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Effects of Taurine Supplementation on Mitochondrial Function in Chronic Ethanol Administered Rats

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ABSTRACT

The present investigation was undertaken in vivo to determine whether the functional alterations of hepatic mitochondria induced by ethanol might be prevented by taurine. We examined the effects of supplementation of taurine on hepatic mitochondrial oxidative phosphorylation in the chronic ethanol-administered rats. Isolated hepatic mitochondria from three groups of rats were functionally tested by an analysis of β-hydroxbutyrate-supported respiration and the coupling of this process to ATP synthesis in the presence of ADP. The three groups were control group (CO), ethanol (60g/L) administered group (AL), and ethanol (60g/L) + taurine (5g/L) supplemented group (AT). Ethanol and/or taurine were given in drinking water for 10 weeks. The mitochondria from AL group had lower state 4 respiratory rate, respiratory control (RC) ratio and ADP : O (P/O) ratio than those from CO and AT group. It showed that the ethanol administered rats were less coupled and thus less efficient with respect to mitochondrial ATP synthesis than both control rats and ethanol + taurine supplemented rats. It suggests that taurine supplementation might improve the impaired oxidative phosphorylation efficiency in mitochondrial dysfunction that is recognized as a cause of liver diseases in chronic ethanol consumption. (*J Community Nutrition* 7(3) : 163~168, 2005)

KEY WORDS : taurine · oxidative phosphorylation · ethanol · liver · mitochondria.

Introduction

Ethanol ingestion produces a wide variety of pathological disturbances affecting a number of organs. Being a small molecule and soluble in both water and lipids, ethanol permeates all tissues of the body and affects most vital functions of virtually all organs including liver, kidney, brain, heart and pancreas (Lieber 1995).

Chronic intake of large quantities of ethanol is associated with marked alterations in mitochondrial structure and function, both in humans and in experimental animals. The most prominent site of ethanol-induced mitochondrial changes is the liver (Piquet et al. 2000 ; Tabouy et al. 1998). Abnormalities in mitochondrial morphology, e.g. deformed and enlarged mitochondria, often with paracrystalline inclusions, are evident in liver, muscle and other tissue (Marcinkeviciute et al. 2000). Along with these cytological changes a dramatic decrease in key mitochondrial inner membrane enzymes occurs. Some of these enzymes participate in electron movement through the electron transport chain and in it's coupling to oxidative phosphorylation (OXPHOS) to produce ATP. One of the chief functions of mitochondria is to supply the cell with ATP made via OXPHOS (Wallace 1994). Mitochondrial ATP production via OXPHOS is essential for normal function and maintenance of human organ systems (Matschinsky 1996). Slightly decreased efficiency of OXPHOS might result in a small but significant decrease in the amount of ATP. If there is a reduction in the amount of ATP available for ATP-dependent cell functions, that cell's function will be impaired.

Recently, there are various known and proposed roles for taurine. Taurine (2-amino ethane sulfonic acid) is a sulphur containing amino acid present in many tissues of man and animals. Conjugation with bile acids, neurotransmitter and

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reaction with certain xenobiotics are its firmly established functions (Park 2001). Taurine has protective properties also when administered therapeutically. Supplementation studies have documented antihypertensive, antidiabetic and antioxidative properties of taurine (Dawson et al. 2000 ; Green et al. 1991 ; Trachtman et al. 1995). Recent studies have implicated taurine as an inhibitor of hepatic damage induced by chronic ethanol consumption (Balkan et al. 2002 ; Dogru-Abbasoglu et al. 2001 ; Kerai et al. 1998 ; Kerai et al. 1999).

In turn, it is suggested that taurine may improve hepatic mitochondrial function in chronic ethanol consumption. The present investigation was undertaken in vivo to determine whether the functional alterations of liver mitochondria induced by ethanol might be prevented by taurine.

Materials and Methods

1. Animals and diets

Twenty-four female Sprague-Dawley rats (140 − 150g) were divided into three groups (8/group) : control group (CO), ethanol (60g/L) administered group (AL), and ethanol (60g/L) + taurine (5g/L) supplemented group (AT). The rats were fed AIN-76 diet for 10 weeks. Ethanol and/or taurine were given in drinking water for 10 weeks (Chang 1999 ; Kim, Park 2002 ; Park, Choi 1997). Differences in food intake and water intake were corrected by weighing food intake and by measuring water intake, which occurred over the 24-hour period. The rats were housed individually in hanging, wire mesh cages in a room in which room temperature (21 ± 1℃), humidity (45 − 50%), and lighting (lights on 06 : 00 AM to 6 : 00 PM) were controlled. Rats were weighed every week.

2. Mitochondrial preparations

The rats were euthanized by decapitation and the livers were quickly excised, chilled in Tris-buffered (pH 7.2) 0.25 M sucrose, and weighed. Mitochondria were prepared by the procedure of Johnson, Lardy (1967). The liver was homogenized in cold, Tris-buffered 0.25M sucrose and the mitochondria were isolated from the homogenate by differential centrifugation. The mitochondria were then washed and re-suspended three times. After the final wash, the mitochondria were re-suspended in the buffer. Mitochondrial protein content was determined by the Biuret method using bovine serum albumin as a standard.

3. Determination of oxidative phosphorylation

Oxygen consumption was determined with oxygen meter (Yellow Springs Instrument Co., Yellow Springs, USA). The reaction chamber was fitted with a magnetic stirrer and temperature was controlled at 25℃. Respiration buffer (75mM glycine ; 10mM phosphate buffer, pH 7.4 ; 75mM KCl ; 5 mM $MgSO_4$; 10mM Tris-HCl, pH 7.2) was pre-equilibrated with air by shaking in a water bath at 25℃ and introduced into the chamber by syringe (Trounce et al. 1994). All subsequent additions to the chamber were made with Hamilton syringes passed through the capillary on top. The 14mM β-hydroxbutyrate was stored frozen (−80℃) in small aliquots. In a typical experiment, freshly isolated mitochondria were added to the chamber containing respiration buffer and β-hydroxbutyrate. After 2 min, ADP was added to stimulate state 3 respiration. State 3 (O_2 consumed by isolated mitochondria upon addition of ADP) and state 4 (O_2 consumed once all the added ADP is phosphorylated to ATP) oxygen consumption rates were calculated according to Chance, Williams (1955) using assumptions from Reynafarje et al. (1985). Respiratory control (RC) ratios, a criterion useful in evaluating coupling, were calculated as the ratio of oxygen consumption rate for state 3 to that for state 4. The ADP : O (P/O) ratios were calculated as the amount of added ADP to the amount of oxygen used during the state 3 respiration. Subsequently, ATP contents were calculated as P/O × state 3 respiration (Estabrook 1967).

4. Statistical analysis

SAS was used for statistical analysis. All values are expressed as group mean values with their standard error. Three group means were compared using ANOVA. Significance of the difference among groups was determined by Duncan's multiple range test at a probability level of 0.05.

Results

The body weight, liver weight and relative liver size (RLS) are presented in Table 1. The effects of ethanol ingestion on body weight and liver weight were observed. The ethanol administered group (AL) and ethanol + taurine supplemented group (AT) gained less body weight and had less liver weight than the control group (CO). However, there were no differences in relative liver size (RLS) among the three groups. This is a typical response to alcohol. The effects of taurine sup-

Table 1. Effects of alcohol and/or taurine on body weight, liver weight and RLS

	Control	Alcohol	Alcohol + taurine
Final body weight(g)	371 ± 3.89[a]	349 ± 4.30[b]	349 ± 4.22[b]
Gained body weight(g)	248 ± 3.87[a]	227 ± 4.04[b]	227 ± 4.00[b]
Liver weight(g)	10.4 ± 3.87[a]	9.8 ± 0.31[b]	9.5 ± 0.20[b]
RLS[1)](%)	2.81 ± 0.04[a]	2.51 ± 0.09[a]	2.73 ± 0.04[a]

All values are Mean ± SE(n = 8), values with different superscripts are significantly different($p < 0.05$)
1) RLS, relative liver size(liver weight/body weight) × 100

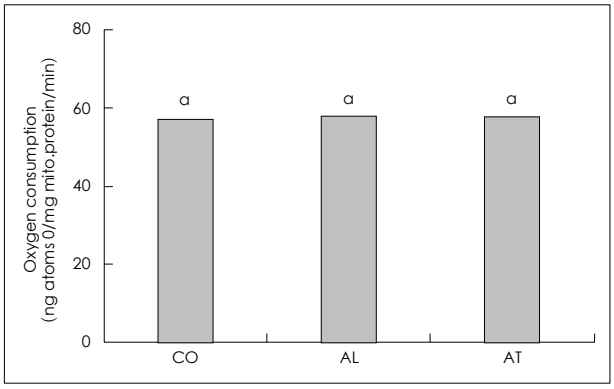

Fig. 1. Effects of taurine supplementation on state 3 respiration of hepatic mitochondria in chronic ethanol administered rats. Each bar with different letter is significantly different($p < 0.05$). CO : control group ; AL : alcohol administered group ; AT : alcohol + taurine supplemented group, n = 8.

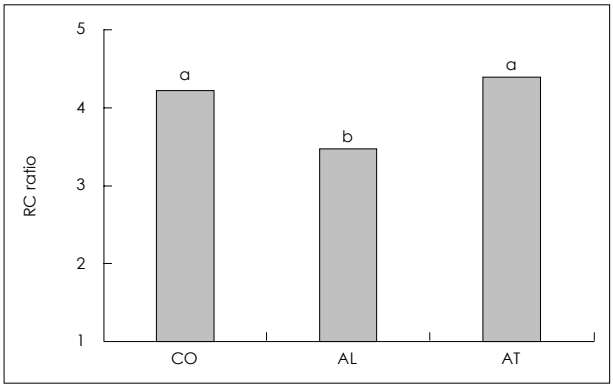

Fig. 3. Effects of taurine supplementation on respiratory control (RC) ratio of hepatic mitochondria in chronic ethanol administered rats. Each bar with different letter is significantly different ($p < 0.05$). CO : control group ; AL : alcohol administered group ; AT : alcohol + taurine supplemented group, n = 8.

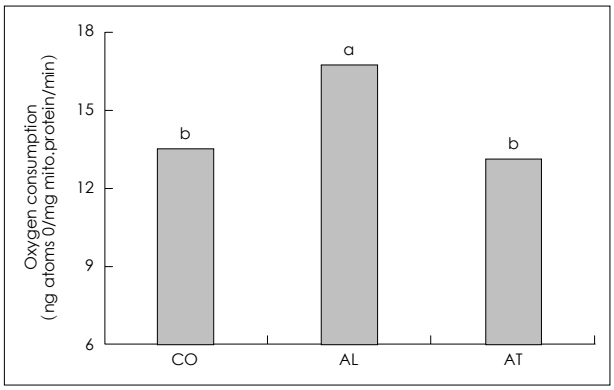

Fig. 2. Effects of taurine supplementation on state 4 respiration of hepatic mitochondria in chronic ethanol administered rats. Each bar with different letter is significantly different($p < 0.05$). CO : control group ; AL : alcohol administered group ; AT : alcohol + taurine supplemented group, n = 8.

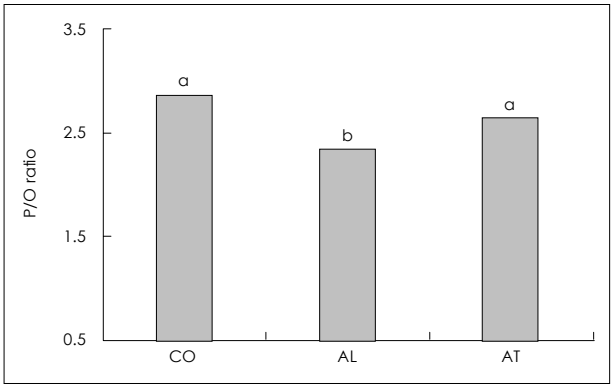

Fig. 4. Effects of taurine supplementation on ADP : O(P/O) ratio of hepatic mitochondria in chronic ethanol administered rats. Each bar with different letter is significantly different($p < 0.05$). CO : control group ; AL : alcohol administered group ; AT : alcohol + taurine supplemented group, n = 8.

plementation on body weight and liver weight were not observed.

Mitochondrial respirations are presented in Fig. 1−5. In control group, the state 3 respiratory rate, the state 4 respiratory rate, the RC ratio, P/O ratio and ATP contents were similar to those previously reported (Kim 1999 ; Kim, Kim 2000) and were an evidence of well prepared mitochondria. With respect to state 3 respiratory rate, there were no differences in oxygen consumption among the three groups (Fig. 1). However, with respect to state 4 respiratory rate (Fig. 2) mitochondria from AL group consumed significantly more oxygen than those from CO and AT group. It means that the mitochondria from AL group had a lower state 4 respiratory rate than those from CO and AT groups. This led to a difference in the calculated RC ratio (Fig. 3). The mitochondria from AL group had a lower RC ratio than those from CO and AT groups. P/O ratio (Fig. 4) was lower in the mitochondria from AL group than were those from CO and AT groups. Characteristically, there were significant differences in ATP

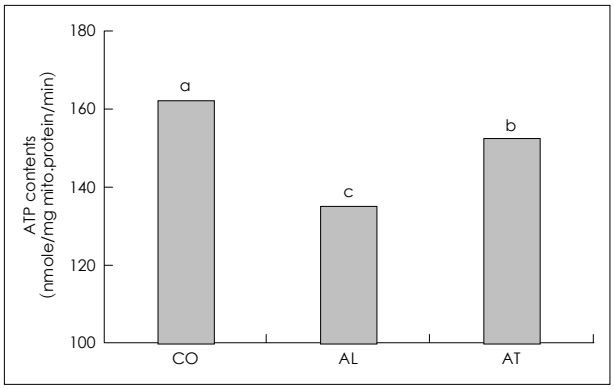

Fig. 5. Effects of taurine supplementation on ATP contents of hepatic mitochondria in chronic ethanol administered rats. Each bar with different letter is significantly different($p < 0.05$). CO : control group ; AL : alcohol administered group ; AT : alcohol + taurine supplemented group, n = 8.

contents among the three groups (CO>AT>AL) (Fig. 5).

Discussion

Hepatic mitochondria are one of the main targets for ethanol-induced injury. One of the chief functions of mitochondria is to supply the cell with ATP made via OXPHOS. The present investigation was undertaken in vivo to determine whether the functional alterations of liver mitochondria induced by ethanol might be prevented by taurine. OXPHOS of hepatic mitochondria isolated from control rats, ethanol administered rats and ethanol + taurine supplemented rats. State 3 and state 4 respiratory rate, RC ratio, P/O ratio and ATP contents were generated.

There were the effects of ethanol administration on decreasing mitochondrial OXPHOS. Mitochondria from the ethanol administered rats showed a lower state 4 respiratory rate as compared to control rats and ethanol + taurine supplemented rats. This finding indicated that chronic ethanol consumption decreases state 4 energy wastage. This led to a difference in the calculated RC ratio and P/O ratio. The differences in the state 4 respiratory rate, RC ratio, and the P/O ratio showed that the mitochondria from ethanol administered rats were more uncoupled and less efficient with respect to mitochondrial ATP synthesis than the mitochondria from control rats and ethanol + taurine supplemented rats.

The question of a change in the efficiency of ethanol-induced oxidative phosphorylation is important for understanding the pathophysiology of alcoholic liver disease but also for the general understanding of the mechanisms of regulation of ATP synthesis and yield. In this study, slightly decreased efficiency of ATP synthesis resulted in a small but significant decrease in the amount of ATP (Fig. 5). If there is a reduction in the amount of ATP available for ATP-dependent cell function, that cell's function will be impaired. Disturbances in ATP synthesis are accompanied by disturbances in lipid metabolism (Berdanier et al. 1979). Ethanol administered rats are characterized by elevated blood lipid levels and by fatty livers (Berdanier 1982 ; Lakshmanan et al. 1977). Our study suggests that decreased efficiency of ATP synthesis due to ethanol could have relevance to the characteristics of liver dysfunction.

Two main mechanisms of energy waste have been reported, namely, a proton leak across the mitochondrial inner membrane or a decrease in proton-pump efficiency. Both proton pumps and mitochondrial inner membrane are responsible for the fine-tuning of oxidative phosphorylation (Marcinkeviciute et al. 2000 ; Piquet et al. 2000). In this study, ethanol administration showed disturbance in active proton conductance, indicated by lower RC ratios. It was demonstrated that the defect in ethanol administered rat resided in active proton conductance, indicating defective ATP synthase (F_1F_0ATPase). The membrane of the mitochondrion contains phospholipids that are specific to the particular membrane and may affect its function of the various proteins embedded in it (Cross, Duncan 1996). This is probably of importance to the F_0 which must have some degree of flexibility within its lipid environment for ATP synthase characteristics. The change in structure of the mitochondrial membrane could alter the properties of ATP synthase (Kim, Kim 2000). Ethanol by its property of generating free radicals during the course of its metabolism causes damage to cell structure and function (Pushpakiran et al. 2004). Free radical induced damage to membrane is associated with increased permeability to ions and water. Ethanol has been shown to reduce ionic transfer through alterations in the monovalent cation pump and the antiport system (Guiet-Bara et al. 1995). This study gives clear indication that the decreased efficiency of ATP synthesis of the defects in ATP synthase, which in turn accounts for uncoupling, has relevance to the characteristics of liver dysfunction.

This study was undertaken to determine the role of taurine in ethanol-fed rats. The results of the present work were of interest because they documented differences between mitochondria from ethanol administered rats and mitochondria from ethanol + taurine supplemented rats. This result showed

that there was an improvement in mitochondrial function for taurine supplemented rat in ethanol induced uncoupling. However, characteristically, the mitochondria from ethanol + taurine supplemented rats could not be compensated and the responsiveness of the mitochondria from ethanol + taurine supplemented rats to induce inefficiency of ATP synthesis was far and above that observed in the mitochondria from control rats. This is manifested in ATP contents (Fig. 5). Slightly decreased efficiency of OXPHOS resulted in a small but significant decrease in the amount of ATP. The main findings in this work are the functional alterations of liver mitochonria induced by ethanol may not be prevented but may be improved by taurine.

Various workers have shown that taurine has protective properties in vivo in several different organs, particularly the liver. Taurine administration may contribute to protect cell structures by avoiding ion overloading and the subsequent water accumulation (Pasantes, Cruz 1985). It is also evidenced that taurine exerts a restorative effect on hepatic lipids and attenuates oxidative stress, reverses fatty liver and hepatic lipid peroxidation in liver in ethanol fed rats (Balkan et al. 2002 ; Kerai et al. 1998 ; Kerai et al. 1999). We speculated that an antioxidant effect and/or membrane stabilization were responsible. However, the mechanism underlying these protective effects is not clear. Much further work is required in order to determine the bases for the protective effects of taurine and its importance in susceptibility to toxicity.

Summary and Conclusion

The present investigation was undertaken to determine in vivo whether the functional alterations of liver mitochondria induced by ethanol might be prevented by taurine. We examined the effects of supplementation of taurine on hepatic mitochondrial oxidative phosphorylation in the chronic ethanol-administered rats. Isolated hepatic mitochondria from 3 groups of rats were functionally tested by an analysis of β-hydroxbutyrate-supported respiration and the coupling of this process to ATP synthesis in the presence of ADP. The three groups were control group (CO), ethanol (60g/L) administered group (AL), and ethanol (60g/L) + taurine (5g/L) supplemented group (AT). The results are as follows :

1) The mitochondria from AL group had a lower state 4 respiratory rate than those from CO and AT group.

2) The mitochondria from AL group had a lower RC ratio than those from CO and AT group.

3) The mitochondria from AL group had a lower P/O ratio than those from CO and AT group.

4) There were significant differences in ATP content among the three groups (CO > AT > AL).

This result showed that there was an improvement in mitochondrial function for taurine supplemented rat in ethanol induced uncoupling, although, in ATP contents, the mitochondria from ethanol + taurine supplemented rats could not be compensated to the level of control rats. It suggests that taurine supplementation might improve impaired OXPHOS efficiency in mitochondrial dysfunction that is recognized as an important cause of ethanol induced liver disease.

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