TAURINE MODULATES CATALASE, ALDEHYDE DEHYDROGENASE, AND ETHANOL ELIMINATION RATES IN RAT BRAIN

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INTRODUCTION

Ethanol metabolism shows heterogeneity in normal individuals, which, in part, is caused by the multiple isoenzymes of both alcohol dehydrogenase (ADH; Bosron and Li, 1986; Crabb et al., 1987; Hittle and Crabb, 1988) and aldehyde dehydrogenase (ALDH; Hempel et al., 1985) and possibly also by other ethanol-metabolizing enzymes such as catalase (Gill et al., 1996), which may play a major role in brain ethanol metabolism. Inherited mutations of genes which encode for the isoenzymes of either ADH or ALDH have been implicated in susceptibility to both alcoholism and alcohol-related organ damage (Sherman et al., 1994), whereas increases in blood catalase activity, assayed in chronic abusers of alcohol, are associated with the propensity to consume large amounts of alcohol (Koechling and Amit, 1992).

Catalase is reputed to play an important role in brain ethanol metabolism, since the $K_m$ of ADH present in the brain is too high to be activated at the concentrations of ethanol achieved in the brain after either an acute dose of ethanol or chronic alcoholization (Lands, 1998). When the activity of catalase is reduced in experimental animals after administration of cyanamide or 3-amino-1,2,4-triazole (AT), certain behavioural characteristics associated with ethanol intoxication, such that acetaldehyde-induced alterations in brain neurotransmitter function might not occur (Ward et al., 1997).

It is of interest that supplementation of rats with the sulphonated amino acid taurine prior to ethanol administration, will also modify ethanol-induced behaviour, such as ethanol-conditioned taste aversion (Aragon and Amit, 1993) and hypnosis (Boggan et al., 1978; McBroom et al., 1986; Ferko, 1987). The biochemical explanations for such effects remain unknown, although their striking similarities to the modification of ethanol-induced behaviour by AT might suggest that experimentally induced catalase inhibition might be the common pathway. Therefore, in this present communication, the activity of catalase has been estimated in the liver and brain after chronic administration of either the catalase inhibitor AT or taurine supplementation. In addition, the activities of ADH and ALDH have been assayed in the livers and brains of these animals. Finally the EERs have been calculated in the liver, brain, and blood of these two treatment groups after acute administration of ethanol.

MATERIALS AND METHODS

Male Wistar rats (200–250g) bred in the breeding colony at the Catholic University of Louvain, Belgium, were maintained in a temperature and light-controlled environment (12 h light: 12 h dark cycle) with food and water available ad libitum. Rats ($n = 68$) were administered taurine in their drinking water, at concentrations of either 12.5 g/l ($n = 34$) or 6.25 g/l ($n = 34$) for a period of 2 weeks. The amount of liquid consumed each 24 h was noted. In another group of rats ($n = 68$), the catalase inhibitor AT was injected daily between 08:00 and 08:30 (0.5 or 1.0 g/kg, i.p.) to rats, ($n = 34$) each for a period of 5 days.

At the end of this period, the rats from each treatment were divided into three groups. One group ($n = 8$) was administered absorption, redistribution, and elimination will all contribute to the resultant ethanol concentration. However, if the ethanol elimination rate (EER) was reduced, particularly in the brain, together with increased ALDH activity, less acetaldehyde would be generated, which would be removed more rapidly, such that acetaldehyde-induced alterations in brain neurotransmitter function might not occur (Ward et al., 1997).

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ethanol i.p. (2 g/kg), between 10:00 and 10:30 and blood was removed from the tail vein of each rat at 30, 60, 90, and 180 min for ethanol assay. In the second group (n = 18), the rats were also administered ethanol (2 g/kg) at a comparable time of day to experiment 1, and were then anaesthetized at 30, 60, and 90 min prior to removal of blood by cardiac puncture (n = 6 for each time point), after which the rats were killed by cervical dislocation and the brain and liver removed for ethanol analysis. In the final group (n = 8), the rats were killed, the livers and brains were removed and homogenized with 0.25 M sucrose (10%, w/v), and stored at −20°C prior to analysis of catalase, ADH and ALDH activities.

AT-injected rats were killed 3 h after the last injection of AT, since this has previously been shown to be the time for the maximal reduction of catalase activity after a single dose of AT (1 g/kg; Aragon et al., 1991). Appropriate controls were studied for each of the experimental groups.

These experiments were approved by the Belgian Governmental agency order, authorized No. LA 1220028, as well as the European Communities Council Directive concerning the use of Laboratory Animals.

**Catalase activity**

Catalase activity was assayed in each of the brain and liver homogenates after dilution (1:50 and 1:1000, respectively) with 0.25 M sucrose.

The catalase assay procedure was based on the method of Peters and Batt (1976), which is a micromodification of the method of Badhuin et al. (1964). The substrate was prepared by dissolving 50 mg bovine serum albumin in 0.2 M imidazole buffer, pH 7.0. Hydrogen peroxide (0.1 ml 38% solution) and Triton X-100 (5 ml of 2% solution) were added and the substrate made up to 50 ml with the imidazole buffer. The colour complex, which reacts with residual hydrogen peroxide remaining after incubation of the sample with the buffered substrate, was titanium sulphate. This was prepared by dissolving 6.75 g titanium oxy sulphate — sulphuric acid complex hydrate (TiO(SO₄) × H₂SO₄ × H₂O) in boiling H₂SO₄, 1 M, and boiling for 2 h. At the end of this time, the solution was made up to 1 l and filtered through No. 43 Whatman paper. Prior to its use, the titanium oxy sulphate was diluted 1:1 with 1 M sulphuric acid. Catalase standards in the range of 0.013–13000 mU/ml were used in the assay. Results were expressed as mU/mg of protein.

**ADH activity**

ADH activity was assayed in the brain and liver supernatants by the method of von Wartburg et al. (1965). Essentially, an aliquot was added to glycine buffer/NaOH 0.1 M pH 10.8 which contained NAD⁺ at a final concentration of 10 mM. The reaction was started by the addition of ethanol, 0.016 M, and the rate of conversion to NADH was assayed over a period of 4 min at 340 nm. For the calculation of ADH activity, the molar extinction coefficient of NADH was utilized; i.e. 0.1 mM NADH has an optical density of 0.622. The results were expressed as µmol/min/mg of protein.

**ALDH activity**

ALDH activity was assayed in each of the brain and liver homogenates after dilution (1:10 and 1:100, respectively) with 0.25 M sucrose by the method of Gill et al. (1996). The solution for the assay was methyl pyrazole 0.2 mM, magnesium chloride 1 mM, NAD 1 mM, rotenone 2 µM, Triton X-100 1% in 50 mM sodium pyrophosphate buffer pH 8.8. After addition of the suitably diluted homogenate, the reaction was initiated by the addition of acetaldehyde, 5 mM, and the rate of production of NADH measured in a spectrophotometer at a wavelength of 340 nm at 30°C for 20 min. The molar extinction coefficient of NADH was utilized for the calculation of ALDH activity i.e. 0.1 mM NADH has an optical density of 0.622. The results were expressed as mU/mg protein.

**Alcohol concentration**

The ethanol content in blood, brain, and liver samples after acute administration of ethanol was assayed by the Boehringer kit method, where NAD⁺ is reduced to NADH in the presence of ethanol and ADH at alkaline pH. In each sample, the proteins were precipitated with trichloroacetic acid (2%, w/v) prior to the enzymatic analyses. The results for blood ethanol concentration are given as g/l while both the liver and brain are given as g/mg of protein.

**Protein analyses**

The protein content of the liver and brain homogenates was assayed by the Biorad method. Suitably diluted samples were added to the diluted Biorad reagent and the intensity of the colour measured at 595 nm. Standards within the range 10–100 µg/ml were prepared.

**Estimation of ethanol elimination**

Ethanol elimination curves were constructed for plasma, liver, and brain by taking the ethanol concentrations assayed at 30, 60, 90, and 180 min and constructing a ‘best fit line’ through these points, such that a zero time, T₀ and time for total ethanol elimination from each of these tissues was obtained. The EER Vₘₐₓ, was then calculated from each graph, and results are given as mmol of ethanol eliminated/min/kg.

**Statistical analysis**

All results are presented as mean ± SD. Statistical analysis was by ANOVA with statistical significance calculated by the Fisher LSD (protected t-test).

**RESULTS**

The initial weights of the rats (means ± SD) were 221 ± 13.9 g and 220 ± 11.9 g respectively which had increased to 261 ± 15.0 g and 254 ± 13.5 g after taurine supplementation for 2 weeks. Such increases in weight were not significantly different from the controls (221 ± 9.8 vs 251 ± 14.7). The consumption of liquid was approximately 25 ml/day in both the taurine-supplemented and control groups. The weight increase in the AT-injected rats and controls was similar (224 ± 14.3 to 241 ± 16.0 and 221 ± 9.8 and 246 ± 10.3, respectively). After 2 weeks of taurine supplementation, the rats administered the higher taurine dose achieved intakes of 0.62 ± 0.08 g taurine/kg/day while the lower supplemented rats’ intakes were 0.31 ± 0.04 g taurine/kg/day; the plasma taurine concentration also increased in a dose-dependent manner, 230 ± 17 µmol/l vs 184 ± 11 µmol/l, respectively, in comparison to controls, 168 ± 17 µmol/l.
Catalase activity

*In vitro* incubation of the catalase standards, range 0.00013–130 mU/ml, with AT (20 μg), for 30 min before the assay for the standard curve inhibited the enzyme activity dramatically, whereas incubation with taurine (10 μg) for a comparable time period decreased the catalase activity only marginally (data not shown).

In the control rats, catalase activity was over fourfold higher in the liver than in the brain (Table 1), which is comparable to other studies where the same tissues were assayed for their catalase activities by the release of oxygen with an oxygen electrode, after addition of hydrogen peroxide. After administration of AT at dose levels of 0.5 and 1.0 g/kg for 5 days, brain catalase activity was decreased significantly in a dose-dependent manner, by 65% and 84% respectively (Table 1). Aragon and Amit (1993) found a similar decrease (70 and 81%, respectively) in perfused rat brains after five daily injections of 0.5 and 1.0 g/kg AT. In the liver, an even more dramatic decrease of catalase activity was observed with these two doses of AT.

The higher dose of taurine (0.62 g/kg for 2 weeks) significantly decreased catalase activity in both the brain and the liver, with the former exhibiting the greatest fall of 86%, whereas hepatic catalase activity was reduced by approximately 25% by this dose of taurine (Table 1).

ADH activity

Total hepatic activity of ADH was not altered significantly after either dose of AT (13.7 ± 2.6 and 15.1 ± 4.0 mU/min/mg protein for 0.5 and 1.0 g/kg respectively) or taurine (22.1 ± 1.1 and 30.0 ± 6.6 mU/min/mg protein for 0.31 and 0.62 g/kg/2 weeks respectively) when compared to the controls (21.03 ± 11.1 mU/min/mg protein), where the range of results was wide. In the brain, ADH activity was 4000-fold lower than that of the liver, and no significant differences were apparent between the different treatment groups and controls (data not shown).

Total ALDH activity

The activity of ALDH increased significantly (*P* < 0.01) in the brain after administration of 0.31 g/kg taurine, and also with both doses of AT (*P* < 0.05) (Table 2). No significant alteration of the liver enzyme was observed, although there was a tendency for the activity of the enzyme to decrease after AT administration and to increase after taurine supplementation (Table 2).

EER

Administration of either 0.5 or 1.0 g/kg AT reduced the EER in the plasma in a dose-dependent manner, reaching significance with the higher (1 g/kg) dose of AT (Table 3). Taurine supplementation had little effect on the EER in the plasma (Table 3). In the brain *T₀* was significantly lower in the AT rats after administration of 2 g/kg ethanol (Fig. 1). In addition, the EERs were reduced in both the AT and taurine-treated rats in comparison to the controls (Fig. 1). In the liver, *T₀* was significantly lower in the AT group in comparison to the taurine-supplemented and control groups. The EER was again significantly reduced in the AT rats, while, in contrast, the taurine-supplemented rats showed a marked increase in EER (Fig. 1).

<table>
<thead>
<tr>
<th>Table 1. Catalase activity in rat brain and liver after AT administration or taurine supplementation</th>
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<tr>
<td>Brain</td>
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<td>-------</td>
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<tr>
<td>(mU/mg of protein)</td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>AT</td>
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<tr>
<td>0.5 g/kg</td>
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<tr>
<td>1.0 g/kg</td>
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<tr>
<td>Taurine</td>
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<tr>
<td>0.31 g/kg</td>
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<tr>
<td>0.62 g/kg</td>
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<tr>
<td>For details, see Materials and methods. AT, 3-amino-1,2,4-triazole; significance by ANOVA with Fisher test: <em>P</em> &lt; 0.05; **P &lt; 0.01; <em>n</em> = 8/group.</td>
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<th>Table 2. Aldehyde dehydrogenase activity in rat liver and brain after administration of AT or taurine</th>
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<td>Brain</td>
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<td>(mU/mg of protein)</td>
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<td>0.5 g/kg</td>
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Table 3. Ethanol elimination rates (EER) in taurine-supplemented and AT treated rats after i.p. injection of 2 g ethanol/kg

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EER (mmol/kg/min)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.552 ± 0.088</td>
</tr>
<tr>
<td>AT</td>
<td>0.500 ± 0.084</td>
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<tr>
<td>0.5 g/kg</td>
<td>0.488 ± 0.052</td>
</tr>
<tr>
<td>1.0 g/kg</td>
<td>0.384 ± 0.088*</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.500 ± 0.084</td>
</tr>
<tr>
<td>0.62 g/kg</td>
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AT, 3-amino-1,2,4-triazole; significance by ANOVA with Fisher test: *P < 0.05; n = 8 rats/group.

Fig. 1. Effects of taurine and 3-amino-1,2,4-triazole (AT) on ethanol elimination rates (EER) in brain and liver of rats.

EERs were calculated in the brain (upper half) and the liver (lower half) after i.p. administration of 2 g/kg ethanol to controls, rats supplemented for 2 weeks with 0.62 g taurine/kg or daily injection of AT, 0.5 g/kg, for 5 days; n = 8 rats/group.

Discussion

There has been considerable interest in the role of catalase in the development of many aspects of alcoholism: alterations in its activity cause many different effects (as reviewed in the Introduction), although the exact biochemical and molecular basis for such changes is unknown. Taurine supplementation will similarly alter many ethanol-induced effects, such that, in the present study the effect of taurine supplementation on the activities of catalase as well as ADH and ALDH have been investigated. The results obtained were compared to those of rats in which catalase activity had been inhibited by AT administration for 5 days, and also to controls.

Oral supplementation with taurine at a dosage of 0.62 g/kg for 2 weeks reduced catalase activity in the brain to a similar extent as AT, although in the liver the reduction was not so large (only to 64% of the controls). It is not known how taurine decreases catalase activity; our in vitro studies showed that, in contrast to AT (which rapidly produces an irreversibly inhibited enzyme, catalase–H₂O₂), taurine has no direct effect upon catalase activity. Whether such excess taurine could form a complex with hypochlorous acid, present in both macrophages and glial cells, to form N-chlorotaurine, which in turn could deplete the cells of hydrogen peroxide, and thereby the stimulus for catalase synthesis, remains unknown.

The hepatic activity of ADH was not altered significantly by either taurine or AT administration. Although a high pH was used in the assay buffer, which might preclude the detection of minor forms of ADH, the major isoenzymes responsible for ethanol metabolism in the liver would have been quantified. Other studies where ethanol metabolism has been investigated after AT administration have also inferred normal ADH activity, since plasma ethanol concentrations were not altered (Aragon et al., 1985, 1989, 1992). Lower doses of taurine (0.4 mM/kg and 2.4 mM/kg), when administered either orally or i.p. to rats, did not significantly alter ADH activity (Theofanopoulos and Lau-Cam, 1998). Brain ADH activity was 4000-fold less than that of liver, which is attributable to the isoform of ADH present, i.e. ADH3. With the method used in this present study, there were no significant changes in the mean activity of ADH in the brains of controls, AT- or taurine-supplemented rats.

Previous studies have indicated that AT reduces acetaldehyde production (Aragon et al., 1992) in vitro, which could be caused by changes in the activities of the different ALDH isoenzymes. However, the hepatic activity of this enzyme did not alter in the AT-treated rats. In contrast, there was a tendency for the activity of this enzyme to increase in the liver of taurine-supplemented rats, although this was not significant. Previous studies by Watanabe et al. (1985) showed that liver and blood acetaldehyde concentrations were significantly reduced when rats were pretreated orally with taurine (0.5 g/kg), which was attributable to the elevation of ALDH activity. Interestingly, both doses of AT and the lower dose of taurine significantly increased the activity of ALDH in the brain. Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton et al., 1977), which may be one of the important factors in modifying ethanol-induced behaviour.

Clearly AT interfered with ethanol absorption, the calculated T₀ was significantly lower in both the liver and brain. However, despite the lower ethanol concentration, there was still a significant decrease in EER in both of these tissues and also in the plasma. This latter result is in contrast to previous studies which investigated EERs in AT-administered rats (Aragon et al., 1989, 1992), but since assessment was only during the first 30 min after ethanol administration, contributions from the absorptive and distribution phase of alcohol...
kinetics would also be present. Previous studies also showed that catalase plays a role in ethanol metabolism; deermice lacking ADH, were still able to eliminate ethanol up to 60% as fast as deermice with ADH activity (Handler et al., 1986).

Taurine supplementation over a 2-week period did not significantly alter the EER in the plasma. In the study by Theofanopoulos and Lau-Cam (1998), who administered lower doses of taurine (55 and 10 mg/kg, 15 min before an acute ethanol injection), a decrease in blood-ethanol concentration was observed, indicating an increased plasma EER. Analysis of liver ethanol content in the taurine-supplemented rats in the present study did indicate an increase in EER in the liver. The explanation for this is unclear; it is unlikely that taurine would induce the microsomal-ethanol oxidizing system, although this must be confirmed in future studies. In the brains of taurine-supplemented or AT-administered rats, the EERs were decreased, which could be responsible for some of the ethanol-induced behavioural modifications induced by these two compounds.

The present study has identified a further biochemical property of taurine, an alteration in catalase activity, to add to its multitude of functions that include osmoregulation and modification of calcium availability. Further studies are currently in progress to identify the biochemical basis for the taurine-induced inhibition of catalase activity.

REFERENCES
