Effects of anaphylaxis mediators on partitioned pulmonary vascular resistance during ragweed shock in dogs

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Mink, S. N., A. Becker, H. Unruh, and W. Kepron. Effects of anaphylaxis mediators on partitioned pulmonary vascular resistance during ragweed shock in dogs. J. Appl. Physiol. 84(3): 782–790, 1998.—We examined the effect of anaphylactic shock on the longitudinal distribution of pulmonary vascular resistance (PVR) in ragweed-sensitized dogs in which PVR was partitioned into an upstream arterial component (Rus) and a downstream venous and capillary component (Rds). We also assessed whether Rus and Rds would be reduced by pretreatment with histamine H1- and H2-receptor blocking agents and with cyclooxygenase and lipoxygenase pathway inhibitors. Anesthetized animals were examined on separate occasions 3 wk apart in which one of the treatments was randomly given. The pulmonary arterial occlusion technique was used to determine segmental pressure drops. During ragweed challenge, PVR increased ~4 times compared with the preshock value (3.04 vs. 12.07 mmHg·l−1·min; P < 0.05). Although both Rus and Rds increased postshock, the greatest relative increase occurred in Rds. None of the treatments reduced partitioned resistances compared with no treatment. Our results show that, under conditions of anaphylactic shock, increases in Rus and Rds could not be ascribed to release of histamine or products of the cyclooxygenase and lipoxygenase pathways.

histamine; asthma; thromboxane; leukotrienes

IN ANAPHYLACTIC SHOCK, the release from basophils and mast cells of preformed and newly generated mediators leads to pulmonary vasoconstriction (19, 26, 32). Among others, histamine and products of the cyclooxygenase [i.e., prostacyclin, prostaglandin (PG) F2α, and thromboxane (Tx)] and lipoxygenase pathways [leukotrienes (LTs): LTB4, LTC4, LTD4, LTE4] may all modulate pulmonary vascular resistance (PVR) (1–4, 9, 17, 20, 24, 29, 30). Histamine, Tx, PGF2α, and the sulfidopeptide LTs cause vasoconstriction (1, 2, 4, 20, 24, 30), whereas prostacyclin causes pulmonary vasodilation (17, 29). These mediators may also act on different segments of the pulmonary vasculature. Histamine causes predominantly postcapillary vasoconstriction (2, 8), whereas LTB4 may cause precapillary constriction (20). Effects of mediators on the pulmonary vasculature may also be species specific (4).

In most studies, the effects of mediators on PVR have been examined under nonanaphylactic conditions (1, 2, 4, 20, 24, 30). In those instances, one or more mediators of anaphylaxis have been intravenously infused in vivo or intact preparations at arbitrary concentrations after which changes in segmental pulmonary vascular resistances were determined. However, the relevance of such preparations to anaphylactic shock, in which an array of mediators is released during anaphylaxis, is not clear.

In the present study, we used a ragweed model of anaphylactic shock (9, 18, 19) to determine the effect of allergen challenge on segmental pulmonary vascular resistances in which PVR was partitioned into an arterial upstream component (Rus) and a downstream (Rds) venous and capillary component (25). We further determined whether changes in Rus and Rds could be modified when pretreatment was undertaken with histamine H1-receptor blockade, histamine H2-receptor blockade, and cyclooxygenase and lipoxygenase pathway inhibition in this anaphylactic model.

METHODS

Anaphylaxis model. This protocol was approved by the University Animal Care Committee. Our canine model of ragweed anaphylaxis has been previously described (18, 19). Newborn dogs received their first dose of antigen (0.5 mg ragweed pollen extract) mixed with 30 mg Al(OH)3 intraperitoneally within 24 h after birth. Injections were repeated at weekly intervals for 8 wk, at biweekly intervals for ~30 wk, and then at monthly intervals. After 8 wk, some animals received airway challenges with ragweed (under pentobarbital anesthesia, 30 mg/kg) rather than injections to maintain hypersensitivity. This protocol was previously described in detail (3). Both sensitizing regimens result in mean immunoglobulin E (IgE) anti-ragweed antibody titers of >256 dilutions when measured ~2 wk before study by passive cutaneous anaphylaxis, whereas nonsensitized littermate controls show no IgE titers by this test. There were no differences in the anaphylactic response between the different sensitization methods so that the animals were analyzed as a single group. The animals were examined at ~1 yr of age.

Anaphylaxis protocol and measurements. Five treatment studies were performed on each animal ~3 wk apart in a randomized design. In the control treatment study (n = 9), no treatment was given. In the histamine H1-receptor-blocker study (n = 7), chlorpheniramine maleate (10 mg/kg iv) was administered (26); in the histamine H2-receptor-blocker study (n = 6), the animals were pretreated with ranitidine HCl (20 mg/kg iv) (18); in the cyclooxygenase-inhibition study (n = 7), this pathway was inhibited by indomethacin (2 mg/kg iv) (15); and in the lipoxygenase-inhibition study (n = 5), this pathway was inhibited by the 5-lipoxygenase-activating protein antagonist MK-0591 (see below) (5). Although in all dogs a control treatment study was performed, all treatments could not be performed in each dog; four dogs were withdrawn at various intervals for use in another study. Of the nine dogs studied, five completed the entire five treatments. However, the results were the same whether these five dogs were analyzed separately or whether the entire group of nine dogs was analyzed.

During each treatment, four conditions were examined that included baseline, treatment, shock, and after treatment. The animals were examined at ~1 yr of age.
PULMONARY VASCULAR RESISTANCE IN ANAPHYLAXIS

Because no "a" wave is observed at the high heart rates found
artery incision and positioned into the left ventricle (LV).
Instruments, Houston, TX) was advanced through a carotid
analysis and Ppw under normal conditions (13) (see rationale in
and because others have shown little difference between Pla
(i.e., left atrial pressure; Pla) because at end diastole we have
measurements were obtained at end expiration so that
on an eight-channel recorder (Astro-Med, West Warwick, RI).
the basis of previous studies (22). All signals were displayed
CO) was determined by thermodilution techniques (Colum-
pressure (Ppa), mean pulmonary wedge pressure (Ppw), and
BP was measured with a polyethylene catheter inserted into
while the animals were anesthetized with pentobarbital
sodium (30 mg/kg iv; Ref. 28). The animal was ventilated (12
ml/kg; Harvard Apparatus, Natick, MA) in the supine posi-
tion with the rate adjusted as necessary to maintain pH
between 7.3 and 7.4. Supplemental oxygen was given to
maintain arterial Po2—100 Torr throughout the study.

All vascular catheters were placed under sterile conditions.
BP was measured with a polyethylene catheter inserted into
the femoral artery. A Swan-Ganz catheter was advanced into
the pulmonary artery to measure mean pulmonary arterial
pressure (Ppa), mean pulmonary wedge pressure (Ppw), and
right atrial pressure and to determine pulmonary capillary
occlusion pressure (Ppc0; see Data analysis). Cardiac output
(CO) was determined by thermodilution techniques (Colum-
bus Instruments), with the average of four determinations
reported. All of the fluid-filled catheters were connected to
transducers (Cobe Instruments) and were referenced relative
the left atrium. The left atrium was determined as the
tissue was found preshock. If, in a particular study, this dose of
antigen was not sufficient to produce shock, then the antigen
dose was doubled until shock occurred because the presence
of shock was the primary end point of the study. After shock
measurements were obtained, 10% pentastarch in normal
saline solution (500—600 ml) was given to return LVEDP back
to baseline condition.

Systemic and pulmonary hemodynamics were determined
while the animals were anesthetized with pentobarbital
sodium (30 mg/kg iv; Ref. 28). The animal was ventilated (12
ml/kg; Harvard Apparatus, Natick, MA) in the supine posi-
tion with the rate adjusted as necessary to maintain pH
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(CO) was determined by thermodilution techniques (Colum-
bus Instruments), with the average of four determinations
reported. All of the fluid-filled catheters were connected to
transducers (Cobe Instruments) and were referenced relative
the left atrium. The left atrium was determined as the
lower one-third distance between the spine and sternum on
the basis of previous studies (22). All signals were displayed
on an eight-channel recorder (Astro-Med, West Warwick, RI).
Measurements were obtained at end expiration so that
respiratory variation would not affect interpretation of the
results.

LVEDP was used to estimate venous downstream pressure
(i.e., left atrial pressure; Pla) because at end diastole we have
observed little difference between mean Ppw and LVEDP (11)
and because others have shown little difference between Pla
and Ppw under normal conditions (13) (see rationale in
Data analysis). A high-fidelity transducer-tipped catheter (Millar
Instruments, Houston, TX) was advanced through a carotid
artery incision and positioned into the left ventricle (LV).
Because no "a" wave is observed at the high heart rates found
in the present study, LVEDP was defined as the pressure at
which the rate of pressure development increased by 150
mmHg/s and was sustained for at least 50 ms (11, 28, 31).

During each condition, plasma concentrations of histo-
mine, Lts (LTE4), as well as the breakdown products of
prostacyclin and TxA2 (i.e., 6-keto-PGF1a, and TxB2, respec-
tively) were obtained. It is recognized that many other
mediators may be released during shock, but these were
considered the important ones in terms of the present study.
Mediators were measured by radioimmunoassay techniques.
Histamine immunoanalysis (ImmuneTech International) was
performed by competition between modified histamine in the
sample and the iodinated histamine tracer for the binding to
the antibody coated on tubes (23). 6-Keto-PGF1a, TxB2, and
LTE4 were measured by NEN Research Products (Boston,
MA) NEK-008, NEK-007, and NEK-043, respectively (15).
For each mediator, the standard curves were generated in
which a known amount of the tracer was placed into an
aliquot of pooled canine plasma and not just the buffer
solution alone. In the LT assay, because in vitro conversion of
LTC4 and LTD4 to LTE4 occurs spontaneously, measurements
of all Lts were obtained after enzymatic conversion (by
-y-glutamyl transpeptidase and microsomal leucine aminopep-
tidase) to LTE4 as described by Heavey et al. (16). For all
mediators, samples were stored at −70°C and analyzed in
duplicate.

Supplemental protocols. Other protocols were performed as
a part of this study to control for the effects of time and other
methodological concerns on our measurements. In the volume-
infusion time-control protocol, volume infusion was not given
after shock, and another set of shock measurements
was obtained over this interval. The objective of this study was to
determine what changes in partitioned resistances would
occur over this interval without the effect of volume infusion.

In the sham-shock protocol, we controlled for the effects of
time and the ragweed diluent (i.e., normal saline solution) on
our hemodynamic measurements. The ragweed diluent alone
was administered during the interval of antigen challenge,
and there was no need for volume infusion.

In the volume-depleted control study, the objective was to
examine what the changes in PVR and partitioned resis-
tances would be when, in nonshocked dogs, LVEDP and CO
were lowered to an extent found in the anaphylaxis protocol.
As previously stated, PVR is dependent on the extent of
hydration (33), and, because LVEDP during allergen chal-
denge would fall compared with the preshock value, this effect
might by itself increase PVR and segmental resistances. The
magnitude of this effect was determined in the volume-
depleted control study. Pulmonary hemodynamics were deter-
mined when the animals were slightly dehydrated and main-
tained without water for 18 h before being studied. This gave
LVEDP and CO values comparable with values found in the
anaphylaxis protocol.

In the lung mechanics study, the objective was to deter-
mine whether the increases in PVR and other resistances
observed during allergen challenge may be related to broncho-
striction and accompanying hyperinflation. In that case,
evascular factors, such as increases in end-expiratory
pleural pressure (Pp) and intrinsic positive end-expiratory
pressure (PEEP) (i.e., alveolar pressure (P A) >0 cmH2O
measured at end-expiration with the expiratory port of the
ventilator occluded) may have contributed to the higher Ppa
found during challenge.

Lung mechanics were measured on a separate occasion at
the end of the anaphylactic protocol in five sensitized dogs
under pentobarbital anesthesia (30 mg/kg iv). The animals
were placed into a volume-displacement plethysmograph as
previously described (21). Lung volume was measured by a
Krogh spirometer mounted on the plethysmograph. Flow was
estimated from a pneumotachograph (model 3800, Hans
Rudolph, Kansas City, MO) placed between the spirometer and the plethysmograph. Airway opening pressure (Pao) was obtained from a lateral pressure tap placed in the endotracheal tube. The lateral pressure tap was connected to a Validyne pressure transducer (Validyne, Northridge, CA). Ppl was estimated by an esophageal balloon, which was placed 5 cm from the gastroesophageal junction and connected by polyethylene tubing to the other port of the Validyne pressure transducer. The output of the pressure transducer could be displayed as Pao, Ppl, or transpulmonary pressure (Ptp). Signals for flow, volume, and pressure were displayed on the oscillograph. During tidal inflation, lung compliance (Cl; ml/cmH2O) was measured from change in lung volume/ΔPtp. The animals were studied at baseline, during shock, and after volume infusion as described in the anaphylaxis study.

Data analysis. In the different protocols, PVR was calculated as (Ppa – LVEDP)/CO. The rationale for the use of LVEDP rather than Ppw was as follows. Before allergen challenge, there was no difference between LVEDP and Ppw (see RESULTS), and thus both could be used interchangeably to calculate PVR. However, during allergen challenge, the time course of the Ppa decay was so slow (>5 s; Fig. 1) that frequently Ppw > LVEDP. This indicated that a true LV filling pressure was never reached, and thus use of LVEDP rather than Ppw represented a more accurate picture of PVR (see DISCUSSION).

To partition PVR into Rus and Rds segments, we estimated Ppco by arterial occlusion in which a biexponential model of pulmonary vascular pressure decay was used (8). As described by Gilbert and Hakim (10), time 0 could be ascertained by the change in pressure obtained from the Ppa tracing. From the slow exponential component of pressure decline, the pressure was plotted on semilog scale and was extrapolated to time 0, which represented Ppco. This was done by “best-visual-fit line” (see DISCUSSION). Gilbert and Hakim indicated that fitting the data points to an exponential between 0.2 and 2 s was sufficient to obtain Ppco. In the present study, we tried to allow 5–8 s because the time for the pressure decay was so slow in anaphylactic shock. Rus, which predominantly represented arterial resistance, was calculated from (Ppa – Ppco)/CO; Rds, which predominantly represented venous and capillary resistance, was calculated from (Ppco – LVEDP)/CO (25).

Statistics. A one-way analysis of variance for repeated measures with missing numbers (ANOVA1R) was used when conditions for a specific treatment in the anaphylaxis protocol or conditions in the respective supplementary protocols were compared. ANOVA1R was also used when a given condition (i.e., shock, volume infusion) was compared between treatments and interventions in the anaphylaxis and supplementary protocols. When multiple means were compared, a Duncan’s multiple-comparison test was used. Results are reported as means ± SD.

RESULTS

In the anaphylaxis protocol, the hemodynamic measurements obtained in the control treatment study are shown in Table 1. At baseline, LVEDP was nearly identical to Ppw, and Ppco was just slightly greater than Ppw. In Fig. 1A, the Ppa decline found during occlusion was analyzed in terms of a biexponential model. After occlusion, there was a initial rapid decline in Ppa and a plateau in pressure (i.e., Ppw) was reached in =2 s. PVR averaged ~2 mmHg·l−1·min (Fig. 2). Because Ppco was calculated to be very close to Ppw (Table 1), Rus accounted for most of PVR (Figs. 3 and 5), whereas the contribution of Rds was fairly small (Figs. 4 and 6).

When antigen was given in the control treatment study, mean BP fell =50% during shock (Table 1), and PVR increased approximately fourfold (Fig. 2). During shock, Ppw (Table 1) was often higher than LVEDP, and thus Ppw did not represent LV filling pressure in many experiments. As shown in Fig. 1B, the profile of occlusion pressure during shock was very slow. Although both Rus and Rds increased during shock compared with preshock values (Figs. 3 and 4, respectively), the relative increase in Rds was much greater than in Rus (Figs. 5 and 6 in the control treatment study).

In the control treatment study, slightly more volume was given than what was intended so that LVEDP and Ppco obtained post-volume infusion were slightly higher than preshock values (Table 1). In general, however, during volume infusion, indexes of pulmonary hemodynamics returned to preshock values, although the distribution of resistance still favored a relative increase in Rds (Fig. 6). During volume infusion, the return in PVR (Fig. 2) toward preshock values was not due to an effect of time alone because, in the volume-infusion protocol (Table 2), the changes in parameters over this interval were much less than what was observed in the control treatment study (compare volume condition found in Figs. 2–6 with the sham volume-infusion values in Table 2).

Moreover, in the sham-shock protocol, ragweed diluent was administered during the “shock interval,” and this study also served as a time control study. In the sham-shock protocol, there was no decrease in BP when the diluent was administered. BP measured 152 ± 6 mmHg at baseline, 148 ± 10 mmHg at sham shock, and 157 ± 13 mmHg during volume infusion.

In the control treatment study (n = 9), the dose of antigen required to produce shock in the individual
dogs (Table 1) ranged between 0.1 and 85 mg and averaged 25 ± 28 mg. Only in the histamine H<sub>1</sub>-receptor study (n = 7) was there a different antigen dose required to produce shock which doubled to 55 ± 50 mg (P < 0.05 vs. control study). In the histamine H<sub>2</sub>-receptor study (n = 6), the mean dose was 35 ± 16 (SD) mg; in the cyclooxygenase inhibition study (n = 7), the mean dose was 34 ± 20 mg; and in the lipoxygenase inhibition study (n = 5), the mean dose was 31 ± 15 mg.

The hemodynamic measurements obtained with the different treatments are shown in Tables 3–6. Relative to baseline values, hemodynamics were unchanged when any of the treatments was administered. Although, by design, BP fell approximately one-half during shock compared with preshock value, in the histamine H<sub>2</sub>-receptor study (Table 4), the fall in BP occurred to the greatest extent, and BP was significantly less than that found in either the histamine H<sub>1</sub>-receptor study (Table 3) or the cyclooxygenase inhibition study (Table 5).

### Table 1. Hemodynamics in the control treatment study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sham Treatment</th>
<th>Shock</th>
<th>Volume Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mmHg</td>
<td>153 ± 17</td>
<td>152 ± 21</td>
<td>80 ± 26*</td>
<td>128 ± 15*</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>20 ± 7</td>
<td>19 ± 8</td>
<td>17 ± 9</td>
<td>28 ± 7*</td>
</tr>
<tr>
<td>Ppw, mmHg</td>
<td>10 ± 5</td>
<td>10 ± 4</td>
<td>5 ± 4*</td>
<td>14 ± 7*</td>
</tr>
<tr>
<td>Ppc, mmHg</td>
<td>11 ± 5</td>
<td>11 ± 5</td>
<td>10 ± 7</td>
<td>21 ± 7*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>9 ± 3</td>
<td>9 ± 2</td>
<td>1 ± 1*</td>
<td>14 ± 6*</td>
</tr>
<tr>
<td>LVEDP – Ppw, mmHg</td>
<td>−0.4 ± 2.8</td>
<td>−0.4 ± 2.8</td>
<td>−3.6 ± 3.6</td>
<td>−0.3 ± 6</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.4 ± 1.4</td>
<td>3.4 ± 1.4</td>
<td>1.6 ± 1*</td>
<td>4.8 ± 2.5*</td>
</tr>
</tbody>
</table>

Values are means ± SD for 9 dogs. BP, mean blood pressure; Ppa, mean pulmonary arterial pressure; Ppw, mean pulmonary wedge pressure; Ppc, pulmonary capillary occlusion pressure; LVEDP, left ventricular end-diastolic pressure; CO, cardiac output. *P < 0.05 from other conditions, by repeated-measures analysis of variance (ANOVA) and Duncan’s multiple-comparison test.

In the different treatment studies, the increases in PVR (Fig. 2), Rus (Fig. 3), and Rds (Fig. 4) found between pre- and postshock conditions were similar to those in the control treatment study, although in the cyclooxygenase inhibition study, during volume infusion, Rds as well as percent Rds/PVR were significantly lower than values found in the control treatment study (Figs. 4 and 6).

The concentration of mediators found in the different studies are shown in Table 7. In the histamine H<sub>1</sub>-receptor study, the concentration of histamine released during shock was higher than values in the other studies, and this finding reflected the higher antigen dose given in the histamine H<sub>1</sub>-receptor study. In the cyclooxygenase-inhibition study, the concentrations of TxB<sub>2</sub> and 6-keto-PGF<sub>1α</sub> were lower, whereas LTE<sub>4</sub> concentrations were higher, than values in the other studies.

**Fig. 2.** Pulmonary vascular resistance (PVR) vs. conditions for different treatment studies. *P < 0.05 from other conditions in a study by analysis of variance (ANOVA) and Duncan’s multiple-comparison test.

**Fig. 3.** Resistance of upstream arterial component (Rus) vs. conditions for different treatment studies. By ANOVA and Duncan’s multiple-comparison test: *P < 0.05 vs. all other conditions in a study; †P < 0.05 vs. preshock in a given study.

**Fig. 4.** Resistance of downstream venous and capillary component (Rds) vs. conditions for different treatment studies. By ANOVA and Duncan’s multiple-comparison test: *P < 0.05 vs. all other conditions in a study; †P < 0.05 cyclooxygenase vs. control study.
In the volume-depleted protocol, LVEDP averaged 2.2 ± 2.1 mmHg, Ppw averaged 2.6 ± 2 mmHg, and CO averaged 1.5 ± 1 l/min. These values compared well with those found in Table 1. However, there was a different pattern of response in PVR, Rus, and Rds between what was found in the anaphylaxis protocol and the volume-depleted protocol. In the volume-depleted protocol, PVR during volume depletion (4.26 ± 1.81 mmHg·l⁻¹·min⁻¹) increased slightly (but not significantly; p < 0.11) compared with baseline (PVR = 3.0 ± 0.94 mmHg·l⁻¹·min⁻¹), but the increase was much smaller than that found between baseline and shock in the control treatment study (p < 0.05 between control and volume-depleted studies).

Moreover, in the volume-depleted protocol, although both Rus (3.49 ± 1.39 mmHg·l⁻¹·min⁻¹; p < 0.11) and Rds (0.78 ± 0.74 mmHg·l⁻¹·min⁻¹; p < 0.01) were higher compared with baseline values (2.42 ± 0.94 and 0.58 ± 0.83 mmHg·l⁻¹·min⁻¹, respectively), most of the increase was in Rus rather than in Rds. With volume depletion, Rus represented 84 ± 13% of PVR whereas Rds represented 16 ± 13% of PVR, and these values were not different from baseline percentages (78 ± 18 and 22 ± 19%, respectively). In the volume-depleted control study, percent Rus/PVR was significantly higher, whereas percent Rds/PVR was lower, than corresponding values found during shock in the control study.

Finally, in the lung mechanics study, the results showed that air trapping did not occur at end expiration. At end expiration, Ppl and Ptp were unchanged in the three conditions, and intrinsic PEEP was not present (Table 8). There were no changes in Ppl or PA during breath holding when measurements were obtained in any of the conditions. Although peak Ptp increased during shock, which indicated that bronchoconstriction was present, there was no change in Cl (either static or dynamic) during allergen challenge.

Table 2. Hemodynamics in the volume-infusion time-control study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sham Treatment</th>
<th>Shock</th>
<th>Sham Volume Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mmHg</td>
<td>168 ± 25</td>
<td>167 ± 23</td>
<td>68 ± 29*</td>
<td>114 ± 51†</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>21 ± 4</td>
<td>21 ± 8</td>
<td>18 ± 9</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>Ppw, mmHg</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td>3 ± 2*</td>
<td>3 ± 2‡</td>
</tr>
<tr>
<td>Pcco, mmHg</td>
<td>9 ± 5</td>
<td>9 ± 5</td>
<td>9 ± 7</td>
<td>9 ± 7‡</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>7 ± 3</td>
<td>7 ± 2</td>
<td>1 ± 1*</td>
<td>1 ± 6*†</td>
</tr>
<tr>
<td>LVEDP − Ppw, mmHg</td>
<td>−0.4 ± 2.8</td>
<td>−0.4 ± 2.8</td>
<td>−2.6 ± 3.6</td>
<td>−2.3 ± 6</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.3 ± 1.4</td>
<td>4.3 ± 1.4</td>
<td>1.6 ± 1*</td>
<td>2.0 ± 2.5‡</td>
</tr>
<tr>
<td>PVR, mmHg</td>
<td>3.26 ± 0.94</td>
<td>3.26 ± 0.94</td>
<td>10.06 ± 6.97*</td>
<td>7.11 ± 6.47‡</td>
</tr>
<tr>
<td>Rus, mmHg</td>
<td>3.79 ± 0.94</td>
<td>2.79 ± 0.94</td>
<td>5.625 ± 2.9*</td>
<td>3.00 ± 2.02‡</td>
</tr>
<tr>
<td>Rds, mmHg</td>
<td>0.47 ± 0.83</td>
<td>0.47 ± 0.83</td>
<td>5.00 ± 5.91*</td>
<td>4.00 ± 1.39‡</td>
</tr>
<tr>
<td>Rds/PVR, %</td>
<td>86 ± 18</td>
<td>86 ± 18</td>
<td>56 ± 22*</td>
<td>42 ± 27*</td>
</tr>
<tr>
<td>Rds/PVR, %</td>
<td>14 ± 19</td>
<td>14 ± 19</td>
<td>44 ± 23*</td>
<td>58 ± 28*</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 dogs. PVR, total pulmonary vascular resistance; Rus, resistance of upstream arterial component; Rds, resistance of downstream venous and capillary component. By repeated-measures ANOVA and Duncan's multiple-comparison test: *p < 0.05 from preshock conditions; †p < 0.05 vs. shock and preshock conditions; ‡p < 0.05 vs. control study.

Table 3. Hemodynamics in the H1-receptor-blocker study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Treatment</th>
<th>Shock</th>
<th>Volume Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mmHg</td>
<td>151 ± 16</td>
<td>164 ± 9</td>
<td>96 ± 43*</td>
<td>136 ± 21*</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>18 ± 2</td>
<td>18 ± 2</td>
<td>15 ± 3*</td>
<td>25 ± 5*</td>
</tr>
<tr>
<td>Ppw, mmHg</td>
<td>10 ± 2</td>
<td>11 ± 3</td>
<td>6.5 ± 2*</td>
<td>13 ± 4*</td>
</tr>
<tr>
<td>Pcco, mmHg</td>
<td>11 ± 3</td>
<td>12 ± 4</td>
<td>9 ± 1.5*</td>
<td>16 ± 4*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>11 ± 3</td>
<td>11 ± 3</td>
<td>4 ± 2*</td>
<td>15 ± 5*</td>
</tr>
<tr>
<td>LVEDP − Ppw, mmHg</td>
<td>0.6 ± 2.2</td>
<td>0.9 ± 1.7</td>
<td>−2.6 ± 2.5*</td>
<td>2.2 ± 3.3</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4 ± 1</td>
<td>3.3 ± 1</td>
<td>1.7 ± 1.2*</td>
<td>4 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 dogs. By repeated-measures ANOVA and Duncan's multiple-comparison test: *p < 0.05 from other conditions; †p < 0.05 vs. preshock; ‡p < 0.05 vs. volume and treatment.
Table 4. Hemodynamics in the H₂-receptor-blocker study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Treatment</th>
<th>Shock</th>
<th>Volume Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mmHg</td>
<td>143 ± 1</td>
<td>144 ± 10</td>
<td>72 ± 34*</td>
<td>113 ± 33*</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>22 ± 4</td>
<td>23 ± 3</td>
<td>29 ± 13</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>Ppw, mmHg</td>
<td>11 ± 3</td>
<td>13 ± 3</td>
<td>7 ± 4P</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Pco, mmHg</td>
<td>15 ± 5</td>
<td>16 ± 4</td>
<td>16 ± 8</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>11 ± 4</td>
<td>14 ± 3</td>
<td>2 ± 3P</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>LVEDP - Ppw, mmHg</td>
<td>0 ± 1</td>
<td>1.3 ± 1.3</td>
<td>-5.5 ± 3.8</td>
<td>0.7 ± 2.9</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>5.6 ± 1.5</td>
<td>5.5 ± 1</td>
<td>2.4 ± 0.5</td>
<td>6.1 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SD for 6 dogs. By repeated-measures ANOVA and Duncan’s multiple-comparison test: *P < 0.05 from other conditions; †P < 0.05 vs. volume and treatment; ‡P < 0.05 vs. histamine H₁-receptor-blocker study and cyclooxygenase-inhibition study.

DISCUSSION

In our ragweed model, the results showed that during allergen challenge PVR increased approximately fourfold compared with the prechallenge measurement. Although both Rus and Rds increased during shock, the greatest relative increase occurred in Rds. With volume hydration, Rus and Rds returned toward baseline, the greatest relative increase occurred in Rds. Although both Rus and Rds increased during shock, approximately fourfold compared with the preshock measurement.

In our ragweed model, the results showed that during allergen challenge PVR increased approximately fourfold compared with the prechallenge measurement. Although both Rus and Rds increased during shock, the greatest relative increase occurred in Rds. With volume hydration, Rus and Rds returned toward baseline, the greatest relative increase occurred in Rds. Although both Rus and Rds increased during shock, approximately fourfold compared with the preshock measurement.

During shock, the increases in PVR and partitioned resistances observed in our model were not simply due to a passive effect of lower flows resulting from a fall in LVEDP (33). During allergen challenge, as LVEDP fell, zone II conditions of West et al. (33) (Ppa > Pp > LVEDP) would evolve in the upper lung regions. PVR and segmental resistances would increase due to zonal changes as flow decreased during shock. The magnitude of this contribution was examined in the volume-depletion protocol in which the distribution of zones of West et al. and CO were similar to values found during anaphylaxis. We found that, in anaphylactic shock, the increase in PVR was much greater than that found during volume depletion and that the primary increase in resistance was in Rds. In contrast, in the volume-depletion model, the primary increase in resistance was in Rus.

The increase in pulmonary vascular pressures in the anaphylactic protocol was also not due to air trapping because there was no evidence to suggest that, at end expiration, increases in Ptp, Ppl, or intrinsic PEEP were present during allergen challenge (Table 8). In all conditions, expiratory flows at end expiration returned to zero, and there were no changes in Ppl and PA during breath holding when measurements were obtained. This would agree with our previous study in which histamine aerosolization failed to produce air trapping in a canine model of severe bronchostenosis (12). Furthermore, it is not possible to exclude that perivascular interstitial edema caused by release of inflammatory mediators from mast cells led to an increase in vasoconstrictor by means of compression of the pulmonary vasculature. However, this explanation appears unlikely as the sole cause of our results because pulmonary vasoconstriction occurred within seconds of allergen challenge and was reversed within minutes by intravascular volume expansion.

We considered that the most likely mechanism for the increase in PVR found during shock was related to a direct effect of mediators on the pulmonary circulation (1, 2, 4, 20, 22, 24, 30). Histamine has been shown to cause vasoconstriction, in canine lungs, that has been most pronounced in Rds (2, 4, 14). This has been shown in many preparations, including isogravimetric and double-occlusion determinations of pulmonary capillary pressure (7, 8, 14). Because histamine was released in large quantities in our model (Table 7), we expected that the increase in Rds would be attenuated by histamine H₁- and H₂-receptor blockade. However, we did not find that H₁- or H₂-receptor blockade modified the increases in PVR or partitioned resistances found in our model.

Such results support those of Silverman et al. (26), who examined the effects of antihistamines on cardio-pulmonary changes in an Ascaris suum canine model of anaphylaxis. During challenge, H₂-receptor blockade, H₂-receptor blockade, or combined H₁- and H₂-receptor blockade, had no effect on PVR in their model. Silverman et al. hypothesized that one possibility for this finding was that the local concentrations of histamine were too high for the dose of chlorpheniramine used during shock in their model.

Although an increase in the dose of chlorpheniramine may produce a greater degree of histamine

Table 5. Hemodynamics in the cyclooxygenase-inhibition study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Treatment</th>
<th>Shock</th>
<th>Volume Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mmHg</td>
<td>157 ± 3</td>
<td>157 ± 5</td>
<td>92 ± 23*</td>
<td>128 ± 24*</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>22 ± 5</td>
<td>23 ± 4</td>
<td>15 ± 6*</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Ppw, mmHg</td>
<td>11 ± 2</td>
<td>13 ± 3</td>
<td>5 ± 3*</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Pco, mmHg</td>
<td>12 ± 3</td>
<td>14 ± 5</td>
<td>8 ± 3*</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>11 ± 2</td>
<td>14 ± 6</td>
<td>2 ± 0.2*</td>
<td>14.0 ± 4</td>
</tr>
<tr>
<td>LVEDP - Ppw, mmHg</td>
<td>-0.2</td>
<td>1.1 ± 1.9</td>
<td>-2.9 ± 2.6*</td>
<td>1.4 ± 3.1</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.5 ± 1.3</td>
<td>4.4 ± 2</td>
<td>1.7 ± 1*</td>
<td>4.5 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 dogs. By repeated-measures ANOVA and Duncan’s multiple-comparison test: *P < 0.05 from other conditions.

Table 6. Hemodynamics in the lipoxygenase-inhibition study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Treatment</th>
<th>Shock</th>
<th>Volume Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mmHg</td>
<td>149 ± 16</td>
<td>153 ± 13</td>
<td>74 ± 12*</td>
<td>141 ± 27</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>21 ± 4</td>
<td>21 ± 4</td>
<td>18 ± 4</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Ppw, mmHg</td>
<td>9 ± 2</td>
<td>12 ± 2</td>
<td>5 ± 2*</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Pco, mmHg</td>
<td>13 ± 3</td>
<td>14 ± 2</td>
<td>12 ± 2</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>10 ± 2</td>
<td>11 ± 3</td>
<td>3.5 ± 0.6*</td>
<td>14 ± 14</td>
</tr>
<tr>
<td>LVEDP - Ppw, mmHg</td>
<td>0.9</td>
<td>0.4 ± 4.4</td>
<td>-1.4 ± 2.4</td>
<td>0.7 ± 3.9</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.3 ± 1.1</td>
<td>4.3 ± 1.7</td>
<td>1.5 ± 0.4</td>
<td>4.3 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SD for 5 dogs. By repeated-measures ANOVA and Duncan’s multiple-comparison test: *P < 0.05 from other conditions; †P < 0.05 from baseline.
Table 7. Mediators of anaphylaxis in the treatment studies

<table>
<thead>
<tr>
<th></th>
<th>Control Study (n = 9)</th>
<th>Histamine H1-Receptor Blockade</th>
<th>Histamine H2-Receptor Blockade (n = 6)</th>
<th>Cyclooxygenase Inhibition (n = 7)</th>
<th>Lipoygenase Inhibition (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>T</td>
<td>S</td>
<td>V</td>
<td>B</td>
</tr>
</tbody>
</table>
| Hista-
mine, nM       | ±3.4 ± 5* | ±3.4 ± 60* | ±32* | 1.2 ± 10* | ±1* | 1.2 ± 175* | ±70* | 1.3 ± 10* | ±1.2 ± 126* | ±51* | 3 ± 2* | ±2 ± 56* | ±23* | ±6 ± 1 ± 72* | ±29* |
| 6-KetoPGF1α, pg/ml | ±6 ± 6 | ±6 ± 11 | ±7 | ±6 ± 11 | ±7 | ±3 ± 1 ± 8* | ±5 | ±5 ± 3* | ±3* | ±3 | ±1 ± 3* | ±2* |
| TXB2, pg/ml       | ±69 | ±69 | ±161 | ±115 | ±48 | ±57 | ±142 | ±102 | ±83 | ±67 | ±165 | ±119 | ±33 | ±5 | ±6 | ±4 | ±57 | ±51 | ±131 | ±93 |
| LTE4, pg/ml       | ±66 | ±66 | ±150 | ±112 | ±45 | ±45 | ±128 | ±93 | ±46 | ±46 | ±165 | ±121 | ±41 | ±41 | ±345 | ±251 | ±62 | ±61 | ±68 | ±70 |

Values are means ± SD; n, no. of dogs. 6-KetoPGF1α, 6-ketoprostaglandin F1α, breakdown product of prostacyclin; TXB2, thromboxane B2, breakdown product of thromboxane A2; LTE4, leukotriene E4, composite leukotrienes. B, T, S, and V, baseline, treatment, shock, and volume infusion, respectively. *P < 0.05 vs. baseline and treatment and †P < 0.05 vs. baseline by repeated-measures ANOVA. ‡P < 0.05 vs. all other studies. §P < 0.05 vs. all other studies except lipoxygenase-inhibition study by repeated-measures ANOVA.

H1-receptor blockade during challenge, we found that an unwanted effect of this higher dose was that a higher allergen dose was needed to produce shock (18) because H1-receptor blockers inhibit mediator release from mast cells (27). With a higher antigen dose, more histamine was released during challenge in the H1-receptor blocker study than in the control study (Table 7). At constant antigen dose in our previous study (18), mediator release and shock under histamine H1-receptor blockade were attenuated compared with no treatment. Thus, in the present study and in the Ascaris model, a demonstrated effect of histamine on PVR during shock remains difficult to discern.

In the cyclooxygenase inhibition study, indomethacin was infused to suppress both the vasodilating (i.e., prostacyclin) and vasoconstricting (i.e., Tx) products of the cyclooxygenase A2, LT4, and LTD4, leukotriene B4, and cyclooxygenase pathways. In isolated perfused guinea pig lungs, Noonan et al. (24) found that infusion of either LT4 or LTD4 caused an increase in PVR that occurred predominantly in the venous segment. In the present study, although MK-591 prevented the increase in LTs otherwise found during shock, we could not find that partitioned resistances decreased with lipoxygenase inhibition.

Although many mediators are released during anaphylaxis (i.e., among others, platelet-activating factor, eosinophil and neutrophil chemoattractants, cytokines, etc.; Refs. 6, 32), in terms of the objectives of the present study, we concentrated on those mediators that, on the basis of previous experiments, have assumed important roles in causing pulmonary vasoconstriction in anaphylaxis (1, 17, 20, 24, 30). Most work on mechanisms of pulmonary hypertension in anaphylaxis has been based on simulated models of this condition (1, 20, 24). However, in this in vivo model of anaphylaxis, we could not relate pulmonary vasoconstriction to histamine release or products of the cyclooxygenase or lipoxygenase pathways.

Interestingly, however, our results showed that, during volume infusion, PVR and partitioned resistances decreased in all treatments compared with shock values and also compared with the volume-infusion time-control study in which volume was not infused over this interval. Once shock is produced in our model, there is a general recovery of hemodynamics over time, although PVR does not usually return to the preshock value for

Table 8. Lung mechanics study

<table>
<thead>
<tr>
<th></th>
<th>Ppl, cmH2O</th>
<th>Peak Ptp, cmH2O</th>
<th>Intrinsic PEEP, cmH2O</th>
<th>Cl, ml/cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>−2.0 ± 1.4</td>
<td>10 ± 3</td>
<td>0</td>
<td>58 ± 28</td>
</tr>
<tr>
<td>Shock</td>
<td>−1.9 ± 1.3</td>
<td>14 ± 2*</td>
<td>0</td>
<td>63 ± 32</td>
</tr>
<tr>
<td>Volume Infusion</td>
<td>−1.8 ± 0.7</td>
<td>12 ± 2*</td>
<td>0</td>
<td>64 ± 32</td>
</tr>
</tbody>
</table>

Values are means ± SD for 5 dogs. Ppl, pleural pressure measured at end expiration; PEEP, positive end-expiratory pressure; Cl, lung compliance measured during tidal inflations; Peak Ptp, peak transpulmonary pressure. *P < 0.05 vs. baseline by ANOVA.
Mediators are metabolized postshock and would further be diluted by volume expansion. Moreover, with volume infusion, pulmonary vascular pressure would increase, and this could provide greater stretch on vascular smooth muscle. By reversal of the bronchoconstrictive effect of the mediators, an increase in pulmonary vascular pressures would return PVR toward preshock values at a faster rate than without volume infusion. Alternatively, saline infusion could affect PVR by altering blood viscosity and by affecting cytokines, vasodilator peptides, or other mediators not measured in the present study.

We also recognize multiple techniques could be used to determine Ppco in our model (7, 8, 10, 25). The gold standard is probably the double-occlusion technique (14) in which the pulmonary artery or vein can be acutely occluded to obtain the respective pressure drops across the arterial, venous, and middle segments. However, this technique would be difficult to use in our chronic in vivo preparation. Because multiple treatments were performed in the same animal over a 4- to 5-mo period, it would be difficult to implant a catheter into one of the pulmonary veins over this time period and to keep the animal healthy.

Furthermore, many mathematical models have been proposed to explain the decay in arterial occlusion pressure (8), which has been explained in terms of monoexponential or biexponential equations. In addition, many approaches have been offered as to when the occlusion should be initiated relative to the arterial pressure tracing. Gilbert and Hakim (10) found little difference in the results when occlusion was initiated between 0 and 150 ms after an initial pressure change was noted and further showed that the extrapolated exponent was relatively insensitive to the exact number of data points used, for instance from 0.2 to 1.5 s or from 0.2 to 2.5 s. In the present study, we chose the time at which a pressure change was first detected and tried to include data obtained over 5–8 s because the pressure decay was so slow once shock was produced.

In the different treatment studies, we found that at baseline Rus accounted for ~70% of PVR whereas Rds accounted for 30%. In the literature, a wide range of values has been reported for these fractions with Rds ranging between ~30 and 50% of PVR. Much of variability may depend on the preparation used, open- or closed-chest, isolated lobe, zones of West et al. (33), etc. (7, 8, 14). In a closed-chest canine preparation with similar methodology, Cope et al. (7) reported that at baseline Rus averaged 71% of PVR whereas Rds averaged 29% of PVR. They also did not find much of a difference in parameters when determined by graphic or visual fit.

In the control treatment study, we compared values of Ppco obtained by best-visual-fit line with those in which the slow component of pressure decay was fitted to an exponential function by regression analysis. Between the two methods, the coefficient of correlation was >0.98 in all conditions (0.99 at baseline, 0.995 at shock, and 0.985 during volume infusion) with a slope of nearly =1 (1.06 at baseline, 1.08 during shock, and 1.08 during volume infusion) and with a y-intercept of nearly zero (~0.7 vs. ~3.5 vs. ~0.38 cmH2O in which the visual method is plotted on the ordinate). Because the methods gave similar results, we do not think that different ways of analyzing the data would have yielded conclusions different from those already discussed.

In the anaphylactic protocol, another point to consider in our measurement of Ppco is that, during the five treatment studies, occlusion of vessels in different parts of the lung may have affected calculation of segmental vascular resistances in the various conditions. In a previous study (22), it was noted that the pulmonary catheter always occluded one of the lower lobes during inflation in the dog. We could not check the catheter location by fluoroscopy under the different conditions to ensure that it was at a constant location. However, preshock measurements were obtained in the same animal multiple times at baseline (~5) before any treatment was given. Accordingly, we could compare the percent coefficient of variation [CV = (SD/mean value) × 100%] of the segmental resistances measured (i.e., percent Rus) to determine the extent to which variability may have contributed to our results. For all dogs, percent CV was 14% with a range of 9.7–29%. Moreover, when the percent Rus measurement was repeated in the control treatment study ~1 h apart, the percent CV was also small at 15%. In contrast, because the changes in percent Rus observed during shock were so large, we do not believe that measurement variability in partitioned resistances, possibly reflecting different positions of the catheter, altered the interpretation of our results.

We further recognize that to some extent PVR may be dependent on flow and that differences in CO may have confounded interpretation of changes in PVR, Rus, and Rds that were found pre- vs. post-volume infusion (33). In the control treatment study, we found that, post-volume infusion, the mean CO was slightly higher than the preshock value (Table 1). However, in other treatment studies, for instance, in the lipoxygenase treatment study, CO values were the same at baseline and post-volume infusion (Table 6), and similar findings in PVR, Rus, and Rds were obtained.

Our study also indicates that Ppw may not reflect LV filling pressure during anaphylaxis. Because the decay in arterial occlusion pressure may be so slow, a true PLa may not be observed. In the present study, we used LVEDP to reflect mean atrial pressure. Previous results showed that, under normal conditions, there was good agreement between these Ppw and LVEDP (11), and other authors have reported no difference between Ppw and PLa under normal conditions (13). It would be difficult to stop the ventilator for the length necessary to ensure that a true Ppw had been reached in the present study. Our results show, therefore, that, in the clinical situation of anaphylactic shock, one must be careful in the interpretation of Ppw as representing LV filling pressure.

In summary, our results show that, in a ragweed model, there was a large increase in PVR observed during shock and that the largest relative increase in
resistance occurred in the venous and capillary segments. None of the treatments used in this model could reverse pulmonary vasoconstriction during shock: either the local concentration of mediators released during challenge were too great for the doses used, combinations of treatments were required, or mediators not thus far examined were more important in causing vasoconstriction. Although previous studies in nonallergic preparations (1, 2, 4, 20, 24, 30) have indicated that histamine release and products of the lipooxygenase and cylooxygenase pathways may be important in producing pulmonary vasoconstriction in anaphylaxis, none of these treatments were able to attenuate the pulmonary vascular effects found in this in vivo allergic model.

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