Ragweed sensitization alters pulmonary vascular responses to bronchoprovocation in beagle dogs

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Theodorou, Andreas, Natalie Weger, Kathleen Kunke, Kyoo Rhee, David Bice, Bruce Muggenberg, and Richard Lemen. Ragweed sensitization alters pulmonary vascular responses to bronchoprovocation in beagle dogs. J. Appl. Physiol. 83(3): 912–917, 1997.—In ragweed (RW)-sensitized beagle dogs, we tested the hypothesis that reactivity of the pulmonary vasculature was enhanced with aerosolized histamine (Hist) and RW. Seven dogs were neonatally sensitized with repeated intraperitoneal RW injections, and 12 dogs were controls (Con). The dogs were anesthetized with intravenous chloralose, mechanically ventilated, and instrumented with femoral arterial and pulmonary artery catheters. Specific lung compliance (Clsp), specific lung conductance (Gsp), systemic vascular resistance index, and pulmonary vascular resistance index (PVRI) were measured before and after bronchoprovocation with Hist and RW. After Hist inhalation (5 breaths of 30 mg/ml), both Con and RW dogs had significant decreases in C\text{Lsp} (−51 ± 4 and −53 ± 5\% respectively) and G\text{sp} (−65 ± 5 and −69 ± 3\%, respectively), but only RW-sensitized dogs had a significant increase in PVRI (38 ± 10\%). After RW inhalation (60 breaths of 0.8 mg/ml), only RW-sensitized dogs had significant increases (62 ± 20\%) in PVRI and decreases in G\text{sp} (−77 ± 4\%) and C\text{Lsp} (−65 ± 7\%). We conclude that, compared with Con, RW-sensitized beagle dogs have increased pulmonary vasoconstrictive responses with Hist or RW inhalation.

pulmonary hypertension; pulmonary circulation; asthma; airway reactivity; LabView

DOGS NEONATALLY SENSITIZED to ragweed (RW) have been demonstrated previously by us (23) and others (5, 9) to develop specific and nonspecific airway hyperresponsiveness. The mechanism by which RW sensitization enhances airway responsiveness is unknown. However, release of bronchoconstricting mediators (such as platelet-activating factor, substance P, leukotrienes, and others) and upregulation of ganglionic muscarinic receptors are proposed by current theories (5, 11). We reasoned that pulmonary vascular responses, especially of vessels in close proximity to airways, also may be influenced by these mechanisms and contribute to the pathophysiology of human asthma. Thus we evaluated the hemodynamic effects of histamine (Hist) and RW bronchoprovocation in dogs that were neonatally sensitized to RW. Our studies suggest that the pulmonary vascular smooth muscle, like airway smooth muscle, is hyperresponsive to Hist or RW.

METHODS

Preparation. All protocols were approved by the University of Arizona Laboratory Animal Care Committee in accordance with guidelines for the humane care of laboratory animals established by the National Institutes of Health. Beagle puppies were obtained from a research breeding colony at the Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM. Each litter (usually 4–6 puppies) was assigned at birth to either the control (Con) or RW-sensitized group.

Our RW-sensitization protocol is a modification of the protocol described by Halonen et al. (14) and Becker et al. (5), as follows. Puppies in the RW-sensitized group received injections (500 mg ip) of short ragweed (Ambrosia artesimisiifolia; Miles, Elkhart, IN) and 0.5 ml ip Al(OH)\textsubscript{3} (Alhydrogel 1.3\%, Accurate Chemical, Westbury, NY) within 24 h of birth. These injections were repeated weekly until age 8 wk, every other week until age 14 wk, and then monthly for the duration of the study. Dogs in the control group did not receive RW injections.

On the study day, the dogs were anesthetized with thiopental sodium (15 mg/kg iv; Abbott, N. Chicago, IL) and immediately intubated with a 6- to 7-Fr cuffed endotracheal tube. They were further anesthetized with chloralose (13) (80 mg/kg iv; Sigma Chemical, St. Louis, MO) dissolved in warm saline (70°C, then allowed to cool to 37°C), followed by a constant infusion of chloralose (40–90 mg·kg\textsuperscript{-1}·h\textsuperscript{-1} for 3.5–5 h) with the use of a syringe pump (model 1001, Medfusion Systems, Norcross, GA). The endotracheal tube cuff was inflated with the minimum pressure required to prevent leaks when the lungs are hyperinflated with twice normal tidal volume for 4–5 s. The dogs were ventilated with a constant-volume ventilator (model 607, Harvard Apparatus, South Natick, MA) at a tidal volume of 15 ml/kg, and the rate was adjusted to maintain baseline arterial PCO\textsubscript{2} (PaCO\textsubscript{2}) between 35 and 45 Torr throughout the studies (usually 12–16 breaths/min). End-tidal CO\textsubscript{2} was measured continuously by using a Novametrix end-tidal CO\textsubscript{2} monitor (model 1260/7000, Novametrix Medical Systems, Wellingford, CT).

Supplemental O\textsubscript{2} was delivered (0.5–2.0 l/min) to maintain O\textsubscript{2} saturations > 95. O\textsubscript{2} saturation was monitored via pulse oximetry using a pulse oximeter (model N-100, Nellcor, Hayward, CA) and a VetStat O\textsubscript{2} transducer and sensor (Nellcor) clipped to the tongue.

An arterial catheter was inserted into the aorta via the femoral artery for monitoring systolic, diastolic, and mean arterial blood pressure (MAP). Swan-Ganz thermodilution catheters (model 93–132–5F, Baxter Healthcare, Irvine, CA) were placed under pressure monitoring via femoral or external jugular veins for measurement of pulmonary arterial P\textsubscript{pa} (P\textsubscript{pa}), central venous (CVP), and pulmonary artery wedge pressure. Cardiac output (CO in l/min) was measured in triplicate at 3- to 4-min intervals with a CO monitor (model 9520A, Edwards Laboratory, Santa Ana, CA) and indexed to weight [cardiac index (CI); l·min\textsuperscript{-1}·kg\textsuperscript{-1}]. Pulmonary vascular resistance index (PVRI) was calculated as mean P\textsubscript{pa} minus pulmonary capillary wedge pressure (PCWP) divided by CI (P\textsubscript{pa} − PCWP)/CI. Systemic vascular resistance index (SVRI) was calculated as femoral MAP − CVP/CI. All the
catheters were connected to transducers (model DT-4812, Ohmeda Medical Services Division, Oxnard, CA) zeroed at the level of the left atrium. Continuous recordings were made by using an eight-channel recorder (model 7758D Hewlett-Packard, Waltham, MA).

Protocol. After anesthesia and instrumentation of the dogs, baseline (BL1) pulmonary function and hemodynamic variables were measured, and Hist bronchoprovocation was performed as described below. The ventilator circuit and nebulizer were rinsed with tap water to remove residual Hist.

Bronchoprovocation was performed as described below. The ventilator circuit and nebulizer were rinsed with tap water to remove residual Hist. When pulmonary function and hemodynamic variables returned to the baseline (BL3) levels (usually 45–60 min), RW bronchoprovocation was performed as described below.

Hist bronchoprovocation. In Con dogs (n = 12) and RW-sensitized dogs (n = 7), baseline (BL1) pulmonary function and hemodynamic measurements, arterial blood gases, and body temperature were measured 2 min after the dogs’ lungs were hyperinflated to twice the tidal volume. These measurements were repeated after the dogs received five breaths of normal saline (Sal1) aerosolized by using an ultrasonic nebulizer (model 25, DeVilbiss, Somerset, PA) in the inspiratory limb of the ventilator. After a second baseline (BL2), the dogs received five breaths of aerosolized Hist (30 mg/ml, Sigma Chemical); measurement of the hemodynamic and pulmonary function variables followed.

RW bronchoprovocation. Con (n = 8) and RW-sensitized (n = 7) beagle dogs received RW bronchoprovocation. BL3 pulmonary function and hemodynamic measurements were obtained 2 min after the lungs are hyperinflated to twice the tidal volume. The measurements were repeated after the dogs received 60 breaths of normal saline (Sal2) aerosolized with the use of the ultrasonic nebulizer in the inspiratory limb of the ventilator. After repeat baseline (BL4) measurements, the dogs received 60 breaths of aerosolized RW (30 mg/ml short RW), followed by measurement of the hemodynamic and pulmonary function variables.

Pulmonary function. We measured pulmonary function by methods developed and routinely used in our laboratory (20, 22, 23). Briefly, airflow was measured with a heated pneumotachograph (Fleisch no. 1, Instrumentation Associates, New York, NY) coupled with two equal sections of polyethylene tubing connected to each side of a differential pressure transducer (Validyne model MP 45-14-871 ± 2 cmH2O). A 3-cm-long esophageal balloon attached to a 40-cm-long catheter was inserted into the midesophagus. It was filled with the appropriate volume of air and positioned at the point where thoracic pressures are most negative, as described by Lemen et al. (19). The transpulmonary pressure was measured by another transducer (Validyne model MP45-32-871 ± 100 cmH2O), with the esophageal balloon catheter connected to one side and the endotracheal tube to the other. Pressure-transducer signals were recorded with an eight-channel recorder (model 7758D, Hewlett-Packard) and continuously monitored. Pressure and flow signals from the recorder were processed with a National Instruments NB-MIO-16 A/D card integrated by computer (Macintosh IIci), by using the software (DqgiVIEW) that we developed from LabView II software (version 2.2, National Instruments, Austin, TX). Our program uses the flow and transpulmonary pressure signals and the Von Neergaard-Wirz equation (28) to measure lung resistance (Rl), and lung dynamic compliance. Lung dynamic compliance and conductance, the reciprocal of Rl, are divided by body weight (in kg) to calculate specific compliance (Cdyn) and specific conductance (Gsp), respectively.

Data analysis. Changes in pulmonary function and hemodynamics after saline, Hist, or RW bronchoprovocation are compared within groups by using paired t-tests. Unpaired t-tests were used to compare the between-group differences. Differences were considered statistically significant at P < 0.05.

RESULTS

Baseline ages, weights, and pulmonary function in Con and RW dogs were not significantly different, as illustrated in Table 1. We had a predominance of female dogs in the RW-sensitized group.

Pulmonary functions for both groups are illustrated in Fig. 1, A and B. After five breaths of saline (Sal1), neither Con nor RW-sensitized dogs had a significant change in Gsp (P = 0.08 and 0.27, respectively) or Cdyn (P = 0.14 and 0.63, respectively). On the other hand, after inhaling aerosolized Hist, both Con and RW-sensitized dogs had significant (P < 0.001) decreases in Gsp (−65 and −69%, respectively) and Cdyn (−51 and −53%, respectively). After 60 breaths of saline (Sal2), Gsp decreased significantly in Con (−19%, P = 0.02) and in RW (−10.5%, P = 0.004) compared with BL3, but these changes in Gsp were not significant (P > 0.05) between groups. Comparing BL3 with Sal2 data, Cdyn also decreased significantly (−13%, P = 0.035) in Con dogs but not in RW-sensitized dogs (−5%, P = 0.21). However, these differences were again not significantly different between groups (P = 0.9), and both groups recovered pulmonary function (BL4) to pre-Sal2 levels.

Lung Gsp and Cdyn decreased significantly (−20%, P = 0.006 and 13%, P = 0.034, respectively) in Con dogs after RW bronchoprovocation, but these decreases were not different from values after 60 breaths of saline (Sal2) bronchoprovocation (P = 0.20). On the other hand, in RW-sensitized dogs, both Gsp and Cdyn decreased markedly (−77%, P = 0.00001, and −66%, P = 0.0003, respectively), and these decreases were three- to fourfold larger (P = 0.004) than from saline alone (Sal2 compared with BL3).

Data on arterial blood gases after Hist and RW bronchoprovocation are illustrated in Table 2. After Hist bronchoprovocation, arterial PO2 (Pao2) decreased significantly (P < 0.05) within groups compared with BL2 and between groups. On the other hand, Pao2 remained >130 Torr for both groups because we gave supplemental O2. The Pao2 in Con did not change after RW bronchoprovocation, but RW-sensitized dogs had a

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Table 1. Baseline physical and pulmonary function data

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>RW-Sensitized</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age, days</td>
<td>30±15</td>
<td>228±31</td>
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<tr>
<td>Range</td>
<td>140–432</td>
<td>154–343</td>
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<tr>
<td>Weight, kg</td>
<td>9.1±0.5</td>
<td>9.3±0.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Range</td>
<td>7.0–11.4</td>
<td>7.7–10.5</td>
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<tr>
<td>Rl, cmH2O·ml⁻¹·s</td>
<td>8.34±1.2</td>
<td>8.71±0.6</td>
<td>0.81</td>
</tr>
<tr>
<td>Range</td>
<td>3.9–13.8</td>
<td>6.7–11.1</td>
<td></td>
</tr>
<tr>
<td>Cdyn, ml/cmH2O</td>
<td>40.3±4.6</td>
<td>37.4±4.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Range</td>
<td>18.0–68.8</td>
<td>26.0–53.4</td>
<td></td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>7.5</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE or ranges; n, no. of dogs; RW, ragweed; Rl, lung resistance; Cdyn, dynamic compliance; M, male; F, female.
significant decrease (P < 0.05) in PaO₂, although the group mean remained >100 Torr.

The pH and PaCO₂ had statistically significant (P < 0.05) changes in both Con and RW-sensitized dogs after Hist bronchoprovocation, although there was no significant difference between Con and RW-sensitized groups. RW bronchoprovocation in RW-sensitized dogs led to a decrease in pH and increase in PaCO₂ (P < 0.05) in the RW-sensitized dogs, with no change in Con dogs.

As illustrated in Fig. 2A, CI is not significantly different at BL1, Sal1, or BL2 between or within Con and RW groups. On the other hand, CI increased significantly after Hist bronchoprovocation in Con dogs compared with pre-Hist levels (BL2) and RW-sensitized dogs. The CI in RW-sensitized dogs did not significantly increase after Hist compared with BL2. After Hist bronchoprovocation, CI in the RW group returned to previous BL levels within the group but remained significantly lower than Con at BL3 (P = 0.007), Sal2 (P = 0.006), and BL4 (P = 0.003). After RW bronchoprovocation, CI did not change significantly in Con or RW-sensitized dogs compared with their preceding baseline (BL4). However, CI in Con dogs remained significantly larger (P = 0.02) than in RW-sensitized dogs as found in their preceding BL values. HR, Fig. 2B, increased significantly (P = 0.0008) in Con dogs and approached a significant increase (P = 0.08) in RW-sensitized dogs after Hist bronchoprovocation compared with BL2 but was not different between groups.

Ppa (Fig. 3A) was not different (P > 0.05) between or within groups for BL1, Sal1, or BL2 data. After Hist bronchoprovocation, Ppa increased significantly compared with BL2 data in the Con group (47%, P = 0.0002) and the RW group (41%, P = 0.006), but Ppa was not different between groups (P 0.4). After recovery, Ppa returned to baseline levels (BL3) and was not significantly different for BL3, Sal2, or BL4 between groups. In contrast, after RW bronchoprovocation, Ppa increased markedly in RW-sensitized dogs (49%, P = 0.001) compared with BL4 values and was significantly larger than for Con dogs (P = 0.03), and Ppa in Con dogs did not change compared with BL4 levels.

As illustrated in Fig. 3B, PVRI in Con dogs is unchanged during Hist and RW bronchoprovocations. In the RW-sensitized dogs, PVRI increased markedly after Hist bronchoprovocation (38%, P = 0.01) compared with BL2 data and was significantly increased compared with Con dogs (P = 0.04). The PVRI also increased markedly (62%, P = 0.03) in RW-sensitized dogs after RW bronchoprovocation compared with BL4 data and was significantly increased compared with Con dogs (P = 0.02).

MAP (Fig. 4A) in Con or RW groups was not different between groups for BL1, Sal1, and BL2. The MAP was lower in RW-sensitized dogs than in Con dogs but was not significantly lower (P = 0.08) at baseline (BL1);
however, it decreased further after five breaths of saline (Sal1) in RW-sensitized dogs and reached significance (P = 0.04) but recovered spontaneously (BL2). Hist bronchoprovocation significantly decreased MAP compared with BL2 data in Con (−13%, P = 0.005) and RW groups (−32%, P = 0.02) and within groups, and these decreases were significantly different (P = 0.049) between groups. After spontaneous recovery in each group, BL3 data were not different within or between groups compared with BL1 and BL2 data. After RW bronchoprovocation, MAP decreased (−21%, P = 0.051) in RW-sensitized dogs compared with BL4 data and was significantly different (P = 0.02) between groups.

As illustrated in Fig. 4B, SVRI decreased markedly after Hist bronchoprovocation in both groups and was not significantly different between groups. This was associated with a decrease in MAP (Fig. 4A) in both groups but an increase in CI (Fig. 2A) in only Con dogs. On the other hand, the RW bronchoprovocation resulted in a large decrease in SVRI in RW-sensitized dogs (−44%, P = 0.006) compared with BL4 data but did not change in Con dogs and approached significant differences (P = 0.06) between groups.

**DISCUSSION**

Our study indicates that RW-sensitized beagle dogs have increased airway and pulmonary vascular responses to Hist and RW bronchoprovocation. Although both Con and RW groups had similar changes in pulmonary function (i.e., Gsp and CLsp) after Hist bronchoprovocation, only RW-sensitized dogs had pulmonary vasoconstrictive responses to RW inhalation. Thus pulmonary vasoconstrictive responses to RW bronchoprovocation appear to be independent of the changes in pulmonary functions in RW-sensitized dogs.
Pulmonary hypertension occurs in asthma patients (7, 8, 10, 12, 21, 25). In one report (25), two children with congenital heart defects and asthma were found to have unexplained pulmonary hypertension on cardiac catheterization. Ppa decreased after aggressive asthma treatment. Thus alterations in pulmonary vascular responses may be an important component of asthmatic attacks.

We (23) and others (5, 9) have demonstrated that repeated RW injections in beagle puppies beginning on the first day of life sensitize them to RW, as illustrated by positive skin tests, anti-RW immunoglobulin (Ig) G and IgE, and airway hyperresponsiveness to RW bronchoprovocation. In our present studies, we used an intact-chest preparation to study the cardiopulmonary interactions induced in vivo by Hist or RW bronchoprovocation in RW-sensitized dogs. Our previous data (23) suggest that the airways of both Con and RW-sensitized beagle dogs respond to Hist (5 breaths of 30 mg/ml) with bronchoconstriction. Because we observed decreases in Gsp and Clsp in both control and RW-sensitized dogs after Hist bronchoprovocation, we believe that the changes in PVRI and SVRI indicate changes in pulmonary vascular smooth muscle responses in RW-sensitized dogs.

We are not aware of previous studies of the effects of aerosolized Hist or RW on pulmonary vascular responses. In studies by others (1, 3, 16, 24, 26) intravenous Hist increases pulmonary vascular resistance by small- and large-vein constriction in isolated blood-perfused dog lungs. This response is thought to be mediated via H₂ receptors on the vascular smooth muscle. Hist may also have pulmonary artery vasodilator actions due to stimulation of H₂ receptors if pulmonary vascular tone is elevated (2). In addition, there are H₁ receptors located on the vascular endothelium, which, when stimulated, may lead to the release of other mediators, such as nitric oxide. After aerosolized Hist, RW-sensitized dogs increased PVRI, presumably due to stimulation of H₁ receptors. Thus we speculate that Hist or other mediators released from the lungs, as well as absorbed across the alveolar surface, could have contributed to the effects of aerosolized Hist on the pulmonary circulation in our studies.

We also noted that only RW-sensitized dogs increased PVRI after RW inhalation, suggesting that RW-sensitized dogs had pulmonary vascular smooth muscle hyperresponsiveness compared with Con dogs. It is difficult to compare our in vivo data with other studies (26, 27) where blood flow is held constant. Increased CI in Con dogs could result from decreased SVRI and the release of catecholamines, with subsequent increase in HR. We think this may have hidden a small increase in PVRI, because CO and PVR are inversely related when vascular tone is constant (30). Despite large changes in SVRI and HR in both groups, RW-sensitized dogs had no change in CI. Thus the increase in PVRI reflects an intrinsic increase in pulmonary vascular tone in RW-sensitized dogs that is clearly not present in Con.

We anticipated changes in pulmonary functions and arterial blood gases to Hist. Therefore, we took precautions to minimize their effects on PVRI. We gave supplemental O₂ throughout the bronchoprovocation studies and continuously monitored O₂ saturation and end-tidal CO₂. Although we observed significant changes in blood gases with Hist bronchoprovocation in both groups and with RW in the RW-sensitized dogs, we feel the differences were not clinically important. Therefore, we do not think changes in PaO₂ or PaCO₂ contributed to the pulmonary vasoconstrictor response we observed. Furthermore, Hist bronchoprovocation led to similar changes in Clsp and Gsp in both Con and RW-sensitized dogs. Despite this similarity, the Con dogs did not develop increased PVRI. Again, this finding supports our conclusion that RW sensitization altered the normal responsiveness of the pulmonary circulation to Hist and RW inhalation.

It has been well established that allergic bronchoprovocation results in the release of numerous media-
tors, including products of arachidonic acid metabolism (prostaglandins, thromboxanes, and leukotrienes) and histamine. These substances are vasoactive, with predominantly pulmonary vasoconstrictor and systemic vasodilator properties. In our RW-sensitized dogs, the RW bronchoprovocation resulted in airway and pulmonary vascular changes that were similar to those observed after histamine. Therefore, our data support the conclusion that histamine release after RW bronchoprovocation may have contributed, at least in part, to our findings. Endothelin-1 (15) also may be a significant mediator in human asthma. Because our RW-sensitized dogs exhibit several similarities to human asthma (i.e., anti-RW IgE and skin-test responses), we speculate that changes in endothelin-1 may also play a role in our model.

In summary, our results indicate that RW sensitization is associated with increased responsiveness of the pulmonary vasculature to RW bronchoprovocation in beagle dogs. Increases in pulmonary vascular resistance were observed after nonallergic (Hist) and allergic (RW) bronchoprovocation in RW-sensitized dogs and were independent of acute changes in pulmonary functions or arterial blood gases. We speculate that hyperresponsiveness of the pulmonary vasculature may be an important mechanism in severe asthmatic attacks in humans.

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Received 17 June 1996; accepted in final form 29 April 1997

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