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Effects of taurine on pulmonary responses to antigen in sensitized Brown–Norway rats

Julio Cortijo [a], Sebastian Blesa [a], Magdalena Martinez-Losa [a], Manolo Mata [a], Enrique Seda [a], Francesco Santangelo [b], Esteban J. Morcillo [a,*]

[a] *Department of Pharmacology, Faculty of Medicine, University of Valencia, Avenida Blasco Ibanez 15, E-46010 Valencia, Spain*
[b] *Zambon Group Spa, Via Lillo del Duca 10, 20091 Bresso, Milan, Italy*

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Abstract

Oxidative stress appears relevant to asthma. Therefore, the effects of the antioxidant taurine (oral, 1 and 3 mmol kg^{-1} day^{-1} for 7 days before challenge) were examined on antigen-induced responses in sensitized Brown–Norway rats. Taurine did not reduce the bronchospasm produced by aerosol antigen but prevented airway hyperreactivity to 5-hydroxytryptamine (5-HT) at 24 h after antigen exposure, and reduced the eosinophils (from $0.178 \pm 0.038 \times 10^6$ to $0.044 \pm 0.014 \times 10^{6*}$ and $0.048 \pm 0.013 \times 10^{6*}$ cells ml^{-1} in antigen and antigen + taurine 1 or 3 mmol kg^{-1}, respectively; $^*P < 0.05$ vs. antigen), lipid hydroperoxides, and Evans blue dye extravasation in bronchoalveolar lavage fluid. Taurine levels in bronchoalveolar lavage fluid from antigen-challenged rats were higher than control values but treatment with taurine failed to further increase these levels. In conclusion, oral taurine showed beneficial effects in an in vivo model of experimental asthma, which confirm and extend the previous positive findings obtained in other models of lung injury.

Keywords: Experimental asthma; Brown–Norway rat; Taurine

1. Introduction

Asthma is a chronic inflammatory disease characterized by reversible bronchial obstruction and airways hyperreactivity, eosinophil accumulation and plasma exudation. There is increasing clinical and experimental evidence that an excess production of reactive oxygen species and defective endogenous antioxidant defence mechanisms may be present in asthma (Barnes, 2000). Therefore, antioxidant therapy might be of clinical benefit for asthma management.

Taurine is an ubiquitous β-amino acid found in most animal species including man. It is in fact the most abundant free amino acid in many tissues and, in particular, in proinflammatory cells like polymorphonuclear leukocytes and tissues exposed to elevated levels of oxidants (Stapleton et al., 1998). It has been proposed that taurine, either intracellularly or released into the extracellular medium, may protect cells against attack by oxidants and have anti-inflammatory and immunomodulator activities. The antioxidant activity of taurine and its protective effect against lipid peroxidation is exerted through direct and indirect mechanisms including the formation of its chlorinated derivative taurine-chloramine (Stapleton et al., 1998). In vitro studies have shown that taurine and taurine-chloramine depress the release of inflammatory mediators by neutrophils and macrophages and modulate T-cell activation (Timbrell et al., 1995; Marcinkiewicz et al., 1998). Furthermore, orally administered taurine has been reported to reduce lung oxidant damage from exposure to ozone, nitrogen dioxide, paraquat, amiodarone and bleomycin exposure in animal models (Schuller-Levis et al., 1995; Timbrell et al., 1995; Gordon et al., 1998; Gurujeyalakshmi et al., 2000).

By contrast with the number of these in vivo studies, to our knowledge, the activity of taurine on experimental models of asthma has not yet been reported. However, there is preliminary clinical evidence of the beneficial effects of taurine administration in asthmatics (Covarrubias, 1994). Therefore, the objective of the present work was the study of the effects of orally administered taurine on antigen-induced bronchoconstriction, airways

* Corresponding author. Tel.: +34-96-386-4623; fax: +34-96-386-4622.
E-mail address: Esteban.Morcillo@uv.es (E.J. Morcillo).

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hyperreactivity and eosinophilia, lipid hydroperoxide and airway microvascular leakage in actively sensitized Brown–Norway rats. In addition, since taurine levels in bronchoalveolar lavage fluid and airway secretions were altered in asthma (Hofford et al., 1997) and other inflammatory lung diseases (Cantin, 1994; Witko-Sarsat et al., 1995), the levels of taurine in bronchoalveolar lavage fluid were also examined in this experimental model of allergic asthma.

2. Methods

2.1. Sensitization procedure

Male Brown–Norway rats weighing 220–300 g were actively sensitized by intraperitoneal injection of 1 ml of a suspension of 1 mg ovalbumin and 100 mg of aluminium hydroxide [$Al(OH)_3$] in 0.9% (wt./vol.) saline for three consecutive days. The sensitized animals were used for experiments 21 days after the initial intraperitoneal injection. This procedure has previously been shown to result in the development of immunoglobulin E-type antibody (Elwood et al., 1992). The protocol and experimental design of this study was approved by the local Ethics Committee and complies with the regulations established by the European Community and by the Spanish and Regional Governments.

2.2. Experimental groups

Animals were randomly distributed into five groups. The untreated groups were a negative control (group A) consisting of sensitized animals receiving drug vehicle and exposed to aerosol saline, and a positive control (group B) comprising sensitized animals subsequently exposed to aerosol antigen and receiving drug vehicle. The treated groups (groups C1 and C2) were sensitized animals treated with taurine 1 and 3 mmol kg^{-1} day^{-1}, respectively. An additional group of sensitized animals received taurine (3 mmol kg^{-1} day^{-1}) but were challenged with saline instead of antigen. Treatments (vehicle or taurine) were administered orally by gavage as a single dose on a daily basis (at 9:00 a.m.) from 7 days prior to the antigen or saline challenge with the last dose given 1 h before the challenge. Oral taurine was selected as the usual way of administration in the clinical setting. The dose level and schedule were based on previous studies (Cortijo et al., 2001).

2.3. Animal preparation and assessment of the effects of taurine on antigen-induced acute bronchoconstriction

Animals were anaesthetised and instrumented as previously outlined (Advenier et al., 1972). The trachea was cannulated for mechanical ventilation with room air by means of a Ugo Basile ventilator at a rate of 60 breaths min^{-1} with a stroke volume of 1 ml 100 g^{-1} body weight. A heated (37 °C) pneumotachograph (Fleish 000) was positioned in the ventilator circuit to measure inspiratory and expiratory flow rate. A side arm from the tracheal cannula was attached to the positive port of a differential pressure transducer (Celesco model LCVR) and used to measure pulmonary inflation pressure, while the negative port of the transducer was attached to a cannula inserted into the intra-pleural space to measure intra-thoracic pressure. The difference between these two pressures is the transpulmonary pressure. Arterial blood pressure was measured by a transducer (Spectramed Statham P23XL) connected to a saline-filled cannula inserted into the carotid artery. Body temperature was maintained at 37 $\pm$ 0.5 °C by a heated blanket. Signals for airflow, transpulmonary pressure and arterial blood pressure were amplified (PMS 800, Mumed) and fed via an analogue to digital converter to a personal computer. Lung resistance (R_L; cm H_2O ml^{-1} s) was calculated according to the method of Amdur and Mead (1958) by the use of PMS dual software. After 10 min stabilisation, animals were challenged with inhaled antigen (100 mg ml^{-1}, 5 min). This dose of antigen was selected from results obtained in preliminary experiments (Olivenstein et al., 1997).

2.4. Assessment of the effects of taurine on airway hyperresponsiveness, eosinophil infiltration, and lipid hydroperoxide

A separate group of sensitized conscious rats were exposed to antigen aerosol in a clear plastic chamber (approximate volume: 4 l) which was connected to the output of a DeVilbiss ultrasonic nebuliser. The nebuliser chamber was filled with an ovalbumin (1% in saline) or saline solution. Nebuliser output was approximately 8–10 ml h^{-1}. The duration of the antigen challenge was 60 min. The time course of airway hyperreactivity in antigen-exposed rats has been previously examined (Elwood et al., 1992) and the response at 24 h was selected on this basis. Twenty-four hours after exposure to the aerosol, airway reactivity was determined from dose–response curves to 5-hydroxytryptamine (5-HT), administered intravenously (6.25, 12.5, 25, 50 and 100 μg ml^{-1}) to animals anaesthetized and instrumented as indicated above. 5-HT has been used to assess airways hyperreactivity in other studies in rats since it yields a reproducible bronchoconstrictor response and does not require pretreatment with propranolol (Pauwels et al., 1990; Carvalho et al., 1999).

After measurement of airway reactivity, animals were killed by an overdose of urethane. Bronchoalveolar cells were collected in 10 successive lavages using 6-ml aliquots of sterile saline with heparin 10 IU ml^{-1}, at room temperature, injected and recovered through a polyethylene tracheal cannula. Cell suspensions were concentrated by low speed centrifugation, and the cell pellet resuspended. Total

cell counts were made in a haemocytometer. Differential cell counts were determined from cytospin preparations by counting 300 cells stained with May-Grünwald-Giemsa. Because the yield of the injected fluid was equivalent in all experimental groups ($\geq 85\%$), the results are expressed as cell number ml^{-1}.

The amount of lipid hydroperoxide in bronchoalveolar lavage fluid was measured as a marker of oxidative stress (Hagiwara et al., 2000) by the Lipid Hydroperoxide Assay kit as indicated by manufacturer (Cayman Chemical, Ann Arbor, MI, USA).

2.5. Assessment of the effect of taurine on microvascular leakage after antigen challenge

Preparation of animals and experimental protocols were derived from Olivenstein et al. (1997). The sensitization, anaesthesia and instrumentation procedures were as noted above. After 10 min stabilisation, the animals received the injection of Evans blue dye (30 mg kg^{-1}, i.v.), and 1 min later, aerosol antigen were administered (100 mg ml^{-1}, 5 min). Five minutes after antigen inhalation, the animals were hyperinflated with twice the tidal volume by manually blocking the outflow of the ventilator. Then, the animals were disconnected from the ventilator and subjected to bronchoalveolar lavage (two aliquots of 1 ml saline, as outlined by Olivenstein et al., 1997) for measurement of Evans blue dye extravasation into the airway lumen. Animals pretreated with aerosol drug vehicles and then receiving antigen or its vehicle were used as controls. Tissues (trachea, bronchi) were not examined since bronchoalveolar lavage fluid is preferable for detecting the presence of dye in this rat model (Olivenstein et al., 1997).

2.6. Taurine determination in bronchoalveolar lavage fluid

Taurine levels were measured in supernatant of bronchoalveolar lavage fluid. Taurine was measured by a fluorometric technique that uses a derivatization with *o*-phthalaldehyde prior to high performance liquid chromatography (Trautwein and Hayer, 1991). The limit of detection of this technique has been established at 5 pmol per analysis, which is adequate for levels found in bronchoalveolar lavage fluid according to previous work in this laboratory (Cortijo et al., 2001).

2.7. Statistical analysis of results: drugs and solutions

Data are presented as mean ± S.E.M. Statistical analysis of results were carried out by analysis of variance (ANOVA) followed by appropriate post-hoc tests including Bonferroni correction and Student's *t*-test (GraphPad Software, San Diego, CA, USA). Significance was accepted when $P < 0.05$.

The following drugs were used: Evans blue dye, formamide, 5-hydroxytryptamine, *o*-phthalaldehyde, ovalbumin (Grade V, fatty acid-free), taurine and urethane were all purchased from Sigma-Aldrich Química (Madrid, Spain). Water purified on a Milli-Q (Millipore Iberica, Madrid, Spain) system was used throughout. Taurine was dissolved in distilled water immediately before use. Evans blue dye was dissolved in isotonic saline (20 mg ml^{-1}) and filtered through a 5-μm Millipore membrane.

3. Results

3.1. Effects of taurine on antigen-induced acute bronchoconstriction

Challenge of sensitized untreated animals with aerosol antigen provoked an acute rise in lung resistance with a peak in about 2–3 min. The baseline values for pulmonary resistance were 0.146 ± 0.008 ($n = 5$), 0.142 ± 0.004 ($n = 7$), 0.149 ± 0.009 ($n = 7$), and 0.155 ± 0.012 ($n = 8$) cm H_2O ml^{-1} s in the negative and positive controls and taurine low and high dose treated groups, respectively, with no statistically significant difference found among these values. The challenge with saline in the negative control group did not significantly alter the pulmonary resistance ($0.76 \pm 1.17\%$ change from baseline values) but antigen exposure significantly increased the pulmonary resistance ($142 \pm 23\%$). Taurine (1 or 3 mmol kg^{-1}) failed to inhibit the antigen-induced bronchoconstriction (the increase from baseline amounted to $133 \pm 9\%$ and $127 \pm 13\%$, respectively; $P > 0.05$ from antigen-exposed untreated animals). Taurine (3 mmol kg^{-1}) did not alter the pulmonary resistance values in saline-challenged rats ($0.57 \pm 1.38\%$ change from baseline values of 0.150 ± 0.010 cm H_2O ml^{-1} s; $n = 5$, $P > 0.05$ from values in the negative control group). No significant changes in arterial blood pressure were observed in taurine-treated animals compared to controls (data not shown).

3.2. Effects of taurine on antigen-induced hyperreactivity

The intravenous administration of 5-HT (6.25–100 μg kg^{-1}) at 24 h after saline (group A) or antigen (group B) exposure resulted in a dose-dependent increase of pulmonary resistance in sensitized untreated rats. Maximal effects were observed for 50 μg kg^{-1} in the antigen-exposed group (positive control), and a similar response level was attained with 100 μg kg^{-1} in the saline group (negative control). Statistical difference between groups, i.e. 'hyperreactivity', was reached from 6.25 to 25 μg kg^{-1} (Fig. 1). When the dose–response curve to 5-HT was reproduced in taurine-treated groups exposed to antigen, a decreased responsiveness (i.e. an anti-hyperreactivity effect) was apparent (Fig. 1). Treatment with taurine (3 mmol kg^{-1}) did not modify the 5-HT dose–response curve obtained in sensitized rats challenged with saline (Fig. 1).

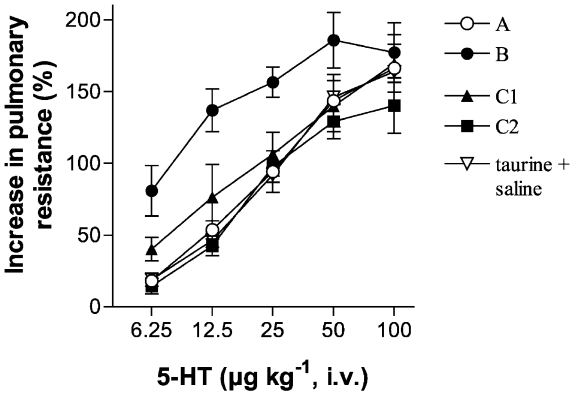

Fig. 1. Dose–response curves for 5-hydroxytryptamine (5-HT) administered intravenously (i.v.) to sensitized rats previously (24 h before) exposed to aerosol saline (group A; negative control) or antigen (1%, 60 min; group B; positive control). The animals received drug vehicle (groups A and B) or taurine (1 or 3 mmol kg^{-1} day^{-1} for 7 days; groups C1 and C2, respectively). In addition, the dose–response curve for 5-HT in animals treated with taurine (3 mmol kg^{-1}) and challenged with saline is shown (taurine + saline). The increase in pulmonary resistance is presented as percent change from baseline values that were 0.160 ± 0.011 (group A), 0.158 ± 0.09 (group B), 0.176 ± 0.011 (group C1), 0.169 ± 0.08 (group C2), and 0.145 ± 0.009 (taurine + saline) cm H_2O ml^{-1} s, respectively, with no statistically significant difference found among these values. Points are mean ± S.E.M. of 11 (group A), 12 (group B), 13 (group C1), 13 (group C2), and 7 (taurine + saline) animals. The responses for 6.25 to 25 μg kg^{-1} 5-HT in group B were significantly ($P < 0.05$) different from the corresponding values in group A, and the responses to 5-HT (6.25–25 μg kg^{-1}) in taurine-treated groups C1 and C2 were significantly different ($P < 0.05$) from their corresponding values in group B.

3.3. Effects of taurine on antigen-induced increased cellularity and lipid hydroperoxide in bronchoalveolar lavage fluid

Bronchoalveolar lavage was carried out 24 h after saline or antigen exposure of sensitized rats. In rats exposed to antigen, there was a great increase in the total number of cells in bronchoalveolar lavage fluid. This increase in total cell number was not significantly modified in treated animals. (Fig. 2). The differential cell count showed an increase of each of the different cell types present in bronchoalveolar lavage fluid of the antigen-challenged rats compared to saline-exposed animals. The increase in the eosinophil number was reduced by taurine while no inhibitory effect was produced on the rest of cell types (neutrophils, lymphocytes and macrophages) (Fig. 2). No significant correlation was found between eosinophil numbers in bronchoalveolar lavage fluid and airway responsiveness to 5-HT in control and treated groups (Fig. 3).

The amount of lipid hydroperoxide in bronchoalveolar lavage fluid was significantly increased in sensitized rats challenged with antigen (3.6 ± 0.4 nmol ml^{-1}; $n = 5$) compared to saline control (0.8 ± 0.3 nmol ml^{-1}; $n = 5$). Treatment with taurine (3 mmol kg^{-1}) did not alter the control values in saline-challenged animals (0.8 ± 0.4; $n = 3$) but attenuated the levels detected in antigen-exposed rats (1.8 ± 0.5 nmol ml^{-1}; $n = 5$, $P < 0.05$ compared to drug vehicle + bleomycin).

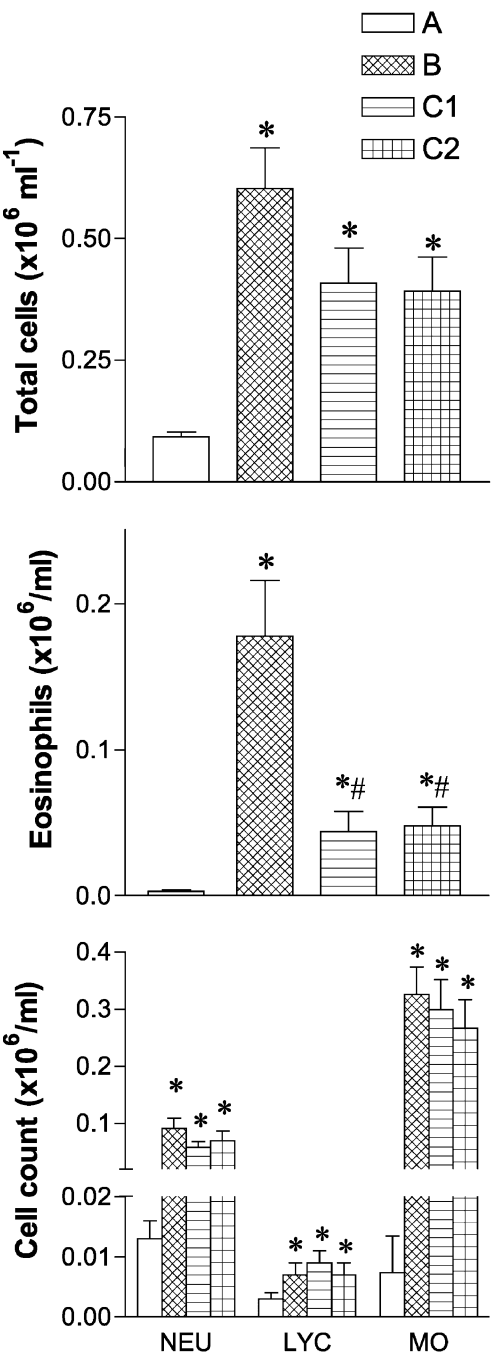

Fig. 2. Total cell numbers (upper panel), eosinophil counts (middle panel), and neutrophils (NEU), lymphocytes (LYC) and macrophages (MO) (lower panel) in bronchoalveolar lavage fluid of sensitized rats previously (24 h before) exposed to aerosol saline (group A) or antigen (1%, 60 min) (groups B and C). The animals received drug vehicle (groups A and B) or taurine (1 or 3 mmol kg^{-1} day^{-1} during 7 days; groups C1 and C2, respectively). Points are mean ± S.E.M. of 11 (group A), 12 (group B), 13 (group C1), and 13 (group C2) animals. $^{*}P < 0.05$ compared to group A; $\#P < 0.05$ from group B.

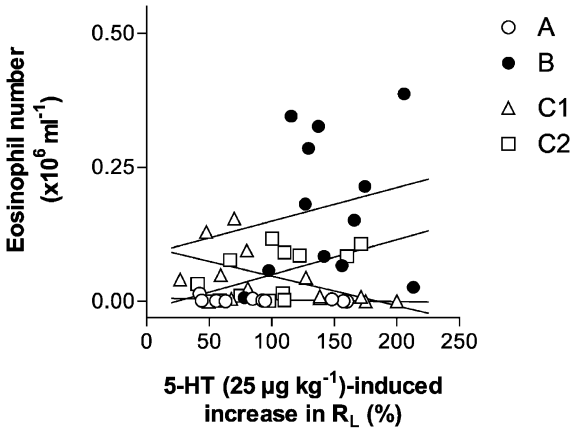

Fig. 3. Correlation analysis between individual values for airway responsiveness, expressed as the increase in pulmonary resistance (R_L) in response to 5-HT (25 μg kg^{-1}, i.v.), and the eosinophil number in bronchoalveolar lavage fluid. The correlations between 5-HT responsiveness and eosinophil number for the different experimental groups were not significant.

3.4. Effects of taurine on Evans blue levels in bronchoalveolar lavage fluid

Antigen challenge produced an increase in the concentration of Evans blue in bronchoalveolar lavage fluid. This increase was reduced by taurine at 1 and 3 mmol kg^{-1} (Fig. 4).

3.5. Taurine levels in bronchoalveolar lavage fluid

The taurine levels in bronchoalveolar lavage fluid of antigen-challenged but untreated rats were higher than the levels observed in saline-exposed rats (Fig. 5). The taurine levels in bronchoalveolar lavage fluid from antigen-challenged rats treated with taurine were also higher than the values in saline-exposed rats but not significantly higher than the taurine levels found in antigen-exposed, untreated rats (Fig. 5). No significant correlation was found between

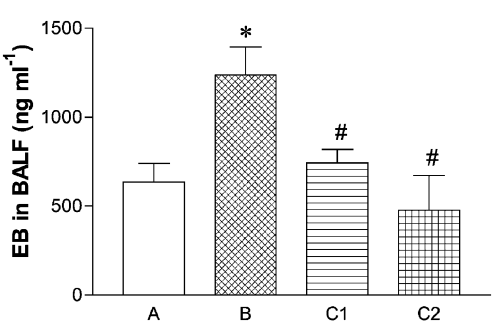

Fig. 4. Effect of taurine on antigen (100 mg ml^{-1}, 5 min)-induced airway extravasation in sensitized rats. Extravasation of Evans blue dye was measured in bronchoalveolar lavage fluid (BALF). The experimental groups are as previously indicated. Taurine inhibited the antigen-induced extravasation. Columns are means ± S.E.M. of 6 (groups A and B), and 5 (groups C1 and C2) animals for each group. $^{*}P < 0.05$ compared to group A; $\#P < 0.05$ from group B.

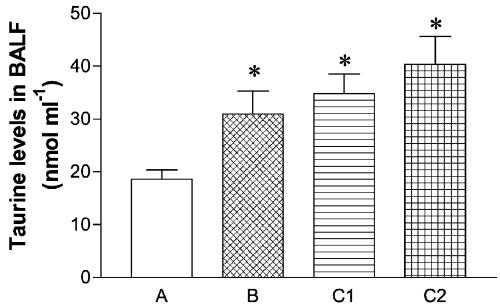

Fig. 5. Taurine levels in bronchoalveolar lavage fluid (BALF) in the different experimental groups as indicated. Data are mean ± S.E.M. of 5 (group A), 8 (group B), 9 (group C1) and 9 (group C2) animals. $^{*}P < 0.05$ from group A.

taurine values in bronchoalveolar lavage fluid and either the airways responsiveness to 5-HT or the neutrophil and eosinophil numbers (data not shown).

4. Discussion

Taurine is an amino acid endowed with antioxidant, anti-inflammatory and immunomodulatory properties (Stapleton et al., 1998) that reduces oxidant-induced lung damage in a variety of experimental models (Timbrell et al., 1995; Gordon et al., 1998; Gurujeyalakshmi et al., 2000). In this study, we have extended these observations to show the anti-hyperreactivity and anti-inflammatory effects of oral taurine in an established experimental model of allergic asthma.

The presence of airways hyperreactivity at 24 h after antigen challenge of sensitized animals is well established in the literature (Pauwels et al., 1990; Elwood et al., 1992; Carvalho et al., 1999). This hyperreactivity was accompanied by increased total and differential cell numbers as previously described (Elwood et al., 1992). However, the level of airways hyperreactivity is not correlated to eosinophil numbers, a finding consistent with other studies in guinea-pig (Pretolani et al., 1994; Pons et al., 2000). Taurine was effective to reduce the airways hyperreactivity to 5-HT as well as the eosinophil counts in bronchoalveolar lavage fluid. To our knowledge, this is the first report in the literature on this anti-hyperreactivity effect of taurine. The mechanisms underlying hyper-reactivity to spasmogens in airways have not been fully elucidated. Airway anaphylaxis produces reactive oxygen species (Weiss and Bellino, 1986) and airways hyperreactivity has been related to oxidative stress (Hulsmann et al., 1994). Furthermore, an alteration in glutathione balance was reported in sensitized guinea-pigs (Grant and Rodger, 1991). Therefore, it is possible that the beneficial effect of taurine may be related to its antioxidant properties. In fact, there is experimental evidence that the activation of the redox-sensitive transcription factor nuclear factor-κB (NF-κB) is decisive for the expression of various inflammatory genes

in this experimental model of asthma (Liu et al., 1997). Although we have not studied the effect of taurine on NF-κB activation, it has been recently shown that oral taurine in combination with niacin blocked the activation of NF-κB in a murine model of pulmonary fibrosis induced by oxidant stress (Gurujeyalakshmi et al., 2000). Reactive oxygen species modulate also the sequential expression of cell adhesion molecules, which mediate cell trafficking into inflammatory sites (Chiu et al., 1997). The antioxidant properties of taurine may therefore contribute to decrease pulmonary eosinophilia through an alteration of the oxidant-sensitive expression of cell adhesion molecules. In addition to reduced numbers of inflammatory cells, taurine may lessen the oxidant burden by diminishing the generation by these cells of superoxide anion and other cytotoxic mediators (Schuller-Levis and Sturman, 1992; Timbrell et al., 1995), and could also protect airway cells from oxidative damage as demonstrated for other antioxidants (Cortijo et al., 1999). To provide further experimental evidence in favour of the antioxidant properties of taurine in this model, we measured the amount of lipid hydroperoxide in bronchoalveolar lavage fluid, which is considered an indicator of oxidative stress (Hagiwara et al., 2000), and found that taurine significantly reduced the augmented levels existing in antigen-exposed animals. This finding is consistent with other reports that show less lipid peroxidation in taurine-treated animals subjected to lung injury mediated by oxidant stress (Giri and Wang, 1992; Venkatesan and Chandrakasan, 1994).

Oxygen radicals are involved in plasma extravasation elicited by antigen challenge in rats, and antioxidants such as superoxide dismutase have an anti-exudative effect in this model (Jia et al., 1999). Consistent with this report, we found that taurine was able to reduce the airway microvascular leakage that follows immediately to the antigen provocation. This result would be also in keeping with reports showing that taurine and other antioxidants like *N*-acetylcysteine are capable to reduce airway extravasation in different animal models (Leff et al., 1993; Stapleton et al., 1998; Bernareggi et al., 1999). However, the acute increase in pulmonary resistance that follows antigen challenge was not reduced by taurine (this study). Bronchoconstriction to antigen is related to the immediate release of mediators from mast cells degranulating in the airways (Advenier et al., 1979), thus suggesting that this amino acid was not effective to inhibit mediator release from mast cells. This result is consistent with the lack of inhibition of antigen-induced bronchospasm showed for taurine (2.4 mmol kg^{-1}, i.v.) in sensitized guinea-pigs (Kurachi et al., 1987).

The levels of taurine found in the bronchoalveolar lavage fluid of rats not challenged with antigen are within the range of levels reported in a previous study with a different rat strain (Cortijo et al., 2001). Antigen exposure of sensitized rats resulted in augmented levels of taurine in bronchoalveolar lavage fluid at 24 h post-challenge. This observation would be consistent with the increased level of taurine found in bronchoalveolar lavage fluid from asthmatic patients (Hofford et al., 1997), although animal models of asthma are not fully equivalent to the human disease (Coleman, 1999). Taurine levels in airway secretions were also augmented in patients of chronic bronchitis and cystic fibrosis (Cantin, 1994; Witko-Sarsat et al., 1995), with a negative correlation found between taurine levels in sputum and respiratory parameters for cystic fibrosis (Witko-Sarsat et al., 1995). We have not found any correlation between taurine levels in bronchoalveolar lavage fluid and airways reactivity to 5-HT or neutrophil and eosinophil numbers in bronchoalveolar lavage fluid. Whether the increase of taurine in bronchoalveolar lavage fluid is due simply to its release from damaged cells or is a compensatory rise in response to oxidant stress as described for other endogenous antioxidants (Smith et al., 1993) is presently unknown. Further studies are required to precise the role of endogenous taurine in the asthmatic process. Treatment with taurine further augmented the taurine levels in bronchoalveolar lavage fluid compared to untreated rats, but this increase failed to reach statistical significance. Therefore, the participation of the enhanced levels of taurine in the beneficial effect of this amino acid is not sufficiently proved.

In summary, the results from this study demonstrate that oral treatment with taurine produced anti-hyperreactivity and anti-inflammatory effects in an animal model of allergic asthma. Yet, the antioxidant effects of taurine may be relevant to this beneficial effects, the precise mechanisms involved remain uncertain.

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