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RAPID COMMUNICATION

ALCOHOL-INDUCED EUPHORIA: EXCLUSION OF SEROTONIN

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Abstract — During the first 30 min after acute ethanol consumption by three fasting normal male volunteers, no increase in circulating tryptophan availability to the brain occurred. On the contrary, a small decrease was observed, which became stronger subsequently. We conclude from this preliminary study that brain serotonin levels are not increased after alcohol intake by normal subjects and that, consequently, this indolylamine is unlikely to mediate the euphoric effects of alcohol.

INTRODUCTION

Euphoria, the feeling of well-being, has been reported during the early (10–15 min) phase of alcohol consumption, i.e. at the time of rising blood-ethanol concentration (Lukas *et al.*, 1986*a*), and can be correlated with transient changes in the brain electroencephalographic activity (Lukas *et al.*, 1986*b*). The neurochemical basis of euphoria in general and that induced by alcohol in particular remains unclear. At least four neuronal mechanisms have so far been implicated, namely those involving the dopaminergic, γ-aminobutyric (GABA)-ergic, opioidergic, and serotonergic systems. For example, it has been suggested that ethanol may reinforce its own intake by activating GABA receptors, thus causing relaxation, and induce euphoria by releasing dopamine from mesolimbic structures and/or endogenous opioids through activation of the right prefrontal cortex (Littelton and Little, 1994; Tiihonen *et al.*, 1994; DiChiara *et al.*, 1996). It has also been suggested (Dakis and Gold, 1985, 1990) that central stimulants induce euphoria and reinforcement by activating dopamine circuits in mesolimbic neurones. The euphorigenic and other subjective effects reinforcing drug intake have led to development of abuse-liability tests for a variety of addictive and other drugs, including serotonin agonists and antagonists (Jasinski, 1991). One such agonist, *m*-chlorophenylpiperazine, has been shown to cause an increase in self-rating of euphoria by normal volunteers (Mueller *et al.*, 1985), although such an effect could be demonstrated in another study (Schwartz *et al.*, 1997) only in depressed subjects and not in normal volunteers.

One approach with which to demonstrate the possible involvement of serotonin in the euphoric effects of alcohol is if consumption of the latter can be shown to increase brain serotonin concentration. We have examined this possibility in the present work by studying the availability to the brain of the serotonin precursor tryptophan (Trp) during the first 30 min after acute ethanol consumption by normal male volunteers. Previously, we showed (Badawy *et al.*, 1995) that acute ethanol consumption by human volunteers actually decreases circulating Trp concentration and availability to the brain, thus decreasing, rather than increasing, brain serotonin synthesis, over a 3-h period. The first time interval of observation was 30 min after ethanol intake, at which the above decreases were already evident. In the present work, it was hypothesized that only an earlier increase in Trp availability to the brain could implicate serotonin in the euphoric effects of alcohol. A summary of this work has appeared in abstract form (Morgan and Badawy, 1999).

SUBJECTS AND METHODS

Subjects

Healthy male volunteers, recruited from hospital staff and science and medical students, were all moderate social drinkers with no family history of alcoholism. Upon screening, all subjects had normal liver function and haematological profiles, were free from any organic or psychiatric disease, and not under any medication. The three volunteers studied in the present work were part of a larger group of subjects who took part in our previous detailed study of the effects of alcohol consumption on Trp metabolism and disposition (Badawy *et al.*, 1995), for which ethical approval by the local Ethics Committee and informed consent by the subjects themselves were both obtained. The subjects' age range was 22–35 years. All subjects fasted overnight (12 h) and did not consume any alcoholic beverages for the 24 h preceding the experiments. Other experimental details have been described previously (Badawy *et al.*, 1995).

Ethanol administration and blood sampling procedures

Ethanol (99.7% pure, Hayman Ltd, Witham, Essex, UK) was mixed with fresh orange juice and administered orally in a dose of 0.8 g/kg body wt, in the form of a 25% (v/v) solution (total vol.: 4 ml/kg body wt) spaced over 20 min. Subjects arrived at the Academic Unit of this hospital at 09:00 and had a 30-min bed rest, after which a venous blood sample (10 ml) was withdrawn. Immediately thereafter, the subjects started consuming the ethanol solution over the 20-min period. Further blood samples (10 ml each) were then withdrawn at 10, 20, and 30 min after the end of the 20-min drinking session. Subjects remained supine throughout the experiment. Serum was isolated immediately after venesection and frozen at –40°C,

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along with an ultrafiltrate prepared at the same time from a 1-ml portion as described below. Both the ultrafiltrates and their original sera were analysed for the laboratory parameters described below on the following day.

Laboratory and statistical procedures

Free (ultrafiltrable) and total (free + albumin-bound) serum Trp concentrations were determined fluorimetrically as described by Badawy and Evans (1976). Ultrafiltration was performed using the Amicon Micropartition MPS-1 assembly on fresh serum, to avoid the effect of freezing on Trp binding (Morgan and Badawy, 1994). The following additional parameters of importance to Trp disposition were also measured, albumin (Doumas and Biggs, 1972), the physiological binder of Trp; non-esterified fatty acids (NEFA) (Mikac-Dević *et al.*, 1973), the physiological displacers of albumin-bound Trp; and glucose (Slein, 1963), which can cause an insulin-mediated modulation of Trp entry into the brain. Results are expressed as means ± SD and were analysed statistically by one-way analysis of variance (ANOVA) for repeated measures followed by Tukey's multiple comparison test, using the STAT-100 programme (Biosoft 1995/96, 37 Cambridge Place, Cambridge CB2 1NS, UK).

RESULTS AND DISCUSSION

Previous studies

The dose of ethanol used in the present work (0.8 g/kg body wt) has previously been shown by us (Badawy *et al.*, 1987) to produce a maximum blood-ethanol concentration of 78 ± 7 mg/dl at 1 h, with a value at 30 min of 59 ± 4 mg/dl (means ± SEM for 10 subjects). The present experiments were thus conducted early during the ascending phase of the blood-ethanol concentration curve, i.e. at the time euphoria is experienced (Lukas *et al.*, 1986*a*).

In our previous investigation of Trp metabolism and disposition after acute ethanol administration to fasting male volunteers, we found (Badawy *et al.*, 1995) that ethanol (0.8 g/kg body wt) decreased free and total serum Trp concentrations over a 3 h period. The maximum decreases occurred at 1.5–2 h after ethanol intake and were 28 and 24% for free and total [Trp] respectively. At 30 min, the first time interval to be examined, the decreases were already evident (10 and 12% respectively).

Brain Trp concentration is the most important single determinant of cerebral serotonin synthesis, because the rate-limiting enzyme of this synthesis, Trp hydroxylase, is unsaturated with its Trp substrate under physiological conditions (Fernstrom and Wurtman, 1971; Carlsson and Lindqvist, 1978; Curzon, 1979). It follows therefore that peripheral factors influencing Trp availability to the brain must play important roles in cerebral serotonin synthesis. These factors include activity of liver Trp pyrrolase at the primary level (Badawy, 1977), and, at the secondary, but more immediate, level, Trp binding to albumin (Curzon, 1979) and competition with Trp by five other circulating amino acids (Val, Leu, Ile, Phe, and Tyr) collectively known as the competing amino acids (CAA) for entry into the brain (Fernstrom and Wurtman, 1971). In human studies, the most accurate predictor of changes in brain Trp, and hence 5-HT, is therefore the ratio of serum [Trp]/[CAA]. In our earlier study (Badawy *et al.*, 1995), we found that ethanol decreased both the free and total Trp ratios significantly as early as 30 min and up to the 2 h time point. Because Trp binding was not altered by ethanol and due to other considerations, we then concluded that the decreases in free and total serum [Trp] are likely to be caused by activation of liver Trp pyrrolase by acute ethanol intake.

Present study

From these earlier studies, it appears therefore that acute ethanol consumption by fasting males causes decreases in circulating Trp concentrations and availability to the brain, which are almost certain to lead to inhibition of cerebral serotonin synthesis. Thus, at no time during the 3-h observation period did we observe an increase in the serum concentrations of the serotonin precursor Trp. It could, however, be argued that an increase in circulating [Trp] could have occurred earlier than 30 min after ethanol consumption, i.e. at a time euphoria is experienced (Lukas *et al.*, 1986*a*). For this reason, the experiments whose results are shown in Table 1 were performed. Free serum and total serum Trp concentrations showed a gradual decrease during the first 30 min after consumption of

Table 1. Effects of acute ethanol consumption on concentrations of serum tryptophan, albumin, non-esterified fatty acids, and glucose in fasting male volunteers

	Time after ethanol consumption (min)			
Serum parameter	0	10	20	30
Free Trp (μg/ml)	1.21 ± 0.24	1.13 ± 0.23	1.08 ± 0.24	1.06 ± 0.23
Total Trp (μg/ml)	8.43 ± 1.32	8.03 ± 1.42	7.68 ± 1.32	7.57 ± 1.40
Free Trp (%)	14.29 ± 1.44	14.15 ± 2.17	13.99 ± 1.29	13.99 ± 1.71
Albumin (g/l)	45.8 ± 0.5	45.8 ± 0.5	45.9 ± 0.3	45.8 ± 0.5
NEFA (mM)	0.49 ± 0.07	0.36 ± 0.05	0.36 ± 0.06	0.34 ± 0.07
Glucose (mg/dl)	102 ± 2	105 ± 10	114 ± 21	117 ± 13

The above parameters were determined before, and at the time intervals indicated after, oral intake of a 0.8 g/kg body wt dose of ethanol as described in the Subjects and methods section. Values are means ± SD for three subjects. The changes observed at 30 min are quantitatively similar to those previously obtained at the same time interval with 10 other subjects (Badawy *et al.*, 1987). One-way ANOVA for repeated measures between subjects (Tukey's Multiple Comparison Test) showed no significant group (time) differences for the percentage free serum Trp or serum albumin or glucose concentrations. For free serum and total serum Trp and serum NEFA concentrations, there were also no significant group differences between the data obtained at 10 min, 20 min, and 30 min. The only significant differences were those between the zero time data and those obtained at 20 min and 30 min.

a 0.8 g/kg body wt dose of ethanol by fasting male volunteers. We showed previously (Badawy *et al.*, 1995) that the decrease at 30 min was significant, presumably because of the larger number (10) of subjects tested. The percentage free serum Trp (an expression of Trp binding to albumin) was also not significantly altered by ethanol, nor was the concentration of the Trp binder albumin (Table 1). The concentration of the physiological displacers of albumin-bound Trp, namely NEFA, was, however, significantly decreased by ethanol (Student's *t*-test) (Table 1), as noted previously by us (Badawy *et al.*, 1987) and others (Jones *et al.*, 1965; Hannak *et al.*, 1985). However, this decrease does not seem to have influenced Trp binding. The increase in serum glucose concentration by ethanol was not significant. A one-way ANOVA with repeated measures (Tukey's multiple comparison test) revealed no significant group (time) differences for the percentage free serum Trp or serum albumin or glucose concentrations. There were also no significant group differences in free serum and total serum Trp and serum NEFA concentrations at 10 min, 20 min, or 30 min after ethanol intake. The only significant differences in these three latter parameters were observed between the zero time group and those at 20 min and 30 min after ethanol consumption.

In conclusion, although only three subjects were investigated in the present experiments, their response to acute ethanol consumption was both adequate and clear-cut in demonstrating a decrease in serum tryptophan concentrations consistent with our previous findings with larger numbers of observation, with no evidence of an increase in circulating Trp availability to the brain. Although the results with this small number of subjects do not support the idea that serotonin mediates the euphoric effects of alcohol, they must be considered as preliminary and therefore still requiring confirmation and replication in a larger number of subjects, in whom euphoria needs to be assessed simultaneously with the biochemical measurements. A number of previous studies have examined the effects of administration of the serotonin precursor Trp on self-rated euphoria and mood. In one such study (Greenwood *et al.*, 1975), Trp did not induce euphoria, whereas in the others, it either enhanced (Smith and Prockop, 1962; Charney *et al.*, 1982), or exerted no effect (Lieberman *et al.*, 1982–83; Cowen *et al.*, 1985) on mood. In relation to alcohol consumption, it is difficult to consider whether euphoria is a mood state, an expression of drug reward, or both. The present results, however, suggest that, even if serotonin has a recognized role in mood regulation, it is unlikely to be involved in the euphoric effects of alcohol. Other neuronal mechanisms are therefore a more likely explanation.

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