DISCUSSION

The major findings of the present study are that in a guinea pig model of asthma created by prior sensitization followed by challenge with ovalbumin and evidenced by an increase in airway responsiveness to histamine, there is:

- An increase in the basal activity of RARs
- An intense stimulation of RARs during ovalbumin challenge
- An increase in sensitivity of RARs to histamine
- A partial reversal of airway hyperresponsiveness and increased sensitivity of RARs to histamine following oral intake of antioxidants.

Though to a lesser degree, the SARs also responded in a similar manner as RARs. The results indicate the differential roles of these two groups of sensory receptors connected to myelinated vagal afferents, in the respiratory symptoms associated with asthma. As there was oxidative and nitrosative stress which got reversed partially by oral intake of antioxidants in this model and since in vivo generation of reactive oxygen species stimulated the airway sensory receptors, it is proposed that oxidative/nitrosative stress contributes significantly to the observed responses.

8.1. Guinea Pig as a Model

Guinea pigs have been the most commonly used small animal species in preclinical studies related to asthma (Canning, 2003). Many fundamental processes, mediators and regulators of airways disease pathogenesis were discovered or demonstrated first in guinea pigs, including the Schultz–Dale (immediate type hypersensitivity) reaction, the actions of histamine, the cysteiny1-leukotrienes and their two receptors, beta adrenoceptor subtypes, thromboxane, vascular endothelial growth factor (VEGF), eotaxin, alveolar macrophage derived neutrophil chemotactic factor(s) (leukotriene B4 and/or IL-8) and the roles of cAMP and inositol triphosphate in signal transduction (Auer and Lewis, 1910; Schultz, 1910; Dale, 1913; Brocklehurst, 1960, Hartley et al., 1968; Piper and Vane, 1969; Hunninghake et al., 1978; Snyder and Krell,
Receptor pharmacology in guinea pigs more closely matches that of human receptor pharmacology than most other commonly used species (Auer and Lewis, 1910; Muccitelli et al., 1987). Several breakthroughs in measuring lung mechanics were developed first in studies using this species, while models of the late phase response following an allergen challenge have been perfected in guinea pigs (Pennock et al., 1979; Iijima et al., 1987; Hutson et al., 1988; Meurs et al., 2006).

The anatomy and physiology of the guinea pig lung resembles that of humans (Muccitelli et al., 1987; Rogers, 2001; Ressmeyer et al., 2006; Canning, 2006). A pseudo-stratified epithelium lines the trachea, mainstem bronchi and large intrapulmonary bronchi of both species (Dalen, 1983; Jeffery, 2001). Vagal afferent nerves, including C-fibers and mechanoreceptors, innervate the epithelium and subepithelial spaces (Canning et al., 2006). Goblet cells and mucus glands are found in the large airways and their function is regulated both neurally and by locally released autacoids (Poblete et al., 1993; Rogers, 2001). A subepithelial vasculature is found between the epithelium and smooth muscle layer (Miodonski et al., 1980; Tanaka et al., 2003).

Airway smooth muscle in guinea pigs is both anatomically and functionally similar to that of human airway smooth muscle. Contractile and relaxant agonists of human airway smooth muscle have nearly identical potency and efficacy in guinea pig airway smooth muscle. Smooth muscle hyperplasia has been observed in models of allergic inflammation (Gosens et al., 2005). Airway smooth muscle (and the epithelium) is also a major source of eotaxin in human and guinea pig airways (Li et al., 1997; Ghaffar et al., 1999).

The autonomic innervation of airway smooth muscle in guinea pigs closely resembles that of humans (Canning, 2006). Parasympathetic cholinergic nerves mediate contractions of human and guinea pig airway smooth muscle through the actions of acetylcholine acting on post-junctional muscarinic M3 receptors (Roffel et al., 1990; Ten Berge et al., 1993). Sympathetic-adrenergic relaxant innervation is sparse and/or nonexistent in the intrapulmonary airways of both species (the guinea pig trachea is densely innervated by sympathetic adrenergic nerves). The primary functional relaxant innervation in both species is parasympathetic and noncholinergic in nature. VIP (and
related peptides) and the gaseous transmitter nitric oxide (NO, synthesized from arginine by the neuronal isoform of NO synthase) have been implicated in nonadrenergic–noncholinergic nerve-mediated relaxations of human and guinea pig airway smooth muscle (Fischer and Hoffmann, 1996; Canning, 2006). Stimuli initiating reflex bronchospasm in human subjects evoke similar reflexes in the guinea pig (Canning, 2006). Stimuli evoking cough in humans evoke cough in guinea pigs also (Canning et al., 2006).

The immediate hypersensitivity response of human and guinea pig airways to allergen (smooth muscle contraction, mucus secretion, vasodilatation and plasma exudation) is attributable in large part to the actions of histamine and leukotrienes acting on histamine H1 receptors and leukotriene cysteinyltide (cysLT1) receptors (Muccitelli et al., 1987; Guhlmann et al., 1989; Hasday et al., 2000). Resident mast cells in the airways likely mediate this acute response to allergen challenge. Dissimilar to human lung mast cells, cross-linking of either IgE or IgG1 receptors activates guinea pig mast cells, whereas only IgE receptors appear to be functional in humans (Ishizaka et al., 1971; Fahy et al., 1997; Regal, 1984). Other mediators associated with the acute allergic response in humans including bradykinin, substance P, Prostaglandin –D2 (PGD2) and platelet activating factor (PAF) have been recovered or demonstrated (through antagonism or inhibition) acutely following allergen challenge in guinea pigs (Dahlen et al., 1983; Murray et al., 1986; Schleimer et al., 1987; Undem et al., 1988; Page, 1990; Taylor et al., 1991; Bertrand et al., 1993).

These mediators implicated in the pathogenesis of asthma produce inflammation and physiological responses in guinea pigs predictive of their role in these human respiratory diseases. These autacoids and neurotransmitters evoking bronchospasm in human subjects, evoke bronchospasm in guinea pigs also (Canning and Chou, 2008). In both species, a component of the response to these constricting agonists is reflex in nature (Canning, 2006). Inflammatory autacoids, chemokines and cytokines including LTD4, LTB4, TNFa, IL-5, IL-8, IL-13, PAF and eotaxin induce inflammation and cellular infiltration into the airway wall and the airspaces of guinea pigs as they are likely to do or have been shown to produce in human subjects (Hunninghake et al., 1978; Page, 1990; Sehmi et al., 1991; Underwood et al., 1996; Hay, 1997; White et al., 1997).
Due to these resemblances with humans, we used guinea pigs to create an animal model of asthma. We sensitized the guinea pigs with ovalbumin as allergen. After 28 days, the sensitized animals showed an increase in basal tracheal pressure as compared to that in control animals (Group 1). Upon challenge with ovalbumin, the animals showed bronchospasm that was documented by changes in airway mechanics, with increases in tracheal pressure and airway resistance along with fall in the dynamic compliance. Further, the animals showed increased airway responsiveness to histamine with a significant reduction in $ED_{50}$ dose of histamine. The level of oxidative/nitrosative stress was also found to be increased in these animals. There was moderate infiltration of inflammatory cells such as neutrophils, lymphocytes and macrophages into the pulmonary interstitium. In the late asthmatic response group, 24 h after ovalbumin challenge, the basal tracheal pressure was still higher compared to that in Group 1. Also there was an increase in airway responsiveness to histamine as compared to that in Group 1 evidenced by a significant reduction in $ED_{50}$ dose of histamine. Moderate to high infiltration of eosinophils along with a low to moderate infiltration of other inflammatory cells such as monocytes and neutrophils was also observed within the lung. These changes demonstrated the successful development of asthma model in these animals.

8.2. Choice of the Receptor

There have not been many studies which examined the behavior of airway sensory receptors in an animal model of asthma. A study performed in Brown Norway rats sensitized with ovalbumin reported that there was not only an increase in the baseline activity of pulmonary C-fiber receptors but also an increase in their sensitivity to capsaicin injections especially after ovalbumin challenge (Zhang et al., 2008). Another study performed simultaneously again in Brown Norway rats sensitized with ovalbumin reported a similar finding excepting that the response to capsaicin injection was not tested after ovalbumin challenge and in the sensitized state there was no significant change in baseline activity (Kuo and Lai, 2008).

Both the studies did not investigate the behavior of SARs and RARs in this model of asthma. However, in a study performed on guinea pigs sensitized with
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ovalbumin, along with C-fiber receptors, the behavior of RARs was also investigated. In the sensitized but not challenged preparation, as observed on the c-fiber receptors, the RAR responses to intravenous capsaicin administration were found to be enhanced. This study also did not investigate the behavior of SARs (Bergren, 2001).

Though these studies provide some evidence that an increased sensitivity of pulmonary C-fiber receptors may contribute to the symptoms of asthma, they do not address the precise roles of other airway sensory receptors viz. RARs and SARs in causing asthma symptoms.

Preliminary studies performed in rabbits (Mills et al., 1969b) and guinea pigs (Koller and Ferrer, 1973) demonstrated that during an anaphylactic shock there could be stimulation of RARs and SARs. Till date, there is no study which has made a systematic investigation on the behavior of RARs and SARs in an animal model of asthma both in the immediate phase and in the late phase, the mechanism behind the responses and their reversal by therapeutic interventions. The present study is the first of its kind to investigate these aspects.

8.3. Changes in Pulmonary Mechanics and Vagal Afferent Activity

8.3.1. Basal Afferent Activity

Following sensitization with ovalbumin (Group 2a, Group 2b and Group 4a), a significant increase in basal RAR activity was observed. The increase in basal RAR activity in ovalbumin sensitized animals could be due to a change in pulmonary mechanics that might have stimulated the RARs. That there was an alteration in pulmonary mechanics is supported by the observations that there were significant increases in tracheal pressure in Groups 2a and 2b. These results are in accordance with previous studies in guinea pigs showing that the baseline values of resistance and elastance were increased in lung parenchymal strips from the early asthmatic response group as well as late asthmatic response group (Lancas et al., 2006; Angeli et al., 2008). The basal Penh values in ovalbumin sensitized mice (Vieira et al., 2007) were also found to be increased. An increase in tracheal pressure (resistance) and decrease in dynamic compliance are known to activate the RARs (Jonzon et al., 1986; Bergren, 2001; Vidruk et al. 1977).
However, the observation that there was an increase in the baseline activity of RARs without a significant increase in tracheal pressure in Group 4a suggests that other mechanisms may also contribute to the increase. It is known that in asthmatics, there is persistent airway inflammation even when there are no symptoms (Laitinen et al., 1985). When an antigen is presented to the host, sensitization occurs through the liberation of IL-4 that helps in the development of TH$_2$ cell from TH cell. TH$_2$ cell secretes various cytokines such as IL-3, IL-4, IL-5, IL-9, IL-13 etc, and stimulate the B cells to produce plasma cells producing IgE. They also tend to activate the eosinophils. These activated eosinophils cause airway inflammation by releasing various mediators such as leukotrienes, major basic protein, platelet-activation factor, eosinophil cationic protein (ECP) and oxidants (Kindt, 2006). Airway inflammation per se can stimulate the RARs resulting in an increase in basal activity (Ravi and Kappagoda, 1990).

In contrast to RARs, there was no change in the basal SAR activity in Groups 2a, 2b and 4a. Since the SARs are located in smooth muscles of the airways (Miserocchi and Sant'Ambrogio, 1974; Bartlett, 1976; Ravi 1986), it is generally believed that conditions which promote bronchoconstriction would stimulate the SARs (Paintal, 1973). Thus it is expected that their basal activity should go up in Groups 2a and 2b where there were significant increases in tracheal pressure. These observations once again support the claim that mechanisms other that changes in tracheal pressure could have accounted for the stimulation of RARs discussed above. These mechanisms could be airway inflammation, oxidative stress and release of mediators.

8.3.2. Control (Group 1)

In Group 1, the responses of RARs to inhalation of different doses of histamine were investigated. Histamine increased the RAR activity significantly at the dose of 0.032 mg/ml (Fig. 5). There have been several studies performed previously, which examined the effect of histamine inhalation on RAR activity. It was reported that, the RARs were stimulated upon histamine inhalation (Vidruk et al., 1977, Bergren, 1997, Schlegale, 2000, Mohammed et al., 1993). Bolus injections of histamine given intravenously also stimulated the RARs (Ravi et al., 1989, Yu and Roberts, 1990). The results observed in the present study are in agreement with the previously published results.
Histamine inhalation increased the SAR activity also significantly at the dose of 0.032 mg/ml (Fig. 34). These results are in agreement with the observation of other investigators which showed increased SAR activity in conjunction with the bronchoconstrictive effect of histamine (Widdicombe, 1954). In both the types of receptors, the histamine responses are blocked by H₁ - receptor blocker (Ravi, et al. 1989; Matsumoto et al., 1992, 1993).

**8.3.2.1. Mechanism**

The RAR responses can be explained by the exquisite mechanosensitivity of the RARs, and by the fact that they respond secondarily to changes in the properties of the mucosa. Thus they 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 (Ravi et al., 1989; Bonham et al., 1996; Widdicombe, 1996b), and by mucus secreted into the airway lumen (Rogers, 2001).

Histamine administration tends to affect the airway mechanics by increasing the tracheal pressure (Ravi et al., 1989, Yu and Roberts, 1990, Bergren, 1997, Vidruk, 1977; present study), reducing the compliance (Sellick and Widdicombe, 1971; Ravi et al., 1989; Yu and Roberts, 1990, Schlegle et al. 2000; Adcock et al. 2003; present study) and increasing the airway resistance (Sellick and Widdicombe, 1971; Adcock et al. 2003; present study). All these factors lead to the activation of RARs.

Like RARs, the SARs are also mechanoreceptors responding to changes in pulmonary mechanics (Widdicombe 1954, Matsumoto et al., 1992). Thus it is not surprising that they are also stimulated by histamine inhalation.

**8.3.3. Group 2a**

In this group, following ovalbumin challenge, there were significant changes in pulmonary mechanics. These included increases in tracheal pressure and airway resistance and fall in dynamic compliance. These observations are in agreement with previously published reports showing changes in airway mechanics, such as increase in tracheal pressure (Bergren, 1997), increase in airway resistance (Lamm et al., 1984;
Everitt and Moore, 1992; Ricciardolo et al., 1994; Montuschi et al., 1999; Aoki et al., 2000) and fall in compliance (Lamm et al., 1984; Montuschi et al., 1999). Similarly, fall in the specific airway conductance was also reported after ovalbumin challenge in guinea pigs (Chhabra et al., 2010).

In this background, it was observed that the $ED_{50}$ dose of histamine was found to be significantly reduced compared to that in Group 1. This observation clearly indicates airway hyperresponsiveness as a lower dose of histamine ($0.10 \pm 0.01$ mg/ml) produced an identical response as the higher dose ($0.42 \pm 0.06$ mg/ml).

Along with these changes, ovalbumin inhalation stimulated the RARs and to a lesser extent SARs significantly. These findings indicate that both these groups of receptors would be activated during an attack of asthma. At the $ED_{50}$ dose, there was significant increase in the activity of RARs. The increase was not only similar but greater too, compared to their response to the $ED_{50}$ histamine dose in Group 1. This observation suggests that along with an increase in airway resistance (and tracheal pressure) other factors may also contribute to the stimulation of RARs. Such an exaggeration was not evident in SARs suggesting thereby that these additional factors may not influence the activity of SARs. Even then, with a lower $ED_{50}$ dose, the SARs were stimulated as much as that with the higher $ED_{50}$ dose in Group 1.

8.3.3.1. Mechanism

The changes in tracheal pressure, airway resistance and dynamic compliance following antigen challenge in sensitized subjects have been studied extensively. It has been reported that IgE produced following sensitization binds with high affinity IgE receptor FceRI on mast cells and basophils, thereby sensitizing these cells to allergen exposure. Crosslinking of adjacent IgE-FceRI receptors complexes by allergens triggers the release of many inflammatory mediators such as histamine and leukotrienes from these cells. These mediators cause bronchoconstriction, increased permeability of venules, and increased mucus secretion (Barnes et al., 1998).

There are several mechanisms which could have contributed to the increase in RAR activity following ovalbumin challenge. These include contraction of airway
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smooth muscles near the site of receptors (Sant’ Ambrogio, 1982) and the effects of
inflammatory mediators on RAR activity (Sellick and Widdicombe, 1971; Bergren et
al., 1984; Bergren and Myers, 1984; Hargreaves et al., 1993; Mohammed et al., 1993;
mediators released commonly during an antigen challenge are histamine (Lamm et al.,
1984), leukotrienes (Lamm et al., 1984), tachykinins (Ricciardolo et al., 1994),
bradykinins, substance P, calcitonin gene related peptide (CGRP) (Myers et al. 2002)
etc. Besides providing changes in pulmonary mechanics (Lamm et al., 1984; Everitt
and Moore, 1992; Ricciardolo et al., 1994; Bergren, 1997; Montuschi et al., 1999; Aoki
et al., 2000; Chhabra et al., 2010), these mediators are known to stimulate the RARs
(Ravi et al., 1989; Hargreaves et al. 1992; Mohammed et al. 1993; Bonham et al. 1996;
Bhagat et al. 2011). Additionally, these agents promote airway inflammation, plasma
exudation and mucus secretion (Barnes et al., 1998). These factors also lead to
stimulation RARs (Ravi and Kappagoda, 1990). Among the various mediators released,
histamine alone has been reported to stimulate the SARs and this stimulation was
always associated with an increase in tracheal pressure (Widdicombe, 1954a; Bartlett et
al., 1976; Davenport et al., 1981b; Matsumoto et al., 1990).

There is new increasing evidence for release of reactive oxygen species such as
hydroxyl radicals (OH) following ovalbumin challenge, thereby indicating oxidative
stress (Shvedova et al., 1995; Almolki et al., 2004; Masini et al., 2005; Chhabra et al.,
2010). An oxidant antioxidant imbalance has also been reported in this situation as
evident by a decrease in the levels of antioxidants (Shvedova et al., 1995; Masini et al.,
2005; Chhabra et al., 2010). In agreement with these observations, in the present study
also, in this Group, there were significant increases in the production of superoxide
anion and lipid peroxidation products. These occurred with a significant fall in the
levels of the antioxidant enzyme. Till date there is no study which has investigated the
effects of reactive oxygen species generated in vivo on the activity of vagal sensory
receptors. The results from such an investigation are discussed under Group 3.

There is negligible information on the effect of oxidative stress and nitrosative
stress on airway sensory receptors. The reports available are mostly indirect. For
instance the responses of RARs to circulatory endotoxin (Lai et al. 2005) and inhaled
wood smoke (Lai and Kou, 1998) were found to be reduced by free radical scavengers suggesting involvement of ROS in the observed response. Finally, to provide evidence whether oxidative stress/nitrosative stress contributed to the changes in pulmonary mechanics and vagal sensory receptor activity, we repeated the study after administering the antioxidants, the results of which are discussed below in Groups 4a and 4b.

8.3.4. Group 2b

The airway responses which occur after 6-9 h following antigen challenge in sensitized subjects are usually termed as late asthmatic response (Bousquet et al., 2000). These responses persist for 24 h (Underwood et al., 1995) and lead to chronic airway inflammation characterized by an inflammatory response consisting of airway perivascular edema, mucus plugging, and infiltration that is characterized by eosinophils and other inflammatory cells, such as neutrophils and monocytes (Larsen et al., 1987). All these factors result in an increase in the basal tracheal pressure as observed in this Group (Table 4) compared to Group 1. In this background, the ED$_{50}$ dose of histamine was also reduced significantly compared to Group 1 suggesting that airway hyperresponsiveness persisted in the late asthmatic phase. In this phase, there is heavy infiltration of eosinophils (Sanjar, 1990; Pretolani et al., 1994) that has been associated with increased expression of IL-5 (Chand et al., 1992; Humbles et al., 1997), very late antigen 4 (Sagara et al., 1997, Pretolani et al., 1994) and eotoxin (Humbles et al., 1997) production. Moderate to high eosinophilic infiltration was evident in the present study also (Table, 14 Fig. 69). There are reports that there is increased production of neuropeptides such as substance P, CGRP, and NKA (Fischer et al., 1996) which promote plasma exudation and bronchoconstriction (Barnes, 2001). Eosinophils promote protein oxidative damage in both allergic asthma and severe asthma via the formation of reactive halogen species such as bromotyrosine (Andreadis et al., 2003) via eosinophil peroxidase (EPO) dependent reaction. EPO-generated oxidants can interact with RNS present in asthmatics and promote protein nitration (MacPherson et al., 2001). Thus, oxidative modification of critical biological targets in asthmatic airways may contribute to the pathophysiological features of asthma, such as epithelial cell damage, airway hyperreactivity, bronchoconstriction, β-adrenergic receptor dysfunction, mucus
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hypersecretion, microvascular leak and edema (Motojima et al., 1992; Yoshikawa et al., 1993). An increased oxidative stress/nitrosative stress was evident in the present study also. All these factors may contribute to the increase in basal activity of RARs in this Group. The absence of an increase in the basal activity of SARs suggests that the increase in tracheal pressure was not sufficient enough to stimulate them. At the ED$_{50}$ dose of histamine, there was significant stimulation of RARs. This response was significantly greater than the response of RARs to the ED$_{50}$ dose of histamine in Group 1. The finding once again suggests that as a lesser dose produces a greater response, besides the changes in airway resistance and tracheal pressure, additional factors might have contributed to the RAR responses. Some of these factors could be neuropeptides such as substance P, CGRP and NKA, liberated during late asthmatic response (Fischer et al., 1996). There are studies which report that substance P and CGRP stimulate the RARs (Bonham et al., 1996; Bhagat et al., 2009, 2011).

As observed in RARs, at the ED$_{50}$ dose of histamine, the SARs were also stimulated significantly and this stimulation was similar to that for the ED$_{50}$ histamine dose in Group 1, suggesting that the response was dependent upon increases in tracheal pressure and airway resistance. It must be remembered that as was the case with RARs, here also the ED$_{50}$ histamine dose was significantly lower compared to that in Group 1.

8.3.5. Group 3

Xanthine xanthine oxidase inhalation resulted in increases in tracheal pressure and resistance and a decrease in dynamic compliance. These responses were significantly greater than the response to saline inhalation in Group 1 (Table 7). In this background, the ED$_{50}$ dose for histamine was also reduced significantly compared to the ED$_{50}$ histamine dose in Group 1. Determination of the oxidative stress parameters demonstrated the involvement of ROS. There were increases in lipid peroxidation products and superoxide anion and decreases in antioxidant enzymes (Fig. 58-66). These results are in agreement with previous studies performed in our institute (Jain et al., 2004). Several other investigations have also demonstrated that following xanthine-xanthine oxidase inhalation, there is in vivo generation of reactive oxygen species resulting in oxidative stress (Katsumata et al., 1990).
ROS damages airway epithelium, resulting in increased epithelial shedding and promotes bronchoconstriction responses (Yukawa et al., 1990). In vitro, hydrogen peroxide induces an increase in the responsiveness of human airways to histamine and methacholine (Hulsmann et al., 1994a). Formation of peroxynitrite also increases airway responsiveness to irritants in guinea pigs in vitro and in vivo (Sadeghi-Hashjin et al., 1996), but its effect in human airways is not yet known. Hydroxyl radical potently induces plasma exudation in rodent airways (Lei et al., 1996). In rats, oxidative stress increases airway mucus secretion, an effect that is blocked by COX inhibitors (Adler et al., 1990). In rat airways, oxidant stress increases cholinergic nerve-induced bronchoconstriction, an effect that may be the result of oxidative damage to acetylcholinesterase (Ohru et al., 1991). Oxidants 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, result 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-PGF2α, which is a potent constrictor of human airways in vitro, acting predominantly via thromboxane (Tx) receptors (Kawikova et al., 1996). All these mechanisms contribute to the changes in pulmonary mechanics observed in situations where there is oxidative stress e.g. asthma. Hence, it is not surprising that the ED_{50} dose of histamine was significantly lower in this Group compared to that in Group 1.

The evidences presented above show that during oxidative stress there is bronchoconstriction, mucus secretion and plasma exudation. Thus it is expected that there would be stimulation of RARs. True to this expectation, there was RAR activation during xanthine-xanthine oxidase inhalation. In this background, at the ED_{50} histamine dose, the RARs were activated significantly. The activation was similar to the RAR responses to the ED_{50} histamine dose in Group 1. A similar observation was noted in the case of SARs also.
These findings lend experimental support to the suggestion that oxidative stress may be one of the contributing factors for stimulation of vagal sensory receptors connected to myelinated afferents during an attack of asthma.

8.3.6. Group 4a

As has been discussed in the preceding chapters, if oxidative stress played a significant role in the early as well as late asthmatic response, it is logical to expect that there should be some reversal in pulmonary mechanics and vagal afferent activity following antioxidant supplementation. True to this expectation, following antioxidant intake, the basal tracheal pressure in this group did not change significantly when compared to that in Group 1. This observation suggests that the persistent inflammation following sensitization might be oxidative stress mediated. Unlike the basal tracheal pressure, the basal RAR activity still remained significantly higher compared to that in Group 1. Though less, it was not significantly different from that in Group 2a. These observations suggest that oxidative stress induced inflammation may not be the main mechanism for the increase in basal activity of RARs. As observed previously in Group 2a, there was no significant change in basal activity of SARs.

As evident from the lung histology data (Fig. 70) there was only partial reversal of airway inflammation after antioxidant intake. Thus, the other factors discussed previously could still be causing airway inflammation which does not lead to a measurable change in pulmonary mechanics. At the same time, these factors and the inflammation though reduced would activate the RARs and raise their basal activity.

Following ovalbumin challenge, the tracheal pressure and airway resistance were increased. These changes were significantly different from responses to saline inhalation in Group 1, suggesting that antioxidant intake does not prevent an asthma attack when exposed to the same antigen. However, when compared to Group 2a, the changes in pulmonary mechanics were significantly reduced suggesting thereby that antioxidant intake may reduce the severity of the attack.

Additionally, antioxidant intake seems to reduce the airway responses to other triggering factors. For instance, the ED$_{50}$ dose of histamine which was higher compared
to Group 2a, became similar to Group 1. This observation also suggests that airway hyperresponsiveness in this model of asthma may also be oxidative stress mediated as antioxidant supplementation restored the normal response of airways to histamine.

In line with the changes in pulmonary mechanics following ovalbumin challenge, there was a significant increase in RAR activity. However, this response was significantly less compared to Group 2a but significantly greater than the response to saline inhalation Group 1, suggesting that at least part of the response was oxidative stress mediated. It could be due to the direct effects of the oxidants on the receptors or due to the indirect effect through changes in pulmonary mechanics. As observed in pulmonary mechanics, following antioxidant intake, there was a reversal in the RAR response to histamine inhalation.

These findings suggest that antioxidant intake may reduce the reflex bronchoconstriction produced by stimulation of RARs by other triggering factors such as histamine. The reversal in their response to histamine was similar as noted in the case of SARs. Even though there is no study till date which has examined the effect of antioxidants on the activity of these afferents in the ovalbumin sensitized animals. It has been 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). The reversals in the sensitivity of RARs and SARs to histamine are in agreement with these observations.

8.3.6.1. Mechanisms

Despite the abundance of evidence indicating elevated oxidative stress and reduced antioxidant defences in asthma, antioxidant supplementation studies to date have been limited (Wood et al., 2003). The only longer term (>1 week) supplementation trials have been encouraging, involving vitamin C (Anah, 1980) and Selenium (Hasselmark et al., 1993), both of which led to a positive improvement in asthma symptoms. There is increasing epidemiological evidence that a lack of dietary antioxidants may be an important determinant of asthma (Greene, 1999). 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; Cook et al., 1997). 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., 1994). Another study reported an association between low vitamin E intake and asthma (Troisi et al., 1995).

Exposure to ozone has been shown to enhance the airway response to ovalbumin in sensitized guinea pigs. Dietary supplementation with antioxidant vitamins E and C, affords variable degree of protection against this enhancement (Chhabra et al., 2010). Vitamin E suppresses the increase in airway reactivity following sensitization and has membrane stabilizing actions (Jain et al., 2005). In another study on isolated tracheal preparations from ovalbumin-sensitized guinea pigs, vitamin E was found to reduce the contractile effects of ovalbumin (Kilic and Erol, 2003). Similar effects were also seen upon the pulmonary mechanics in the present study also.

Epigallocatechin-3-Gallate, a main polyphenol present in the green tea leaves has been shown to reduce severe respiratory abnormalities appearing soon after the ovalbumin challenge in guinea pigs, such as bronchoconstriction, alveolar inflation, release of Eosinophilic Major Basic Protein and myeloperoxidase, free radical such as nitrotyrosine induced lung injury, and release of proinflammatory molecules (TNF-α) in BAL fluid (Bani et al., 2006).

A high dose vitamin C administration was recently reported to decrease eosinophilic infiltration (Chang et al., 2009), and airway hyperreactivity to methacholine (Joeng et al. 2010) in ovalbumin sensitized mice and to decrease inflammatory cells in BAL fluid, not only eosinophils but also neutrophils and lymphocytes in ovalbumin sensitized guinea pigs (Haines et al., 2011; Joeng et al. 2010). It was shown that SOD mimics given before antigen challenge of sensitized guinea pigs attenuated allergen-induced asthmatic bronchospasm (Masini et al., 2002).

Mohsenin et al. (1983) showed that ascorbic acid treatment significantly reduced airway reactivity to methacholine in asthmatic subjects, and Ogilvy et al. (1981) demonstrated that ascorbic acid decreased both the intensity and duration of the
bronchoconstriction induced by methacholine aerosol in subjects with normal respiratory function.

8.3.7. Group 4b

In this Group unlike the observation in Group 2b, the basal tracheal pressure was not significantly different from that in Group 1 suggesting that antioxidant intake was able to reverse the chronic inflammation. There have been a few studies which have shown the reversal of chronic inflammation by antioxidant supplementation. The SOD mimetic compound M40403 counteracts the ovalbumin induced bronchoconstriction, eosinophilic lung infiltration, lipid peroxidation and myeloperoxidase activity in a guinea pig model of allergic asthma-like reaction (Masini et al., 2005). Similarly, Ebselen, a glutathione peroxidase mimic has been shown to reduce the late asthmatic response (Zhang et al., 2002). TA-270, an antioxidant that potently scavenges reactive oxygen species, has been reported to reduce the bronchoconstriction evident in late asthmatic response. TA-270 inhibits the pulmonary accumulation of eosinophils and airway hyperresponsiveness to acetylcholine in the sensitized guinea pigs (Aoki et al., 2000).

Our results are in agreement with these findings. Further evidence in support is provided by the histological findings wherein it was observed that following antioxidant intake, there was reversal in airway infiltration of eosinophils and neutrophils (Fig. 71).

As observed in Group 4a, in this Group also, the ED$_{50}$ histamine dose was similar to the ED$_{50}$ histamine dose in Group 1. These findings suggest that antioxidant intake reduces airway responses to histamine inhalation in the late asthmatic phase also. The basal RAR activity in this Group was not significantly different from that in Group 1. The ED$_{50}$ histamine response of the RARs in this Group was significantly lower when compared with that in Group 2b even though the ED$_{50}$ dose per se was higher in this Group than in Group 2b. The RAR responses were comparable to those elicited in Group 1.

These results once again lend support to the claim that antioxidant intake reduces the sensitivity of RARs to histamine as discussed previously. Antioxidants may
inhibit the direct effect of oxidants in the nerve endings, reduce bronchoconstriction and reduce the fluid exudation - stimuli which normally stimulate RARs (Ravi and Kappagoda, 1990). When the ED$_{50}$ dose of histamine in this Group became similar to that in Group 1, the SARs were stimulated to a similar degree as in Group 1. These results suggest that the SAR activity is regulated by changes in bronchomotor tone.

Thus, the observations of the present study demonstrate clearly that oxidative stress plays a significant role in the airway hyperresponsiveness induced by allergen challenge in guinea pigs sensitized previously with ovalbumin. In this model of asthma, there is significant attenuation of the activities of vagal sensory receptors connected to myelinated afferents, especially RARs. Since airway hyperresponsiveness and airway inflammation are the hallmarks of allergic diseases such as asthma, the modulation of these receptor activities may play a crucial role in the symptomatology of asthma. It has been documented previously that stimulation of RARs causes various reflex responses such as bronchoconstriction, mucus secretion, cough, tachpnea etc. (Coleridge and Coleridge, 1986; Widdicombe, 1986, 1999, 2003; Karlsson et al., 1988). The RARs have been considered to give rise to dyspneic sensation associated with asthma (Ravi and Kappagoda, 2009). Thus, the present study provides an experimental basis for the clinical symptoms observed in asthmatics. A simultaneous stimulation of SARs may prove to be beneficial as the SARs cause bronchodilation (Paintal, 1973) and relieve dyspeneic sensations. Since there is attenuation of airway hyperresponsiveness, airway inflammation and RAR activity by antioxidant intake, it is tempting to postulate that antioxidant intake may be a general treatment option for asthmatics. However, it is emphasized that since allergic disorders such as asthma are multifactorial, blocking oxidative stress alone is unlikely to lead to complete resolution of asthma symptoms. Perhaps, as claimed by other investigators, they may be useful as adjunct therapy.