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For more than 100 years, it has been known that vagal reflexes from the lungs can influence ventilation and the pattern of breathing. Even though the Hering-Breuer reflex was described as early as 1868, there were no dramatic advances in our understanding of lung reflexes until the development of recording action potentials in single vagal fibers. Analysis of the respiratory reflexes was greatly facilitated by Adrain’s development of the techniques for recording activity from single nerve fibers (Adrain, 1933).

Afferent nerve endings are the natural starting point of all reflex activity. The airway sensory receptors constantly monitor the mechanical and chemical changes in the lungs and transmit this information primarily via vagal afferent pathways to the central nervous system (CNS), which through the motor pathways evokes a host of reflex responses. Some afferent inputs go through the sympathetic nervous to CNS and reflex effects mediated by sympathetic afferents are believed to be moderate and of less importance than vagal pathways (Paintal, 1973).

Receptors in the lower airways and alveoli have two main functions 1) to regulate the pattern of breathing and other motor systems (such as bronchomuscular tone) in healthy conditions and in response to physiological changes and 2) to evoke appropriate changes in breathing and related functions as a protective mechanism, reacting to harmful invasion of the lungs and during diseases of the airways and lungs (Coleridge and Coleridge, 1986).

The Inhalation of irritants gases and vapours elicits respiratory defense reflexes such as apnea, cough, laryngeal constriction and bronchoconstriction. These reflexes tend to limit the access of the irritants into lung and minimize their harmful effects.

2.1. Airway Sensory Receptors

Different studies on pulmonary afferent endings in dogs (Sampson and Vidruk, 1975; Coleridge and Coleridge, 1977), cats (Knowlton and Larrabee, 1946;
Widdicombe, 1954a) and rabbits (Mills et al., 1969c) established the various categories of pulmonary receptors. On the basis of their morphology, adaptation behavior to maintained stimulus and conduction velocity, presently there are mainly three categories of pulmonary sensory receptors, 1) slowly adapting receptors (SARs) 2) rapidly adapting receptors (RARs) and 3) C-fiber receptors (pulmonary and bronchial) (Paintal, 1973; Sampson, 1977; Coleridge and Coleridge, 1984; Widdicombe, 1986a). In addition to the above receptors, vagal Aδ receptors have been reported in the tracheal epithelium of guinea pigs (Ricco et al., 1996; Undem and Carr, 2001; Undem et al., 2002). These receptors adapt rapidly to a maintained mechanical stimulus and their sensitivity to some chemicals varies from the conventional RARs (Widdicombe, 2003).

2.1.1. Slowly Adapting Pulmonary Stretch Receptors (SARs)

The SARs are a category of neural afferent endings that innervate the tracheobronchial tree. Their original designation was based on the observation that an increase in airway wall tension increased receptor discharge. They have a rhythmic pattern of discharge during eupneic breathing (dynamic property) and this discharge adapts slowly as airway wall tension is maintained (static property) (Sant'Ambrogio et al., 1974). The rhythmic discharge of eupneic breathing is characterized by a mounting discharge during inspiration and a declining discharge during expiration. The pattern of slowly adapting stretch receptor discharge during eupneic breathing is a direct consequence of their anatomical location within the tracheobronchial tree and the orientation of their receptor endings in relationship to airway wall structures (Schelegle, 2003).

The first documented role for SARs was to provide the afferent ‘input’ for evoking the Hering–Breuer inflation reflex. This reflex is characterized by an early termination of inspiration when the lungs are inflated during inspiration and a prolongation of the expiratory pause when a prolonged inflation is applied at the end of inspiration (Widdicombe, 1954b). In addition, these receptors have been implicated in the regulation of airway smooth muscle tone, the regulation of systemic vascular tone and heart rate, and the pathophysiology of restrictive lung disease (Schelegle, 2003).
2.1.1.1. Morphology and Anatomical Distribution

The SARs are located within the airway smooth muscle layer (Bartlett et al., 1976) and correspond to the myelinated afferent nerve fibers innervating airway smooth muscle in numerous mammalian species (Larsell, 1921; Larsell and Dow, 1933; Elftman, 1943; Fisher, 1964; Spencer and Leof, 1964) The conduction velocity recorded for these vagal afferents are in the range of 14 –59 m/sec (Paintal, 1973).

A considerable variation between mammalian species exists in the distribution of SARs along the tracheobronchial tree. They are distributed in the extrathoracic trachea, intrathoracic trachea, bronchus and in the intrapulmonary airways (Widdicombe, 1954a; Miserocchi et al., 1973; Bartlett et al., 1976; Sant'Ambrogio, 1982; Ravi, 1986; Keller et al., 1989).

On the basis of their location in the tracheobronchial tree and the different forces acting upon them during the ventilatory cycle, the extra-/intrathoracic and extra-/intrapulmonary SARs have different patterns of discharge. Intrathoracic SARs have a rhythmic pattern of discharge during eupneic breathing. In contrast, extrathoracic tracheal receptors exhibit a more irregular pattern of discharge and may exhibit in a few receptors a mounting discharge during expiration. The large group of SARs with inspiratory rhythmic discharge pattern has been further characterized based on whether they continue to discharge during expiration. The SARs with discharge activity during expiration are called “low-threshold” receptors and those that are silent during expiration but discharge only during inspiration are called “high-threshold” receptors (Paintal, 1973; Ravi, 1986). SARs are classified as type I and type II also according to their responses to constant pressure inflation of the lungs (Sant'Ambrogio, 1982). Type I receptors tend to saturate in their response when inflation pressure is above 10 cmH₂O and are believed to be mainly in larger and proximal airways, whereas type II receptors have a relatively linear response to lung inflation, increasing activity with increasing pressure and are believed to be more in distal airways (Sant'Ambrogio, 1982).

2.1.1.2. Reflex Responses of SARs

SAR Activity on Tidal Volume and Inspiratory Time

The stimulation of SARs during inspiration acts as an ‘off-switch’ in the respiratory centers of the brain stem to terminate inspiration (Coleridge and Coleridge,
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1986). If peak phasic SAR activity is increased during inspiration, then tidal volume is decreased and inspiratory time is shortened (Clark and von Euler, 1972; Trenchard, 1977).

SAR Activity on Expiratory Time

As hypothesized by Hering and Breuer (1868a, b) that tonic input from the vagus nerves during expiration when the lung is not in motion contributes to breathing frequency by affecting expiratory time, the SAR activity during lung deflation also contributes to determine the expiratory time. When the tonic impulse activity of SARs increases during expiration the expiratory time is lengthened and if tonic SAR activity is decreased during expiration the expiratory time is shortened (Hering and Breuer, 1868a; Bartoli et al., 1974; Zuperku et al., 1982). In addition, the dynamic SAR activity during lung deflation also contributes to determining the expiratory time (Zuperku et al., 1982). Zuperku et al. (1982) attributed this contribution of dynamic and tonic expiratory SAR activity to the long time constant for summation of SAR input in the expiratory centers within the brainstem.

An increase in the phasic discharge of SARs during inspiration indirectly acts to shorten expiratory time (Cohen, 1979) most likely by shortening the period of activation of inspiratory muscle early in expiration (von Euler, 1997). This possibility is supported by several studies that have shown a linear relationship between the inspiratory and expiratory time for a given breath (Grunstein et al., 1973; D’Angelo and Agostoni, 1975; Bartoli et al., 1975; D’Angelo, 1978).

Response to Carbon Dioxide

CO₂ has an inhibitory effect on SARs (Mustafa and Purves, 1972; Schoener and Frankel, 1972; Sant'Ambrogio et al., 1974; Bradley et al., 1976; Kunz et al., 1976; Coleridge et al., 1978; Mitchell et al., 1980) with intrapulmonary higher threshold receptors being the most affected (Ravi, 1985).

The inhibition of SARs discharge by CO₂ has been shown to be independent of changes in pulmonary mechanics (Mustafa and Purves, 1972; Sant'Ambrogio et al., 1974; Coleridge et al., 1978; Green et al., 1986). They provided an afferent input that was inversely related to pulmonary CO₂ and exerted an inhibitory influence on the
respiratory center. Hence an increase in lung CO$_2$ would increase breathing. The treatment with acetazolamide attenuates the CO$_2$-induced inhibition thereby indicating that an elevated PCO$_2$ produces inhibition by increasing hydrogen ion concentration at the receptor location (Sant’Ambrogio et al., 1974; Ravi, 1985). The CO$_2$-induced inhibition of SARs does not involve a reduced influx of Na$^+$ through voltage-gated Na$^+$ channels, but may involve the activation of 4-aminopyridine sensitive K$^+$ channels at the nerve terminals of SARs (Matsumoto et al. 1999, 2000).

Effect of Chemical Substances upon SAR Activity

SARs are not very chemosensitive. Following the administration of bronchoconstrictive agents an increase in peak inspiratory and/or mean expiratory discharge activity of SARs located below the trachea in several species has been observed (Widdicombe, 1954a; Bartlett et al., 1976a; Davenport et al., 1981b; Matsumoto et al., 1990) along with vagal parasympathetic efferent stimulation (Matsumoto, 1996). These interventions have been shown to act through the contraction of airway smooth muscle and not by a direct stimulation of the SAR terminal ending (Matsumoto et al., 1990, 1992, 1993; Matsumoto and Shimizu, 1994; Matsumoto, 1996). It has been shown that an increase in SAR activity relaxes airway smooth muscle by reducing parasympathetic tone to the airway (Widdicombe and Nadel, 1963a, b). SARs have been suggested to play a role in a negative feedback mechanism that acts to limit increases in parasympathetic tone to the airway and optimize the reciprocal relationship between dead space and airway resistance (Widdicombe and Nadel, 1963b). Volatile anesthetics such as halothane may inhibit or stimulate the receptors depending on concentration and type of SAR receptor (Nishino and Koch, 1994). Veratrum alkaloids are strong stimulants for SARs (Ravi, 1986) and their activation can be blocked by ouabain and flecainide (Matsumoto et al., 1998b). Wood smoke inhibits SARs, but not due to an irritant effect but because of the high concentration of CO$_2$ in the smoke (Lai and Kou, 1998). Surfactants also inhibit the SARs by changes in lung compliance (Chen et al., 1998).

2.1.1.3. SARs and Lung Disease

The role that phasic activity of SARs plays in determining normal airway tone and how this may be altered in disease states, such as asthma, has become an area of active study. The SARs play important roles in the regulation of breathing pattern
and/or airway tone in pathological conditions where their sensitivity to normal stimuli is increased due to bronchoconstriction (Widdicombe, 1954a; Bartlett et al., 1976a; Davenport et al., 1981b; Matsumoto et al., 1990), airway obstruction (Davenport et al., 1981a) or decreases in lung compliance (Yu et al., 1991). Koller and Ferrer (1973) made a brief mention that there was an increase in SAR activity following allergen challenge in sensitized guinea pigs.

2.1.2. C-fiber Receptors

Paintal (1955b), first recorded impulses in vagal afferent non-myelinated fibers from the lungs of cats and called them ‘specific deflation receptors’ which were subsequently renamed as type J-receptors by him (Paintal, 1969). Later Coleridge et al. (1965) extended these studies to the dog and designated them as pulmonary C-fiber receptors. In the eupneic anaesthetized animal, C-fiber receptors usually have a sparse irregular discharge. They are not very sensitive to lung volume changes, but can be stimulated by large lung inflations in dogs and by forced lung deflations in cats (Coleridge et al., 1965). Pulmonary vascular congestion is a strong stimulus. The main 'physiological' stimulus to these receptors is believed to be an increase in pulmonary interstitial fluid (Paintal, 1969). Drugs that cause pulmonary edema strongly stimulate the pulmonary C-fiber receptors. The receptors are excited by an increase in pulmonary blood flow, which raises the possibility that they may play a role in the respiratory changes in exercise and an inhibition of exercise itself (J- Reflex) (Paintal, 1973; Anand and Paintal, 1980).

2.1.2.1. Morphology and Anatomical Distribution

Morphologic evidence shows that approximately 75% of the afferent fibers in the vagal branches innervating the respiratory tract are non-myelinated (C-fibers) (Agostoni et al., 1957). These afferent fibers can be subdivided into two groups based upon circulatory accessibility: 1) bronchial C-fibers are those endings, which are located in the extrapulmonary airways which are accessible primarily from bronchial circulation (Coleridge et al., 1984) 2) Pulmonary C-fibers are those arising from the endings located in the lungs which are accessible primarily from the pulmonary circulation. Pulmonary C-fiber endings are located close to the pulmonary capillaries in
the interstitium and hence are called as ‘juxta-pulmonary capillary receptors’ or ‘type J receptors’ (Paintal, 1969). These are non-myelinated vagal afferents (mostly) with conduction velocity in the range of 0.8-7 m/sec (Paintal, 1973). These two groups of afferents also appear to have different sensitivities to chemical and mechanical stimuli (Coleridge and Coleridge, 1984).

Many chemicals have been shown to stimulate the receptors. These include foreign substances such as phenyl diguanide and capsaicin and also natural mediators such as histamine, bradykinin, prostaglandins and 5-hydroxytryptamine. The c fibers can be stimulated by a number of inhaled irritants such as cigarette smoke, ozone, sulfur dioxide, ammonia, acrolein, and volatile anesthetics (Paintal, 1973; Coleridge and Coleridge, 1984, 1994).

2.1.2.2. Reflex Responses of C-fiber Receptors

Activation of C-fiber receptors in various pathophysiological conditions evokes pulmonary defense reflexes.

Rate and Depth of Breathing

The moderate levels of pulmonary C-fiber discharge increase the respiratory rate and decrease tidal volume. Bolus intravenous administration of standard doses of capsaicin or phenyl diguanide results in apnea in expiration, often followed by rapid shallow breathing (Paintal, 1973; Coleridge and Coleridge, 1984). These responses are mediated by the pulmonary C-fiber receptors as demonstrated by vagal cooling and vagotomy (Coleridge and Coleridge, 1984). At the threshold dose, these chemicals produce rapid shallow breathing only (Anand and Paintal, 1980) suggesting that the primary respiratory response of pulmonary C-fiber receptor stimulation is tachypnea.

Pulmonary Congestion and Edema

The pulmonary C-fibers are activated by pulmonary congestion and edema (Paintal, 1973; Coleridge and Coleridge, 1984). They are sensitive to an increase in pulmonary interstitial fluid volume or pressure as a result of changes in pulmonary
vascular pressures or an increase in the fluid filtration across the capillary wall (Paintal, 1973). The bronchial C-fiber receptors are also stimulated pulmonary congestion/edema (Coleridge and Coleridge, 1977, Ma et al., 2003).

**Bronchoconstriction**

Stimulation of bronchial and pulmonary C-fibers causes contraction of the smooth muscle of airways (Coleridge and Coleridge, 1984; Coleridge and Coleridge, 1986). The bronchoconstriction is greatly attenuated by neurokinin A and B receptor antagonists, thereby suggesting that local release of bronchoconstrictor peptides from C-fiber terminals is responsible for this response. Pulmonary and bronchial C-fibers also activate a non-adrenergic, non-cholinergic bronchodilator pathway (Inoue et al., 1991).

**Submucosal gland secretion**

Stimulation of pulmonary and bronchial C-fibers in dogs increases submucosal gland secretion in the trachea (Coleridge and Coleridge, 1994) and larynx by an atropine-sensitive (cholinergic) reflex.

**Cough**

Inhalation of capsaicin aerosol reproducibly causes cough in humans (Lammers et al., 1988; Fuller, 1990) and conscious guinea pigs (Karlsson, 1996). In guinea pigs, cough to capsaicin aerosol is abolished by prior treatment with high dose systemic capsaicin, which destroys capsaicin-sensitive (C-fiber) afferents (Karlsson, 1996). This suggests that stimulation of lower airway C-fibers can evoke cough in humans and guinea pigs.

**Cardiovascular system**

The cardiovascular effects of pulmonary and bronchial C-fiber stimulation include bradycardia, and systemic and pulmonary hypotension. The cardiac slowing is prominent, often beginning with a period of asystole. The bradycardia is largely mediated by activation of cardiac vagal efferents (Coleridge and Coleridge, 1984), and is usually absent after cholinergic blockade with atropine (Brender and Webb-Peploe, 1969; Daly and Kirkman, 1988).
Skeletal Muscle Inhibition

Stimulation of pulmonary C-fibers by injection of phenyldiguanide in cats inhibits spinal motor neurons by a reflex that can be abolished by lesions in the caudate nucleus (Paintal, 1973). Based on these observations, Paintal (1970) hypothesized that pulmonary C-fibers act physiologically as a brake on exercise in the presence of pulmonary congestion (J - reflex).

Conscious Perception

It has been suggested that C-fiber stimulation gives rise to a sensation of breathlessness during exercise or high altitude pulmonary edema (Paintal, 1995). In humans, intravenous lobeline or intravenous or aerosolized capsaicin produces unpleasant sensations in the throat and midsternum that are described as burning, choking or irritation (Paintal, 1995; Raj et al., 1995). Indeed, it has been suggested that the cough elicited with these sensations is a behavioral response to the unpleasant sensations (Widdicombe, 1998).

2.1.3. Rapidly Adapting Receptors (RARs)

Airway rapidly adapting receptors (RARs) were first identified by Keller and Loeser (1929). Using multifiber records from the vagus nerves of rabbits, they showed that some receptors responded with a rapidly adapting discharge to lung inflation and deflation and to mechanical stimulation. Knowlton and Larrabee (1946) recorded single fiber activity and were the first to quantitate “adaptation index (AI)” defining it as the percentage decrease in impulse frequency from a receptor after 1 sec of maintained stimulus.

\[
\text{AI} = \frac{\text{Peak frequency} - \text{Average frequency during 2}^{\text{nd}} \text{sec of inflation}}{\text{Peak frequency}}
\]

On the basis of rapid adaptation (≥70%), Knowlton and Larrabee (1946) described them as rapidly adapting receptors (RARs). They are also referred as “irritant receptors” (Mills et al., 1969c).
2.1.3.1. Morphology and Anatomical Distribution

RARs are located mainly in and under the airway epithelium. They are found in the airway wall from the nasopharynx to the larger bronchi (Sant'Ambrogio and Widdicombe, 2001; Widdicombe, 2001). In the tracheobronchial tree they are concentrated at the carina and hilar regions (Widdicombe, 1996a; 2001). Based upon their conduction velocities (8–35 m/s), the nerve fibers originating from these receptors indicated that they were myelinated (Kappagoda and Ravi, 2006).

It has been reported that, RARs are closely associated with mucosal venules, which lose their myelin sheaths terminally and end as encapsulated bodies containing non-myelinated fibers (Kappagoda et al., 1990). RARs have a wide range of properties (Ravi and Kappagoda, 1990). These vagal afferents are stimulated by mechanical as well as chemical stimulus. They respond secondarily to the changes in properties of mucosa and are stimulated by airway smooth muscle contraction (Coleridge and Coleridge, 1986; Coleridge et al., 1989; Canning, 2002), by mucosal vasodilatation with resultant interstitial extravasation of plasma (Bonham et al., 1996; Widdicombe, 1996b) and by mucus secreted into the airway lumen (Rogers, 2001). An increase in hydrostatic pressure and fall in plasma oncotic pressure or vasoactive substances such as histamine and SP will increase extra vascular fluid volume and stimulate RARs (Ravi and Kappagoda, 1990; Bonham et al., 1996).

2.1.3.2. Stimulus for RARs

Mechanical Stimulation

Being a mechanoreceptor, RARs are stimulated by punctate stimulation of the receptor area. A nylon catheter introduced into the trachea has been used as a technique to stimulate the RARs in the proximal areas (Widdicombe, 1954).

Rapidly Adapting Receptors and the Pulmonary Circulation

Pulmonary congestion stimulates the RARs in the experiments performed in artificially ventilated, anaesthetized dogs (Kappagoda et al., 1987). The increase in left atrial pressure by partly obstructing the mitral valve stimulated the RAR without any discernible evidence of adaptation thereby suggesting that the activity of RAR is
modulated by factors, which influence fluid fluxes from the microcirculation of the airways. During partial obstruction of the mitral valve, the increase in hydrostatic pressure in the pulmonary veins raises pressure in the bronchial venules of the proximal airways (Wagner et al., 1998), promotes fluid flux and activates RARs.

**Starling Forces and the Microcirculation of the Lung**

The RAR are responsive to changes in extravascular fluid volume in the lung. Therefore, besides hydrostatic pressure, their activity can be influenced by the other factors in the Starling equation, i.e. plasma oncotic pressure and microvascular (capillary) permeability. In addition, they can be influenced by lymphatic obstruction (Kappagoda and Ravi, 2006).

**Effect of Altering Oncotic Pressure on RAR Activity**

Reduction in the oncotic pressure in plasma by plasmapheresis (by removing 10–12% of the estimated blood volume of an animal and returning the red blood cells suspended in saline) enhances the transfer of fluid from the vascular compartment to the extravascular one. This increase in fluid flux is reflected in pulmonary lymph flow. As expected from the Starling equation, the flow of lymph is sensitive to both an increase in hydrostatic pressure caused by increments in left atrial pressure and a reduction in oncotic pressure of plasma. Thus, it could be anticipated that raising the left atrial pressure would increase the extravascular fluid volume in the respiratory system and that this increase would be enhanced further after plasmapheresis (Kappagoda and Ravi, 2006). It was found that the increase in the RAR activity observed during the period of increased left atrial pressure was enhanced further by plasmapheresis (Kappagoda and Ravi, 1989).

**Effect of Lymphatic Obstruction on the Activity of RAR**

Lymph is sensitive to an increase in hydrostatic pressure. Obstruction of lymph drainage in both dogs (Ravi et al., 1988) and rabbits (Hargreaves et al., 1991; Ravi et al., 1994) stimulates the RARs. The activity was sustained for the duration of obstruction without adaptation. Further, the obstruction of pulmonary lymphatic drainage against a background of pulmonary congestion showed additive effects on stimulation of RARs.
2.1.3.3. Reflex Responses of RARs

RARs act as the first line of defense and evoke the defense reflexes similar to C-fiber receptors in pathological conditions (Ravi and Kappagoda, 1990). In eupnoeic breathing they are usually silent and produce occasional discharges, but they are sensitive to mechanical stimulation. RARs are stimulated in hyperpnoea and by vigorous inflations and deflations of the lungs.

Ventilatory Reflex

The activation of RARs can accelerate or augment breathing (Davies et al., 1978; Davies and Roumy, 1982). That tachypnea can result from RAR stimulation has been demonstrated by injection of histamine in cats (Glogowska et al., 1972) and by pulmonary congestion in dogs (Kappagoda et al., 1989).

Bronchoconstriction

The stimulation of RARs can alter the airway tone by causing the bronchoconstriction (Mills et al., 1969b). The bronchoconstriction associated with pulmonary congestion has been reported to be due to stimulation of RARs (Kappagoda et al., 1988).

Airway Secretions

During discrete stimulation of RARs by reduction in pulmonary compliance there is a reflex increase in bronchial secretion (Yu et al., 1989).

Coughing

Mechanical stimulation of RARs located at the carina can induce cough (Widdicombe, 1998). Many tussigenic irritants such as bradykinin, capsaicin and prostaglandin E2 stimulate the RARs thereby causing cough (Hargreaves et al., 1992; Mohammed et al., 1993). Hence the coughing that occurs in diseases of airways e.g., asthma has been considered to be due to stimulation of RARs (Korpas and Widdicombe, 1991). The cough accompanying angiotensin converting enzyme inhibitor therapy has also been reported to be due to RAR stimulation (Hargreaves et al., 1992).
Other Reflexes

Other reflexes evoked from RAR stimulation, include airway vasodilation, and laryngoconstriction (Coleridge and Coleridge, 1986; Widdicombe, 1986; Karlsson et al., 1988). These reports show that the RARs are an important afferent mechanism mediating the airway defense reflex.

2.1.3.4. Responses of RARs to Lung Autacoids

Histamine

Histamine is known to constrict the airway smooth muscle. The stimulation of RARs by histamine has been considered to be secondary to the constriction of the airways which causes the distortion of tissues in the vicinity of the receptors (Mills et al., 1969b; Dixon et al., 1979). Vidruk et al (1977) suggested that histamine has an additional direct effect on RAR, independent of the effect on smooth muscle. This effect was no longer evident after the administration of H₁ receptor antagonist chlorpheniramine (Sampson and Vidruk, 1977).

Histamine also increases the permeability of the systemic venules and enhances lymph flow (Haddy et al. 1972). In the bronchial circulation, it promotes bronchovenular permeability and causes bronchial oedema (Pietra et al. 1972; Fishman and Pietra, 1976). It has also been shown that infusion of histamine into the right atrium increases pulmonary lymph flow (Ravi et al. 1989). Thus, histamine may stimulate the RARs by fluid flux in the extravascular space of the airways. It was found that even sub-threshold doses of histamine enhanced the responses of RAR to pulmonary congestion caused by small elevations of left atrial pressure or by pulmonary lymphatic obstruction (Ravi et al. 1989). Also, against a background of pulmonary lymphatic obstruction, the stimulus–response curve relating histamine and RAR activity was shifted to the left when compared with that elicited in the control state (Ravi et al. 1989).

Histamine infusion also produced a sustained stimulation of RAR without producing any detectable change in airway pressure (Ravi et al. 1989). These results indicate that some of the effects of histamine on RAR are likely to be mediated by changes in the permeability of the bronchial vasculature.
Bradykinin

Bradykinin stimulates the RARs indirectly by increasing the permeability of bronchial vasculature (Hargreaves et al., 1992). It was observed that enalapril, an angiotensin converting enzyme (ACE) inhibitor, when given i.v. increased the resting activity of RAR by increasing the bradykinin. Additionally, in presence of enalapril, the dose-response curve relating receptor activity to bradykinin was shifted towards the left, suggesting that the sensitivity of RAR to bradykinin was enhanced by enalapril (Hargreaves et al., 1992).

Substance P

Exogenous administration of substance P has been shown to cause airway microvascular leak (Lei et al., 1992; Lembeck et al., 1992) and increase the RAR activity (Bonham et al., 1996; Bhagat et al., 2011). Increased microvascular leak is a potent stimulus of RARs (Kappagoda et al., 1987; Kappagoda and Ravi, 1989; Ravi and Kappagoda, 1990; Ravi and Kappagoda, 1992). SP produces its effect by binding to NK-1 or NK-2 receptors. It is reported that the NK-1 receptors are located in the blood vessels and NK-2 receptors in the airway smooth muscle (Iversen, 1985). Administration of CP-96345 (NK-1 receptor antagonist) has been shown to block the SP induced effect on RAR activity (Bonham et al., 1996, Bhagat et al., 2011).

Prostaglandins

Several prostaglandins stimulate the RARs. Prostaglandin F$_2$α, a potent constrictor of airway smooth muscle, when injected into the right atrium in dogs or given as aerosol in guinea pigs stimulates the RARs (Coleridge et al., 1976; Bergren et al., 1984). Administration of prostaglandin E$_2$ into the right atrium of dogs produces only minor effects on RARs (Coleridge et al., 1976). However, in cats, it has been demonstrated that administration of increasing concentrations of prostaglandin E$_2$ as an aerosol causes a dose-dependent increase in RAR activity and bronchoconstriction (Mohammed et al., 1993). Even though bronchoconstriction by itself can activate the RARs, the prostaglandin induced stimulation may be due to an increase in the fluid flux.
also as during bronchoconstriction, there would be passive compression of the veins draining the bronchial mucosa (Ravi, 2002).

**Irritant Vapours**

The RARs are stimulated by vapours of ammonia, cigarette smoke, alcohol, acetone, ether, inhaled wood smoke and carbon dust (Bergren and Sampson, 1982; Sellick and Widdicombe, 1971; Widdicombe, 1954; Sampson and Vidruk, 1975; Ravi et al., 1995; Lai and Kuo, 1998). Irritant vapours can stimulate the RARs directly as isoproterenol had no effect on the ability of the vapors to stimulate the rapidly adapting receptors (Bergren and Sampson, 1982). This stimulation can occur in the absence of significant changes in peak intratracheal pressure. In fact, inhalation of ether reduced the intratracheal pressure and yet stimulated the receptors (Bergren and Sampson, 1982). Even though the mechanism behind these stimulations can be the concurrent increase in tracheal pressure as well as the direct stimulatory effect of the irritant vapors upon the receptors, there are evidences which show that they may be due to the fluid flux in the vicinity of the receptors (Ravi, 1998).

There is a close interaction between airway nerves and inflammation. The neurogenic inflammation occurs due to the activation of airway sensory receptors associated with the vagal afferents (Coleridge and Coleridge, 1986). Excess production of ROS is a common feature of various pulmonary pathophysiological conditions including asthma (Emelyanov et al. 2001). The concept that capsaicin-sensitive vagal lung afferent fibres may detect the existence of excess ROS in the lungs is relatively new (Soukhova et al., 1999). This concept is indirectly supported by the findings that C fiber-mediated airway reflexes are evoked by cigarette smoke (Lee, 1990), inhaled wood smoke (Kou et al. 1997) or pulmonary air embolism (Chen & Kou, 2000) and that the afferent responses of pulmonary C fibers to the latter two insults (Chen et al. 1997; Lai & Kou, 1998a) or endotoxin (Lai et al., 2005) are greatly attenuated by ·OH scavengers. The sensory nerves in turn may also amplify inflammation in the airways through the release of neurotransmitters. The idea that sensory nerves may amplify and spread the inflammatory response has attracted considerable attention as it may contribute to the inflammation in airway diseases such as asthma.
2.2. Asthma

Asthma is a major chronic respiratory disease affecting millions of people all over the world, both in the developed and developing countries. There is a wide variation in prevalence of asthma across the globe and even within each country.

The Global Strategy for Asthma Management and Prevention Report (WHO/NHLBI Workshop Report, 1995) stated that “Asthma is a chronic inflammatory disease of the airways in which many cell types play a role, in particular mast cells, eosinophils and T lymphocytes. In susceptible individuals the inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or early morning. These symptoms are usually associated with widespread but variable airflow obstruction that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli.”

For many years, the basic alterations in asthmatics were considered to be bronchospasm, edema, and hypersecretion. Evidences of bronchial inflammation were provided from the studies of nonspecific bronchial hyperresponsiveness, bronchoalveolar lavage (BAL) (Godard et al., 1982), bronchial biopsies (Jeffery, 1996), and induced sputum (Maestrelli et al., 1995) and observations made from the postmortem of patients who died from an attack of asthma (Ellis, 1908; Jeffery, 1996).

The genetic predisposition to develop asthma is now well recognized (Holgate, 1997) and the IgE-mediated response to common allergens represent the most common form of the disease in childhood and early adulthood (Platts-Mills, and Wheatley, 1996).

2.2.1. Acute Inflammation and Brief Symptoms

Precipitous symptomatic attacks of asthma may be caused by several known or unknown factors such as exposure to allergens (Platts-Mills, and Wheatley, 1996), viruses (Busse and Gern 1997), or indoor and outdoor pollutants (Wardlaw, 1993) and each of these may induce an acute inflammatory response.
2.2.1.1. Early-phase Reaction

Inhaled allergen challenge in allergic patients leads to an early inflammatory allergic reaction and in some cases, this may be followed by a late-phase reaction. The early-phase reaction is initiated after the activation of cells bearing allergen-specific IgE. It is characterized by the rapid activation of airway mast cells (Murray et al., 1985, Liu et al., 1991) and macrophages (Tonnel et al., 1983, Calhoun et al., 1992). The activated cells rapidly release proinflammatory mediators such as histamine (Jarjour et al., 1997), eicosanoids (Wenzel et al., 1989), and ROS which induce contraction of airways smooth muscle, mucous secretion, and vasodilatation. The bronchial microcirculation has a central role in this inflammatory process (Persson et al., 1995). Inflammatory mediators induce microvascular leakage with exudation of plasma into the airways (Greiff et al., 1993, Van-Vyve et al., 1995). Acute plasma protein leakage induces a thickened engorged and edematous airway wall and a resultant narrowing of the airway lumen. In addition, plasma may also traverse the epithelium, pass through the tight junctions, and collect in the airway lumen. Plasma exudation may compromise epithelial integrity, and its presence in the lumen may reduce clearance of mucus (Wanner et al., 1996). Plasma proteins may also promote the formation of viscid luminal plugs of exudates mixed with mucus and inflammatory and epithelial cells. Together, these effects contribute to airflow obstruction.

This bronchoconstrictive response associated with acute inflammation is characterized by brief symptoms including wheezing, dyspnea, and shortness of breath which usually do not persist for more than a day or so.

2.2.1.2. Late-phase Reaction

Cellular Events and Release of Proinflammatory Mediators

The late-phase inflammatory reaction occurs between 6 to 9 h after allergen provocation and involves the recruitment and activation of eosinophils (De Monchy et al., 1985), CD4+ T cells (Robinson et al., 1993), basophils (Guo et al., 1994), neutrophils (Koh et al., 1993, Montefort et al., 1994), and macrophages (Calhoun et al., 1993). There is selective retention of airway T cells (Gratziou et al., 1996), the
expression of adhesion molecules (Lassalle et al., 1993; Georas et al., 1992; Bentley et al., 1993), and the release of selected proinflammatory mediators (Liu et al., 1991, Smith et al., 1992) and cytokines involved in the recruitment and activation of inflammatory cells (Jarjour et al., 1997, Zangrilli et al., 1995). The activation of T cells after allergen challenge leads to the release of T helper cell, type 2 (Th2)-like cytokines which may be a key mechanism of the late-phase response (Kay, 1992). The release of preformed cytokines by mast cells is the likely initial trigger for the early recruitment of cells (Bradding et al., 1994) and induces the more persistent involvement by T cells.

Recruitment of Inflammatory Cells into the Airways

Inflammatory cells mature and are released by bone marrow and traffic in the circulation before being recruited into the airway wall. The recruitment of peripheral blood cells including eosinophils, lymphocytes, and monocytes into inflamed airways is the result of adhesive interactions between circulating inflammatory cells and microvascular endothelial cells via the production of proinflammatory mediators, cytokines, and chemokines, and the expression of cell surface adhesion molecules. Upregulation of distinct adhesion molecules such as CD11a, CD11b, CD18, or Very Late Antigen (VLA-4) on blood cells and intercellular adhesion molecule-1 (ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells is a critical step for the induction of the inflammatory response (Georas et al., 1992, Granger and Kubes, 1994, Bochner and Schleimer, 1994). The ligand VLA-4 is not present on neutrophils (Neeley et al., 1993), which may, in part, explain the selective recruitment of eosinophils in asthma (Ohkawara et al., 1995). An increase of such airway vascular adhesion molecules has been observed in asthma (Wardlaw et al., 1992, Fukuda et al., 1996). Recruitment of cells into the walls of the airways is associated with their priming and activation (Nagata et al., 1995) and is also dependent on cytokines such as IL-5 (Sur et al., 1995) and GM-CSF acting to enhance eosinophil recruitment, terminal maturation (Lopez et al., 1988), and expression of their adhesion molecules (Neeley et al., 1993, Sedgwick et al., 1995). Chemokines such as RANTES (regulated upon activation, normal T-cell expressed and secreted) (Alam, et al., 1993, Teran et al., 1996) and eotaxin (Garcia-Zepeda et al., 1996, Elsner et al., 1996) also act on eosinophils and T cells (Sallusto et al., 1997) to enhance markedly their recruitment and
possibly their activation. RANTES (Holgate et al., 1997) and IL-16, a lymphocyte chemoattractant factor, and macrophage inflammatory protein 1 α (MIP-1 α) are found in BAL fluid (BALF) of antigen-challenged asthmatics (Cruikshank et al., 1995) and may also participate in the process.

The enhancement of nonspecific bronchial hyperresponsiveness can usually be demonstrated after the late-phase reaction but not after the early-phase reaction following allergen or occupational challenge (Cockcroft and Murdock, 1987; Fabbri et al., 1991).

**2.2.2. Airway Inflammation**

The airway inflammation has been widely demonstrated in asthma, and an association between the extent of inflammation and the clinical severity of asthma has been demonstrated (Bousquet et al., 1990; Vignola et al., 1993).

**2.2.2.1. Characteristics of Airway Inflammation**

Inflammation in asthma appears to be far more complex than a simple eosinophilic inflammation alone (Jeffery, 1992). All cells of the airways, including T-cells, eosinophils, mast cells, macrophages, epithelial cells, fibroblasts, and even bronchial smooth muscle cells are involved in asthma and become activated. Nonetheless, eosinophils play an effector role by release of proinflammatory mediators (Schwartz, 1992; Henderson, 1994; Racke et al., 1996; Barnes, 1996), cytotoxic mediators (Gleich et al., 1993), and cytokines (Holgate, 1993; Shah et al., 1995; Maestrelli et al., 1995; Jarjour and Busse, 1995; Drazen et al., 1996; Mueller et al., 1996), resulting in vascular leakage, hypersecretion of mucus, smooth muscle contraction, and epithelial shedding and bronchial hyperresponsiveness. These cells are also involved in the regulation of the airway inflammation and initiate the process of remodeling by the release of cytokines (Holgate, 1993; Shah et al., 1995; Maestrelli et al., 1995; Jarjour and Busse, 1995; Drazen et al., 1996) and growth factors.

**2.2.2.1.1. Role of Epithelium**

For many years, bronchial epithelial cells were considered to act mainly as a barrier participating in mucociliary clearance and removal of noxious agents. More
recently, epithelial cells have been found to participate in inflammatory reactions by the release of eicosanoids, peptidases, matrix proteins, cytokines, and nitric oxide (NO). In asthma, epithelial cells are activated thereby releasing greater amounts of mediators such as 15-HETE, prostaglandin E2 (PGE2), fibronectin, cytokines, growth factors, and endothelin spontaneously or after stimulation (Campbell et al., 1993, Bradding et al., 1995) which can induce bronchial obstruction, inflammation, and airways remodeling (Nakamura, et al., 1995).

In asthma, epithelium is partly shed, ciliated cells appear swollen, vacuolized and there is often loss of cilia (Laitinen et al., 1985, Montefort et al., 1993). When epithelium is reconstituted there are greater numbers of goblet cells than normal. In fatal asthma, extensive epithelial shedding is commonly observed (Ellis, 1908). Epithelial cells of asthmatics are also significantly less viable than those of normal subjects (Campbell et al., 1993). Epithelial shedding can be caused by plasma exudation (Persson, 1996), toxic inflammatory mediators such as eosinophil granule proteins (Gleich et al., 1979, Takafuji et al., 1996), free radicals, tumor necrosis factor-alpha (TNF-α) (Kips et al., 1993), mast cell proteolytic enzymes (Schwartz, 1992), metalloproteases from epithelial cells (Rickard et al., 1992) or macrophages (Mautino et al., 1997). Epithelial damage may lead to heightened airways responsiveness (Jeffery et al., 1989, Ohashi et al., 1992), the destruction of a diffusion barrier altering permeability of the airway mucosa (Sparrow and Mitchell, 1991), the depletion of epithelial-derived relaxant factors (Rabe, et al., 1995) and loss of enzymes (neutral endoproteases) responsible for degrading proinflammatory neuropeptides including substance P (Lilly et al., 1993).

2.2.2.1.2. Inflammatory Cells

Eosinophils

Tissue eosinophilia is a characteristic of asthma (Lacoste et al., 1993). Eosinophils found in the airways of symptomatic asthmatics are activated (Broide et al., 1991; Laitinen et al., 1991). Most allergic and nonallergic asthmatics, including those with mild asthma, have a bronchial eosinophilia and there is a significant association between eosinophil activation and asthma severity (Bousquet et al., 1990) as well as
Bronchial hyperresponsiveness (Bradley et al., 1991). The biological properties of eosinophils include the release of toxic granule proteins, oxygen free radicals, eicosanoids (sulfido-peptide leukotrienes) (Busse and Sedgwick, 1994), Th2-like cytokines (Broide et al., 1992; Ying et al., 1995) and growth factors (Weller, 1991; Gleich et al., 1993). Once activated, products from eosinophils contract human bronchial smooth muscle (Rabe et al., 1994), increase vascular permeability (Collins et al., 1993) and induce airway hyperresponsiveness (Leff, 1994). Eosinophils are deleterious in asthma by the release of highly toxic products such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and oxygen free radicals which induce the shedding of the surface epithelium in keeping with the hypothesis of eosinophil-induced damage of the bronchi (Gleich et al., 1993). Eosinophils can be important cells of airways remodeling. Eosinophils can release growth factors (Ohno et al., 1992; Walz et al., 1993), elastase (Lungarella et al., 1992), and metalloproteases (Ohno et al., 1997) involved in the process of tissue remodeling and fibrosis. Eosinophil products also stimulate fibroblasts (Pincus et al., 1987).

**Lymphocytes**

Increased numbers of T lymphocytes are found in the airway mucosa of patients with fatal asthma (Azzawi et al., 1992) or in asthmatics of variable asthma causation (Azzawi et al., 1990; Bradley et al., 1991, Bentley et al., 1992; Saetta et al., 1992). The majority of lymphocytes bear CD4-receptors whereas CD8-positive cells are more rarely identified, even during exacerbations of asthma (Corrigan et al., 1995). After allergen challenge there is an increase, in bronchial biopsies of asthmatics, of activated T cells and Th2 cytokines (Robinson et al., 1993.; Bentley et al., 1993). There appears to be an association between the severity of asthma and the number of CD4-positive cells in BALF (Walker et al., 1991; Robinson et al., 1993). Few B cells are present in the bronchi of asthmatics. T cells are likely to play a role in controlling the chronic inflammation of allergic and nonallergic asthma by the release of Th2 - cytokines (Robinson et al., 1992; Del Prete et al., 1993; Ying et al., 1995). However, in stable chronic asthmatics T cells in the airways are also of the Th0 or Th1 phenotype (Pene et al., 1993; Krug et al., 1996).
Mast Cells

Mast cells are found in the bronchi of normal subjects and asthmatics (Djukanovic et al., 1990; Bradley et al., 1991; Pesci et al., 1993; Koshino et al., 1995). They are often degranulated in the airways of asthmatics in both their stable phase and after allergen challenge (Laitinen et al., 1985; Beasley et al., 1989). Mast cells appear to be critical “trigger” cells during episodes of acute asthma (Broide et al., 1991) eliciting acute bronchoconstriction, edema, and mucus secretion by the release of histamine and other vasoactive mediators such as PGD2 and cysteinyl leukotrienes. However, the role of mast cells is not confined to acute asthma and they may release neutral proteases including tryptase (Schwartz, 1992) and chymase. Tryptase has proinflammatory mast cell function (Imamura et al., 1996) and potentiates histamine-induced contraction in human sensitized bronchus (Johnson et al., 1997), whereas chymase exhibits procollagen proteinase activity (Kofford et al., 1997; Welle, 1997). Mast cells store preformed Th2-like cytokines which can be released during activation (Bradding et al., 1992; Bradding et al., 1994; MacGlashan et al., 1994; Ochensberger et al., 1996). Mast cells may be involved in airway remodeling because they appear to have an important role in pulmonary fibrosis (Kawanami et al., 1979; Jordana, 1993; Chanez et al., 1993). Mast cells are potential sources of products stimulating migration and proliferation of fibroblasts (Ruoss et al., 1991; Nagata et al., 1992).

Macrophages

Mononuclear phagocytes are likely to be involved in the pathogenesis of asthma because macrophages are among the cells present in the airway inflammatory infiltrate (Poulter et al., 1990; Bentley et al., 1992), particularly in asthma of the nonatopic form (Bentley et al., 1992). Alveolar macrophages (AM) have been recovered from BAL and most studies have revealed their increased activation (Godard et al., 1982; Joseph et al., 1983; Fuller et al., 1988) and shown a significant correlation between their activation and the severity of asthma (Cluzel, et al., 1987; Kelly et al., 1988). Endobronchial challenge with allergen induces the activation of AM (Tonnel et al., 1983; Calhoun et al., 1993); they are also activated during the late-phase reaction after allergen challenge (Gosset et al., 1991). Macrophages may also be involved in the generation of the airway
obstruction and the regulation of the airway inflammation through release of enzymes (Joseph et al., 1983), eicosanoids (Damon et al., 1989), platelet-activating factor (PAF) (Arnoux et al., 1982), oxygen free radicals and cytokines (Borish et al., 1992; Chanez et al., 1994), and mucus secretion that are likely to be deleterious for the bronchi. Macrophages may also be involved in the regulation of the airway remodeling through the secretion of growth-promoting factors for fibroblasts, cytokines, and growth factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), or TGFβ (Kovacs and DiPietro, 1994). They also express PDGFβ mRNA In vivo in asthma (Taylor et al., 1994).

**Polymorphonuclear Neutrophils**

The role of neutrophils in stable asthma remains unclear. However, neutrophils are increased in the airways during the late-phase reaction after an allergen challenge (Koh et al., 1993, Montefort et al., 1994), in some patients who died within hours after an asthma exacerbation (Sur et al., 1993, Carroll et al., 1996), in nocturnal asthma (Martin et al., 1991), in some patients with long-standing asthma (Foresi et al., 1990), or in patients with corticosteroid-dependent asthma (Tanizaki et al., 1993).

**2.2.1.3. Inflammatory Mediators**

**Histamine**

Histamine is synthesized and released by mast cells in the airway wall and by circulating and infiltrating basophils and is stored in granules within mast cells and basophils. Histamine has multiple effects on airway function that are mediated by specific surface receptors on target cells namely, H1, H2 and H3 receptors (Barnes, 1991). It exerts many effects that are relevant to the pathophysiological mechanisms of asthma, including bronchoconstriction, plasma exudation, and mucus secretion (Barnes et al., 1998).

**Adenosine**

Adenosine is a purine nucleoside that is produced by dephosphorylation of 5’-AMP by the membrane-associated enzyme 5’-nucleotidase and is liberated intracellularly
by cleavage of the high energy bonds of adenosine triphosphate, adenosine diphosphate, and cyclic 5’-AMP. Adenosine acting as a vasodilator can function synergistically with several inflammatory mediators, leading to increased vascular permeability. Adenosine is a potent mediator of mast cell degranulation and therefore may contribute to the inflammatory changes observed in asthma (Barnes et al., 1998).

**Prostanoids**

Prostanoids include prostaglandins and thromboxane (Tx), which are generated from arachidonic acid, usually by the action of cyclooxygenases (COX). Prostanoids are produced in asthmatic airways and appear to have several effects on the airways, including bronchoconstriction (Coleman and Sheldrick, 1989; Kawikova et al., 1996), plasma exudation (Okazawa et al., 1997; Tokuyama et al., 1992), sensitization of nerve endings, and effects on the release of inflammatory mediators from inflammatory cells (Giembycz et al., 1990; Peters et al., 1982), which are mediated by prostanoid receptors.

**Leukotrienes**

There is increasing evidence that leukotrienes play an important role in the pathophysiological changes of asthma. There is now substantial evidence that cysteinyl-leukotrienes play an important role in asthma. Cysteinyl-leukotrienes production is increased in asthma in response to various challenges that worsen asthma (Taylor et al., 1989; Wenzel et al., 1995). Cysteinyl-leukotrienes are potent mediators of bronchoconstriction (Drazen, 1988), plasma exudation (Arakawa et al., 1993; Henderson, 1994), and mucus secretion (Marom et al., 1982; Hoffstein et al., 1990), and there is now a growing body of evidence that they may also increase eosinophilic inflammation (Wegner et al., 1993; Underwood et al., 1996).

**Platelet-Activating Factor (PAF)**

PAF has long been implicated in the pathophysiological mechanisms of asthma. PAF is produced by any of the cells that are activated in asthmatic airways and has a profound effect on airway function, producing bronchoconstriction (Barnes et al., 1989; Spencer et al., 1991), inducing airway hyperresponsiveness (Spina et al., 1991; Perretti
and Manzini, 1993), plasma exudation (O’Donnell and Barnett, 1987; Evans et al., 1989), and mucus hypersecretion (Steiger et al., 1987), and recruiting and activating eosinophils (Sanjar et al., 1990; Evans et al., 1996b).

**Peptide Mediators**

Several peptides, including bradykinin, tachykinins, CGRP, endothelins (ETs), and complement, are involved in asthma. They are usually cleaved from larger precursors and are released in an active form. They are subject to degradation by peptidases (such as NEP) both in the circulation and in the airways (Barnes et al., 1998).

**Bradykinin**

Bradykinin has long been considered to be a mediator involved in asthma. Inhaled bradykinin is a potent bronchoconstrictor in asthmatic patients (Fuller et al., 1987b; Polosa and Holgate, 1990). Bradykinin is a potent inducer of airway microvascular leakage and causes prolonged leakage at all airway levels (Rogers et al., 1990). Bradykinin stimulates airway mucus secretion from human submucosal glands in vitro, and these effects are mediated by B₂ receptors (Nagaki et al., 1996), indicating a direct effect of bradykinin on submucosal glands.

The most important property of bradykinin is its ability to activate nociceptive nerve fibers in the airway, because these may mediate the cough and chest tightness that are such characteristic symptoms of asthma. This effect of bradykinin may be enhanced by hyperesthesia of sensory nerves in the airways that have been sensitized by inflammatory mediators. Inhalation of bradykinin by asthmatic patients rather closely mimics an asthma attack; in addition to wheezing, patients experience chest tightness and coughing which are common sensory manifestations during asthma exacerbation. Bradykinin is also a potent bronchoconstrictor in asthmatic patients, and after allergen challenge there is a disproportionate increase in responsiveness to bradykinin, compared with methacholine, which may not be maximal until several days after allergen challenge and may persist for several days (Berman et al., 1995). This may be a reflection of airway sensory nerve hyperesthesia.
Tachykinins

Tachykinins are increased in the secretions of asthmatic patients and produced by sensory nerves (Barnes et al., 1991; Uddman et al., 1997), although there is increasing evidence that inflammatory cells such as macrophages may release SP (Ho et al., 1997). Tachykinins are potent bronchoconstrictors (acting via NK2 receptors) (Evans et al., 1988) and stimulate mucus secretion (Rogers et al., 1989; Meini et al., 1993), plasma exudation (Rogers et al., 1988; Lötvall et al., 1990a), and cholinergic neurotransmission (Undem et al., 1991; Myers and Undem, 1993).

Endothelins

Endothelins are potent constrictor peptides that were originally described as vasoconstrictors released from endothelial cells. They are involved in the pathophysiological mechanisms of asthma (Barnes, 1994b; Hay et al., 1996). Endothelin-1 (ET-1) is abnormally expressed in asthma. ET-1 is a potent bronchoconstrictor (Takahashi et al., 1997; Goldie et al., 1995) and induces plasma exudation (Sirois et al., 1992) and mucus secretion (Shimura et al., 1992).

Cytokines

Cytokines are small protein mediators that play an integral role in the coordination and persistence of inflammation in asthma. The chronic airway inflammation of asthma is unique, in that the airway wall is infiltrated by T lymphocytes of the Th2 phenotype. Th2 lymphocytes produce a panel of cytokines, including IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, and GM-CSF. The primary signals that activate Th2 cells are unknown but may be related to the presentation of a restricted panel of antigens in the presence of appropriate cytokines. Dendritic cells are ideally suited to act as the primary contacts between the immune system and external allergens. Interaction of co-stimulatory molecules on the surface of antigen-presenting cells (in particular, the B7.2/CD28 interaction) may lead to proliferation of Th2 cells, thus perpetuating mast cell activation and eosinophilic inflammation. This may lead to the production of specific IgE by B lymphocytes under the influence of IL-4, which plays a critical role in the isotype switching of B lymphocytes from IgG to IgE.
production. Other cytokines, including TNF-a and IL-6, may also be important. The IgE produced in asthmatic airways binds to FceRI on mast cells, priming them for activation by antigen (Barnes et al., 1998).

The differentiation, migration, and pathobiological effects of eosinophils may occur through the effects of GM-CSF, IL-3, and IL-5. Once recruited from the circulation, mature eosinophils in the presence of these cytokines change phenotype into hypodense eosinophils, which show increased survival in bronchial tissue. These eosinophils are primed for ligand-initiated generation of increased amounts of cys-LTs and for cytotoxicity to other cells, such as those of the airway epithelium. Eosinophils themselves may also generate other cytokines (Barnes et al., 1998).

2.2.3. Remodelling of the Airways

Asthma represents a chronic inflammatory process of the airways followed by healing whose end result may be an altered structure referred to as a remodeling of the airways (Rennard, 1996). Repair usually involves two distinct processes: regeneration, which is the replacement of injured tissue by parenchymal cells of the same type; and replacement by connective tissue and its eventual maturation into scar tissue. In many instances both processes contribute to the healing response. In asthma the processes of cell dedifferentiation, migration, differentiation, and maturation as well as connective tissue deposition can be followed either by complete or altered restitution of airways structure and function, the latter often seen as fibrosis and increase in smooth muscle and mucus gland mass (Bousquet et al., 1992).

2.2.4. Neural Mechanisms

There is considerable evidence that neural mechanisms contribute to the pathophysiology and symptomatology of asthma. There is complex interaction between inflammation and neural control of airways, along with effects of inflammatory mediators on neurotransmission and neurotransmitters, which in turn modulate the inflammatory responses in the airways (Barnes, 1986). Autonomic nerves regulate many aspects of airway function, including airway smooth-muscle tone, secretions, blood flow, microvascular permeability, and the migration and release of inflammatory...
cells (Barnes, 1986, 1990). In addition to classical cholinergic and adrenergic neural pathways, NANC neural mechanisms are present in the airways. Various neuropeptides along with nitric oxide are being shown to mediate the NANC effects.

Because the changes in bronchomotor tone in asthma occur rapidly, it was suggested that there might be an abnormality in autonomic neural control of the airways, with an imbalance between excitatory and inhibitory pathways, resulting in excessively reactive airways. Several types of autonomic defects have been proposed in asthma, including enhanced cholinergic, α-adrenergic and non-adrenergic non cholinergic (NANC) excitatory mechanisms, or reduced β adrenergic and NANC bronchodilator mechanisms.

2.2.4.1. Autonomic Innervation

Cholinergic Pathway (Parasympathetic)

The cholinergic nerves are the dominant neural bronchoconstrictor pathway in animal and human airways. Many triggers, which induce bronchospasm such as histamine, prostaglandins also stimulate afferent receptors leading to reflex bronchoconstriction. Inflammatory mediators may lead to enhanced cholinergic neurotransmission in the airways because of facilitation of acetylcholine release in parasympathetic ganglia within the airways or from post-ganglionic nerve terminals binding to muscarinic receptors and producing bronchoconstriction (Barnes, 1996).

Adrenergic Pathway (Sympathetic)

This is a bronchodilatory mechanism comprising of sympathetic nerves that release noradrenaline and adrenal medulla which secretes adrenaline. These mediators activate adrenoceptors namely α and β on the target cells (Ahiquist, 1948). β - agonists have a dramatic effect in relieving bronchoconstriction, suggesting a defect in adrenergic mechanisms to be responsible for the pathophysiology of asthma. It has been proposed that there is an impairment of β-receptor function in asthma (Szentivanyi, 1968). Reduction in β-adrenergic responsiveness in asthmatics was found to correlate well with the severity of the disease (Brooks et al., 1979).
Non-adrenergic Non-cholinergic Pathway (NANC)

Non-adrenergic Non-cholinergic effects are mediated by the release of co-transmitters from classic adrenergic and cholinergic nerves which can be neuropeptides along with nitric oxide and vasoactive intestinal peptide (VIP). Two types of NANC nerves are found in airways. i-NANC or inhibitory NANC relaxes the airway smooth muscles whereas e-NANC or excitatory NANC causes bronchoconstriction. The mediators of i-NANC are nitric oxide and vasoactive intestinal peptide (VIP) both of them causing the relaxation of airway smooth muscle. Substance P and related tachykinins are the neurotransmitters of e-NANC nerves and causes bronchoconstriction. It has been suggested that abnormal function of NANC nerve may be involved in asthma (Barnes, 1986).

2.2.4.2. Afferent nerve fibers

C-fiber Receptors

Stimulation of C-fiber endings results in local release of tachykinins such as substance P, NKA and calcitonin gene related peptide (CGRP) at the site of inflammation. Retrograde release of peptides from C-fiber receptors via axonal reflex produces neurogenic inflammation in asthmatic airways (Barnes, 1995). Stimulation of C-fiber endings produces reflex bronchoconstriction and increased mucus secretion (Karlsson, 1996).

Rapidly Adapting Receptors

The RARs have been implicated in asthma because RARs are stimulated by agents which promote bronchoconstriction. When the smooth muscles of the airways contract, there is distortion of the RARs which lie nearby. Their stimulation promotes bronchoconstriction that further stimulates the RARs. Thus, there is a positive feedback mechanism operating in the airways (Widdicombe, 1974a). It is evident that there is increased inflammation in asthma and increased release of inflammatory mediators and neuropeptides from inflammatory cells and from C-fiber endings (Barnes, 1995). RARs can be activated directly by neuropeptides and inflammatory mediators (Kappagoda et al., 1987; Ravi and Kappagoda, 1990).
Taken together, these studies indicate that many asthmatics have physiologic abnormalities even when they are asymptomatic and their symptoms can be brought under control with anti-inflammatory treatment.

2.3. Reactive Oxygen Species (ROS)

For many years, the science of free radicals was the preserve of physical and inorganic chemists. In the last 25 years, the free radicals in the form of reactive oxygen species (ROS) have become increasingly recognized as playing a major role in many disease processes (Kirkham and Rahman, 2006).

Airways are exposed to a higher PO$_2$ than other organs, and this particular environment may possibly enhance the production of ROS leading to oxidative stress. Oxidative stress has been defined as a disturbance in the balance between the production of ROS and antioxidant defenses which may lead to tissue injury (Betteridge, 2000). There is extensive damage that occurs when ROS overwhelm the antioxidant defenses of the host. Oxidative stress may play 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. 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 into surrounding cells. During the respiratory burst, a NADPH-dependent superoxide-generating system is activated and releases superoxide (O$_2^\cdot$) into the cell.

\[
2\text{O}_2 + \text{NADPH} \xrightarrow{\text{NADPH Oxidase}} 2\text{O}_2^\cdot + \text{NADP}^+ + \text{H}^+
\]

A dismutation reaction, catalyzed by superoxide dismutase (SOD) then results in the production of hydrogen peroxide (H$_2$O$_2$), which, in the presence of halide ions (i.e. $\Gamma^-$, $\text{Cl}^-$, $\text{Br}^-$), will react to form a hypohalous acid (e.g. HOCl/HOBr). In eosinophils, this reaction is catalyzed by eosinophil peroxidase (EPO). In neutrophils, this reaction is catalyzed by myeloperoxidase.
Review of Literature

\[ 2O_2^- + H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \]

\[ H_2O_2 + Cl^- + H^+ \xrightarrow{\text{EPO/MPO}} HOCl + H_2O \]

HOCl/HOBr may then react with \( O_2^- \) or \( Fe^{2+} \) to produce another strong oxidant, probably the hydroxyl radical (\( \cdot OH \)).

\[ HOCl + O_2^- \rightarrow \cdot OH + Cl^- + O_2 \]

\[ HOCl + Fe^{2+} \rightarrow \cdot OH + Cl^- + Fe^{3+} \]

Thus, during this "respiratory burst", the inflammatory cells release high concentrations of \( O_2^- \), \( \cdot OH \), HOCl/HOBr and \( H_2O_2 \) that may leak into surrounding cells resulting in increased quantities of free radicals in airway tissues (Wood et al. 2003). Furthermore, the inflammatory cells of asthmatics have an increased capability to generate free radicals compared to controls, which further contributes to high concentrations of ROS (Neyens et al. 1984; Cluzel et al. 1987; Jarjour and Calhoun 1994).

ROS such as superoxide anion (\( O_2^- \)) and the hydroxyl radical (\( \cdot OH \)) are unstable molecules with unpaired electrons, capable of initiating oxidation. This can result in the oxidation of proteins, DNA, and lipids that may cause direct tissue injury or induce a variety of cellular responses, through the generation of secondary metabolic reactive species. The lung exists in a high-oxygen environment and together with its large surface area and blood supply is highly susceptible to injury mediated by oxidative stress. Consequently, the lung contains many antioxidant defenses in order to protect itself from oxidant-induced tissue damage.

Lipid peroxidation is perhaps the most extensively studied consequence of free radical attack (Betteridge, 2000). Lipid peroxidation is the mechanism by which unsaturated lipids react with molecular oxygen to undergo auto-oxidation and if once initiated, proceeds as a chain reaction. 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).
Oxygen derived free radicals not only direct tissue injury but also stimulate arachidonic acid metabolism to produce lipid mediators of inflammation such as isoprostanes, and platelet activated factor (PAF) (Shvedova et al., 1995). Many of the effects of the ROS in the airways may be mediated by these lipid mediators. The lipid mediator PAF stimulates eosinophils to release $O_2^{-}$ via a magnesium ion dependent process. 8-iso-PGF$_{2\alpha}$ has been found to be a potent constrictor of smooth muscle. This effect has been observed in vitro in human and guinea pig airways (Kawikova et al. 1996) as well as in cultured rat aortic smooth muscle (Fukunaga et al. 1993). 8-iso-PGF$_{2\alpha}$ has also been shown to elicit airway hyperresponsiveness in isolated perfused mouse lungs (Held and Uhlig, 2000), and cause airway obstruction and airway plasma exudation in guinea pigs in vivo (Okazawa et al. 1997).

ROS appear to oxidize certain amino acids in proteins such as methionine and cysteine and may thus profoundly alter the function of proteins. Many enzymes such as alpha-1 protease and neutral endopeptidase may also be inhibited, leading to profound changes in cell function. Oxygen metabolites may also stimulate inflammatory cells themselves. This is therefore a self-perpetuating cascade. Oxygen radicals stimulate the release of histamine from mast cells (Barnes, 1990). ROS may be delivered to the airway by inhalation. One of the major sources of oxygen radicals in a living body is xanthine oxidase which catalyzes the oxidation of xanthine to uric acid (Ikuta et al., 1992). In vivo inhalation of xanthine/xanthine oxidase causes bronchoconstriction in anesthetized cats and also increases BHR to inhaled acetylcholine which reflects epithelial damage (Katsumata et al., 1990). Infection and inhaled pollutants activate leukocytes to produce oxidants. ROS contracts airway smooth muscle preparations directly and this effect is enhanced when the epithelium is injured or removed (Pennings et al., 1999). Pretreatment of this preparation with antioxidants inhibits the bronchoconstriction significantly (Katsumata et al., 1990). Exogenous oxidants or endogenous ROS from airway inflammatory cells may contribute to mucus hypersecretion occurring in asthma.

### 2.3.1. Effect of ROS on Airways

ROS can influence airway function by several ways.
Airway Smooth Muscle

Hydrogen peroxide directly constricts airway smooth muscle in vitro, and this effect is mediated partly via the release of prostanoids (Rhoden and Barnes, 1989). ROS may damage airway epithelium, resulting in increased epithelial shedding and increased bronchoconstriction responses (Yukawa et al., 1990). In vitro, hydrogen peroxide induces an increase in the responsiveness of human airways (Hulsmann et al., 1994a).

Vessels

Little is known regarding the effects of ROS on the bronchial vasculature. Hydroxyl radical potently induces plasma exudation in rodent airways (Lei et al., 1996).

Secretions

The effects of ROS on mucus secretion have not yet been investigated in human airways. In rats, oxidative stress increases airway mucus secretion, an effect that is blocked by COX inhibitors (Adler et al., 1990).

Nerves

Allergen impairs the function of bronchodilating nerves in guinea pig airways in vivo by an effect that is blocked by SOD, suggesting that superoxide anions may scavenge NO released from motor nerves (Miura et al., 1997). In rat airways, oxidant stress increases cholinergic nerve-induced bronchoconstriction, an effect that may be the result of oxidative damage to acetylcholinesterase (Ohrui et al., 1991).

Inflammatory Cells

Oxidants also activate NF-kB (which orchestrates the expression of multiple inflammatory genes that undergo increased expression in asthma), thereby amplifying the inflammatory response (Barnes and Karin, 1997). Many of the stimuli that activate NF-kB appear to do so via the formation of ROS, particularly hydrogen peroxide (Schreck et al., 1991). ROS activate NF-kB in an epithelial cell line (Adcock et al., 1994) and increase the release of proinflammatory cytokines from cultured human airway epithelial cells (Rusznak et al., 1996). ROS and peroxynitrite induce lipid
peroxidation, resulting in the formation of additional mediators. Isoprostanes are derived from lipid peroxidation of arachidonic acid (Morrow and Roberts, 1996). The most prevalent isoprostane is 8-epi-PGF-2α, which is a potent constrictor of human airways in vitro, acting predominantly via thromboxane (Tx) receptors, (Kawikova et al., 1996).

2.3.2. Role in Asthma

Bronchoalveolar lavage fluid cells from asthmatic patients show increased production of superoxide anions, compared with cells from normal individuals (Jarjour and Calhoun, 1994), and this production is increased further after allergen challenge (Calhoun and Bush, 1990). Increased generation of superoxide has also been reported in circulating monocytes and neutrophils from asthmatic patients (Vachier et al., 1994), and there is evidence for increased oxidative stress in the circulation (Rahman et al., 1996). Circulating eosinophils from asthmatic patients produce excessive superoxide after activation (Chanez et al., 1990), and this is increased even further after allergen challenge (Evans et al., 1996b). Hydrogen peroxide levels in exhaled condensates are increased in asthmatic adults and children (Dohlman et al., 1993; Jobsis et al., 1997; Antczak et al., 1997; Horvath et al., 1998) and are increased further during exacerbations (Dohlman et al., 1993). Thiobarbituric acid-reactive substances, which are produced as a result of lipid peroxidation are increased in exhaled condensates from asthmatic patients (Antczak et al., 1997). Pentane, another product of lipid peroxidation, is also increased in the exhaled air from asthmatic patients during exacerbations of asthma (Olopade et al., 1997).

2.4. Anti-oxidant Defence Mechanisms

Within the lung, powerful anti-oxidant enzymes are present and their levels may increase 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 alkoxy and peroxyl species (e) breaking the chain of an initiated sequence and (f) quenching /scavenging singlet oxygen. Anti-oxidants are of three types (Gutteridge,
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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 and Vitamin C etc.

SOD is a ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress (McCord and Fridovich, 1969). It catalyzes dismutation of \( \text{O}_2^- \) radicals to \( \text{H}_2\text{O}_2 \). There are three forms of SOD. The copper-zinc SOD is located in the cytosol, the manganese SOD is primarily a mitochondrial enzyme, and extracellular SOD is usually found on the outside of the plasma membrane (Folz et al. 1997).

Catalase is a tetrameric hemoprotein that metabolizes the \( \text{H}_2\text{O}_2 \) and leads to the production of \( \text{H}_2\text{O} \) and \( \text{O}_2 \). It does not metabolize large molecular peroxides such as lipid hydroperoxide products of lipid peroxidation (Webster and Nunn, 1988). Catalase is most effective in the presence of high \( \text{H}_2\text{O}_2 \) concentrations. However, in the presence of low concentrations of either \( \text{H}_2\text{O}_2 \) or other peroxides, the glutathione system plays a critical role (Cantin, 1999).

The glutathione system is a central mechanism for reducing \( \text{H}_2\text{O}_2 \). It complements catalase as a reducing system for \( \text{H}_2\text{O}_2 \) but exceeds catalase in its capacity to eliminate additional varieties of toxic peroxides (Ross et al. 1985). The key enzyme in the redox cycle responsible for the reduction of \( \text{H}_2\text{O}_2 \) is Glutathione peroxidase (GPx). This reaction specifically requires reduced glutathione (GSH) to serve as the electron donor. The glutathione disulfide (GSSG) formed in the course of the reaction is subsequently reduced back to GSH by glutathione reductase. All GPx contain a selenium atom in the active site in the form of selenocysteine.

There are several small molecules that provide anti-oxidant defense in vivo. These include Vitamin C, Vitamin E, GSH, Urate and β-carotene. They appear to be of some importance in the prevention of pulmonary damage mediated by oxidant stress (Hatch, 1995). Uric acid is hydrophilic and has chain-breaking properties and also stabilizes vitamin C. It also appears to function as a potent scavenger of inhaled oxidizing air pollutants, such as ozone and nitrogen dioxide, in the upper respiratory tract (Greene, 1999). Vitamin E is the chief non-enzymatic anti-oxidant present in the
lipid structure of the cells. Vitamin E acts as a chain breaking anti-oxidant (Mates et al., 2000). A deficiency of Vitamin E leads to progressive damage of cellular and subcellular membranes leading to pathological conditions. Vitamin E contributes to membrane stability (Meydani, 1995). α-tocopherol, a form of Vitamin E inhibits protein kinase C which activates O₂⁻ producing enzymes NADPH oxidase. Dietary intake of vitamins may play a role in host defense against oxidative lung damage and Vitamin E supplementation in diet has been found to suppress O₂⁻. Animal studies have shown that circulating cells exhibit membrane alterations where there is a lack of vitamin E in the diet (Mates et al., 2000).

Vitamin C is the major anti-oxidant substance present in the airway surface liquid of the lung, where it could be important in protecting against both endogenous and exogenous oxidation. It appears to function both as a water soluble scavenger and as a regenerative agent for Vitamin E. The fat soluble Vitamin E and water soluble Vitamin C are thought to act co-operatively in a system whereby Vitamin E, which is mainly sequestrated in cell membranes and other lipid structures is maintained in a reduced state by interaction with water soluble Vitamin C (Malo and Tessier, 1990).

Free radical scavengers and anti-oxidant enzymes prevent histamine release from rat mast cells (Ikuta et al., 1992). ROS and peroxidation products can deplete endogenous anti-oxidant pools and oxidatively damage membranes of resident lung cells (Shvedova et al., 1995). Decreased peripheral blood GSH peroxidase activity has been documented in asthmatic patients suggesting reduced anti-oxidant defences and increased susceptibility to ROS (Marrades et al., 1997). It has been shown that anti-oxidant capacity is decreased in plasma of patients both in stable and acute asthma (Pennings et al., 1999). SOD activity is significantly lower in patients with asthma and decreases further during an asthmatic exacerbation. Levels of GSH in epithelial lining fluid decrease rapidly in patients with mild asthma during an asthma exacerbation (Comhair et al. 2000). It is likely that decreased anti-oxidant protection of the respiratory epithelium contributes to the pathogenesis of asthma. Due to their large size, lipid insolubility and short circulating half-lives in plasma, anti-oxidant enzymes are unable to gain access to the cell interior in sufficiently large concentration to increase cellular anti-oxidant defenses.
There is increasing epidemiological evidence that a lack of dietary antioxidants may be an important determinant of asthma (Greene, 1995). Population surveys have shown that a low dietary intake of the antioxidant vitamin C is associated with poorer lung function and increased prevalence of wheezing (Britton et al., 1995). A low intake of vitamin C is associated with increased bronchial reactivity (Soutar et al., 1997), consistent with the proposal that the increased prevalence of asthma may be a result of reductions in the dietary intake of antioxidants (Seaton et al., 1995). Another study reported a weak association between low vitamin E intake and asthma (Troisi et al., 1995). Asthmatic patients have been reported to have lower than normal concentrations of Vitamin C in their plasma and blood leukocytes. Studies have shown significant improvements in respiratory measurements as a result of supplementation of Vitamin C. Studies suggest the potential for a modest effect of a large supplementation of Vitamin C in reversing hyper-responsiveness and other symptoms of ongoing asthma (Hatch, 1995).

Several antioxidants have also been administered to asthmatic patients, to explore the effects of these compounds on lung function and airway reactivity. There have been several short term studies with vitamin C showing small beneficial effects on either lung function or airway reactivity, but no measurements of inflammation have been made (Bielory and Gandhi, 1994). Selenium administered for a 3-month period to patients with chronic asthma produced a small but significant improvement in clinical symptoms but no improvement in lung function or airway reactivity (Hasselmark et al., 1993).

2.5. Nitric Oxide and Reactive Nitrogen Species

Nitric oxide (NO) has long been considered as an atmospheric pollutant. Since the discovery in 1987 that NO was the endothelium derived relaxing factor (EDRF), its importance in the regulation of body functions, including the respiratory tract, has become apparent (Palmer et al. 1987). Nitric oxide 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).

Nitric oxide is synthesized from the semi-essential amino acid L-arginine by the enzyme NO synthase (NOS), and three isoforms of this enzyme have been identified
Two constitutive NO synthase isoforms (collectively called cNOS) are expressed in inhibitory nonadrenergic noncholinergic neurons (neuronal NOS or nNOS), endothelial cells (endothelial NOS or eNOS), and epithelial cells (nNOS and eNOS) of the airways (Ward et al. 1995). These isoforms are activated by depolarization - or agonist-induced intracellular Ca$^{2+}$ changes and small (picomolar) amounts of NO are generated. This activation is short-lived, and the NO produced serves as a diffusible signaling molecule mediating various processes, including relaxation of airway and vascular smooth muscle (Moncada and Higgs, 1993). These effects are mainly mediated by activation of soluble guanylyl cyclase by binding of NO to its prosthetic heme group (Mayer, 1994).

An inducible isoform of NO synthase (iNOS) is induced by pro-inflammatory cytokines in a variety of cells, including macrophages and epithelial cells (Barnes and Belvisi, 1993). iNOS is distinguished from the cNOS isoforms by its prolonged Ca$^{2+}$-independent production of relatively large (nanomolar) amounts of NO, while its expression, unlike that of cNOS, is inhibited by glucocorticosteroids (Knowles et al. 1990). In these high concentrations, iNOS-derived NO not only activates soluble guanylyl cyclase, but may additionally have cytostatic and cytotoxic effects, which may be important in the host defense against invading micro-organisms and malignant cells (Morris and Billiar, 1994; Kolb and Kolb-Bachofen, 1992). However, this comes with a price, since high levels of NO can also cause respiratory tract injury and thus contribute to the pathobiology of respiratory tract disease. These 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. At present, the best understood of these reactions is the reaction with O$_2^{-}$ to form peroxynitrite ONOO$^-$ (Parks et al. 1981), a strong oxidant (Singh and Evans, 1997). Although ONOO$^-$ is relatively stable, it can be protonated to yield peroxynitrous acid (ONOOH) (Conner and Grisham, 1996), which then rapidly decomposes to NO$_3^-$ via the intermediate formation of ·OH and NO$_2$-like species. ONOOH is very unstable, highly reactive, and capable of both oxidizing and nitrating reactions. For instance, irreversible ONOOH
modifications include nitration of aromatic amino acids, lipids, or DNA bases (Van der Vliet et al. 1994). The amino acid tyrosine appears to be particularly susceptible to nitration and the formation of free or protein-associated 3-nitrotyrosine has recently attracted interest as a potential biomarker for the generation of RNS in vivo (Ramezanian, et al. 1996; Van der Vliet et al. 1999).

Reactions with thiol residues leading to the formation of S-nitrosothiols (SNO) have been proposed as a mechanism whereby NO groups are transported and targeted to specific effector sites, a potentially unique signaling mechanism induced by nitrosative stress (Mayer et al. 1995, Moro et al. 1994). The exact mechanism by which S-nitrosation occurs in vivo is still unclear, but it involves the formation of NO-derived intermediates with the redox equivalence of NO⁺ (the primary candidates are N₂O₃ and ONOOH) and (di) nitrosyl iron complex (Kharitonov et al. 1994; Hogg et al. 1996). Nitrosation of amines by these reactive nitrogen intermediates has been implicated in the mutagenic properties of NO, presumably through nitrosative deamination of DNA bases (Van der Vliet et al. 1999). It is also of interest that SNO such as S-nitroso-L-glutathione (GSNO) may inhibit enzymes associated with the response to oxidative stress in eukaryotic cells, including glutathione peroxidase (GPx), glutathione reductase (Becker et al. 1995), glutathione-S-transferase (Clark, and Debnam, 1988), and γ-glutamyl cysteine synthase (Han et al. 1996).

High concentrations of iNOS-derived NO are also produced in asthmatic airway inflammation. Thus, NO is detectable in the exhaled air from humans and various experimental animals (Gustafsson et al. 1991), and its concentration is increased in exhaled air of patients with chronic asthma (Alving et al. 1993). The increase in exhaled NO can be normalized after the administration of oral glucocorticosteroids (Yates et al. 1995) or inhalation of the selective iNOS inhibitor aminoguanidine (Yates et al. 1996), indicating that high exhaled NO concentrations in these patients may reflect inflammation-induced enhanced expression of iNOS. Indeed, immunohistologic studies have indicated increased expression of iNOS in the airway epithelium and some inflammatory cells in airway biopsies from patients with asthma (Hamid et al. 1993). In addition, both in allergic patients with asthma (Kharitonov et al. 1995) and in sensitized rats (Yeadon and Price, 1995) and guinea pigs (Yan et al. 1995), it has been
demonstrated that allergen provocation results in enhanced endogenous NO production during the late asthmatic response, which is due to the induction of iNOS during this response (Yan et al. 1995).

2.5.1. Effects on Airways

NO has many effects on airway function, although the effects of endogenous NO depend on the site of production and the amount produced (Barnes, 1996b).

Airway Smooth Muscle

NO and NO donor compounds relax human airway smooth muscle in vitro via activation of guanylyl cyclase and increases in cyclic GMP levels (Ward et al., 1995a; Gaston et al., 1993). High concentrations of inhaled NO produce bronchodilation and protect against cholinergic bronchoconstriction in guinea pigs in vivo (Dupuy et al., 1992). In humans, inhalation of high concentrations of NO (80 ppm) has no effect on lung function in normal subjects and produces only weak and variable bronchodilation in asthmatic patients (Hogman et al., 1993; Kacmarek et al., 1996). NO may, however, be the major neurotransmitter of bronchodilating nerves in human airways. In proximal human airways, there is a prominent inhibitory NANC (i-NANC) bronchodilating neural mechanism, which assumes particular functional importance because it is the only endogenous bronchodilating pathway in human airways. The neurotransmitter of this i-NANC pathway in human airways is NO, because NOS inhibitors virtually abolish this neural response (Belvisi et al., 1992a, b; Bai and Bramley, 1993).

Blood Vessels

NO is a potent vasodilator in the bronchial circulation and may play an important role in regulating airway blood flow, as in the pulmonary circulation (Higenbottam, 1995; Martinez et al., 1995). Endogenous NO may increase the exudation of plasma by increasing blood flow to leaky postcapillary venules, thus increasing airway edema (Kuo et al., 1992b).

The effect of endogenous NO on plasma exudation may depend on the amount produced and the site of production. In the context of asthma, the increased production
of NO is likely to result in increased plasma exudation. Furthermore, if peroxynitrite is generated in asthma, this may lead to the formation of hydroxyl radicals that also increase airway plasma exudation (Lei et al., 1996).

Additionally, 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).

All the studies discussed above clearly indicate the role of ROS in mediating responses such as bronchoconstriction and airway hyperresponsiveness to various challenges. Yet, till date there is no study which has systematically examined the effect of in-vivo generated ROS in an animal model of asthma on the activity of airway receptors and their modulation by antioxidant intake.