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Effects of *in vivo* taurine depletion on induced-chemiluminescence production in macrophages isolated from rat lungs

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Summary. Alveolar macrophages isolated by pulmonary lavage from partially taurine-depleted rats demonstrated increased (2–2.6 fold) chemiluminescence due to the extracellular reaction between exogenous zymosan and various reactive forms of oxygen compared to macrophages isolated from control animals. Partial taurine depletion was achieved by adding 3% β-alanine to the drinking water of the rats for 5 weeks prior to harvesting the macrophages. Superoxide dismutase activity was not increased in the lung tissue of the taurine-depleted rats. These data suggest that taurine has antioxidant properties and that taurine depletion is potentially deleterious to alveolar macrophages and pulmonary tissue.

Keywords: Amino acids – Taurine – Superoxide anion – Chemiluminescence – Superoxide dismutase – Macrophage

Introduction

Taurine (2-aminoethanesulfonic acid), a naturally occurring non-toxic β-amino sulfonic acid, has been demonstrated in the last few years to be a potentially nutritionally important amino acid in animals and man. Possible functions of taurine include the following: a neurotransmitter or neuromodulator, a modulator of calcium ion uptake, a regulator of protein phosphorylation, a modulator of myocardial contraction, an osmoregulator, a modulator of phospholipid metabolism, a membrane stabilizer, an effector in reproduction and postnatal development, and an antioxidant (Huxtable, 1989, 1992; Lombardini, 1991; Schaffer and Azuma, 1992; Sturman, 1993).

It has also been demonstrated that taurine protects animals against pulmonary toxicity induced by exposure to paraquat, amiodarone, and bleomycin. However, while the mechanism of action of the protective ability of taurine is unknown it is presumed that the antioxidant properties of taurine are involved. Paraquat, a broad spectrum herbicide, bleomycin an antineoplastic agent, and amiodarone, an antiarrhythmic agent, produce pulmonary fibrosis

possibly by forming superoxides and free radicals in the lung (Hussain et al. 1985; Kennedy et al., 1988; Wang et al., 1989; Gordon et al., 1992). Other studies involving taurine as a protecting agent against oxidant injury induced by nitrogen dioxide and ozone in lung tissue have also been reported (Gordon et al., 1986; Banks et al., 1990, 1991, 1992).

In the present study, we investigated the role of taurine in alveolar macrophages and lung tissue preparations obtained from partially taurine-deficient rats. Taurine deficiency was produced by feeding β-alanine in the drinking water to the rats for 5 weeks. Superoxide anion (and/or other reactive forms of oxygen such as hydrogen peroxide, singlet oxygen, and hydroxyl radical) generated in the macrophages was determined by measuring chemiluminescence in the presence of zymosan. Superoxide dismutase activity was also measured.

Materials and methods

Chemicals

β-Alanine, xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), catalase, diethylenetriaminepentaacetic acid (DETAPAC), zymosan, luminol and bovine albumin were purchased from Sigma Chemical Co.

Taurine depletion

Experimental animals were treated with β-alanine (3% in the drinking water) for 5 weeks to partially deplete the tissues of their taurine content (Mozaffari et al., 1986). Control animals were maintained on untreated water. The taurine content of isolated lung tissue for control animals ($N \times 3$) was $8.21 \pm 0.07 \mu$moles/g wet tissue while the taurine context of lung tissue after treatment of animals with β-alanine ($N = 3$) for 5 weeks was $4.02 \pm 0.45 \mu$moles/g wet tissue ($P < 0.01$).

Isolation of alveolar macrophages

Alveolar macrophages were harvested from adult male Sprague Dawley rats by tracheal lavage according to the procedure of Castranova et al. (1980). Rats were anesthetized with sodium pentobarbital (65 mg/kg body weight) and then the abdominal aorta was cut to exsanguinate the animal. Ten ml aliquots (50 ml total) of ice-cold Hepes buffer (145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5 mM glucose, pH 7.4) were used to lavage the lungs from each animal. The lavage fluid contained over 80% macrophages. The macrophages contained in the lavage fluid were then concentrated by centrifugation (500 g, 5 min) and then washed twice (by alternate centrifugation and resuspension) in the above Hepes buffer.

Preparation of lung homogenates

Lungs were removed from anesthetized adult male Sprague Dawley rats and perfused with saline (room temperature) through the pulmonary artery and veins until the lungs were blanched. The tissue was minced with scissors in 3 volumes of ice-cold 50 mM phosphate buffer (pH 7.8) and then homogenized with a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, N.Y.). The homogenate was stored frozen at $-80°C$ until needed for the superoxide dismutase assay. At that time the lung homogenates were thawed and centrifuged at $12{,}000 \times g$ at 4°C for 20 min. The supernatants were used for the enzyme assay.

Chemiluminescence assay

Chemiluminescence was measured as previously described by Banks et al. (1990). Briefly, alveolar macrophages (5×10^5) were suspended in 5 ml of Hepes buffer (above) containing 10 mg% luminol and 2 mg/ml zymosan. The reaction mixtures (5 ml total volume) were incubated at 37°C in a water bath and counted in plastic scintillation vials at timed intervals (total 2 hour time period) in a Packard Tri-Carb (1900TR) scintillation counter operated in the out-of-coincidence mode.

Superoxide dismutase assay

Superoxide dismutase activity was assayed in lung tissue preparations by measuring NBT reduction by superoxide anion at 560 nm according to the procedure of Spitz and Oberley (1989). The incubation mixture contained 50 mM potassium phosphate buffer, pH 7.8; 1 mM DETAPAC; 1 unit catalase; 5.6×10^{-5} M NBT; 0.1 mM xanthine; xanthine oxidase, and lung tissue preparation (above). The rate of NBT reduction in the absence of tissue was used as the reference rate.

Protein assay

The Lowry method (Lowry et al., 1995) was used to quantitate the amount of protein in the tissue homogenates. Bovine albumin was used as the standard.

Results

Zymosan-stimulated (particle-stimulated) chemiluminescence isolated by tracheal lavage as a function of time in rat alveolar macrophages is demonstrated in Fig. 1. In macrophages isolated by tracheal lavage from control animals the chemiluminescence increases, reaching a maximum at 10–15 minutes, and then sustains this level of activity for at least another 20 min at which time the

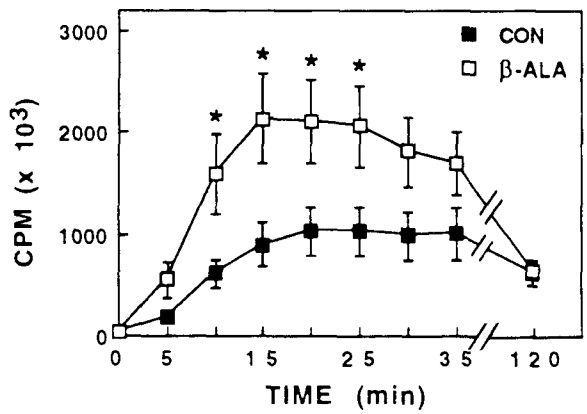

Fig. 1. Zymosan-stimulated chemiluminescence in alveolar macrophages isolated from taurine-depleted and control rats as a function of time. Chemiluminescence was measured as cpm for 5×10^5 cells in a liquid scintillation counter. Data points represent mean values ± SEM (N = 10 for both control and β-alanine-treated animals). Statistical comparisons were performed using the Students t-test (*$P < 0.05$ compared to control data). Experimental conditions are described in the Materials and methods section

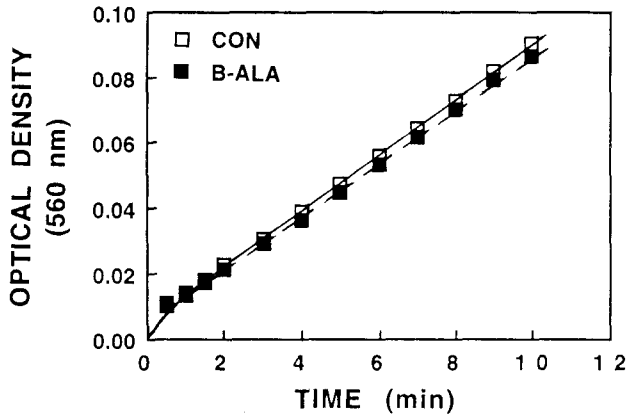

Fig. 2. Superoxide dismutase activity in lung tissue preparations from taurine-depleted and control rats as a function of time. Data are presented as means ± SEM (N = 7 for control animals; N = 8 for β-alanine-treated animals). Statistical comparisons were performed using the Students t-test (No significant differences were noted at $P < 0.05$ compared to control data). Experimental conditions are described in the Materials and methods section

activity decreases. However, in macrophages isolated from β-alanine-treated animals, i.e. partially taurine depleted, there is a significant increase (2–2.6 fold) in chemiluminescence at 4 experimental time points: 10, 15, 20, and 25 minutes. The chemiluminescence returns to control values at 120 minutes.

Superoxide dismutase activity was also measured in centrifuged lung homogenates prepared from both control and β-alanine-treated animals (Fig. 2). The activity of superoxide dismutase was measured at a single protein concentration (20μg/ml) that decreased the reference rate of NBT reduction by approximately 50%. In the β-alanine-treated animals 20μg of protein from the centrifuged lung homogenates demonstrated no significant difference in superoxide dismutase activity (slope = 0.0084 optical density units/min) than 20μg of protein obtained from lung tissue of control animals (slope = 0.0082 optical density units/min).

Discussion

One of the many functions proposed for taurine is as an antioxidant (Gaull et al., 1985; Wright et al., 1986). In this regard taurine has been used to protect animals from pulmonary fibrosis induced by the oxidant moieties, i.e., superoxide anion and free radicals, which increase in lung tissue after exposure to paraquat (Gordon et al., 1992), amiodarone (Kennedy et al., 1988), bleomycin (Wang et al., 1989), nitrogen dioxide (Gordon et al., 1986, 1992), and ozone (Banks et al., 1990, 1991, 1992).

Gordon and colleagues (1992) reported that when Golden Syrian hamsters were injected with paraquat or given an intratracheal instillation of bleomycin it could be demonstrated by light microscopy (after glutaraldehyde-cacodylate fixation of the lungs) at one day after treatment, a

localized, mild edema, and aggregation of large numbers of inflammatory cells in the lung tissue. At seven days post treatment with paraquat or bleomycin there were small focal areas of alveolar bronchiolarization and interstitial fibrosis. At 28 days post treatment with paraquat or bleomycin light microscopy revealed the persistence of alveolar and interstitial inflammatory cells and localized interstitial fibrosis. However, light microscopy of the lung after pretreatment of the animals with taurine in their drinking water (1%) for 28 days prior to injection with paraquat or bleomycin, demonstrated an amelioration of the pathology observed in the non-pretreated animals (Gordon et al. 1992).

On the contrary, if the animals were pretreated with 1% guanidinoethanesulfonic acid (GES) in their drinking water for 28 days prior to injection of paraquat or bleomycin, the pathology was significantly worse (Gordon et al., 1992). (GES is a taurine transport inhibitor and significantly reduces the total body stores of taurine in all tissues of the animal. Usual reduction in taurine content of various tissues is 50–80%.) At one day post treatment with paraquat or bleomycin a generalized, severe edema was present along with the aggregation of large numbers of inflammatory cells. At seven days, the edema was reduced but light microscopy revealed considerable interstitial fibrosis and alveolar bronchiolarization in the lung tissue. At 28 days light microscopy revealed severe interstitial fibrosis and alveolar bronchiolarization along with persistent alveolar and interstitial inflammatory cells in the paraquat treated animals. Unfortunately, no biochemical or functional tests were performed in these studies using GES. Therefore, the degree of additional pulmonary damage associated with taurine depletion was not quantitated in these morphological studies.

Giri and colleagues (Wang et al., 1989) also tested taurine as a protecting agent against lung pathology associated with bleomycin. In these studies it was reported that the quantity of lung collagen was reduced in the taurine-bleomycin treated hamsters compared to the saline-bleomycin treated animals. Also reported was that the lesions associated with bleomycin exposure, i.e., diffuse mononuclear alveolitis and multifocal fibroplasia, were reduced by 60% in the taurine-treated animals.

A diet supplemented with 2.5% taurine was shown by Giri and colleagues (1992) to reduce the pulmonary toxicities associated with amiodarone toxicity. In these studies quantitative assays were performed. Collagen accumulation, phospholipid content, malondialdehyde equivalent (an index of lipid peroxidation) and superoxide dismutase activity, which were increased after administration of amiodarone, were all reduced in the lung tissue of the taurine-treated animals.

Further studies by Giri and colleagues (Wang et al., 1991, 1992; Giri and Wang, 1992) tested the combined effects of niacin and taurine on the fibrotic lesions caused by bleomycin in the hamster. The conclusions of these studies which included morphological (light and electron microscopy) and biochemical tests were that the combination, taurine plus niacin, provide nearly complete protection against the fibrotic lesions induced by bleomycin treatment.

Banks and colleagues (Banks et al., 1990, 1991, 1992) used isolated alveolar macrophages to study the protective effects of taurine against ozone exposure. In their studies isolated alveolar macrophages were preloaded with taurine (varied concentrations up to 500μM) prior to ozone exposure. After a specific exposure time various biochemical tests were performed on the macrophages to determine the beneficial effects of taurine. Their results demonstrated that the macrophages increased in viability when taurine at a concentration of 100μM was added to the cell cultures. Interestingly, it was noted that this is the taurine concentration present in plasma. The viability of the macrophages was increased as indicated by an increase in trypan blue exclusion, a decrease in lipid peroxidation, a decrease in the decline of Na^+-K^+ ATPase activity, and a lessening of the leakage of reduced glutathione and protein into the medium.

It has also been reported by Schuller-Levis and Sturman (1992) that reactive oxygen intermediates increase in cats fed taurine-free diets. Alveolar leukocytes isolated from cats fed diets deficient in taurine produced elevated levels of superoxide anion in response to stimulation by phorbol myristate acetate and also elevated levels of H_2O_2 compared to cats fed normal taurine diets.

In the present studies, we tested both lung tissue and alveolar macrophages isolated from control and taurine-depleted animals for their superoxide anion content by measuring chemiluminescence and superoxide dismutase activity. The increase in chemiluminescence in pulmonary tissue that has reduced taurine content due to the *in vivo* treatment of the animals with β-alanine is most likely indicative of an increase in the superoxide anion (and subsequent, free radical generation) in the extracellular fluid of the macrophages which reacts with zymosan and is then, in turn, responsible for the chemiluminescence (Miles et al., 1978). No change in superoxide dismutase was observed. However, other explanations for the increase in chemiluminescence in the macrophages obtained from the taurine-depleted animals are possible such as the following: 1) a reduction in the scavenging of hypochlorous acid due to the decreased concentration of taurine in the β-alanine treated animals; 2) altered lipid content of the macrophages due to taurine depletion (Harada et al., 1988; Hamaguchi et al., 1991) which may potentially produce changes in chemiluminescence; and 3) stress due to alterations in osmoregulation of pulmonary cells as a result of the decrease in taurine levels which in turn may release various cytokines that could promote the activation of circulating macrophages.

From the above studies it appears that taurine is a necessary component of the diet without which animals may be at higher risk of pulmonary fibrotic disease especially when exposed to noxious oxidizing substances. Thus, it is of interest to speculate that exogenous taurine may have therapeutic applications for individuals who have potential exposure to oxidants, such as nitrogen dioxide, ozone, or paraquat, due to their employment. In addition, individuals treated with the anticancer agent, bleomycin, and the antiarrhythmic agent, amiodarone, may also benefit from concomitant treatment with taurine as a dietary supplement.

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