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Activation of Liver Tryptophan Pyrrolase Mediates the Decrease in Tryptophan Availability to the Brain after Acute Alcohol Consumption by Normal Subjects

Abdulla A-B Badawy[1,*], Donald M. Doughrty[2], Dawn M. Marsh-Richard[2] and Alex Steptoe[1]

[1]Cardiff School of Health Sciences, University of Wales Institute Cardiff (UWIC), Western Avenue, Cardiff CF5 2YB, Wales, UK and [2]Department of Psychiatry, Neurobehavioral Research Laboratory and Clinic, School of Medicine, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

*Corresponding author: Cardiff School of Health Sciences, University of Wales Institute Cardiff (UWIC), Western Avenue, Cardiff CF5 2YB, Wales, UK. Tel: +44-29-2041-6858; Fax: +44-29-2041-6982; E-mail: ABadawy@uwic.ac.uk

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Abstract — Aims: We have previously suggested that acute ethanol consumption by normal subjects decreases the availability of circulating tryptophan (Trp) to the brain by activating liver Trp pyrrolase, the first and rate-limiting enzyme of the (major) kynurenine pathway of Trp degradation. The aim of the present study was to examine this hypothesis further by measuring plasma levels of kynurenine metabolites following alcohol consumption. **Methods:** After an overnight fast and a light breakfast, each of 10 healthy subjects received one of five drinks (placebo and doses of ethanol of 0.2, 0.4, 0.6 and 0.8 g/kg body weight in tonic water) on five different occasions. Blood samples were withdrawn 2 h later and plasma was analysed for concentrations Trp, competing amino acids (CAA) and kynurenine metabolites. **Results:** Along with the depletion of plasma Trp and the decrease in its availability to the brain, as expressed by the ratio of [Trp]/[CAA], plasma kynurenine was elevated by doses of ethanol of 0.2–0.8 g/kg body weight. The ratio% of [kynurenine]/[Trp], an index of the expression of Trp pyrrolase activity, was also increased by all doses of ethanol. **Conclusions:** We conclude that activation of liver Trp pyrrolase mediates the depletion of plasma Trp and the decrease in its availability to the brain induced by acute ethanol consumption.

INTRODUCTION

Alcohol (ethanol) causes profound changes in the metabolism of the essential amino acid L-tryptophan (Trp) in both man and experimental animals (for reviews, see LeMarquand *et al*., 1994a,b; Badawy, 2002, 2005). The effects of ethanol depend on many factors, including its dose, route of its administration, choice of the animal species and/or strain, method of assessment of metabolism or turnover, and whether the changes observed occur after acute or chronic alcohol intake or during the withdrawal phase.

Previous studies of the acute effects of alcohol on Trp disposition in normal human subjects demonstrated a decrease in plasma Trp after acute consumption of whisky or pure ethanol (Siegel *et al*., 1964; Eriksson *et al*., 1983; Badawy *et al*., 1995; Markus *et al*., 2004). This decrease occurs both under fasting conditions and if alcohol is consumed with a meal. As a result of this decrease, the availability of circulating Trp to the brain is impaired when expressed as the ratio of plasma [Trp] to the sum of the five amino acids that compete with Trp for entry into the brain (Val, Leu, Ile, Phe and Tyr), i.e. the [Trp]/[CAA] ratio (Badawy *et al*., 1995; Markus *et al*., 2004). This ratio is the most accurate predictor of Trp entry into the brain and hence the rate of serotonin synthesis (for a detailed discussion, see Badawy, 2002, 2005).

In our previous study (Badawy *et al*., 1995), we suggested that the decrease in circulating [Trp] and its availability to the brain is likely to be mediated by activation of liver Trp pyrrolase (TP) (Trp 2,3-dioxygenase; EC 1.13.11.11), because the decreases in both free and total (i.e. free + albumin-bound) [Trp] were relatively similar with no change in Trp binding to albumin as expressed by the percentage of free Trp. This pattern of effect is typical of TP activation or induction (see, e.g., Badawy and Evans, 1983). In the present work, we provide further evidence in support of this mechanism by demonstrating the elevation of the concentration of the Trp major oxidative product kynurenine after acute ethanol consumption.

SUBJECTS AND METHODS

Subjects

The 10 subjects of the present study were a part of a wider investigation into the effects of alcohol consumption on measures of impulsive behaviour, details of which have appeared elsewhere (Dougherty *et al*., 2008). Subjects were recruited through various media advertisements and those who met the necessary criteria by telephone interview were invited to undergo a rigorous psychiatric and medical screen, using standard instruments (for details, see Dougherty *et al*., 2008). Those who passed the screening tests for exclusion of psychiatric and organic disease, alcohol and drug abuse or dependence, and over the counter or prescribed medication were invited to participate. Of the 10 subjects who participated, there was only one female, three African Americans and seven Caucasians. Their age ranged between 21 and 37 years (mean ± SEM: 27.4 ± 2.0). The subjects gave their written informed consent to participate in the study, which was approved by the Institutional Review Board of Wake Forest University Health Sciences Center, NC, USA and conducted in accordance with the Declaration of Helsinki.

Subjects were instructed to fast overnight but were provided with a light breakfast between 08.00 and 08.30 consisting of two Nutri-Grain® Kellogg bars (total weight 74 g), the main nutrient contents of which were carbohydrate (53.56 g), protein (3.26 g) and fat (5.54 g). Using a repeated-measures design, all subjects consumed each of the four alcohol doses or the placebo drink on separate days: a placebo drink [three cups, each

containing a total of eight fluid oz (240 ml)] of tonic water with 2 ml of 95% ethanol applied to the rim (giving no measurable breath alcohol concentration), or one of four doses of ethanol (0.2–0.8 g/kg body weight) in tonic water. The order of the alcohol dose administration was randomized across subjects. The tonic water was of the regular type (i.e. containing high fructose corn syrup) and provided ~69 g of sugar in the total volume consumed. The three cups were consumed over a total period of 15 min, starting at 09.00, at the rate of one cup every 5 min. A venous blood sample (10 ml) was withdrawn from each subject 2 h after the end of the drinking period. We have previously shown (Badawy *et al.*, 1995) that this is the time interval after alcohol consumption at which maximum decreases in the Trp concentrations and ratios occur. Because the subjects consumed the light breakfast and the tonic water with its high sugar content, the results of the placebo (zero-ethanol dose) condition were compared with those from a control group from a previous study consisting of 10 fasting normal subjects very closely matched for age (27.2 ± 1.9 years), gender and ethnicity.

Methods

Plasma was isolated and frozen at −80°C until transported in the frozen state to Cardiff, UK, for analysis. No plasma ultrafiltrates were prepared in this study. Plasma total [Trp] and those of the five Trp competitors (Val, Leu, Ile, Phe and Tyr) were determined by our recently developed rapid gas-chromatographic method, based on solid-phase extraction and using norvaline as internal standard (Badawy *et al.*, 2008). A plasma perchloric acid extract was also prepared, which was used to measure Trp, kynurenine (K) and other kynurenine metabolites [3-hydroxykynurenine (3 HK), 3-hydroxyanthranilic acid (3 HAA), kynurenic acid (KA), anthranilic acid (AA) and xanthurenic acid (XA)] using our newly developed HPLC procedure. Briefly, Trp and the above six kynurenines were separated isocratically using a Perkin–Elmer LC200 HPLC quaternary system with UV/Vis and fluorescence detection in series. The mobile phase was 10 mM sodium phosphate: methanol (73:27, by vol), final pH 2.8, at 37°C, at a 1.2 ml/min flow rate and using a Synergi 4 μ Fusion-RP80A column (250 × 4.6 mm) with guard column (Phenomenex). Data handling and processing were performed by the associated Total Chrome software with reference to standards of known concentrations run as calibrants. Results (expressed in μM) were corrected for full recovery. Statistical analysis was by one-way analysis of variance (ANOVA) with replicated measures and a two-tailed level of significance (P) was set at 0.05.

RESULTS

Effects of various doses of ethanol on plasma total Trp concentration and availability to the brain

As shown in Table 1, plasma total [Trp] was significantly decreased by all doses of ethanol consumed 2 h earlier. The decreases were 26, 42, 47 and 53% after the 0.2, 0.4, 0.6 and 0.8 g/kg doses, respectively. By contrast, the sum of the five Trp competitors Val, Leu, Ile, Phe and Tyr was not significantly altered by any of the ethanol doses tested. As a consequence, the ratio of [Trp]/[CAA], which reflects Trp availability to the brain, was decreased by doses of ethanol of 0.4–0.8 g/kg body weight, by 35, 38 and 39%, respectively. Only the decrease by the smallest dose (0.2 g/kg) (27%) was not significant. In data not shown here, there were no significant changes in individual concentrations of the five Trp competitors, except Phe, which was decreased by 39% by the 0.8 g/kg dose.

Effects of various doses of ethanol on plasma concentrations of kynurenine and kynurenine metabolites

These effects are shown in Table 2, which lists the Trp values for comparative purposes. As shown, plasma kynurenine concentration was significantly elevated by all doses of ethanol. In fact, the maximum elevation (78%) was observed with the smallest ethanol dose (0.2 g/kg body weight). These elevations, coupled with the decreases in [Trp], led to dramatic increases in the ratio% of [kynurenine]/[Trp], which is an expression of Trp oxidation by Trp pyrrolase. The increases in this ratio% dose-dependently reflected the decreases in plasma [Trp]. Plasma total kynurenines were also measured, but the sum of these was not significantly elevated by ethanol. Total Trp oxidation, expressed as the ratio% of [total kynurenines]/[Trp], was also not significantly altered by ethanol, mainly because of the wide individual variations, except after the largest dose (0.8 g/kg body weight), which increased it by 162% ($P = 0.0004$).

Comparison of parameters of Trp metabolism and disposition between the placebo treatment and a fasting control group

Because the study subjects received a light breakfast to tolerate better the ethanol doses, and additionally a sugar-containing tonic water, it was considered important to ascertain any differences in the parameters reported in Tables 1 and 2 for the placebo condition from those expected under fasting conditions. For example, food intake causes profound changes in plasma amino acids, depending on the nature of the dietary component. The major component of the light breakfast and the tonic water consumed by our subjects, carbohydrate, is known to induce three important changes mediated by insulin: (1) a decrease in plasma-free Trp due to inhibition of lipolysis (Pérez-Cruet *et al.*, 1974; Fernando *et al.*, 1976); (2) an increase in the [Trp]/[CAA] ratio due to a decrease in concentrations of the Trp competitors, particularly the branched-chain amino acids (BCAA) Val, Leu and Ile, through stimulation of their uptake by muscles (Fernstrom and Wurtman, 1972; Ashley *et al.*, 1982; Yokogoshi and Wurtman, 1986; Lyons and Truswell, 1988; Wurtman *et al.*, 2003) and (3) a potential release of Trp from the pancreas into the circulation (Bender *et al.*, 1975). As regards the increase in the [Trp]/[CAA] ratio induced by carbohydrate intake, we expect this ratio to be higher in our placebo-treated subjects, compared with fasting controls, through the above mechanisms. As regards free [Trp], although not measured in the present work, its depletion by carbohydrate intake is likely to be reflected in a decreased entry into the liver and consequent decrease in kynurenine production.

The results in Table 3 confirm these predictions. Thus, as expected, compared to fasting, food intake significantly decreased the sum of the five Trp competitors ([CAA]) and increased the total [Trp]/[CAA] ratio. The decrease in the [CAA] was largely due to depletion of the three [BCAA], rather than of [Phe + Tyr]. Also kynurenine concentration in the placebo treatment condition was nearly half of that in fasting subjects. However, TP activity, expressed as the ratio% of [kynurenine]/[Trp] was

Table 1. Effects of various doses of ethanol on plasma tryptophan concentration and availability to the brain

	Dose of ethanol (g/kg body weight)				
Parameter	0	0.2	0.4	0.6	0.8
[Trp]	38 ± 3	28 ± 3*	22 ± 1*	20 ± 1*	18 ± 1*
[CAA]	405 ± 39	402 ± 44	363 ± 32	344 ± 29	318 ± 24
[Trp]/[CAA]	0.094 ± 0.009	0.069 ± 0.011	0.061 ± 0.006*	0.058 ± 0.006*	0.057±.003*

Values (in μM or ratio) are means ± SEM for $n = 10$ per group.
*$P = 0.0264$ at least (one-way ANOVA with replicated measures).

Table 2. Effects of various doses of ethanol on plasma kynurenine(s) concentrations

	Dose of ethanol (g/kg body weight)				
Parameter	0	0.2	0.4	0.6	0.8
[Trp]	38 ± 3	28 ± 3*	22 ± 1*	20 ± 1*	18 ± 1*
[Kyn]	0.65 ± 0.10	1.17 ± 0.11*	1.09 ± 0.11*	1.13 ± 0.13*	1.16 ± 0.11*
TP (%)	1.72 ± 0.35	4.17 ± 0.50*	4.96 ± 0.35*	5.64 ± 0.75*	6.44 ± 0.12*
[Kyns]	4.57 ± 0.62	4.27 ± 0.80	4.10 ± 0.55	4.30 ± 0.72	5.67 ± 0.72
TTOx (%)	12.0 ± 1.8	15.2 ± 2.5	18.6 ± 2.7	21.5 ± 4.5	31.5 ± 4.3*

Values (in μM or as%) are means ± SEM for $n = 10$ per group.
*$P = 0.0229$ at least (one-way ANOVA with replicated measures).
Trp, tryptophan; Kyn, kynurenine; Kyns, kynurenines; TP, Trp pyrrolase, expressed as the % [Kyn]/[Trp]; TTOx, total Trp oxidation, expressed as the % [Kyns]/[Trp].

Table 3. Comparison of parameters of tryptophan metabolism and disposition between fasting subjects and the non-fasting placebo group

Parameter (year, μM or ratio)	Fasting group ($n = 10$)	Placebo condition ($n = 10$)	Difference %	Significance P
Age	27.2 ± 1.9	27.4 ± 2.0	+0	
[Trp]	43 ± 2	38 ± 3	−12	
[CAA]	653 ± 34	405 ± 39	−38	0.0002
[Trp]/[CAA]	0.066 ± 0.004	0.094 ± 0.008	+42	0.0045
[Phe + Tyr]	139 ± 15	103 ± 13	−26	
[BCAA]	514 ± 27	302 ± 31	−41	0.0001
[Trp]	37 ± 3[a]	38 ± 3	+3	
[Kyn]	1.253 ± 0.200	0.655 ± 0.101	−48	0.0157
TP (% Kyn/Trp)	3.39 ± 0.34	1.72 ± 0.35	−49	0.0112
[Kyns]	4.851 ± 0.956	4.565 ± 0.619	−6	
TTOx (% Kyns/Trp)	13.11 ± 2.07	12.01 ± 1.79	−8	

Values are means ± SEM (one-way ANOVA for replicated measures).
[a]Trp was determined by HPLC.
BCAA, branched-chain amino acids, namely Val, Leu and Ile; CAA, competing amino acids, namely Val, Leu, Ile, Phe and Tyr; Kyn, kynurenine; Kyns, total kynurenines; Phe, phenylalanine; TP, tryptophan pyrrolase; Trp, tryptophan; TTOx, total Trp oxidation; Tyr, tyrosine.

also significantly lower in the placebo condition, suggesting inhibition of the TP activity in addition to decreased Trp flux through the pathway because of decreased entry into the liver. The total kynurenine concentration and total Trp oxidation were, however, not different between the two groups of subjects.

DISCUSSION

Decrease in Trp availability to the brain after acute ethanol consumption by normal subjects

The present study has confirmed previous findings that acute ethanol consumption by normal (non-alcohol-dependent) subjects decreases Trp availability to the brain, expressed by the ratio of [Trp]/[CAA] and that this decrease is caused by depletion of [Trp] and not elevation of [CAA] (Badawy *et al*., 1995; Markus *et al*., 2004). Also in confirmation of findings by these latter authors, the above decrease also occurs after food intake, as well as under our earlier fasting experimental conditions. As shown in Table 1, the decrease in the above ratio was significant after a 0.4 g/kg body weight dose of ethanol, which is equivalent to the intake of 2–3 units of alcohol, depending on the ethanol content of the beverage. As discussed previously (Badawy *et al*., 1995; Badawy, 2003), although a decrease in brain serotonin of the order of 25–35% by moderate doses of alcohol may not be associated with undue behavioural consequences in normal subjects, the same doses may cause a greater depletion in susceptible individuals, which may induce an episode of dysphoria, loss of control and, in the presence of a provocative stimulus or situation, aggressive behaviour.

Mechanism of the decrease in Trp availability to the brain after acute ethanol consumption by normal subjects

We have previously suggested (Badawy *et al*., 1995) that the decrease in Trp availability to the brain is due to the activation of liver TP. This is the first and rate-limiting enzyme of the quantitatively most important pathway of Trp degradation, the hepatic kynurenine pathway. This is because the decreases in both free and total [Trp] were relatively similar with no change in Trp binding to albumin (expressed as the% free Trp). This is a typical feature of TP activation or induction. TP is regulated by several mechanisms, including hormonal induction by glucocorticoids, substrate activation and stabilization by Trp and cofactor activation by haem (Badawy and Evans, 1975; see also Badawy, 2005). Because acute ethanol consumption lowered (rather than increased) serum [Trp] and did not elevate cortisol (Badawy *et al*., 1995), we excluded substrate activation and hormonal induction and concluded that the likely mechanism of the ethanol effect is TP activation by its haem cofactor. It is difficult for obvious ethical reasons to ascertain this mechanism without direct investigation of liver tissue, but a further assessment of the mechanism is possible through determination of the main oxidation product of the TP reaction, kynurenine. The results in Table 2 show clearly that plasma kynurenine concentration was increased significantly by all doses of ethanol, with the smallest dose (0.2 g/kg) causing the maximal increase. This suggests that liver TP is extremely sensitive to activation by ethanol in normal subjects, which may have important physiological implications. The % [kynurenine]/[Trp], an expression of TP activity, was also significantly and dramatically increased by all doses of ethanol, thus providing further evidence for TP activation by ethanol. Although kynurenine was dramatically elevated by ethanol, there were no significant effects on total kynurenines, and total Trp oxidation, expressed as the ratio% of [kynurenines]/[Trp], was significantly increased only by the largest ethanol dose. This suggests that ethanol may also exert effects on other enzymes of the kynurenine pathway in the opposite direction to the kynurenine elevation.

However, the possibility cannot be totally excluded that ethanol may also activate extrahepatic indoleamine dioxygenase (IDO). As far as we could ascertain, however, any likely effect of acute ethanol administration on IDO has not been studied. For this to occur, evidence is also required for an increase in circulating cytokines, particularly interferon-γ, the principal effector of IDO (Pfefferkorn *et al*., 1986; Taylor and Feng, 1991) after acute ethanol consumption. As far as we could ascertain, the acute effects of ethanol on interferon levels have not been reported, although it has been shown that ethanol, in fact, releases various cytokines that inhibit interferon signalling and antiviral defences and that, generally, ethanol inhibits both innate and acquired immunity (for review, see Gao, 2005). The above possibility therefore remains unlikely, at least until evidence to the contrary emerges from future research.

Effects of food intake on parameters of Trp metabolism and disposition

Although plasma-free [Trp] was not measured in the present study, it is likely to have been decreased by food intake in view of the well-known increase in Trp binding through inhibition of lipolysis by insulin (Pérez-Cruet *et al*., 1974; Fernando *et al*., 1976). A decrease in free [Trp] after carbohydrate intake, as is likely in the subjects of the present study, would suggest that, compared to fasting, less Trp would have entered the liver and hence less kynurenine is formed. This notion is supported by the observed lower plasma kynurenine concentration in our placebo treatment group, compared with fasting controls (Table 3). However, a decrease in Trp entry into the liver after inhibition of lipolysis would not by itself be expected to alter TP activity, as assessed by the ratio% of [kynurenine]/[Trp], because the flux of Trp through the kynurenine pathway would not necessarily be impaired. However, the above percentage was actually decreased by food intake, suggesting TP inhibition. We have previously shown that carbohydrates (in the form of glucose, fructose or sucrose) inhibit TP activity (Badawy and Evans, 1976) and therefore conclude that the food and sugar-containing tonic water consumed by our subjects decreases both Trp entry into the liver and hepatic TP activity.

Food intake also exerts effects on Trp availability to the brain. Paradoxically, and unlike the situation in the liver, Trp availability to the brain is actually increased by carbohydrate intake, despite the decrease in plasma free [Trp]. This is due to the lowering of concentrations of the Trp competitors, in particular the three branched-chain amino acids Val, Leu and Ile, following an insulin-mediated increase in their uptake by muscles (Fernstrom and Wurtman, 1972; Ashley *et al*., 1982; Yokogoshi and Wurtman, 1986; Lyons and Truswell, 1988; Wurtman *et al*., 2003) and, additionally, a possible release of Trp from the pancreas (Bender *et al*., 1975). This is clearly demonstrated by the results in Table 3 showing the higher [Trp]/[CAA] ratio and the lower [CAA] and [BCAA] in the placebo treatment condition, compared with fasting controls. However, despite these differences between fasting and food intake, all our subjects received the light meal and the sugar-containing tonic water on each of the five occasions and this validates the placebo treatment as an appropriate control for the ethanol treatments.

General conclusions and comments

The present results and those reported previously by us and others further confirm the ability of acute ethanol consumption by normal subjects to decrease Trp availability to the brain, an effect almost certain to inhibit cerebral serotonin synthesis and may explain alcohol-induced aggression, depression and loss of control in susceptible individuals. Our demonstration of elevated plasma kynurenine adds further support to our previous suggestion that alcohol decreases plasma [Trp] by activating liver Trp pyrrolase. By inference, this important regulatory peripheral enzyme may play a primary role in mediating the effects of alcohol on brain serotonin.

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