Brain transmitter precursors and metabolites in diabetic ketoacidosis

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SUMMARY Patients studied during recovery from an episode of ketoacidotic diabetes had raised blood glucose, plasma free fatty acid and plasma free tryptophan concentrations. Plasma total tryptophan was decreased. Well controlled diabetics showed normal values. The ketoacidotic patients had increased lumbar CSF tryptophan and 5-hydroxyindoleacetic acid concentrations. Plasma tyrosine and CSF tyrosine and homovanillic acid concentrations were normal in both diabetic groups. The results are discussed in relation to somewhat similar findings in uraemic and hepatic encephalopathy and to changes in rats with streptozotocin diabetes.

Abnormalities of brain transmitter metabolism have been reported in the encephalopathies of advanced renal and liver disease¹⁻⁴ and may contribute to some of the associated neurological disturbances. Minor neurological symptoms, for example drowsiness and confusion are encountered in diabetic ketoacidosis, and irreversible coma is a rare but well recognised complication.^{5,6} The finding by Jellinger and Riederer⁷ of raised 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) concentrations postmortem in brains of patients with diabetic coma suggests that brain transmitter metabolism is altered in this condition.

Brain 5HT synthesis is influenced by plasma free tryptophan as this is a major determinant of brain tryptophan concentration^{8,9} and tryptophan hydroxylase, the rate limiting enzyme for the synthesis of brain 5HT is normally unsaturated with tryptophan.¹⁰ Thus, changes of availability of tryptophan influence human brain 5HT turnover^{9,11} Normally, most of the tryptophan in plasma is not free but is bound to albumin.^{12,13} Free fatty acids also bind to albumin and decrease tryptophan binding thus increasing the fraction in the free state.^{8,14} Therefore plasma free tryptophan and thus, brain tryptophan might be expected to increase in diabetic

ketoacidosis as plasma free fatty acids are elevated.^{15,16} However, although rats made diabetic with streptozotocin show the above plasma changes,^{17,18} brain tryptophan concentration is either unaltered or decreased^{17,20} probably because of high plasma concentration of branched chain amino acids which compete with tryptophan for transport to the brain.¹⁹ Furthermore, even when brain tryptophan decreases in these diabetic rats, 5HT synthesis remains normal¹⁸ due to a compensatory rise of tryptophan hydroxylase activity.²⁰

Despite these animal findings, post mortem evidence indicates increased 5HT synthesis in human diabetic coma.⁷ We have therefore determined free fatty acids and free and total tryptophan in plasma of (a) ketoacidotic diabetics, (b) well controlled diabetics, and (c) non-diabetics. CSF tryptophan and 5HIAA were measured in groups (a) and (c) as respective indices of the availability of tryptophan to the central nervous system and of 5HT synthesis therein. Determinations also were made in plasma and CSF of the dopamine precursor tyrosine and in the CSF of the dopamine metabolite homovanillic acid (HVA).

Patients and Methods

Following admission to hospital, ketoacidotic diabetics were clinically assessed and treated with intravenous fluids and insulin by conventional procedures, including soluble insulin 10 units iv, immediately followed by 5 units im per hour thereafter until blood sugar control was achieved. Intravenous rehydration therapy consisted of 0.9% Saline,

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1-2 litres in the first hour followed by 1 litre per hour thereafter usually supplemented with added potassium. During the recovery period informed consent was obtained for blood sampling from 15 patients (six male, nine female; aged 35 ± 18 years) and for lumbar CSF sampling from seven patients (two male, five female; aged 38 ± 17 years). Of those who had CSF sampling one was comatose, two were drowsy, and four were alert at the time of admission. Alert patients were studied within 1 hour of initiation of treatment whereas the others were not sampled until their mental state had become normal, usually after 2-3 hours of treatment. Blood but not CSF was taken from another group of 22 patients with well controlled diabetes (15 male, seven female; aged 50 ± 20 years). Blood, and in most cases CSF was also collected from a control group of 24 non-diabetic patients (13 male, 11 female; aged 42 ± 17 years) undergoing lumbar puncture as part of their neurological investigation. None of these patients had disorders known to alter central transmitter amine metabolism. The study was approved by the County Hospital Ethics Committee.

Blood and CSF samples were taken between 0900 h and 1400 h. Blood samples were collected into cold heparinised tubes, immediately centrifuged and the plasma stored at -20° until analysis. CSF was collected less than 10 min after blood was taken and similarly stored. Plasma free fatty acids total tryptophan and tyrosine and CSF tryptophan, tyrosine, 5HIAA, and HVA were determined as previously described.¹ Plasma free tryptophan was determined by the method of Bloxam *et al*²¹ in which pH is controlled during ultrafiltration. Control values were higher than by the previously used method¹ in which pH was not controlled. Blood glucose was determined by the glucose oxidase method and plasma albumin by the bromocresol green method.

Results

PLASMA TRYPTOPHAN CONCENTRATION AND BINDING (table 1)

Plasma tryptophan (total and free), free fatty acids and tyrosine concentrations of non-diabetic and well

controlled diabetic groups essentially were identical and the well controlled group had a mean blood glucose concentration which was only slightly above the normal range. The ketoacidotic group had grossly raised blood glucose values and moderate decreases and increases of plasma total and free tryptophan respectively so that the percentage of tryptophan in the free state was approximately doubled. The latter change was associated with an increase of free fatty acids concentration and there was a positive correlation between free fatty acids and percentage free tryptophan when the three groups of subjects were combined (fig). The correlation was also statistically significant for the ketoacidotic group but not for the other groups taken alone (table 2). The former group also showed a positive correlation between free fatty

Figure Relationship between percentage free tryptophan and free fatty acids in plasma. Non-diabetic patients $\circ$, well controlled diabetic patients Δ , ketoacidotic diabetic patients $\bullet$. The regression line is for all three groups of patients with the exception of the two outliers indicated by an asterisk. ($r = 0.85$, $n = 35$, $p < 0.001$).

Table 1 Plasma concentrations of tryptophan and related substances in "well controlled" and ketoacidotic diabetes

	Non-diabetics	Diabetics	
		Well controlled	Ketoacidotic
Plasma			
Tryptophan $\mu\text{g/ml}$			
Total	10.73 ± 2.29 (24)	10.73 ± 2.23 (22)	7.63 ± 2.87 (15) ^{†§}
Free	2.73 ± 0.73 (9)	3.02 ± 0.87 (22)	3.90 ± 1.41 (15) ^{*†}
% free	23.58 ± 5.17 (9)	30.03 ± 15.83 (22)	58.35 ± 21.65 (15) [†]
FFA mEq/l	0.51 ± 0.31 (24)	0.45 ± 0.19 (22)	1.02 ± 0.43 (15) [†]
Tyrosine $\mu\text{g/ml}$	13.33 ± 3.31 (23)	13.83 ± 3.83 (22)	14.78 ± 5.73 (15)
Albumin		43.3 ± 2.2 (17)	40.7 ± 8.1 (15)
Blood			
Glucose mmol/l	—	6.42 ± 2.16 (22)	26.59 ± 14.76 (14)
pH	—	—	7.20 ± 0.13 (14)

Values given as means $\pm$ one SD with number of patients in brackets

Differences from control group: * $p < 0.05$; $^{\dagger}p < 0.001$

Differences from well controlled diabetic group: $^{\ddagger}p < 0.05$; $^{\S}p < 0.01$; $^{\parallel}p < 0.001$

Table 2 Correlation coefficients between plasma free fatty acids, tryptophan and glucose (numbers of patients in brackets)

	Correlation coefficients		
	Non-diabetics	Diabetics	
		Well controlled	Ketoacidotic
Free fatty acids vs % free tryptophan	0.66 (9) ns	0.28 (21) ns	0.76 (14) $p < 0.01$
Free fatty acids vs total tryptophan	0.29 (24) ns	- 0.11 (21) ns	- 0.60 (14) $p < 0.05$
Free fatty acids vs glucose	—	- 0.12 (21) ns	0.66 (14) $p < 0.02$

acids and glucose concentration and a negative correlation between free fatty acids and total tryptophan. Plasma albumin concentrations did not differ in the ketoacidotic and well controlled diabetics. The above biochemical abnormalities of the ketoacidotic group were not explicable by age differences between groups. This was apparent from the correlation coefficients of biochemical values vs age for the non diabetic and for the well controlled diabetic groups. These were far below the level of significance for plasma free fatty acids and total tryptophan and of marginal significance ($p = c 0.1$) for free tryptophan, % free tryptophan and tyrosine. Combining the two groups gave positive values for the latter correlations: free tryptophan vs age, $r = 0.41$ (31), $p = 0.02$; % free tryptophan vs age, $r = 0.51$ (30), $p < 0.01$; tyrosine vs age $r = 0.32$ (45), $p < 0.05$. As the ketoacidotic group was somewhat younger than the other two groups, age differences do not appear to have contributed to the abnormally high values shown by the former group for free tryptophan and % free tryptophan. On the contrary, age-related effects would tend to have opposed these abnormalities.

Table 3 CSF concentrations of amine precursors and metabolites in well-controlled and ketoacidotic diabetics

	Non-diabetics	Ketoacidotic diabetics
Tryptophan $\mu\text{g/ml}$	0.45 $\pm$ 0.13 (19)	0.58 $\pm$ 0.09 (7)*
5HIAA $\mu\text{g/ml}$	0.018 $\pm$ 0.009 (19)	0.037 $\pm$ 0.018 (7)†
Tyrosine $\mu\text{g/ml}$	1.79 $\pm$ 0.49 (19)	1.95 $\pm$ 0.53 (7)
HVA $\mu\text{g/ml}$	0.032 $\pm$ 0.015 (16)	0.041 $\pm$ 0.026 (6)

Values given as means $\pm$ one SD with number of patients in brackets. Differences from control group: * $p < 0.05$; † $p < 0.01$.

AMINE PRECURSORS AND METABOLITES IN THE CSF

Results in table 3 show that lumbar CSF tryptophan and 5HIAA concentrations of ketoacidotic diabetics were higher than the values for non-diabetic subjects and that CSF tyrosine and HVA were not raised in the ketoacidotic subjects. As the ketoacidotic group were largely female (5/7) significances were also calculated for females only. Significant differences persisted (tryptophan $p < 0.01$, 5HIAA, $p < 0.05$ when compared with female

Table 4 Correlation coefficients between plasma and lumbar CSF indoles (numbers of patients in brackets)

	Correlation coefficients	
	Non-diabetics	Ketoacidotic diabetics
Plasma total tryptophan vs CSF tryptophan	0.38 (19) ns	- 0.06 (7) ns
Plasma free tryptophan vs CSF tryptophan	0.86 (4) ns	0.40 (7) ns
Plasma total tryptophan vs CSF 5HIAA	- 0.07 (19) ns	- 0.28 (7) ns
Plasma free tryptophan vs CSF 5HIAA	0.58 (4) ns	0.42 (7) ns
CSF tryptophan vs CSF 5HIAA	- 0.09 (18) ns	0.33 (7) ns

Table 5 CSF/plasma ratios for tryptophan and tyrosine

	Non-diabetics	Ketoacidotic diabetics
CSF tryptophan/plasma free tryptophan	0.190 $\pm$ 0.029 (4)	0.146 $\pm$ 0.032 (6)
CSF tyrosine/plasma tyrosine	0.137 $\pm$ 0.027 (10)	0.150 $\pm$ 0.028 (6)

Neither of the differences between the two groups of subjects was significant. Similarity of the tryptophan ratios is substantiated by calculating them from the mean values for plasma free tryptophan (Table 1) and CSF tryptophan (Table 3). Ratios of 0.165 and 0.149 were obtained for the non-diabetic and ketoacidotic groups respectively.

controls). Furthermore, age differences did not appear to contribute to the above biochemical abnormalities as correlations coefficients of CSF tryptophan and 5HIAA vs age for non-diabetic group were far below the level of significance. Compared to alert diabetics those patients who were comatose or drowsy ($n = 3$) on admission tended to have slightly higher (mean $\pm$ SD) CSF tryptophan (0.64 ± 0.06 vs 0.53 ± 0.09 mg/ml) and 5HIAA (0.04 ± 0.01 vs 0.03 ± 0.02) at the time of study. However these changes were not statistically significant though this may be partly due to the small numbers involved.

RELATIONSHIP BETWEEN PLASMA AND CSF VALUES

Results in table 5 show that CSF/plasma ratios for both free tryptophan and tyrosine were essentially normal in the ketoacidotics. None of the correla-

tions within groups between plasma tryptophan (total or free) and CSF tryptophan or 5HIAA concentrations or between CSF tryptophan and 5HIAA attained significance (table 4). However as in previous studies on non diabetic subjects¹ correlations between CSF tryptophan or 5HIAA and plasma free tryptophan concentrations were larger than those with plasma total tryptophan.

Discussion

The results show that the raised plasma FFA concentration which occurs in ketoacidotic but not in well controlled diabetes^{15 16} leads to liberation of plasma tryptophan from albumin so that total and free tryptophan concentrations are decreased and increased respectively. The rise of plasma free tryptophan leads (as in other circumstances^{1 2 4 8 9 22}) to increased tryptophan concentration in the central nervous system, as indicated in the present study by the elevated CSF tryptophan concentration. The brain tryptophan change appears to enhance 5HT synthesis as shown by the raised CSF concentration of the 5HT metabolite 5HIAA. These results are rather similar to earlier findings on patients with advanced renal^{1 4} or hepatic²² encephalopathy and are consistent with necropsy data⁷ and (in the case of the liver findings) with many animal experiments.²

These biochemical changes could be involved in the sensorial clouding progressing to coma which occurs in these disorders. However, altered indole metabolism is only part of the widespread metabolic disturbances they cause. It may well play a part in the altered behaviour but is hardly likely to be solely responsible for it—large increases of tryptophan intake in normal subjects only moderately impair consciousness.^{23 24}

The mechanisms by which brain 5HT synthesis is increased in the three disorders have similarities but they are not identical. Thus, in ketoacidotic diabetes and hepatic encephalopathy, plasma tryptophan binding is weakened because of increased free fatty acids but in renal encephalopathy plasma free fatty acids are normal and the decreased binding of tryptophan (and other albumin bound substances) reflects unknown biochemical changes.^{25 26} Further, while in ketoacidotic diabetes and renal encephalopathy,¹ the central tryptophan increase is entirely explicable by the rise of plasma free tryptophan this may not be so in the liver disturbances as in experimental portocaval anastomosis the central change is more striking than the peripheral one² as a result of blood-brain barrier changes.²⁷

The effects of human ketoacidotic diabetes and of experimental streptozotocin diabetes on plasma free fatty acids and tryptophan are similar—in both

groups free fatty acids and free tryptophan increase.^{17 18} However, CSF tryptophan increased in the human subjects but brain tryptophan concentration was either unaltered or decreased in the diabetic rats. This apparent difference in access of tryptophan to the central nervous system in the two species could indicate that plasma branched chain amino acid changes^{19 28} have relatively little effect on the transport of tryptophan to the human brain but markedly inhibit in the diabetic rat.

Although plasma concentrations of branched chain amino acids increase in both the experimental¹⁹ and human disease,²⁸ recent work⁹ on non-diabetic subjects suggests that the influence of plasma competing amino acids on human brain aromatic amino acid concentration has probably been overemphasised in the past. The present results to some extent strengthens this possibility as ratios of CSF tryptophan to plasma free tryptophan (and of plasma tyrosine to CSF tyrosine) were almost normal in the ketoacidotic group at a time when metabolic consequences of the ketoacidosis were still apparent that is, when blood glucose and plasma free fatty acid concentrations were still raised. However, plasma branched chain amino acids were not determined and therefore a firm conclusion is not possible.

As tryptophan administration increased human CSF 5HIAA,¹¹ the increased availability of tryptophan to the brain appears at least partly responsible for the elevated CSF 5HIAA values (and hence for increased brain 5HT turnover) in the ketoacidotic patients. Increased brain tryptophan may however not be entirely responsible as rats made diabetic with streptozotocin show increased brain 5HT synthesis even though brain tryptophan is not raised²⁰ and it can be seen from table 3 that the ketoacidotic patients had a relatively more marked mean rise of CSF 5HIAA than of tryptophan.

Finally, the normal plasma and CSF tyrosine concentrations in the ketoacidotic diabetics indicate that the tryptophan changes are not merely part of some non-specific change of aromatic amino acid metabolism or disposition. Similarly, the lack of a significant increase of CSF HVA indicates that the increased 5HIAA reflects increased synthesis of 5HT in the central nervous system and is not merely due to inhibition of its egress from CSF by the mechanism which it shares with HVA.²⁹

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