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The reflexes originating in the tracheobronchial tree and lungs fall into two categories: 1) **Regulatory reflexes** that determine the pattern of breathing in normal circumstances and, 2) **Defense reflexes** that protect the respiratory tract from potentially harmful influences. Reflexes evoked by changes in lung volume regulate the pattern of breathing and the tone of airway smooth muscle under normal circumstances. Reflexes evoked by chemical stimuli applied to airways or defense reflexes serve quite a different function. They represent emergency, protective responses brought into play when harmful agents are carried to the respiratory tract in the inspired air or in the bloodstream (Coleridge and Coleridge, 1986). These reflexes comprise of a variety of respiratory maneuvers such as cough, apnea succeeded by rapid shallow breathing, bronchoconstriction and increased secretion by airway glands. These maneuvers expel the noxious agents or at least limit their further entry into the lung thereby reducing their harmful effects. The inputs which initiate the airway defense reflexes are confined mainly in vagus nerves (Coleridge and Coleridge, 1986). The trigger for these responses can be (i) extrinsic agents such as irritant gases and (ii) intrinsic agents such as locally released lung autacoids. The inflammatory mediators released during inflammatory processes such as an asthma attack can affect the activity of vagal sensory receptors. Thus, these receptors can play an important role in the pathophysiology of mucosal inflammation and allergic conditions such as asthma (Ravi, 1998).

1.1. Pulmonary Vagal Sensory Receptors

There are 4 distinct groups of vagal sensory receptors that have been identified in the airways (Coleridge and Coleridge, 1994). These are: the slowly adapting pulmonary stretch receptors (SARs), the rapidly adapting receptors (RARs), the bronchial C-fiber receptors and the pulmonary C-fiber (type J) receptors. The SARs have a characteristic respiratory rhythm in their discharge. They are stimulated by lung expansion and they adapt slowly to a maintained hyperinflation. They are connected to myelinated vagal afferents and are responsible for the Hering-Breuer inflation reflex. Their endings are located in the smooth muscle layer of the proximal airways.
(Coleridge and Coleridge, 1986). On the other hand, RARs commonly referred to as ‘irritant’ receptors and ‘cough’ receptors have an irregular resting discharge. They are also connected to myelinated vagal afferents and adapt rapidly to a maintained hyperinflation of the lung. They are located in the epithelial and sub-epithelial regions of the proximal airways (Coleridge and Coleridge, 1986). However, recently it has been proposed that they are located near bronchial venules (Ravi and Kappagoda, 1990).

The C-fiber receptors (connected to non-myelinated vagal afferents) have been categorized as the ‘bronchial’ and ‘pulmonary’ based upon their vascular accessibility to chemicals. The bronchial C-fiber is activated preferentially by injection of certain chemicals such as bradykinin into the bronchial circulation (Coleridge and Coleridge, 1984); the pulmonary C-fiber is stimulated by chemicals such as phenyl diguanide and capsaicin, when injected into the pulmonary circulation (Paintal, 1973). Both these groups of receptors have negligible spontaneous activity. Several studies have reported that upon inhalation of irritant fumes causing airway irritation, there is activation of RARs (Paintal, 1973). Because of this property RARs have also been referred to as ‘irritant’ receptors (Paintal, 1973). Following their activation, there is a reflex cough, an increase in airway secretion and bronchoconstriction (Ravi, 1998). Thus, the RARs have been considered to play an important role in airway defense reactions. These are initial ‘warning signals’ regarding the condition of the airways. If no treatment is given, the situation will worsen as the RARs will function as a ‘positive feedback’ mechanism – an increase in airway resistance will stimulate the RARs mechanically and their stimulation will cause further bronchoconstriction. RARs can be stimulated by the various inflammatory mediators such as histamine, bradykinin, prostaglandins, tachykinins etc. which are released during the inflammatory process. Increases in the fluid flux either by increase in intravascular hydrostatic pressure (Roberts et al. 1986; Kappagoda et al. 1987) or by plasmapheresis also stimulates the RARs (Ravi et al. 1994). Even though the main function of the SARs is to ‘regulate’ respiration, they get activated by conditions which promote airway constriction (Paintal, 1973). However, unlike the RARs, their stimulation causes airway relaxation (Widdicombe and Nadel, 1963). The C-fiber receptors do not form part of present investigation and the focus of the present study is mainly on the activity of RARs. For comparison, the responses of SARs are also investigated.
1.2. Asthma

Asthma is a chronic inflammatory disease of airways in which there occurs recurrent episodes of wheezing, breathlessness, chest tightness and cough. These symptoms are usually associated with widespread but variable airflow obstruction that is at least partly reversible either spontaneously or with treatment (WHO/NHLBI Workshop Report, 1995). There is a loss of epithelial cell integrity, sub-basement membrane thickening and occlusion of bronchial lumen by mucus. There is hyperplasia and hypertrophy of bronchial smooth muscle and hyperplasia of goblet cells also. Atopy is a pre-disposing factor for asthma and most of these subjects have hyper-reactive airways. It has been suggested that nearly 5-10% of the world’s population is suffering from this disease. Even though asthma is a treatable disorder, its prevalence and severity are increasing and the mortality rate is rising (Barnes, 1989).

An attack of asthma can be precipitated by several triggering factors such as pollens from grasses, weeds and trees, house dust, mite, fungi, viral infections, cigarette smoke, diesel fumes etc. On exposure to the triggering factors, there is the liberation of inflammatory mediators such as histamine, bradykinin, leukotrienes, neuropeptides, prostaglandins, thromboxanes and platelet activating factor from primary effector cells including mast cells, eosinophils, macrophages, lymphocytes, neutrophils, platelets and epithelial cells. These mediators increase the permeability of bronchial blood vessels. Additionally, these chemicals may act directly upon the hyper-reactive airways and promote bronchospasm. Airway hyper-responsiveness (AHR) is an important feature of asthma that involves an enhanced contractile response of the airway smooth muscle to these mediators (Holtzman et al., 1983). AHR monitoring gives an indication of the severity of the disease and accounts for the frequency of the symptoms and the improvement with treatment (Takeda et al., 1997). These inflammatory mediators may also stimulate the underlying sensory receptors and produce reflex mechanisms. However, there is negligible information in the literature regarding the responses of these sensory receptors following allergen challenge in the sensitized animals.
1.3. Role of Reactive Oxygen Species (ROS)

The airways are always exposed to a higher PO$_2$ than other organs, and this particular environment may possibly enhance the production of reactive oxygen species (ROS) leading to oxidative stress. During an oxidative stress there is a disturbance in the balance between the production of ROS and antioxidant defenses which may lead to tissue injury (Betteridge, 2000). There is extensive tissue damage that occurs when ROS overwhelm the antioxidant defenses of the host. The oxidative stress also plays an important role in the pathophysiology of asthma (Doelman and Bast, 1990; Barnes, 1990) and may be a final common pathway leading to tissue damage.

An exposure to a variety of different substances such as allergens, gaseous pollutants, chemicals, drugs, bacteria and viruses (Levine, 1995) leads to the recruitment and activation of inflammatory cells in asthmatic airways, including mast cells, eosinophils, neutrophils, lymphocytes, macrophages and platelets. Activated inflammatory cells respond with a "respiratory burst", which involves the uptake of oxygen and subsequent release of ROS, such as superoxide ion and hydroxyl ion, into surrounding cells. Lipid peroxidation is perhaps the most extensively studied consequence of free radical attack (Betteridge, 2000). The occurrence of this process in biological membranes causes impairment of membrane functioning, decreased fluidity, inactivation of membrane bound receptors and enzymes and increased non-specific permeability of ions such as calcium. Lipid peroxidation makes a significant contribution towards worsening of tissue injury (Gutteridge and Halliwell, 1990).

ROS can influence airway cell function by several ways. Acute exacerbations of asthma are found to be associated with increased oxidative stress (Nadeem et al. 2005). ROS can induce the production of cytokine and chemokine through induction of the oxidative stress-sensitive transcription of nuclear factor-$\kappa$B in bronchial epithelial cells (Biagioli et al. 1999). It may interact with DNA, contract airway smooth muscle and increase vascular permeability (Pennings et al., 1999). ROS may also impair $\beta$-adrenoceptor function. Alveolar macrophages have been shown to impair $\beta$-adrenergic responsiveness in guinea pig trachea in vitro. This effect has been presumed to be due to release of ROS (Barnes, 1990). Exogenous oxidants or endogenous ROS from
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Airway inflammatory cells may contribute to mucus hypersecretion occurring in asthma. Oxygen free radicals have been implicated in AHR after ovalbumin challenge in guinea pigs (Ikuta et al., 1992).

1.4. Anti-oxidant Defence Mechanisms

Within the lung, powerful anti-oxidant enzymes are present and their levels may increase or decrease following chronic exposure to increased levels of ROS (Pennings et al., 1999). Anti-oxidants can act by (a) removing oxygen /decreasing total local oxygen concentrations (b) removing catalytic metal ions (c) removing key ROS such as superoxide radical and hydrogen peroxide (d) scavenging initiating free radicals such as alkoxyl and peroxyl species (e) breaking the chain of an initiated sequence and (f) quenching /scavenging singlet oxygen. Anti-oxidants are of three types (Gutteridge, 1995) (1) Cellular anti-oxidants: superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx); (2) Membrane antioxidants: Vitamin E, β-carotene, Co-enzyme Q; and (3) Extracellular anti-oxidants: Transferrin, Lactoferrin, Albumin, Ceruloplasmin, Bilirubin, Vitamin C etc.

Thus, there is increasing evidence that oxidative stress plays an important role in the pathogenesis of asthma. Normally, there is an anti-oxidant defence mechanism of the body which protects it from free radical attack (Betteridge, 2000). It has been reported that during asthmatic condition, inflammatory cells increase the production of ROS which contribute to the oxidative stress (Barnes, 1990). Thus, it has been proposed that in asthma, there is an increased oxidant generating capacity and a reduced anti-oxidant defense leading to persistent inflammation of the airways (Smith et al., 1993).

1.5. Role of Nitric Oxide and Reactive Nitrogen Species

Nitric oxide (NO) also plays a key role in the physiologic regulation of airway function and has been implicated in the pathophysiology of inflammatory airway diseases, including bronchial asthma (Barnes and Belvisi, 1993). It is synthesized from the semi-essential amino acid L-arginine by the enzyme NO synthase (NOS). Three isoforms of this enzyme have been identified (Moncada and Higgs, 1993) namely
endothelial NOS or eNOS, neuronal NOS or nNOS and inducible NOS or iNOS. The detrimental effects of NO are generally assumed to be related to the formation of more reactive intermediates termed as reactive nitrogen species (RNS). The rapid reaction of NO with free radicals (radical-radical reaction) has emerged as one of the major routes to the formation of RNS such as peroxynitrite.

High concentrations of iNOS-derived NO are produced in asthmatic airway inflammation. The role of iNOS-induced NO in allergen-induced airway hyperreactivity (AHR) is still uncertain. Since NO is a bronchodilator, increased levels of NO could have beneficial effects on the airway reactivity to bronchoconstrictive stimuli. However, high levels of NO could also have detrimental effects by causing edema due to increased bronchial blood flow and plasma exudation in the airways (Kuo et al. 1992). In addition, high concentrations of NO as well as peroxynitrite, which can be generated from NO and oxygen radicals during the asthmatic inflammation (Gaston et al. 1994), may cause epithelial damage and hence AHR (Flak and Goldman, 1996; Sadeghi et al. 1996). Furthermore, the allergic inflammatory response may be exacerbated by a selective suppressive effect of NO on the T helper cells, type 1 (Th1), and this might promote the proliferation of T helper cells, type 2 (Th2), which are specifically involved in asthmatic airway inflammation (Barnes and Liew, 1995).

Even though there is sufficient information on the effects of various inflammatory mediators upon the vagal sensory receptors, there is scanty information on the involvement of ROS in stimulation of vagal sensory receptors and eliciting the defense reflexes (Ruan et al., 2003). There are some studies which have reported that in the presence of hydroxyl radical scavenger dimethylthiourea (DMTU), there is a reduction in the stimulation of RARs to circulatory endotoxin (Lai et al. 2005), pulmonary embolism (Chen et al., 1997) and wood smoke (Lai and Kou, 1998). However, these reports are indirect.

A few other studies reported the modulation of the sensitivity of c-fiber receptors to exogenous chemicals such as capsaicin in the sensitized animals (Zhang et al., 2008; Kuo and Lai, 2008; Bergren, 2001). But these studies did not investigated whether the sensitivity of RARs and SARs were also altered in such a preparation. Further there is no study which has addressed the role of oxidative stress in stimulating
the vagal sensory receptors in animals sensitized and challenged with an allergen. An increase in ROS/RNS production as a part of inflammation in the sensitized animals may stimulate these vagal sensory receptors directly or indirectly. Whether *in vivo* generation of ROS stimulates the airway receptors remains to be seen.

Keeping these lacunae in mind, in the present study, attempts were made first to examine the behavior of vagal sensory receptors connected with myelinated fibers during increased oxidative stress induced AHR and to observe whether partial reversal of inflammation/AHR by dietary supplementation with anti-oxidants - Vitamin C and Vitamin E - would attenuate ROS/RNS generation, and change receptor activity.