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# TRYPTOPHAN AND TYROSINE DISPOSITION AND BRAIN TRYPTOPHAN METABOLISM IN ACUTE CARBON TETRACHLORIDE POISONING

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**Abstract**—Forty-eight hours after injecting rats with carbon tetrachloride there were large increases of plasma non-esterified fatty acid and free tryptophan concentrations. Total plasma tryptophan was not significantly altered. Brain, liver and muscle tryptophan concentrations increased significantly with percentage changes in the order: brain > liver > muscle > kidney. The increased brain tryptophan was associated with a small but significant increase of 5-hydroxytryptamine and a larger increase of its metabolite 5-hydroxyindolylacetic acid. Tyrosine concentrations increased significantly in plasma, brain, liver, muscle and kidney with percentage changes in the same order as those of tryptophan. Food intake was decreased and this correlated significantly with brain tryptophan and 5-hydroxyindolylacetic acid concentrations. Control rats kept for 48 hr on restricted intake did not show the above brain changes though plasma non-esterified fatty acid concentration was increased as in the drug treated rats. Results are discussed in relation to previous findings in acute liver failure and to the possible roles of brain tryptophan and 5-hydroxytryptamine in liver failure and appetite control.

Tryptophan is the only amino acid which is largely bound to human plasma protein [1] and it is similarly bound in at least 13 other warm-blooded vertebrates [2]. Non-esterified fatty acids (NEFA) decrease this binding [3, 4]. Physiological and pharmacological alterations of plasma NEFA are associated with parallel changes of free plasma tryptophan concentration in both the rat [5] and man [6, 7] and brain tryptophan is also usually altered in the same direction [5, 8, 9]. As cerebral tryptophan concentration is a determinant of cerebral 5-hydroxytryptamine (5-HT) synthesis [10–12] it is possible that these changes may influence brain function.

The above observations suggested that in severe hepatic failure, as plasma NEFA concentration is high [13], plasma ultrafilterable tryptophan and hence the brain indoles might also be increased; evidence for such a relationship has indeed been obtained in human fulminant hepatic coma [14] and in an experimental model of acute hepatic failure in the pig effected by surgical devascularization of the liver [15].

A widely employed model of acute hepatic necrosis in the rat is obtained by carbon tetrachloride injection [16]. Carbon tetrachloride treatment provides a convenient means of studying the relationships between liver failure, the metabolism of tryptophan in the brain and its distribution between plasma and brain and other tissues. This paper describes such a study. Tryptophan changes were compared with those of another aromatic amino acid tyrosine which is not bound to plasma protein.

## METHODS

Male Sprague–Dawley rats were obtained from Carworth Europe (Alconbury, Huntingdon, England) at 140–150 g body wt and were individually housed on arrival at the laboratory. They were fed a diet *ad lib.* of Oxoid breeding diet and tap water. Body wt and weight of food eaten per 24 hr were recorded daily between 09.30 and 10.00 hours. When the mean body wt reached 200 g, the rats were poisoned by the i.p. injection of 1·3 ml/100 g of a 10% solution of carbon tetrachloride in arachis oil [17] and killed 48 hr later. Control animals received arachis oil (1·3 ml/100 g i.p.) only. Following decapitation the brain, entire liver and a piece of the right gastrocnemius muscle were rapidly removed. Blood was collected into heparinized tubes for the preparation of plasma which was stored at −20° until determinations were made. Brain tryptophan, tyrosine, 5-HT, 5-hydroxyindolylacetic acid (5-HIAA) and plasma total tryptophan and tyrosine were determined as previously described [12]. Muscle and liver tryptophan and tyrosine were similarly determined. Plasma free tryptophan was determined fluorimetrically following ultrafiltration of 1·0-ml samples of plasma [8] while plasma NEFA was determined [18] in 0·05-ml aliquots of plasma.

## RESULTS AND DISCUSSION

*Plasma changes.* Two days after carbon tetrachloride injection large and significant increases of plasma NEFA, free tryptophan and total tyrosine were found (Table 1). Mean total tryptophan concentration was not significantly altered. Plasma NEFA and per cent free tryptophan correlated positively and significantly for the carbon tetrachloride ($r = 0{\cdot}7534$, $n = 12$, $P < 0{\cdot}01$) but not for the control group ($r = 0{\cdot}5515$, $n = 12$, N.S.). Similar relationships were previously obtained on pigs with acute liver failure following hepatic devascularization [15]. The absence of a significant correlation in the control group probably merely reflects the relatively narrow ranges of NEFA and free tryptophan values.

Other workers [19] have reported that plasma total tryptophan fell strikingly after carbon tetrachloride treatment comparable to that used in the present study in which mean total tryptophan was unaltered. However, results do suggest that plasma total tryptophan would have been low in rats with more severely damaged livers. Thus two with particularly marked accumulations of hepatic fat showed relatively low plasma total tryptophan (10·19 and 11·17 μg/ml) which was almost completely free (92%, 92%). This was associated with particularly high NEFA (1·20 and 1·00 m-equiv/l.). These results are consistent with previous findings when NEFA was increased by drug treatments [5]. It was suggested then that because of the resultant release of plasma tryptophan from binding, its uptake and metabolism by tissues also increased so that the plasma tryptophan equilibrium was disturbed and total tryptophan eventually fell.

*Tissue changes.* Concentrations of both tryptophan and tyrosine were significantly increased in brain, liver and muscle of carbon tetrachloride-treated rats (Table 1). Tissue percentage tryptophan changes were smaller than those of plasma free tryptophan while the tissue percentage tyrosine changes were on the whole comparable to the plasma changes. Percentage increases of tissue concentration of both amino acids were in the order: brain > liver > muscle > kidney. The brain findings agree with other results suggesting that in acute liver failure [15] brain tryptophan and tyrosine changes are a consequence of the plasma changes. In chronic failure altered uptake mechanisms may also be involved [20].

In the carbon tetrachloride-treated rats brain tryptophan concentration was positively and significantly correlated with plasma free but not total tryptophan concentration (Fig. 1a and b). In the control group the ranges of brain tryptophan and plasma free tryptophan concentrations were small and a significant correlation was not found. However, the values for both the control and treated groups can be expressed by a single regression line. This is not the case for the brain tryptophan vs plasma total tryptophan relationship (Fig. 1a). These findings are consistent with earlier observations in pigs with devascularized livers [15].

An apparent result of the increased brain tryptophan concentration is that 5-HT synthesis is enhanced as indicated by the small but significant increase of brain 5-HT and the larger rise of 5-HIAA (Table 1). Brain 5-HIAA was significantly correlated with the cerebral tryptophan concentration in the poisoned group ($r = 0{\cdot}8189$, $n = 12$, $P < 0{\cdot}001$). Similar changes in hepatectomized dogs and rats [20] and in pigs with devascularized livers [15], taken together with the neurological changes following feeding of tryptophan but not of other amino acids to a dog with a portocaval anastomosis [22], have led to the suggestion that altered indole metabolism is involved in the development of hepatic encephalopathy. However, rats with portocaval anastomosis do not exhibit these symptoms although their brain indole metabolism is enhanced [20, 23]. Another possibility is that the biochemical changes may either cause or result from anorexia which is a characteristic early symptom of human liver disease and occurs in rats with portocaval anastomosis [25]. This possibility was investigated as follows.

*Relationships between increased brain tryptophan metabolism and decreased food intake.* During the period 24–48 hr after injection mean food intake by the carbon tetrachloride-treated rats (11·6 ± 4·8 g, $n = 12$) was significantly less ($P < 0{\cdot}001$) than the intake by the control animals (26·8 ± 4·2, $n = 12$) (means are given ± one S.D.). Significant negative correlations were found for the treated animals between the food intakes and both brain tryptophan and brain 5-HIAA (Fig. 2). As the above biochemical changes were qualitatively similar to those following 24 hr food deprivation of normal rats [8], it was possible that they were simply a consequence of reduced food intake.

To investigate this further, eight rats were put on restricted amounts of their normal diet ranging from 5 to 20 g/day for a 48-hr period. Mean plasma NEFA at 48 hr was 0·79 ± 0·26 m-equiv/l. (two control animals; 0·32, 0·26). These values were very similar to corresponding one for the carbon tetrachloride-treated animals (see above and Table 1). However, brain tryptophan did not rise (Fig. 2a). This suggests that when increased NEFA leads to a rise of plasma free tryptophan there is an increase of liver tryptophan catabolism in normal but not in carbon tetrachloride-poisoned rats which gradually counteracts the plasma free tryptophan and hence brain tryptophan

Table 1. Effect of carbon tetrachloride poisoning on plasma non-esterified fatty acids (NEFA) and on tryptophan and tyrosine disposition and metabolism

| | Plasma NEFA (m-equiv/l.) | Plasma tryptophan (μg/ml) Total | Free | Tissue tryptophan (μg/g wet wt) Liver | Muscle | Kidney | Brain | Brain 5-OH indoles (μg/g wet wt) 5-HT | 5-HIAA |
|---|---|---|---|---|---|---|---|---|---|
| Controls | 0·27 ± 0·09 | 14·90 ± 2·60 | 2·47 ± 0·44 | 5·88 ± 1·21 | 4·71 ± 1·03 | 23·42 ± 2·70 | 3·31 ± 0·94 | 0·60 ± 0·06 | 0·46 ± 0·06 |
| Carbon tetrachloride | 0·66 ± 0·27 | 13·73 ± 4·92 | 6·11 ± 2·17 | 9·05 ± 1·51 | 6·19 ± 1·95 | 24·26 ± 3·15 | 6·14 ± 2·35 | 0·67 ± 0·05 | 0·66 ± 0·14 |
| % Change | +142 | −8 | +147 | +54 | +31 | +4 | +85 | +12 | +43 |
| P | <0·001 | N.S. | <0·001 | <0·001 | <0·05 | N.S. | <0·01 | <0·01 | <0·001 |

| | Plasma tyrosine (μg/ml) | Tissue tyrosine (μg/g wet wt) Liver | Muscle | Kidney | Brain |
|---|---|---|---|---|---|
| Controls | 16·81 ± 1·23 | 10·96 ± 1·37 | 13·54 ± 3·13 | 108·2 ± 10·9 | 11·35 ± 2·35 |
| Carbon tetrachloride | 22·64 ± 5·94 | 19·04 ± 2·19 | 20·48 ± 6·79 | 129·9 ± 9·5 | 21·31 ± 4·81 |
| % Change | +70 | +73 | +51 | +20 | +87 |
| P | <0·001 | <0·001 | <0·01 | <0·01 | <0·001 |

Rats were killed 48 hr after injection of carbon tetrachloride (1.3 ml/100 i.p. of a 10% solution in arachis oil). Controls were injected with arachis oil alone. Results are given as means ± one S.D. of determinations on 12 rats/group except for the kidney values when 6 rats/group were used.

increases. Therefore, increased brain tryptophan in the carbon tetrachloride-treated rats is not simply caused by their decreased food intake. These findings also suggest that the increase of brain tryptophan on 24 hr complete food deprivation might not occur on more prolonged deprivation.

It is of interest that amino acids in high dosage decrease food intake and that tryptophan is particularly effective [26]. Also, intake is reduced by intrahypothalamic injection of an amino acid mixture which contains tryptophan [27] and by intraventricular injection of 5-HT [28]. Therefore it is not impossible that increases of brain tryptophan or other amino acids may have a role in determining the decreased food intake in liver disease and other conditions involving anorexia. However, it would be rash at present to assume that the negative correlations of brain tryptophan and 5-HIAA with food intake (Fig. 2) necessarily imply a direct causal relationship between these parameters. Decreased food intake following injections of *p*-chlorophenylalanine [29], an inhibitor of 5-HT synthesis, seems to contradict such a relationship, though it is possible that this drug effect is due to gastric ulceration [30] rather than to 5-HT changes.

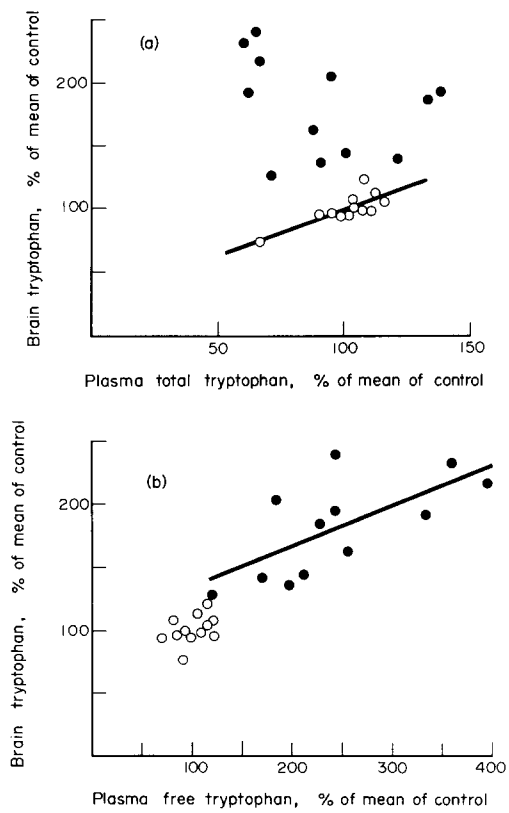


Fig. 1. Relationships between brain tryptophan and plasma tryptophan concentrations. (a) Brain tryptophan vs plasma total tryptophan concentration: ○, control animals; ●, carbon tetrachloride-poisoned animals. Correlation coefficient r for control group = 0·7594 (n = 12, $P < 0·01$); r for poisoned group = −0·3106 (n = 12, N.S.). (b) Brain tryptophan vs plasma free tryptophan concentration: ○, control animals; ●, carbon tetrachloride-poisoned animals: r for control group = 0·4372 (n = 12, N.S.); r for poisoned group = 0·6712 (n = 12, $P < 0·05$).

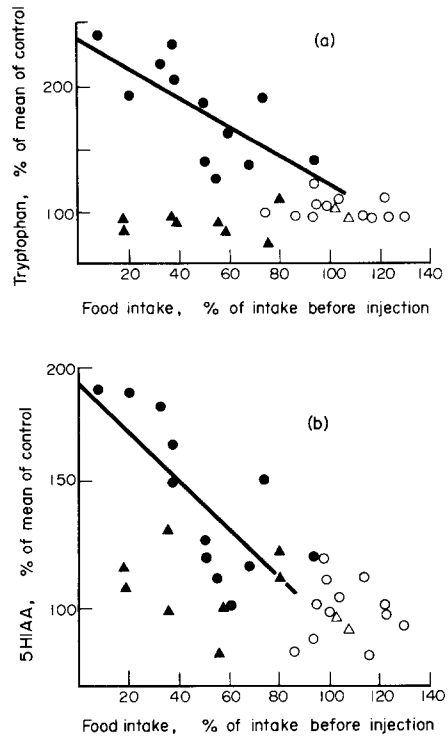


Fig. 2. Relationships between brain tryptophan and 5-HIAA concentrations and food intake. (a) Brain tryptophan vs food intake: ○, control animals; ●, carbon tetrachloride-poisoned animals. Correlation coefficient r for control group = 0·1251 (n = 12, N.S.); r for poisoned group = −0·7128 (n = 12, $P < 0·01$). ▲, 8 rats partially deprived of food during 48 hr before killing; △, 2 rats fed *ad lib.* during 48 hr before killing. (b) Brain 5-HIAA vs food intake: ○, control animals; ●, carbon tetrachloride-poisoned animals. Correlation coefficient r for control group = −0·058 (n = 12, N.S.); r for poisoned group = −0·7371 (n = 12, $P < 0·01$). Brain tryptophan and 5-HIAA concentrations are expressed as percentages of the means of the control group. Food intakes are expressed as percentages of the mean food intake during the 24-hr period before injection.

Results in general provide further evidence that plasma free tryptophan changes influence brain 5-HT metabolism [5, 8, 9, 15, 20] and indicate that carbon tetrachloride intoxication may provide a convenient means by which the relationships between the symptoms of liver failure and altered brain 5-HT synthesis may be studied.

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