Myogenic and neurogenic mechanisms and arachidonate metabolites in bronchial muscle response to allergen

L. J. J. AnsSEN, I. MCGROGAN, J. WATTIE, P. M. O'BYRNE, AND E. E. DANIEL
Asthma Research Group, Departments of Medicine and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Myogenic and neurogenic mechanisms and arachidonic metabolites in bronchial muscle response to allergen. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L1118–L1125, 1997.—We investigated allergen-induced airway hyperresponsiveness (AH) in bronchial tissues obtained from dogs that inhaled Ascaris suum leading to AH (RESP) in vivo or that exhibited no change (NON-RESP) as well as from dogs that inhaled saline (SHAM). RESP tissues were not hyperresponsive to KCl or to carbachol, whereas contractions to electrical field stimulation (EFS) were reduced. This reduction was reversed partially by indomethacin and completely by replacement of the bathing fluid. Radioimmunoassay revealed marked elevation of prostaglandin (PG) E2 generation in RESP tissues compared with SHAM and NON-RESP tissues. EFS-evoked contractions were often followed by a slowly developing secondary contraction in RESP tissues but not in SHAM or NON-RESP tissues. However, indomethacin masked such secondary contractions in many SHAM and NON-RESP tissues and markedly enhanced those in RESP tissues, whereas L-655,240 (thromboxane A2/PGD2 receptor antagonist) abolished such contractions in all groups. We were unable to detect thromboxane using radioimmunoassay. We conclude that allergen-induced AH involves altered generation of cyclooxygenase metabolites of arachidonic acid (particularly PGE2) as well as of a nonprostanoid inhibitory factor; as such, the responsiveness of the tissue in vitro is dependent on the relative levels of inhibitory and excitatory metabolites.

prostaglandin E2; thromboxane A2; cholinergic innervation

Asthma is characterized by bronchoconstriction and hyperresponsiveness to various spasmogens. In early attempts to elucidate the pathophysiological mechanisms underlying asthma, attention was initially focused on the regulation of the airway smooth muscle (ASM) per se. It is now recognized that asthma is secondary to airway inflammation, accompanied by an influx of inflammatory cells and elevated levels of proinflammatory mediators and cytokines in the airways. Changes in other components of the airway wall participate in reversible airway narrowing. A number of models of asthma have been developed that involve induction of an inflammatory response in the airways. For example, dogs exhibit airway bronchoconstriction, airway inflammation, and hyperresponsiveness to inhaled spasmogens after exposure to the aerosolized allergen Ascaris suum (4, 10, 14, 20). The respiratory response was thought to be due to release of bronchoconstrictor inflammatory mediators after cross-reactivity of the A. suum antigen with a parasitic nematode, Toxocara canis, commonly found in dogs. This response is believed to be immunoglobulin E (IgE) mediated, as indicated by a positive skin test (i.e., wheal), although IgE levels have not yet been measured directly. Since then, many studies have been carried out using allergen inhalation to elucidate the mechanisms underlying airway hyperreactivity (5, 6, 8, 15, 16, 19). Nonetheless, the mechanisms underlying allergen-induced airway hyperresponsiveness (AH) are still not completely understood. Many studies indicate the involvement of several arachidonic acid metabolites in the responses to inhalation of allergen. For example, allergen-induced AH is accompanied by elevation of prostaglandin (PG) D2 levels (11) and is antagonized by blockade of thromboxane (Tx) A2 receptors (6, 11) or activation of PGE2 receptors (21).

Another model of asthma involves induction of an inflammatory response by exposure to ozone (12). Like asthma and allergen-induced AH, the ozone-induced inflammatory response is associated with AH to spasmogens in vivo and, in this case, also in vitro; however, this model differs from asthma and allergen-induced AH in that it is not IgE mediated. Ozone-induced AH seems to involve decreased levels of an inhibitory cyclooxygenase metabolite(s) (likely PGE2) and possibly also increased levels of an excitatory cyclooxygenase metabolite(s) (possibly TxA2; see Ref. 12). There are a variety of in vitro data which show that PGE2 in dogs can affect airway responsiveness by either inhibiting release of acetylcholine (ACh) from airway nerves or inhibiting the responsiveness of the airway muscle (2, 18).

In the present study, we investigated whether allergen-induced AH developed in vivo could also be demonstrated in isolated tissues in vitro and examined the roles of various arachidonic acid metabolites in the changes in airway responsiveness. Bronchial airway tissues were used, since these are primarily responsible for determining peripheral resistance to airflow. The data suggest that metabolism of arachidonic acid is markedly altered during allergen-induced inflammation. This manifests primarily as increased generation of PGE2 (as well as a nonprostanoid inhibitory factor) accompanied by reduced mechanical responses; however, there is also evidence that production of an excitatory autacoid is simultaneously increased, leading to a contraction that develops and resolves slowly.

Materials and Methods

In vivo measurements and inhalation of allergen. Dogs (15–30 kg; either sex) were anesthetized using intravenous pentobarbital sodium (30 mg/kg) to induce surgical anesthesia; this level of anesthetization was maintained during the course of the in vivo study by additional injections as required. An endotracheal tube and an esophageal balloon catheter were inserted. The endotracheal tube was connected
to a constant-volume ventilator set at a tidal volume of 10 ml/kg and frequency of 30 min⁻¹. The esophageal balloon catheter and a port at the equipment end of the endotracheal tube were connected to a differential pressure transducer (Hewlett-Packard 267B; Hewlett-Packard, Waltham, MA) and pressure amplifier (Hewlett-Packard 8805C) to monitor transpulmonary pressure. Measurements of peripheral resistance (R_L) were obtained at constant volume using techniques described previously, and airway responsiveness to aerosolized ACh was assessed 30 min after induction of anesthesia using methods that have been described previously (10, 12, 25). Dose-response curves to ACh were constructed by plotting the baseline and peak values of R_L after each concentration of ACh aerosol delivered. From each curve, an ACh-provocative concentration (PC5₀) which is the concentration of ACh that increased R_L by 5.0 cmH₂O·l⁻¹·s⁻¹ above the baseline value was calculated by interpolation as previously described (10, 12, 25).

Dogs were then exposed to allergen (A. suum in 0.9% saline) or saline alone. During this one-time challenge, the concentration of allergen was increased from 10⁻⁵ M in 10-fold increments until R_L was elevated 10 cmH₂O·l⁻¹·s⁻¹ above baseline, after which the dogs were ventilated with air until R_L returned to baseline. Later (24 h), dogs were anesthetized as described above, and the airway responsiveness to ACh was again assessed, after which they were euthanized with pentobarbital sodium (100 mg/kg). Dogs were defined as being hyperresponsive when there was a decrease in the ACh PC5₀ of twofold or more (10, 12, 25).

Tissue dissection and organ bath studies. After euthanization, pulmonary lobes were excised and were pinned out in physiological solution, and the overlying parenchymal tissue and vasculature were dissected away, thereby exposing the bronchial tree from which ring segments were excised (5–10 mm wide; 2–10 mm outer diameter), as described previously (12). Ring segments were mounted vertically in 3-ml organ baths using platinum hooks inserted through the lumen (taking care to not damage the epithelium); one of the platinum hooks was fastened to a force displacement transducer while the other served as an anchor. Throughout the studies, tissues were bathed in Krebs-Ringer buffer (KRB) bubbled with 95% O₂-5% CO₂ at room temperature in standard KRB (bubbled with 95% O₂-5% CO₂) or using one of two protocols. One set of eight tissues was constructed by plotting the baseline and peak values of R_L after each concentration of ACh aerosol delivered. From each curve, an ACh-provocative concentration (PC5₀) which is the concentration of ACh that increased R_L by 5.0 cmH₂O·l⁻¹·s⁻¹ above the baseline value was calculated by interpolation as previously described (10, 12, 25).

Dogs were then exposed to allergen (A. suum in 0.9% saline) or saline alone. During this one-time challenge, the concentration of allergen was increased from 10⁻⁵ M in 10-fold increments until R_L was elevated 10 cmH₂O·l⁻¹·s⁻¹ above baseline, after which the dogs were ventilated with air until R_L returned to baseline. Later (24 h), dogs were anesthetized as described above, and the airway responsiveness to ACh was again assessed, after which they were euthanized with pentobarbital sodium (100 mg/kg). Dogs were defined as being hyperresponsive when there was a decrease in the ACh PC5₀ of twofold or more (10, 12, 25).

RESULTS

In vivo measure of airway responsiveness. Inhalation of allergen (A. suum) caused an immediate bronchoconstrictor response in all 20 dogs assayed, whereas inhalation of vehicle did not. When assayed 24 h later, 10 of the allergen-exposed animals were found to be hyperresponsive to ACh (i.e., exhibited more than a 2-fold increase in R_L).
In dogs exposed to vehicle (SHAM), 1.296 the contractile response to KCl (Fig. 2), the maximal differences between the three groups with respect to mechanisms. The latter acts through both electromechanical exclusively through electromechanical coupling mechanisms, the former acts on the cholinergic agonist CCh. Although the former acts examined the contractile responses to KCl (60 mM) or measured in vivo could also be detected in vitro, we did not significantly alter the response to KCl or unmask any significant differences between the three groups.

In vitro responses to exogenously added KCl or cholinergic agonist. To determine if hyperresponsiveness measured in vivo could also be detected in vitro, we examined the contractile responses to KCl (60 mM) or the cholinergic agonist CCh. Although the former acts exclusively through electromechanical coupling mechanisms, the latter acts through both electromechanical and pharmacomechanical coupling mechanisms.

In the absence of IDM, there were no significant differences between the three groups with respect to the contractile response to KCl (Fig. 2), the maximal decrease in ACh PC5.0; RESP), whereas the other 10 dogs did not (NON-RESP). The mean magnitudes of the change in PC5.0 (i.e., PRE and POST) were 0.84 ± 0.17 in dogs exposed to vehicle (SHAM), 1.29 ± 0.17 in NON-RESP dogs, and 3.56 ± 0.38 in RESP dogs. Figure 1 indicates the range of changes in sensitivity to ACh in these animals. None of the eight SHAM dogs exhibited AH to inhaled ACh.

In vitro responses to nerve-released cholinergic agonist. The frequency–response characteristics of the tissues were examined using trains of pulses with frequencies ranging from 0.1 to 30 pps delivered at 5-min intervals (Fig. 3); tissues were electrically stimulated over prolonged periods without replacement of bath fluid between stimulations. In tissues stimulated in this way in the absence of IDM, there were no statistically significant differences between SHAM and NON-RESP, whereas those in RESP tissues were significantly reduced (Fig. 3A). IDM potentiated the responses in all three groups, particularly at low EFS frequencies, consistent with its prevention of the inhibitory effect of one or more cyclooxygenase metabolites on neurotransmission in this tissue (2). Nonetheless, the responses in RESP tissues exposed to IDM were still significantly smaller than those in the other two groups (Fig. 3B), suggesting that an inhibitory factor unrelated to cyclooxygenase metabolism is generated in RESP tissues.

We obtained somewhat different results when the tissues were washed immediately before electrical stimulation to wash out any accumulated autacoids. In this case, we used trains of pulses (20 pulses at 20 Hz) delivered at 20-min intervals in standard KRB or KRB

Table 1. Carbchol EC50 in the presence or absence of IDM and/or nifedipine

<table>
<thead>
<tr>
<th>Group</th>
<th>EC50 (log M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td></td>
</tr>
<tr>
<td>-IDM</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>+IDM</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>+IDM + NIFED</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>NON-RESP</td>
<td></td>
</tr>
<tr>
<td>-IDM</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>+IDM</td>
<td>6.1 ± 0.7</td>
</tr>
<tr>
<td>+IDM + NIFED</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>RESP</td>
<td></td>
</tr>
<tr>
<td>-IDM</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>+IDM</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>+IDM + NIFED</td>
<td>6.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. IDM, indomethacin (10⁻⁶ M); NIFED, nifedipine (10⁻⁷ M); SHAM, airway tissues exposed to vehicle; NON-RESP, airway tissues exposed to allergen that did not become hyperresponsive; RESP, airway tissues exposed to allergen that became hyperresponsive; EC50, concentration eliciting half-maximal excitatory response.
containing IDM (10^{-5} M) or L-655,240 (10^{-6} M; TxA2/PGD2 receptor antagonist), and the bathing fluid was replaced <5 min before each response. In the absence of pharmacological agents, EFS responses within each group increased during the first 60 min and then decreased over the next 80 min (Fig. 4A). There was no significant difference in the responses at each time point between the three groups. In the presence of IDM (10^{-5} M), the EFS responses at 20 min were not significantly different from those obtained in its absence (compare points at time = 5 min in Fig. 4, A and B), but all subsequent responses within each group showed a progressive increase in magnitude (Fig. 4B). Again, there were no significant differences between the groups at each time point in the presence of IDM. In tissues pretreated with L-655,240, the EFS responses obtained at 20 min in each group were not significantly different from each other (Fig. 4C) or from those obtained in the same group in the absence of L-655,240 (compare Fig. 4, A and C). In SHAM and NON-RESP tissues, the magnitudes of responses to successive electrical stimulations remained relatively constant (as opposed to the progressive changes in control and IDM-treated tissues described above), whereas RESP tissues showed a progressive and marked decrease such that the response at 140 min was almost negligible (Fig. 4C).

In some cases, primarily among tissues from responders, the EFS-evoked twitch contraction was followed by a smaller contraction that developed relatively slowly, reaching a peak ~1–2 min after EFS and then relaxing partially during the period between stimulations (Fig. 5A). These were more prevalent and notably larger in RESP than in NON-RESP or SHAM tissues (Fig. 5B). IDM (10^{-5} M) unmasked small secondary contractions in some SHAM and NON-RESP tissues and markedly enhanced the prevalence, magnitude, and duration of such secondary contractions in RESP tissues (Fig. 5B). L-655,240 (10^{-6} M) did not significantly alter the initial
shown to be sufficient to mediate prejunctional inhibition of cholinergic neurotransmission (2); the concentration of PGE$_2$ within the tissue is likely to be even higher. PGE$_2$ levels were increased by 2–3 ng/ml in all three groups over the subsequent 90 min (Fig. 6). This accumulation was not significantly increased in tissues that were electrically stimulated (Fig. 6). PGE$_2$ accumulation in the electrically stimulated tissues as well as in the time controls was greatly reduced, if not abolished, by IDM (data not shown). Levels of TxB$_2$ never exceeded the detection limit of the assay.

**DISCUSSION**

Allergen-induced AH has been used in many studies as a model to elucidate the mechanisms underlying asthma. However, these mechanisms remain poorly understood, in part because the majority of the studies were carried out using in vivo preparations in which the various effects of allergens on the nervous, immune, and vascular systems in the airways confound interpretation of the data. In the present study, we used isolated airway tissues in which these effects are absent or controlled. We also used CCh as a cholinergic agonist rather than ACh, as was done in earlier studies (11), since ACh is susceptible to degradation by cholinesterases, and it is possible that cholinesterase activity is altered during AH; for example, in a previous study of a canine model of asthma (11), ACh had no effect over the concentration range $10^{-7}$ to $10^{-6}$ M, whereas these concentrations are effective in nonhyperresponsive dogs. CCh, on the other hand, is not susceptible to cholinesterase activity. In contrast to our expectations, we found that bronchial tissues isolated from hyperresponsive dogs demonstrated no change in responses to exogenously added spasmogens and reduced responses to electrical stimulation (this reduction was reversed partially by IDM or by replacement of the bathing medium to remove any inhibitory factors in the bathing medium). The data are consistent with allergen exposure having enhanced generation of inhibitory arachidonic acid metabolites from cyclooxygenase, as well as of a possible nonprostanoid inhibitory factor. Therefore, these data suggest that the responsiveness of the airway tissues in vitro is determined by the relative levels of inhibitory and excitatory factors elicited by exposure to allergen, as discussed below.

Changes in myogenic mechanisms? Contraction of ASM is a Ca$_2^+$-dependent event, primarily involving activation of myosin light chain kinase in response to an elevation in cytosolic Ca$_2^+$ concentration ([Ca$_{i}$]). The latter is a result of agonist-induced release of internally sequestered Ca$_2^+$ and/or depolarization-induced opening of voltage-dependent Ca$_2^+$ channels. A number of Ca$_2^+$ homeostatic mechanisms contribute to restoring [Ca$_{i}$] to basal levels and thereby mediate recovery from excitation, including uptake into the internal store and extrusion from the cell. It has been suggested that AH may involve changes in the function of the ASM per se, such as changes in Ca$_2^+$ handling or increased sensitivity to Ca$_2^+$ (14, 15, 24). We did not obtain any evidence for such changes under the present conditions.

**Fig. 5.** EFS evokes a twitch contraction and a slowly developing secondary contraction. Twitch contractions and secondary contractions were evoked by electrical stimulation; in this experiment, tissues were washed before evoking the mechanical responses. A: original tracing showing twitch contraction evoked by EFS [20 pulses at 0.5 Hz (●) or 20 Hz (○)] in SHAM (left) and HY-P-RESP (RESP, right) tissues. In RESP tissues, twitch contraction was followed by a secondary contraction that developed slowly and relaxed slowly. Dotted lines indicate zero tone. B: mean magnitudes of contractile responses evoked by EFS [20 pulses at 20 Hz] in SHAM (open bars), NON-RESP (hatched bars), and RESP (filled bars) tissues in the absence (NORM) or presence of indomethacin (IDM) or L-655,240 as indicated. Twitch contraction is indicated by the first bar in each pair of bars; secondary contraction, when present, is indicated by the second bar in each pair of bars.

**Fig. 6.** Radioimmunoassay for prostaglandin E$_2$ (PGE$_2$). PGE$_2$ levels in bath fluid before electrical stimulation (BASAL) were not significantly different between SHAM and NON-RESP tissues but were markedly elevated in fluid from RESP tissues. Next, tissues were washed, and accumulation of PGE$_2$ over the course of 90 min was quantified. During this 90-min period, one-half of the tissue in each group was electrically stimulated (FS; see MATERIALS AND METHODS), whereas the other half served as time controls; electrical stimulation did not increase the accumulation of PGE$_2$.
experimental conditions. For example, there seems to be no change in electromechanical coupling mechanisms (i.e., voltage-dependent Ca\textsuperscript{2+} influx), since we found no difference between SHAM and RESP tissues with respect to the sensitivities or magnitudes of responses to CCh in the absence of nifedipine (Table 1) or to KCl (Fig. 2). The lack of a significant difference between the CCh responses in the presence or absence of nifedipine, as well as the KCl responses, also suggests that there is no change in the sensitivity of the contractile apparatus to Ca\textsuperscript{2+} after exposure to allergen. Likewise, there seems to be no change in agonist-induced release of internally sequestered Ca\textsuperscript{2+}, since there was no change in the maximal contractile response or in the EC\textsubscript{50} for CCh (Table 1). Measurements of ionic currents in single cells also did not reveal any difference between SHAM and RESP tissues with respect to membrane currents at rest or during cholinergic stimulation (Janssen, unpublished observation), consistent with our claim that basal [Ca\textsuperscript{2+}] is not elevated in these tissues and that agonist-induced release of internal Ca\textsuperscript{2+} is not altered.

Airway inflammation may involve a change in airway tissue elastance (via lymphocyte-mediated destruction of the matrix), which would result in a change in the optimal preload for these tissues. If this occurred in the present experiments, our use of the same preload tension (=1.25 g) for all tissues could account for the observed decrease in responses in allergen-exposed tissues. However, there was no difference in the magnitude of responses between the three groups when tissues were washed before stimulation (to remove any inhibitory substances; Fig. 4A), which contraindicates an allergen-induced change in the optimal preload. It is worth reiterating that, when this type of experiment is repeated without a wash before each stimulation, there is a marked difference between the three groups (Fig. 3A).

Changes in neurogenic mechanisms? In canine ASM the excitatory innervation is almost exclusively cholinergic in nature. We have demonstrated above that the myogenic response to cholinergic stimulation is unaltered. However, it is possible that allergen-induced airway inflammation may result in changes in the release of ACh from the nerve endings, leading to exaggerated neurogenic excitation. For example, Elbon et al. (8) found that allergen-induced AH in guinea pigs involves release of eosinophil-derived mediators that downregulate postsynaptic inhibitory muscarinic receptors, leading to increased cholinergic neurotransmission. Similarly, Mitchell et al. (19) have shown that basal and histamine-induced release of ACh is increased in ragweed-sensitized dogs, suggesting that immune sensitization facilitates the release of the neurotransmitter from postganglionic parasympathetic nerves. In a different canine model of AH [that induced by inhalation of ozone (12)], we have found that AH seems to involve decreased postsynaptic inhibition (likely due to decreased generation of PGE\textsubscript{2}) and possibly also increased presynaptic excitatory input (perhaps due to increased generation of TxA\textsubscript{2}).

Surprisingly, we found that tissues from dogs that had demonstrated AH in vivo after allergen exposure were not more excitable to EFS than control tissues. In fact, there was a significant decrease in the neurogenic responses in RESP tissues compared with SHAM and NON-RESP tissues. This reduction of the neurogenic responses was reversed partially by IDM (Fig. 3) and completely by replacement of the bathing medium to remove any inhibitory factors (Fig. 4). Thus allergen-induced inflammation may be accompanied by generation of inhibitory factors for mediator release. Radioimmunoassay and the effect of IDM on mechanical responses indicate that one of these factors is PGE\textsubscript{2} (discussed in more detail below); however, the inability of IDM to completely reverse the hyporesponsiveness suggests that a nonprostanoid inhibitory factor is also produced. Studies of epithelium-dependent inhibition of canine tracheals are also consistent with involvement of both PGE\textsubscript{2} and a nonprostanoid factor (McGrogan and Daniel, unpublished observation).

Changes in cyclooxygenase metabolites? Our observations and those of others suggest that allergen-induced airway inflammation is accompanied by marked changes in the metabolism of arachidonic acid. First, we found that basal levels of PGE\textsubscript{2} were markedly increased in RESP tissues (Fig. 6A). The overall bath concentration of PGE\textsubscript{2} reached 10\textsuperscript{-9} M (the effective concentration within the tissue is likely to be higher); this concentration of PGE\textsubscript{2} has been shown to mediate marked inhibition of neurogenic responses, but not those to exogenously added ACh, in canine bronchial smooth muscle (2). Thus PGE\textsubscript{2} accumulation could explain the sensitivity of responses in allergen-exposed animals to neurally released cholinergic agonist but not those to cholinergic agonist added exogenously. The accumulation of PGE\textsubscript{2} concentration was sensitive to the cyclooxygenase antagonist IDM. Similarly, Itabashi et al. (11) have shown that inhalation of allergen by dogs is accompanied by significant accumulation of another prostanoid (PGD\textsubscript{2}), although this was not prevented by IDM.

Second, we found that the responses to EFS in SHAM and NON-RESP tissues were not significantly altered by L-655,240, whereas those in RESP tissues were markedly reduced in the presence of this Tx/PG receptor antagonist (Fig. 4). Itabashi et al. (11) also found that blockade of Tx receptors (using an antagonist distinct from the one used in our study) had no effect on the cholinergic responses in control and nonresponder groups but markedly antagonized those in the hypersponder group. Similarly, Chung et al. (6) have shown that the late phase of allergen-induced AH in dogs is antagonized by yet another TxA\textsubscript{2} receptor antagonist.

Third, the slowly developing secondary contractions that followed the initial EFS-evoked twitch contractions were larger and much more prevalent in RESP tissues than in SHAM or NON-RESP tissues, and these were enhanced in all three groups by IDM but were eliminated by L-655,240 (Fig. 5). Similar secondary contractions have been shown to be induced by leukotri-
enes and to be sensitive to inhibition by PGE₂, although the mechanism involved is as yet unclear (1).

These observations are consistent with allergen exposure initiating changes in the generation of both excitatory and inhibitory arachidonic acid metabolites via cyclooxygenase. As a result, the effect of allergen-induced inflammation on airway responsiveness depends on the relative levels of these metabolites as well as on the sensitivity of the tissue to them (e.g., density of receptors for the metabolites within the particular tissue). For example, allergen causes airway responsiveness to be increased in the small airways (5, 11), decreased in the trachealis (5, 18), and unchanged in the larger bronchi (this study and Ref. 11), perhaps reflecting the relative levels of arachidonic acid metabolites and their receptors throughout the airways.

Distinct mechanisms underlying different models of airway inflammation. The epithelium is the major source of PGE₂ and of a nonprostanoid inhibitory factor (17, 18, 23). Ozone-induced inflammation in canine ASM is accompanied by destruction of the epithelium and increased responsiveness of isolated bronchial tissues, the latter of which seems to be secondary to decreased generation of an inhibitory cyclooxygenase metabolite, likely PGE₂ (12). In the present study, tissues excised from dogs exhibiting allergen-induced hyperresponsiveness demonstrated reduced in vitro mechanical responses (Fig. 3) accompanied by increased levels of PGE₂ (Fig. 6); electron-microscopic examination of the tissues showed no appreciable damage of the epithelium in tissues from hyperresponsive dogs compared with their control counterparts (Daniel, unpublished observation). Thus the mechanisms underlying ozone-induced and allergen-induced AH seem to differ markedly, and this could account for the contrasting observations (i.e., hyperresponsiveness versus hypo-responsiveness) made in excised tissues from these two models. Decreased ASM responsiveness is also seen in the mouse after Toxocara-induced eosinophilic inflammation (5) as well as in the Basenji-Greyhound model of asthma (even though the tissues were excised from dogs that demonstrated hyperresponsiveness in vivo; see Ref. 7).

In the rat, induction of an airway inflammatory response causes increased generation/release of nitric oxide from the airway epithelium (22) and hyporesponsiveness to the smooth muscle, which is secondary to the effects of nitric oxide on the smooth muscle (16). In the present study, Nω-nitro-L-arginine (a blocker of nitric oxide synthesis) had no effect on the magnitudes of contractions evoked by EFS (J. assen, unpublished observation).

The mechanisms underlying other models of asthma may also differ from those described above. For example, in canine airway tissues that have been sensitized to allergen but that are not actually inflamed at the time of study, there are changes in shortening velocity that were interpreted to reflect increased myosin light chain kinase levels within the smooth muscle, although maximum force generation is not increased (14). Moreover, studies of the changes in airway function after acute exposures to allergen or ozone do not take into account the structural changes that accompany repeated/prolonged exposures (which are also seen in the airways of asthmatics). Thus the relationship between these various animal models of asthma and the clinical (i.e., human) condition is equivocal. Studies in humans vary with respect to whether or not smooth muscle force generation is increased; we are not aware of any reports of decreased responsiveness of human ASM after inflammation or in asthma.

We conclude that allergen-induced changes in airway responsiveness studied in vitro reveal altered metabolism of arachidonic acid by cyclooxygenase, leading to increased generation of the inhibitory metabolite PGE₂ and possibly other excitatory prostanoids. In addition, a nonprostanoid inhibitory factor may also be produced. As such, the responsiveness of the tissue is dependent on the relative levels of inhibitory and excitatory autacoids.

This study was supported by grants from the Medical Research Council and the Ontario Thoracic Society.

These data have been presented in abstract form (Am. J. Respir. Crit. Care Med. 153: A741, 1996).

Address for reprint requests: L. J. Janssen, Dept. of Medicine, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3S5.

Received 22 November 1996; accepted in final form 26 August 1997.

REFERENCES


9. Inman, M. D., J. A. Denburg, R. Ellis, M. Dahlbäck, and P. M. O’Byrne. Allergen-induced increase in bone marrow progenitors in airway hyperresponsive dogs: regulation by a...


24. Triggle, D. J. Calcium, the control of smooth muscle function and bronchial hyperreactivity. Allergy 38: 1–9, 1983.