Research Article

Effect of Taurine on The Respiratory System of Rats

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ABSTRACT

The present study was designed to investigate the effect of taurine on isolated trachea and pulmonary artery of rats and the possible mechanism(s) of action. The possible antioxidant effect of taurine was also studied by measuring its protective effect against cyclophosphamide induced lung injury. Taurine produced a concentration dependent relaxation in the isolated tracheal strips and pulmonary arterial rings precontracted by serotonin (2x10^-4 M). The relaxing effect of taurine was not influenced by pretreatment with nitric oxide synthase inhibitor (L-NAME), cysteinyl leukotrienes receptor 1 blocker (montelukast), H1 receptor blocker (chlorpheniramine), β-adrenoceptor blocker (propranolol), potassium channel blocker (amiodarone), cyclo-oxygenase inhibitor (indomethacin) or muscarinic receptor blocker (atropine). Preincubation with adenosine receptor blocker (aminophylline) significantly potentiated the relaxing effect of taurine in the tracheal strips and pulmonary arterial rings. Cyclophosphamide (CYP, 150 mg/kg) administered i.p. in a single dose was used to produce lung injury in rats. CYP caused marked increase in lung lipid peroxides (MDA) and decrease in lung reduced glutathione (GSH). Administration of taurine (1% in drinking water) starting 7 days before CYP and continuing throughout the duration of the experiment (24 hours) improved significantly the lung GSH and MDA. It can be concluded that taurine relaxes precontracted rat tracheal strips and pulmonary arterial rings probably by direct effect on the smooth muscles. Also, the observed antioxidant activity of taurine which may contribute to its relaxant effect suggesting the usefulness of taurine in pulmonary hypertension.

Keyword: taurine, trachea, pulmonary artery, MDA, GSH

1. Introduction

Taurine, a derivative of the sulfur-containing (sulfhydryl) amino acid cysteine, is the only known naturally occurring sulfonic acid (Brosnan and Brosnan, 2006). It is present in high concentrations in the body, which provides the logic for its involvement in a variety of functions from osmoregulation to cardioprotection and from hypertension to the neurotransmitter role. All these actions make taurine a polyfunctional molecule, informing its role in maintenance of various life-forming processes (Gupta, 2006). Antihypertensive effects of taurine have been demonstrated in several experimental models (Fujita and Sato, 1984 and Harada et al., 2004).

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In-vitro studies showed that taurine relaxed precontracted rabbit ear artery (Franconi et al., 1982), rat aorta (Ristori and Verdetti, 1991) and rat mesenteric artery (Li et al., 1996). Thoracic aortic rings isolated from rats that were chronically given beta-alanine to deplete internal taurine showed enhanced contractile responses to norepinephrine and high potassium, and reduced relaxant responses to sodium nitroprusside and acetylcholine (Abebe and Mozaffari, 2003). Thoracic aortic rings isolated from rats that were chronically given taurine showed reduced contractile responses to norepinephrine and high potassium nonspecifically (Abebe and Mozaffari, 2000). These experiments
suggest that taurine plays an important role in the maintenance and regulation of vascular tone in normal and pathological situations. However, the effects of taurine in other vascular beds and the underlying mechanism(s) are not well documented yet.

Taurine has been recognized as an antioxidant long time ago, scavenging both reactive oxygen and nitrogenous radicals. Cyclophosphamide (CYP) is one of most effective chemotherapeutic agents used in treatment of many types of cancers (Gallagher, 2003). Cancer patients usually rank pneumonitis as one of the most distressing side effects after CYP therapy (Blossom et al., 1997). The mechanism of such side effects is possibly the generation of free radicals through CYP activation which may cause direct injury to epithelial cells of the lung. Such initial lung injury may subsequently increase the influx of activated inflammatory cells into lung parenchyma. The inflammatory cells, such as macrophages or polymorphonuclear cells, produce reactive oxygen species (ROS), and these ROS may be important contributors to pathogenesis of CYP-induced pneumonitis (Colvin, 1982).

The present study was undertaken to examine the effect of taurine on isolated pulmonary arterial rings and tracheal strips of rats and to investigate the possible mechanism(s) of its action. This was done by evaluating the influence of autonomic control, arachidonic acid metabolites, potassium ions, and nitric oxide on the effect of taurine. The antioxidant effect of taurine was also investigated.

2. Materials and methods

2.1. Materials

Adult Sprague-Dawley rats of both sexes weighing 200-250g were used. Animals were obtained from local source (Faculty of Pharmacy, Mansoura University). They were maintained under standard conditions of temperature of 25˚C with regular 12h light/12h dark cycle and allowed free access to standard laboratory food and water.

Taurine powder (Fluka Chemie, Switzerland), Serotonin creatinine sulfate monohydrate (5-HT) powder (Fluka Chemie, Switzerland), Propranolol, (lnderal ampoules, 0.1%, Astrazeneca, UK), N⁴-nitro-L-arginine methyl ester hydrochloride (L-NAME) powder (Fluka Chemie, Switzerland), Aminophylline, (Cidophylline ampoules, 250mg/10ml, Chemical Industries Development; CID, Egypt, under license from Schering AG, Germany), Amiodarone HCl, (cordarone ampoules 150mg/3ml, Sanofi Aventis), Atropine sulphate powder (fine-chem. Ltd., India), Chlorpheniramine maleate, (Pirafene ampoules, 5mg/ml, Memphis Co. For Pharma. & Chemical Ind., Cairo, Egypt), Cyclophosphamide, (Cycram vial, 1gm white powder, EIMC Pharmaceuticals Co., Egypt), Indomethacin powder (PHARCO Pharmaceuticals, Alexandria, Egypt) and Montelukast powder (Merck Sharp & Dhom, California, USA).

2.2. Effect of taurine on isolated pulmonary arterial rings and tracheal strips of rats

2.2.1 Preparation of pulmonary arterial rings

Rats were killed by an overdose of diethylether. The chest was opened along the right side of the sternum. The pericardium and the connective tissue between aorta and pulmonary artery were removed. The pulmonary artery was tied gently, separated and rapidly placed in warm physiological salt solution. The composition of the physiological salt solution (PSS) in mM/L was: NaCl, 118; KCl, 4.7; CaCl₂,2H₂O, 2.5; KH₂PO₄, 1.2; MgSO₄.7H₂O, 1.2; NaHCO₃, 25; and glucose, 10 (El-Mazar et al., 1995).

The pulmonary artery was trimmed and cut into rings. The rings were cleaned of connective tissue, and weighed. Each ring was suspended horizontally between two stainless steel parallel hooks, one of which was fixed and the other attached to isometric tension transducer for the measurement of isometric tension in an organ bath filled with 50 ml of the PSS at fixed temperature of 37˚C and continuously bubbled with 100% pure O₂.

The suspended pulmonary arterial rings were allowed to equilibrate for 30 minutes under a resting load of 3 g (Leff et al., 1986) during that time the bath solution was replaced periodically every 5 minutes.

2.2.2 Preparation of rat tracheal zig-zag strips

Rats were killed by an overdose of diethylether. The trachea was removed and placed in PSS, the composition of which in mM/L was: NaCl, 118; KCl, 4.7; CaCl₂,2H₂O, 2.5; KH₂PO₄, 1.2; MgSO₄.7H₂O, 0.62; NaHCO₃, 25; and glucose, 10 (Nials et al., 1997). The trachea was cleaned of extraneous tissues and examined carefully to locate the smooth muscle. The cartilage was cut longitudinally; the trachea was opened on a cork board and kept moist with the PSS. A sharp scalpel was used to cut transverse slits at equally spaced intervals first on one side of the preparation and then on the other side (Emmerson and Mackay, 1979).

The resulting zig-zag strip was then weighed. A cotton thread was tied to one end of the tracheal strip for attachment to a fixed point in the organ bath. Another cotton thread was tied to the other end of the tracheal strip and connected to the isometric transducer. The strip was set up in an organ bath filled with 50 ml of the PSS, maintained at 36˚C and continuously bubbled with 100% pure O₂ (Brunn et al., 1995). The tracheal zig-zag was allowed to equilibrate for 40 minutes under resting load of 1 g (Wessler et al., 1994); during that time the bath solution replaced periodically every 10 minutes.

Isometric tension generated by pulmonary arterial and the tracheal smooth muscles was measured using a displacement transducer (model 50-7905, Harvard Apparatus LTD., South Natick, MA, USA) and recorded on a two-channel oscillograph (model 50-8622, Harvard Apparatus LTD., USA). The responsiveness of the pulmonary arterial rings and tracheal strips to different
agents was recorded and calculated as % relaxation of the initial contraction. The rings and strips were precontracted by \((2\times10^4 \text{ mM})\) 5-HT, when the contraction of the isolated pulmonary arterial rings and tracheal strips reach the maximum, different concentrations of taurine (10, 30 and 100 mM) were added cumulatively to the organ bath.

2.3. Effect of inhibitors on the relaxation-induced by taurine

A contraction was made by 5-HT, after the contraction reaches maximum, the rings and strips were incubated with the inhibitor for the specified time, then the same concentrations of taurine (10, 30 \& 100 mM) were added cumulatively.

The following inhibitors were used: 100 \(\mu\)M L-NAME (nitric oxide synthase inhibitor) for 20 minutes (MacLean and McCulloch, 1998), 0.1 \(\mu\)M montelukast (cysteinyli leukotrienes receptor 1 blocker) for 5 minutes (Ishimura et al., 2006), 1 \(\mu\)M chlorpheniramine (histamine H1 receptors blocker) for 5 minutes (Gruetter et al., 1992), 50 \(\mu\)M propranolol (\(\beta\)-adrenoceptor blocker) for 5 minutes (Rubanyi and Vanhotte, 1985), 10 \(\mu\)M indomethacin (cyclo-oxygenase inhibitor) for 20 minutes (Curzen et al., 1995), 140 \(\mu\)M aminophylline (adenosine receptors antagonist) for 5 minutes (Matsuda et al., 2000), 1 \(\mu\)M atropine (muscarinic receptors blocker) for 5 minutes (Fabiani et al., 1997) and 14 \(\mu\)M amiodarone (non selective potassium channel blocker) for 5 minutes (Krasteva et al., 2007).

2.4. Measurement of protective effect of taurine against CYP-induced lung toxicity in rats

Rats were divided into four experimental groups, each of 6 rats. The groups were treated according to the following schedule:

**Group I:** Control group, injected i.p. with saline (0.001 ml/g body weight for 8 days).

**Group II:** Taurine group, injected i.p. with an equal volume of saline while being administered taurine (1% in drinking water to reach a dose of 1 g/kg per day, orally for 8 days).

**Group III:** Received CYP (150 mg/kg, i.p. on day 7).

**Group IV:** Received CYP (150 mg/kg, i.p.) and taurine (1% in drinking water to reach a dose of 1 g/kg per day, orally) starting 7 days before CYP and continuing throughout the rest of the experiment (24 hours).

2.5. Sampling and biochemical parameters measuring

Twenty four hours after injection of CYP, animals were killed by overdose of ether and the lungs were quickly isolated, washed with saline, blotted dry on filter paper, weighed and then 10% (w/v) homogenates of each sample were made in ice cooled suitable buffer using a homogenizer. The lung homogenates were used for the determination of thiobarbituric acid reactive substances (TBARS), non-protein sulphydryl compounds (NPSH), superoxide dismutase activity and nitric oxide.

2.6. Statistical analysis

Data were expressed as mean \pm S.E.M. (Significance was accepted at \(p<0.05\)). Statistical analysis was carried out using paired Student’s t-test (Daniel, 1991). Statistical calculations were carried out using Instat-2 computer program (GraphPad Software Inc., V2.04, San Diego, CA, USA).

3. Results and Discussion

In the present study, taurine produced a concentration dependent relaxation in the isolated tracheal strips and pulmonary arterial rings of rats precontracted by serotonin. The relaxing effect of taurine was not influenced by pretreatment with nitric oxide synthase inhibitor (L-NAME), cysteinyli leukotrienes receptor 1 blocker (montelukast), H1 receptor blocker (chlorpheniramine), \(\beta\)-adrenoceptor blocker (propranolol), potassium channel blocker (amiodarone), cyclo-oxygenase inhibitor (indomethacin) or muscarinic receptor blocker (atropine) (table 1 and 2).

Several in vitro studies showed that taurine relaxes precontracted arteries. In rabbit ear artery, Franconi et al. (1982) showed that taurine produced a concentration-dependent relaxation in the artery precontracted by high potassium and a slight relaxation when the artery precontracted by noradrenaline. On the other hand, Li and colleague (1996) reported that taurine selectively depressed the vasoconstriction induced by noradrenaline without significant effects on the contraction elicited by other substances in isolated rat mesenteric artery. Similarly, Ristori and Verdetti (1991) reported that taurine reduced the basal tone and caused relaxation of the precontractions induced by depolarization and noradrenaline in isolated rat thoracic aortic rings.

In the present study, the relaxing effect of taurine in the pulmonary arterial rings and tracheal strips was significantly potentiated by preincubation with adenosine receptor blocker, aminophylline. Adenosine has been reported to produce a concentration dependent decrease in pulmonary arterial tension of precontracted pulmonary arterial rings and theophylline reduces the vasodilation induced by adenosine (El-Kashef et al., 1999). In the present study, taurine induced relaxation of the isolated pre-contracted pulmonary arterial rings was significantly potentiated by aminophylline, an adenosine receptor blocker (figure 1).

This observation is puzzling since, it was expected that blocking of adenosine receptor would certainly inhibit the relaxing effect of taurine rather than potentiating the relaxing effects. The possible explanation of this puzzling observation is probably the existence of adenosine receptor subtype that not respond to aminophylline, a non selective adenosine receptor.
Table 1. Effect of L-NAME, montelukast, chlorpheniramine, propranolol, indomethacin, theophylline, atropine or amiodarone on taurine induced relaxation of precontracted isolated pulmonary arterial rings of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Response (% relaxation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before 10 mM</td>
</tr>
<tr>
<td>L-NAME</td>
<td>20.5±0.7</td>
</tr>
<tr>
<td>Montelukast</td>
<td>19.4±1.5</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>23±2.5</td>
</tr>
<tr>
<td>Propranolol</td>
<td>21±1.1</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>20.8±1.0</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>17.8±1.1</td>
</tr>
<tr>
<td>Atropine</td>
<td>20±1.6</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>22.2±1.4</td>
</tr>
</tbody>
</table>

Data are represented as mean ± S.E.M. * Significantly different (p<0.05) compared to respective values (before treatment) using paired student’s t-test.

Table 2. Effect of L-NAME, montelukast, chlorpheniramine, propranolol, indomethacin, theophylline, atropine or amiodarone on taurine induced relaxation of precontracted isolated tracheal strips of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Response (% relaxation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before 10 mM</td>
</tr>
<tr>
<td>L-NAME</td>
<td>22±1.34</td>
</tr>
<tr>
<td>Montelukast</td>
<td>18.8±2.60</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>21.6±2.23</td>
</tr>
<tr>
<td>Propranolol</td>
<td>19.8±1.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>15.8±0.97</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>17.8±1.46</td>
</tr>
<tr>
<td>Atropine</td>
<td>14.4±0.51</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>18.4±1.435</td>
</tr>
</tbody>
</table>

Data are represented as mean ± S.E.M. * Significantly different (p<0.05) compared to respective values (before treatment) using paired student’s t-test.

Figure 1. Effect of aminophylline (140 μM) on taurine-induced relaxation of isolated pulmonary arterial rings of rats. Data are expressed as means ± S.E.M., n=5 * Significantly different (p<0.05) compared with respective values (before treatment) using paired Student’s t-test.

Adenosine had been proposed as a possible mediator involved in the physiopathology of asthma. This effect of adenosine is antagonized by theophylline (Nieri et al., 1997). The bronchoconstrictive action of adenosine is mediated in part via mast cell derived factors such as histamine and in other part via ACh (Nieri et al., 1997). In the present study, taurine induced relaxation of the isolated precontracted tracheal strips was potentiated by aminophylline indicating the involvement of adenosine receptor in mediating the relaxing effect of taurine in tracheal smooth muscles (figure 2). Therefore, taurine produced a concentration-dependent relaxation in the isolated tracheal strips and pulmonary arterial rings of rats precontracted by serotonin probably by direct effect on the pulmonary smooth muscle and induced tracheal relaxation partly via blocking of adenosine receptors existing in the rat trachea and probably by direct effect on the tracheal smooth muscles.

Many studies have focused on the detoxification function of taurine and have demonstrated that taurine reacts with and neutralizes ROS, which are generated receptor blocker, or it may need either higher concentration of the antagonist or more time to be blocked.
during an oxidative cell burst. In this study, an increase in lung weight index was observed after a single i.p. dose of CYP (150 mg/kg) in rat compared with the control group. This observation is in agreement with Sulkowski et al., (2002) who reported that CYP caused edema and inflammation in lung tissues after a single i.p. dose in rats. This inflammation and edema after CYP treatment may be responsible for the increase in lung and liver weight indices.

Taurine (1% in drinking water to reach a dose of 1 g/kg per day for 8 days) did not reduce the increase in lung/body weight ratio induced by CYP indicating that taurine did not modify the inflammation produced by CYP (table 3). This can be explained by the study of Mastaloudis et al., (2004) who demonstrated that supplementation with vitamin E or vitamin C prevents oxidative damage induced by exercise but has no apparent effect on inflammation. Oxidative damage and the inflammatory response may be operating independently or inflammation was great enough to overwhelm the effects of antioxidants. Higher doses of taurine or any antioxidant may be required to elicit the anti-inflammatory effect.

Excessive formation of lipid peroxide is considered to be an indirect in vivo feasible index for free radical generation and in turn, for oxidative stress in the pathogenesis of several lung injuries, particularly those caused by exposure to exogenous oxidants (Parizada et al., 1991; Ryrfeldt et al., 1993). Lipid peroxidation may give rise to several products, most of them are biologically active and cytotoxic. Substances that result from splitting off the two C-C bonds adjacent to the hydroperoxy group are alkanals, typified by MDA. These MDAs are highly reactive compounds that, through reaction with protein thiols and/or cross-linking of amino groups of proteins, can cause considerable intracellular damage (Esterbauer, 1993).

The MDA production, an index of lipid peroxidation, was increased significantly in lung homogenate compared to control groups after CYP administration (table 3). This observation is in line with many reports that demonstrated apparent elevation in lung TBARS following administration of CYP (Kaya et al., 1999; Sulkowski et al., 2002). This increase in MDA may reflect the generation of ROS or FR by CYP (Manda and Bhatia, 2003b).

Administration of taurine to rat before CYP significantly reduced the increase in lung lipid peroxide compared to CYP group. This result is in agreement with Bhat et al., 1994; Gurujeyalakshmi et al., 2000 who found that the antineoplastic agent, bleomycin, forms an intracellular bleomycin Fe2+ complex that generates oxygen free radicals and produces pneumonitis and fibrotic lesions. Addition of taurine and niacin to the drinking water reduced the inflammatory response in animals treated with bleomycin. The protective effect was reflected in the degree of decrease of lipid peroxidation. Pretreatment with taurine ameliorated CYP induced cystitis, as indicated by a significant decrease in lipid peroxides and a marked restoration of glutathione content (Abd-Allah et al., 2004). Taurine supplementation reduced lipid peroxidation in plasma and tissues of ethanol treated rats as evidenced by reduction in TBARS (Pushpakiran et al., 2004).

Reduction of lipid content (GSH) is known as one of the endogenous antioxidants, being identified as a protector against the damaging effect of free radicals (El-Khatib, 1997). Administration of CYP, in the current study, reduced lung content of GSH (table 3). This is in line with other reports that demonstrated GSH reduction or depletion in lung following CYP challenging in animals. Sulkowski et al., (2002) and Manda and Bhatia, (2003a) reported that, CYP is capable to reduce the level of tissue GSH in both mice and rats, respectively.

Taurine led to a significant increase of lung glutathione when administered before CYP in comparison with CYP-injected group. This observation is in agreement with the study conducted by Venkatesan and Chandrakasan, (1994) which showed that pretreatment of rats with daily intraperitoneal injection of taurine plus niacin 7 days prior and 2 days after CYP significantly inhibited the development of lung injury, prevented the alterations in lavage fluid biomarkers associated with inflammatory reactions, with less lipid peroxidation and restoration of reduced glutathione. It was reported that pretreatment with taurine leads to restoration of GSH content in rat urinary bladder treated with CYP (Abd-Allah et al., 2004). Additionally, taurine overcome lead induced oxidative stress as evidenced by an increase in reduced glutathione content (Gurer et al., 2001).
The increase of GSH may be the mechanism of ta urine induced relaxation of smooth muscles where Joris et al., (2002) showed that GSH and other thiols dependentely decreased the tension in isolated guinea pig tracheas. Relaxations by GSH were paralleled with multiple physiological processes that range from aerobic metabolism (Fridovich, 1974). CYP produced no significant change in lung SOD (table 3), a result that agreed with the work of Sulkowska et al., (2002). In contrast, CYP, 100 mg/kg as a single i.p. dose for 5 days in rats decreased lung SOD (Patel and Block, 1985). Such controversy could be explained in virtue of the postulate that the total period of drug administration and the dosage itself seem to regulate the activity of antioxidant enzymes. In the current study, pretreatment with ta urine produce no significant change in SOD activity which may contribute to its antioxidant activity as evidenced by increasing the level of lung GSH and decreasing the lung MDA in rats pretreated by CYP.

### Table 3. Effect of cyclophosphamide(CYP, 150 mg/kg) and its combination with taurine (1%) on lung/body weight ratio, GSH, MDA, SOD & nitrate/nitrite levels in rat lung.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lung/body weight ratio</th>
<th>GSH (mmol/g.tissue)</th>
<th>MDA (nmol/g.tissue)</th>
<th>SOD (U/mg tissue)</th>
<th>Nitrate/nitrite level (µmol/g.tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0062 ± 0.0002</td>
<td>3.45 ± 0.19</td>
<td>345.14 ± 21.41</td>
<td>17.77 ± 1.307</td>
<td>14.39 ± 0.49</td>
</tr>
<tr>
<td>CYP</td>
<td>0.0070 ± 0.0003</td>
<td>1.15 ± 0.09</td>
<td>971.5 ± 43.91</td>
<td>19.87 ± 1.37</td>
<td>15 ± 0.51</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.0065 ± 0.0002</td>
<td>4.45 ± 0.19</td>
<td>361.84 ± 24.02</td>
<td>20.35 ± 1.095</td>
<td>14.82 ± 0.61</td>
</tr>
<tr>
<td>Taurine/CP</td>
<td>0.0077 ± 0.0002</td>
<td>2.12 ± 0.06</td>
<td>676.81 ± 23.38</td>
<td>19.65 ± 0.79</td>
<td>14.68 ± 0.55</td>
</tr>
</tbody>
</table>

- Data are expressed as mean ± SEM, n = 6.
- Taurine was administered orally for 7 days before and 1 day after CYP injection.
- Single dose of CYP was injected i.p.
- $^*$ significantly different from control group using unpaired student’s t test (p<0.05).
- $^*$ significantly different from CYP group using unpaired student’s t test (p<0.05).

4. Conclusion
Taurine relaxes precontracted rat tracheal strips and pulmonary arterial rings probably by direct effect on the smooth muscles. Also, ta urine has antioxidant activity which may contribute to its relaxant effect. Ta urine may be useful in protection and treatment of bronchial asthma.

References


