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Decreases in taurine levels induced by β-alanine treatment did not affect the susceptibility of tissues to lipid peroxidation

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Summary. We aimed to investigate the effect of decreased taurine levels on endogenous and induced lipid peroxide levels in liver, brain, heart and erythrocytes as well as prooxidant and antioxidant balance in the liver of rats administered β-alanine (3%, w/v) in drinking water for 1 month to decrease taurine levels of tissues. This treatment caused significant decreases in taurine levels of liver (86%), brain (36%) and heart (15%). We found that endogenous and ascorbic acid-, NADPH- and cumene hydroperoxide-induced malondialdehyde (MDA) levels did not change in the liver, brain and heart homogenates following β-alanine treatment. Also, H_2O_2-induced MDA levels remained unchanged in erythrocytes. In addition, we did not observe any changes in levels of MDA, diene conjugates, glutathione, α-tocopherol, ascorbic acid and the activities of superoxide dismutase, glutathione peroxidase and glutathione transferase in the liver. According to this, buffering or sequestering capacity of tissues to exogenous stimuli was not influenced by reduced taurine levels in tissues of rats.

Keywords: Taurine – β-Alanine – Lipid peroxidation – Antioxidants – Rats

Introduction

Taurine (2-aminoethanesulfonic acid) is the major intracellular free β-amino acid, which is normally present in most mammalian tissues (Hansen, 2001). Although taurine is not a constituent of any structural mammalian protein, it has various important physiological roles such as antioxidant, osmoregulator, membrane stabilizator, neurotransmitter (Schaffer et al., 2003). Taurine was reported to have protective properties when administered therapeutically. Supplementation studies have documented antiatherogenic (Murakami et al., 1999; Balkan et al., 2002), antidiabetic (Hansen, 2001), neuroprotective (Wu et al., 2005), cardioprotective (Chang et al., 2004) and hepatoprotective (Erman et al., 2004; Hagar, 2004; Balkan et al., 2005) properties of taurine. The beneficial effects of taurine have been attributed to its antioxidant ability (Balkan et al., 2002, 2005; Schaffer et al., 2003; Hagar, 2004; Pushpakiran et al., 2004). Since taurine is an important intracellular antioxidant, its deficiency is believed to augment cellular oxidative stress (Dawson et al., 1999; Messina and Dawson, 2000). However, there is a few knowledge about the effect of decreased taurine levels on oxidative stress (Eppler and Dawson, 2000; Kerai et al., 2001; Golubnitschaja et al., 2003). Therefore, we wanted to investigate the effect of decreased taurine levels on endogenous and induced lipid peroxide levels in liver, brain, heart and erythrocytes as well as prooxidant and antioxidant balance in the liver of rats administered β-alanine (3%, w/v) in drinking water for 1 month to decrease taurine levels of tissues.

Materials and methods

Animals and treatments

Male Wistar rats of 180–200 g body weight were used in this study. They were obtained from Center for Experimental Medical Research Institute of Istanbul University. The animals were allowed free access to food and water and were kept in wire-bottomed stainless steel cages. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of İstanbul. Rats were divided into two groups (n = 6 in each group): 1) The first group was controls. 2) The second group was given β-alanine (Sigma; USA) in drinking water (3%, w/v) for 4 weeks to decrease taurine levels. At the end of treatment period of 1-month, the animals were fasted overnight. Heparinized blood was taken by heart puncture under ether anaesthesia, and liver, brain and heart of rats were quickly removed.

Methods

To determine taurine contents of liver, brain and heart tissues were homogenized in ten volumes of 3% sulfosalisilic acid. After centrifugation, the

supernatant was filtered through 0.45 μM filter and finally was determined with Hewleet Packard amino acid analyzer (Chen et al., 2004). Some part of tissues were homogenized in ice-cold 1.15% KCl in Ultraturrax homogenizer for the determination of the susceptibility of tissues to lipid peroxidation and induced lipid peroxide levels were assayed in tissue homogenates (10%, w/v) in three different media (Devasagayam and Tarachand, 1987). The incubation mixture (1 ml) for the estimation of ascorbate-induced lipid peroxidation, contained 50 μM $FeSO_4$, 1 mM KH_2PO_4, 0.15 M Tris-HCl buffer (pH 7.4), 0.4 mM ascorbic acid and 0.1 ml tissue homogenate. For NADPH-induced system, 50 μM $FeCl_3$ instead of $FeSO_4$, and 4 mM ADP were included and 0.4 mM NADPH was added instead of ascorbic acid. For cumene hydroperoxide-induced system, 0.05 M Tris-HCl buffer (pH 7.4) and 0.1 ml tissue homogenate were included in final mixture (1 ml). After incubation at 37 °C in a shaking water bath for 30 min, the amount of malondialdehyde (MDA) formed was estimated according to Buege and Aust (1978). Erythrocyte susceptibility to lipid peroxidaton was determined according to the method of Stocks et al. (1972). The final composition of the incubation mixture was 5 mM H_2O_2, 2 mM sodium azide and erythrocyte suspension in phosphate-buffered saline (30 mg Hb/ml incubation mixture). Lipid peroxidation was asseyed by measurement of MDA production during 2 h incubation period at 37 °C. Values were expressed as nanomoles of MDA per gram of Hb. Hb concentration of erythrocytes suspensions was measured by Drabkin's reagent (Bauer et al., 1974).

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities and prooxidant and antioxidant status were also measured in the liver of β-alanine treated rats. ALT and AST activities were determined in the plasma by using Roche autoanalyser. The degree of lipid peroxidation was assessed by two different methods in the liver. First, the levels of malondialdehyde (MDA) were measured by thiobarbituric acid test (Ohkawa et al., 1979). Second, diene conjugate levels were determined in hepatic lipid extracts at 233 nm spectrophotometrically and calculated using a molar extinction coefficient of $2.52 \times 10^4\ M^{-1}\ cm^{-1}$ (Buege and Aust, 1978). Liver glutathione (GSH) levels were measured with 5,5-dithiobis-(2-nitrobenzoate) at 412 nm (Beutler et al., 1963). α-tocopherol and ascorbic acid levels were measured in liver homogenates by the methods of Desai (1978) and Omaye et al. (1979) respectively. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) activities were determined in postmitochondrial fraction, which was separated by centrifugation of liver homogenates at $10000 \times g$ for 20 min. SOD activities were assayed by its ability to to increase the effect of riboflavin-sensitized photooxidation of orthodianisidine (Mylorie et al., 1986). GSH-Px (Lawrence and Burk, 1976) and GST (Habig and Jacoby, 1981) activities were measured using cumene hydroperoxides and 1-chloro-2,4-dinitrobenzene as substrates, respectively. Protein levels were determined using bicinchoninic acid (Smith et al., 1985).

Statistical analyses

The results were expressed as mean ± SD. Statistical analysis was performed by Student's t-test.

Results

a) β-alanine treatment caused significant decreases in taurine contents of liver (86%), brain (36%) and heart (15%). The decrease in taurine levels was detected mostly in the liver (Fig. 1).

b) Endogenous and ascorbic acid-, NADPH- and cumene hydroperoxide-induced MDA levels in the liver, brain and heart homogenates did not change following β-alanine treatment. Similarly, H_2O_2-induced MDA levels did not alter in the erythrocytes due to β-alanine treatment (Fig. 2).

c) There were no significant changes in plasma ALT and AST activities and the levels of MDA, DC, GSH, α-tocopherol, ascorbic acid and the activities of SOD, GSH-Px and GST in the liver of taurine-depleted rats as compared to control rats (Table 1).

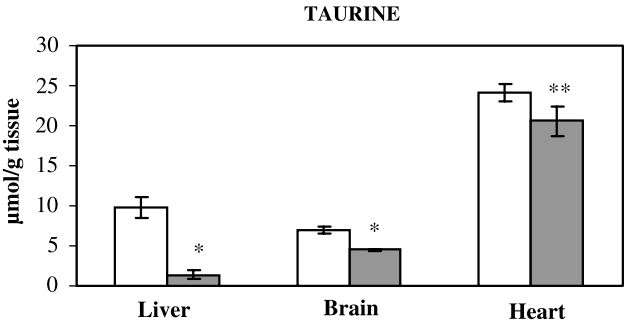

Fig. 1. Taurine levels of liver, brain and heart of control (□) and β-alanine-treated (■) rats (mean ± SD; n = 6 each; $^*p < 0.001$; $^{**}p < 0.01$)

Discussion

β-Alanine is a well known antagonist of taurine transport which produces depletion of tissue taurine levels, since both amino acids share a common transport system, selective for β-amino acids (Shaffer and Kocsis, 1981). However, β-alanine treatment at the similar dose and duration was found to decrease taurine concentrations in different percentages in various tissues (Kerai et al., 2001; Dawson et al., 2002). In the present study, we found that the administration of 3% β-alanine in the drinking water to rats for 1 months decreased taurine levels in the liver (86%), brain (36%) and heart (15%). Our results were very similar to those observed by the previous studies (Kerai et al., 2001; Dawson et al., 2002).

Taurine has demonstrated to act as an antioxidant (Balkan et al., 2002, 2005; Schaffer et al., 2003; Hagar, 2004; Pushpakiran et al., 2004). It has been proposed that severe decreases in taurine levels of tissues may influence their antioxidant power and susceptibility to oxidative damage (Dawson et al., 1999; Messina and Dawson, 2000). The heart, brain and liver have high levels of taurine (Dawson et al., 1999). Since the effectiveness of antioxidant defence system in heart and brain is weaker than liver, taurine may especially gain importance as an

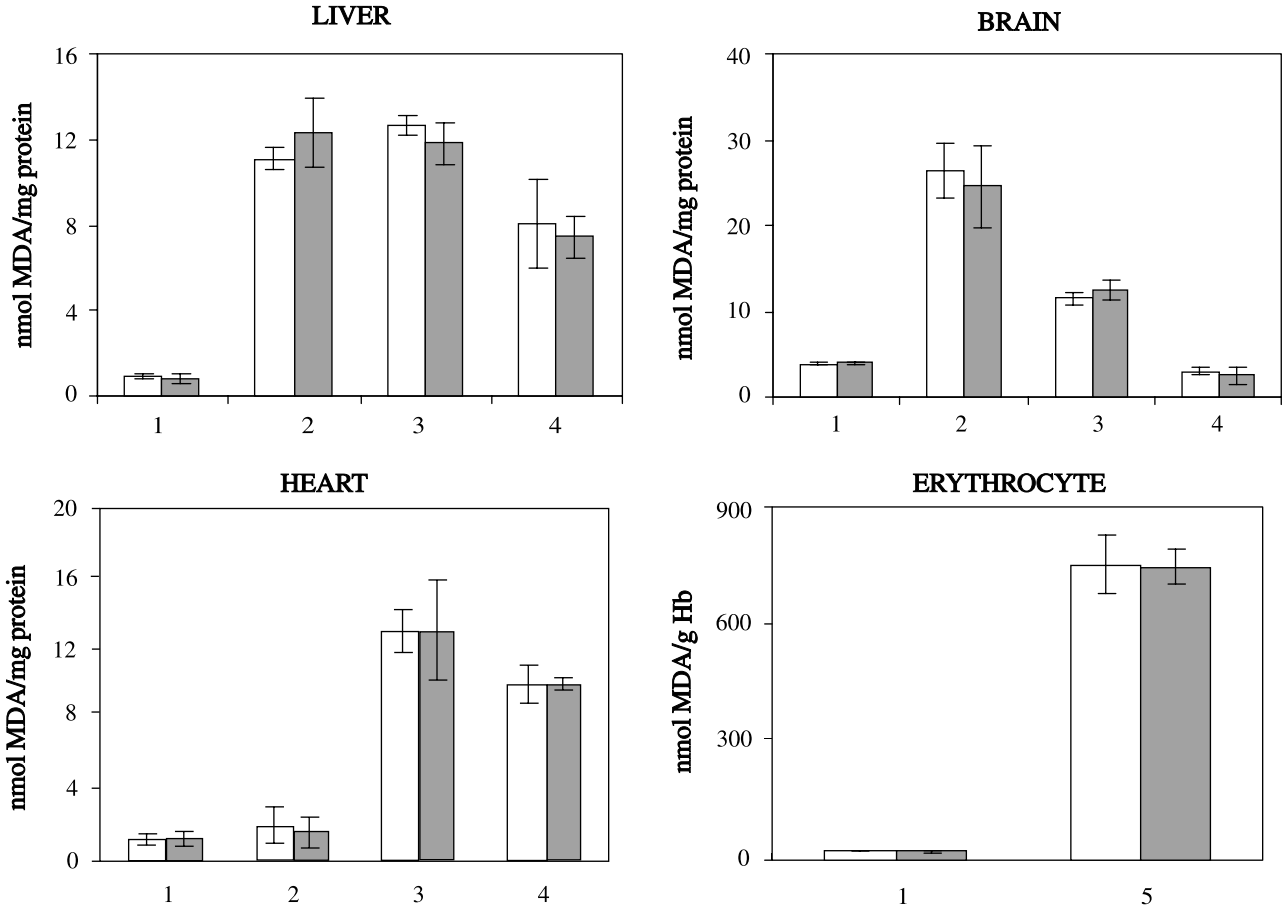

Fig. 2. Endogenous (*1*), ascorbic acid (*2*)-, NADPH (*3*)- and cumene hydroperoxide (*4*)-induced malondialdehyde (MDA) levels in liver, brain and heart homogenates as well as endogenous and H_2O_2 (5)-induced MDA levels in erythrocytes of control (□) and β-alanine-treated (▩) rats (mean ± SD; n = 6 each)

Table 1. Plasma transaminase activities and prooxidant and antioxidant status in the liver of control and β-alanine treated rats (Mean ± SD; n = 6 each)

	Control	β-Alanine
Plasma		
Alanine transaminase (U/L)	34.7 ± 5.28	33.3 ± 6.74
Aspartate transaminase (U/L)	89.3 ± 9.45	85.8 ± 8.15
Liver		
Malondialdehyde (nmol/g tissue)	152.5 ± 12.9	162.5 ± 16.3
Diene conjugate (μmol/g tissue)	1.53 ± 0.15	1.45 ± 0.28
Glutathione (μmol/g tissue)	5.98 ± 0.33	5.70 ± 0.64
α-tocopherol (nmol/g tissue)	46.2 ± 3.04	53.0 ± 4.85
Total ascorbic asit (nmol/g tissue)	471.7 ± 55.2	484.3 ± 45.0
Superoxide dismutase (U/mg protein)	26.9 ± 2.95	23.4 ± 4.07
Glutathione peroxidase (nmol/min/mg protein)	430.0 ± 53.8	408.3 ± 34.8
Glutathione transferase (nmol/min/mg protein)	270.8 ± 26.0	280.7 ± 55.4

antioxidant power in heart and brain (Schaffer et al., 2003). Indeed, taurine depletion was reported to induce shape and size changes (Schaffer et al., 1998) and DNA damage (Golubnitschaja et al., 2003) in cardiomyocytes. Decreased taurine levels were found to be associated with the development of cardiomyopathy (Dawson et al., 2002; Schaffer et al., 2003; Golubnitschaja et al., 2003). However, in taurine transporter knockout mice, chronically lowered muscle taurine levels affected skeletal, but not cardiac muscle function (Warskulat et al., 2004). On the contrary, some investigators have reported that taurine depletion could provide a protection from ischemic injury (Allo et al., 1997) and exercise-induced muscle injury (Dawson et al., 2002). On the other hand, chronically β-alanine treatment also caused neurotoxic pathological changes in adult cats (Lu et al., 1996). However, β-alanine treatment alone did not cause any effect on MDA and GSH levels as well as histopathologic appearance in

the liver, but co-administration of β-alanine with alcohol to rats exacerbate hepatotoxicity of ethanol (Kerai et al., 2001). In addition, when hepatic taurine content was depleted with β-alanine, portal-central fibrosis induced by ethanol plus carbon tetrachloride treatment was observed to proceed cirrhotic structure (Erman et al., 2004).

In our study, we investigated endogenous and induced lipid peroxide levels in liver, brain, heart and erythrocytes. However, endogenous and ascorbic acid-, NADPH- and cumene hydroperoxide-induced MDA levels did not change in the liver, brain and heart homogenates following β-alanine treatment. Similarly, H_2O_2-induced MDA levels did not alter in the erythrocytes after β-alanine treatment. This unexpectedly situation may be related to compensatory mechanisms supplying a protection against exogenous oxidative stimuli in reduced taurine concentrations. However, we could not observe any changes in levels of MDA, DC, GSH, α-tocopherol, ascorbic acid and the activities of SOD, GSH-Px and GST in the liver, although liver taurine levels were almost depleted by β-alanine treatment.

In conclusion, our results show that β-alanine treatment did not affect the susceptibility of liver, brain, heart and erythrocytes to lipid peroxidation as well as prooxidant-antioxidant balance in the liver of rats. According to this, buffering or sequestering capacity of tissues to exogenous stimuli was not influenced by reduced taurine concentrations in tissues of rats.

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