of the shoulder muscles. Preliminary investigations showed normal haematology, normal urea, sodium, potassium, chlorine, uric acid, and creatinine, with a creatinine clearance of 80 ml/min. The plasma immunoreactive parathyroid hormone level was 1,075 pg/ml (normal up to 650 pg/ml), and the plasma 25-hydroxyvitamin D on three occasions was 13, 14, and 16 ng/ml respectively (normal 7-23 ng/ml for April; T. C. B. Stamp, personal communication). The results of routine urine examination were normal. Skeletal radiological screening showed nothing abnormal; in particular there were no Looser zones or subperiosteal erosions. Using copper thiocyanate as internal marker we found faecal fat to be raised on the first determination at

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