Stimulation of Pulmonary Vagal Afferent C-Fibers by Lung Edema in Dogs

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SUMMARY. In anesthetized, open-chest dogs we examined the effect of pulmonary edema on the firing frequency of afferent vagal fibers arising from the lung. We recorded impulses from slips of the cervical vagus nerves and infused isotonic Krebs-Henseleit solution (20% of body weight) intravenously to increase net filtration pressure in the lung microvasculature. Measurement of extravascular lung water (6.0 ± 0.4 g/g dry lung), and morphological examination of lung tissue (revealing various degrees of perivascular and peribronchial cuffing) confirmed that edema was present. At the end of the infusion when the lungs were congested (lung microvascular pressure, 37 cm water) and edematous, the impulse frequency of pulmonary and bronchial C-fibers and rapidly adapting receptors had increased 5-6 times. The only significant change in slowly adapting receptor activity was an increase during deflation. When lung water was still elevated but lung microvascular pressure had been restored to control by withdrawal of blood, impulse activity of rapidly and slowly adapting receptors reverted to or below control. Pulmonary C-fiber activity, although less than during congestion, remained significantly above control, several C-fibers being stimulated by interstitial edema in the absence of alveolar edema. Bronchial C-fibers were stimulated in severely edematous lung showing pronounced peribronchial cuffing and alveolar edema, but were not stimulated in milder grades of edema. Our results support the hypothesis (Paintal, 1969) that pulmonary C-fibers (J-receptors) are stimulated by an increase in interstitial pressure secondary to edema.

AFFERENT vagal endings in the lung are believed to be largely responsible for triggering the dyspnea and airway constriction of acute left ventricular failure in humans. Support for this hypothesis is provided by the observation that acute congestion of the pulmonary vascular bed in dogs and cats evokes vagally mediated tachypnea, bradycardia, and systemic hypotension (Churchill and Cope, 1929; Aviado et al., 1951; Downing, 1957), and that acute congestion and infusion-induced edema cause a vagally mediated bronchoconstriction in the absence of changes in blood gas tension (Jones et al., 1978; Chung et al., 1983).

Pulmonary C-fibers (J-receptors) are thought to play an important part in initiating these reflex effects. They are stimulated by acute pulmonary congestion (Paintal, 1969; Coleridge and Coleridge, 1977a, 1984) and they are known to be capable of triggering ventilatory, bronchomotor, and cardiovascular effects similar to those evoked by lung congestion (Paintal, 1973; Coleridge and Coleridge, 1984). Paintal suggested that these C-fiber endings function as interstitial stretch receptors, and are stimulated when fluid moves out of the pulmonary capillaries and increases volume or pressure in the interstitial spaces in which the endings are thought to be located (Paintal, 1969, 1970). This hypothesis was based on Paintal’s observation that J-receptors in cats were stimulated during the terminal alveolar edema induced by intratracheal administration of chlorine or intravenous injection of alloxan (Paintal, 1969). However, the response of this group of afferents to less severe forms of edema, unaccompanied by chemical injury to the lung, has not been described.

Pulmonary C-fibers are not the only pulmonary afferents likely to contribute to the reflex effects of pulmonary congestion and edema. Bronchial C-fibers (Coleridge and Coleridge, 1977a; Teo et al., 1985), and both slowly adapting and rapidly adapting stretch receptors (Marshall and Widdicombe, 1958; Costantin, 1959; Sellick and Widdicombe, 1969; Teo et al., 1985) are stimulated in acute pulmonary congestion, and all three have important reflex respiratory effects (Coleridge and Coleridge, 1986).

Our purpose in the present experiments was to examine the effects of interstitial lung edema on all four types of pulmonary afferent, avoiding methods that involved administration of noxious chemicals such as chlorine or alloxan. We produced pulmonary edema by infusing isotonic Krebs-Henseleit solution intravenously to increase net filtration pressure in the lung microvasculature (Snashall et al., 1977). In this way, we could produce a measurable degree of pulmonary edema while recording afferent vagal impulses, and examine changes in afferent activity at the end of infusion (stage 1), when lung water
was increased and lung vascular pressures were high, and at a later stage (stage 2), when lung water was still elevated but lung vascular pressures had been restored to control by withdrawing blood from a femoral artery. The degree of edema was assessed by measurement of extravascular lung water and by morphological examination of lung tissue.

**Methods**

**General**

Dogs (12.1-27.7 kg) were given promazine hydrochloride (Sparine, Wyeth Laboratories, 50 mg subcutaneously); 40 minutes later, they were anesthetized with 0.25 ml/kg, iv, of a 1:1 mixture of solutions of Dial Compound (allobarbital 100 mg/ml, urethane 400 mg/ml, Clba) and sodium pentobarbital (50 mg/ml). Supplemental doses were given as needed to maintain anesthesia.

The trachea was cannulated low in the neck, and the chest was opened in the midline. The lungs were ventilated with 50% O2 in air by a Harvard respirator (tidal volume, 15 ml/kg; frequency, usually 10-15/min) whose expiratory outlet was placed under 3-5 cm of water. Tidal CO2 was monitored by a Beckman LB-1 gas analyzer. Tracheal pressure was recorded from a sidearm on the tracheal cannula. Arterial blood pressure and left atrial pressure were recorded through catheters in a femoral artery and the left atrial appendage, respectively. Pressure in the main pulmonary artery was recorded through a Swan-Ganz catheter inserted via the right external jugular vein. Pulmonary arterial and left atrial pressures were measured from a zero reference point at the level of the left atrium.

Pressures were recorded with Statham P23Gb strain gauges. An electrocardiogram (lead II) was recorded. Afferent vagal impulses were recorded, using conventional techniques, and were counted by ratemeters (see below). After amplification, the signals representing end-tidal CO2 pressures, ratemeter outputs, and other variables were recorded by a Grass polygraph, and pressures and action potentials were recorded by an ultraviolet light recorder (SE Laboratories).

**Recording of Afferent Vagal Impulses**

Using conventional techniques, we dissected fine slips of the left cervical vagus nerve and recorded impulses arising from afferent endings in the lower lobe of the left lung. We used two pairs of electrodes to record impulses from two pulmonary afferents simultaneously in 17 of the 31 dogs, from three fibers simultaneously in 13 dogs, and from one fiber in one dog. Slowly adapting pulmonary stretch receptors, rapidly adapting (irritant) receptors, and pulmonary and bronchial C-fibers were identified by their patterns of discharge, by their responses to inflation of the lung and to injection of capsaicin, and by the conduction velocities of their afferent fibers (Coleridge and Coleridge, 1977b). The approximate location of all endings was determined by probing the lung; endings not located in the left lower lobe were discarded.

Impulse frequencies were counted by ratemeters whose window discriminators were set to accept potentials of a particular amplitude. Discharge frequencies of pulmonary and bronchial C-fibers and rapidly adapting receptors, whose firing rarely exceeded 20 impulses/sec, were usually counted by a ratemeter in 1-second bins (Fig. 1). Discharge frequencies of slowly adapting receptors, which may reach 100 impulses/sec or more at the peak of inflation, were usually recorded as a voltage analog of impulse frequency (Fig. 1). In addition, activity in each ventilatory cycle was counted by a ratemeter triggered by the ventilator, and a cumulative record of activity over a longer period was made by a ratemeter counting in 1-minute bins.

**Protocol**

Pulmonary afferent impulses and pulmonary vascular pressures were recorded continuously throughout the experiment. After a control period of 10 minutes, we infused Krebs-Henseleit buffer solution (pH 7.4; 300 mOsm/liter; 37°C; equilibrated with 5% CO2 in O2) intravenously in an amount equivalent to 15-27% of body weight (Snashall et al., 1977). The rate of infusion was adjusted to increase left atrial pressure to about 30 cm H2O in 20-40 minutes (Guyton and Lindsey, 1959). Thereafter, solution was infused to maintain left atrial pressure at this level for a

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Effect of infusing 4.5 liters of Krebs-Henseleit solution (22.2% of body weight) on the afferent discharge of a pulmonary C-fiber (Pulm. C) and a slowly adapting pulmonary stretch receptor (SAPSR). Impulses recorded by ratemeters. Part A: control; mean pulmonary microvascular pressure (Pm), 8.4 cm H2O. Part B: stage 1, infusion completed; Pm 32.2 cm H2O. Part C: stage 2, after Pm was reduced to 8.4 cm H2O by bleeding the dog. Both endings located in left lower lobe; presence of interstitial edema confirmed by morphological examination of lung tissue; lung water, 6.4 g/g dry weight. Pco2, tidal CO2 partial pressure; Ppa, mean pulmonary arterial pressure; Pla, mean left atrial pressure; Pa, mean systemic arterial pressure; Pt, tracheal pressure.
further 30–40 minutes. At the end of infusion (stage 1), left atrial pressure was allowed to decrease spontaneously for about 10 minutes, after which it was restored to the control level by withdrawal of blood from a femoral artery. Recordings of pulmonary afferent activity were continued for a further 5 minutes (stage 2). Finally, the lungs were removed for measurement of lung water and for morphological examination. We measured total plasma protein (Snashall et al., 1977) in blood samples (5–10 ml) drawn from a femoral artery into heparinized syringes in the control period, at the end of infusion (stage 1) and after lung vascular pressures were restored to control (stage 2).

**Measurement of Extravascular Lung Water**

After action potential recordings were completed, and while the dog was still alive, the hilum of the left lung was clamped, and 30 ml of blood were withdrawn from the left ventricle into a heparinized syringe for the estimation of extravascular lung water (see below). The left lower lobe was excised, placed in liquid nitrogen until frozen, and then stored in dry ice overnight (see below).

The right lower lobe was excised, its bronchus cannulated, and a solution of 2% glutaraldehyde and 1% formaldehyde in phosphate buffer (pH 7.4) instilled into the lobe at a pressure of 5–10 cm H2O. The lobe was stored at 4°C in the glutaraldehyde/formaldehyde solution for 3 days. Specimens of the lobe were embedded in glycol methacrylate, sectioned at 2 µm and stained with toluidine blue. We examined the sections for evidence of alveolar edema.

After being stored overnight in dry ice, the left lower lobe was placed in a cryostat at −30°C. A cube of tissue (2.5 cm/side) was cut from the center of the lobe, and its surface was examined under a dissecting microscope (5X) for evidence of perivascular and peribronchial edema. Normal extravascular lung water in dogs is 3.1–3.8 g water/g dry lung (Snashall et al., 1977; Bhattacharya et al., 1984). We defined lung edema as moderate when extravascular lung water was between 4.0 and 5.9 g/g dry lung, and severe when lung water exceeded 5.9 g/g dry lung. Perivascular and peribronchial cuffing was graded in severity (see Results).

**Analysis of Data**

We calculated pulmonary microvascular pressure (Pmv) by the formula

\[ P_{mv} = P_{la} + 0.4 (P_{pa} - P_{la}) \]

0.4 being the fraction of total pulmonary vascular resistance downstream from the microvascular exchange site (Snashall et al., 1977).

The impulse discharge of pulmonary and bronchial C-fibers and rapidly adapting receptors was counted over the complete ventilatory cycle; that of slowly adapting receptors was counted separately in inflation [from the beginning of the upstroke in tracheal pressure (P_{T}) to peak P_{T}] and deflation (from peak P_{T} to beginning of next upstroke). Impulse frequencies per ventilatory cycle were averaged over 1 minute during the control period and expressed as impulses/sec. If firing was regular, a similar procedure was adopted for counting frequencies at the end of infusion (stage 1) and after lung vascular pressures were restored to control (stage 2). If activity was irregular during the latter two periods, impulses were averaged over 5 minutes. All values are expressed as means ± se.

We used Student's paired t-test and Wilcoxon's signed rank test to determine the statistical significance of changes in afferent activity between control and stages 1 and 2.

**Results**

In experiments on 31 dogs (18.5 ± 0.7 kg), infusion of a volume (3.62 ± 0.16 l) of Krebs-Henseleit solution equivalent to 19.8 ± 0.6% of body weight decreased total plasma proteins by 2.0–3.9 g/100 ml (Table 1). Estimated pulmonary microvascular pressure increased approximately 3-fold (Table 1). The expansion of extracellular fluid volume increased tracheal pressure at the peak of inflation by an average of 6.6 cm H2O (Table 1), representing a decrease in dynamic lung compliance of 51.1 ± 2.6% (P < 0.001).

**Table 1**

<table>
<thead>
<tr>
<th>Total Plasma Protein, Vascular Pressures, and Tracheal Pressure at Various Stages of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular pressures</td>
</tr>
<tr>
<td>Pl. prot. (g/100 ml)</td>
</tr>
<tr>
<td>Lung (cm H2O)</td>
</tr>
<tr>
<td>P_{pa} (mm Hg)</td>
</tr>
<tr>
<td>P_{mv} (mm Hg)</td>
</tr>
<tr>
<td>P_{la} (mm Hg)</td>
</tr>
<tr>
<td>P_{T} (cm H2O)</td>
</tr>
<tr>
<td>Control  5.2 ± 0.1</td>
</tr>
<tr>
<td>Stage 1  2.4 ± 0.1</td>
</tr>
<tr>
<td>Stage 2  2.7 ± 0.1</td>
</tr>
</tbody>
</table>

Data (mean ± se) in 31 dogs during the control period, at end of infusion (stage 1), and after lung vascular pressures restored to control (stage 2). Pl. prot. = total plasma protein; P_{pa} = mean pulmonary arterial pressure; P_{mv} = mean pulmonary microvascular pressure; P_{la} = mean left atrial pressure; P_{a} = mean systemic arterial pressure; P_{T} = tracheal pressure measured at peak of inflation.

**Analysis of Data**

We calculated pulmonary microvascular pressure (Pmv) by the formula

\[ P_{mv} = P_{la} + 0.4 (P_{pa} - P_{la}) \]

0.4 being the fraction of total pulmonary vascular resistance downstream from the microvascular exchange site (Snashall et al., 1977).

The impulse discharge of pulmonary and bronchial C-fibers and rapidly adapting receptors was counted over the complete ventilatory cycle; that of slowly adapting receptors was counted separately in inflation [from the beginning of the upstroke in tracheal pressure (P_{T}) to peak P_{T}] and deflation (from peak P_{T} to beginning of next upstroke). Impulse frequencies per ventilatory cycle were averaged over 1 minute during the control period and expressed as impulses/sec. If firing was regular, a similar procedure was adopted for counting frequencies at the end of infusion (stage 1) and after lung vascular pressures were restored to control (stage 2). If activity was irregular during the latter two periods, impulses were averaged over 5 minutes. All values are expressed as means ± se.

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A 20 cm H₂O
B 20 cm H₂O
C 20 cm H₂O

Effect of Infusion on Lung Afferent Activity

Action potentials were recorded from 74 afferent vagal fibers arising from endings in the lung.

Pulmonary C-Fibers

We examined 21 pulmonary C-fibers in 16 dogs. Control activity was sparse and irregular (Figs. 1 and 2; Table 2). Nineteen of the 21 fibers were stimulated as lung vascular pressures increased (Figs. 1 and 2), average impulse frequency increasing more than 6-fold (Table 2) by the end of the infusion (stage 1). Usually, the augmented discharge was irregular, without cardiac or ventilatory modulation; in three fibers, however, firing developed a pronounced ventilatory rhythm, maximum frequencies occurring during inflation. The ventilatory discharge was abolished by switching off the ventilator. In some experiments, phasic bursts of firing were occasionally observed lasting 30–120 seconds and having peak frequencies of 20–30 impulses/sec. These periodic bursts of activity occurred both when the lungs were congested and edematous (stage 1) and also after lung vascular pressures had been restored to control and only edema was present (stage 2; Fig. 1C). They were not accompanied by obvious changes in pulmonary vascular or tracheal pressure.

Table 2

<table>
<thead>
<tr>
<th>Type of afferent</th>
<th>Impulses/sec</th>
<th>Control</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulm. C</td>
<td>21</td>
<td>0.6 ± 0.1</td>
<td>4.6 ± 0.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Br. C</td>
<td>12</td>
<td>0.6 ± 0.2</td>
<td>3.4 ± 0.7</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>RAR</td>
<td>21</td>
<td>1.7 ± 0.5</td>
<td>8.4 ± 1.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SAPSR (infl.)</td>
<td>20</td>
<td>54.4 ± 6.3</td>
<td>59.5 ± 7.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>(defl.)</td>
<td>20</td>
<td>6.0 ± 2.7</td>
<td>18.3 ± 5.0</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± se of impulse frequencies recorded from four types of lung afferent: Pulm. C = pulmonary C-fiber; Br. C = bronchial C-fiber; RAR = rapidly adapting receptor; SAPSR = slowly adapting pulmonary stretch receptor; n = number of fibers. Activity of SAPSR in inflation (infl.) and deflation (defl.) counted separately; activity of other afferents counted in complete ventilatory cycle. Activity of 74 pulmonary fibers at the end of infusion (stage 1) is compared with the initial control activity before infusion; activity of 64 afferents (located in lung tissue with confirmed edema) after vascular pressures restored to baseline level (stage 2) is compared with the initial control activity before infusion.
Bronchial C-Fibers

Twelve bronchial C-fibers were examined in nine dogs. All had a sparse and irregular discharge and all were stimulated during the infusion, activity increasing 5-fold (Table 2) and usually occurring in brief irregular bursts (Fig. 3). Prolonged outbursts of firing lasting 30–120 seconds were sometimes observed, similar to those displayed by pulmonary C-fibers.

Rapidly Adapting Receptors

Twenty-one rapidly adapting (irritant) receptors were examined in 16 dogs. Thirteen had an irregular discharge (Fig. 4A), which usually was most prominent during inflation. The remaining eight receptors were either silent under control conditions (Fig. 5A), or at most fired one or two impulses during occasional ventilatory cycles. All but one of the 21 receptors was stimulated by the infusion, activity increasing 5-fold on average (Table 2). Firing acquired an obvious ventilatory modulation, maximum frequencies, which sometimes reached 30–60 impulses/sec, usually occurring during inflation (Figs. 4B and 5B). The ventilatory discharge was abolished and the overall firing frequency reduced by switching off the ventilator. Several receptors developed a pronounced cardiac rhythm of discharge, usually most obvious during inflation. Stimulation was often associated with irregular and prolonged variations in firing lasting 1–2 minutes (Fig. 4B).

Slowly Adapting Pulmonary Stretch Receptors

Twenty slowly adapting stretch receptors were examined in 16 dogs. The receptors had a wide range of thresholds and sensitivities, activity at the peak of inflation ranging from 9 to 120 impulses/sec. Seven endings were in the "low threshold" category and continued to discharge at functional residual capacity (FRC). In 10 of the receptors, infusion caused a conspicuous increase in activity during inflation (Fig. 4B), firing usually beginning earlier in the ventilatory cycle. In others, however, activity in inflation decreased (Fig. 1), so that, overall, the changes during inflation were not significant (Table 2). Twelve receptors were stimulated during deflation (Fig. 4B). On average, firing during deflation increased 3-fold, an effect that was statistically significant for the whole group of 20 receptors (Table 2).

Unnamed Pulmonary Afferents

In several experiments, the infusion stimulated previously silent fibers that originated in the lung but did not appear to belong to a known category of pulmonary afferent. The potentials were of small amplitude and barely rose above the noise level. Firing was irregular and appeared to be multiferb.
in origin. The endings were stimulated by probing or pinching the lung, but not by injecting capsaicin (5–20 μg/kg) into the right or left atrium or by hyperinflating the lung (2–3 V_{T}). We have not included these fibers in our quantitative analysis of afferent activity.

**Lung Afferent Activity after Relief of Congestion**

After the infusion was completed and lung vascular pressures had been above control for 50–80 minutes, 200–900 ml blood were withdrawn from a femoral artery to restore pressures to their control levels (Table 1). When final recordings of impulse activity were made 5 minutes later (stage 2), 67 of the original 74 fibers were still active. The remaining seven (two pulmonary C-fibers, two slowly adapting, and three rapidly adapting receptors) were no longer active, and could not be stimulated by probing the lung. Since loss of activity was preceded by progressive reduction in spike amplitude, it was probably due to deterioration of the vagal filament on the recording electrodes, rather than to decreased responsiveness of the afferent ending itself.

Peak tracheal pressure decreased when lung vascular pressures were reduced but it did not return to its original level (Table 1), and dynamic lung compliance was still, on average, 31.7% less than during the control period.

**Pulmonary C-Fibers**

Of 19 pulmonary C-fibers still active in stage 2, two were in experiments in which there was no evidence of pulmonary edema (see below). Both these fibers were stimulated by the infusion (stage 1), but firing reverted to control when lung vascular pressures were finally reduced (stage 2). Edema was present (see below) in experiments on the remaining 17 pulmonary C-fibers. Activity in 13 of these 17 fibers remained above control after lung vascular pressures were reduced (Figs. 1 and 2), indeed, in five of them, impulse frequencies were higher than at the end of infusion. The augmented discharge was usually irregular, but one of the three fibers firing with a ventilatory rhythm at the end of the infusion continued to do so after vascular pressures were reduced. On the average, activity in these 13 pulmonary C-fibers increased 9-fold, from an initial control discharge of 0.3 ±0.1 impulses/sec to 2.9 ± 0.7 impulses/sec. The increase in discharge frequency of the 17 endings located in edematous lung was statistically significant (Table 2).

**Bronchial C-Fibers**

All 12 bronchial C-fibers remained active to the end of the experiment, and lung edema was confirmed in all cases (see below). Activity remained above control in eight bronchial C-fibers, and even continued to increase in two of them (Fig. 3F). However, firing decreased in four fibers (Fig. 3C), so that although bronchial C-fiber activity increased overall in edema, the average for the whole group was not significantly different from control (Table 2). Nevertheless, when the grade of edema was taken into account, bronchial C-fibers arising from severely edematous lung were found to be stimulated significantly (see below).

**Rapidly Adapting Receptors**

Eleven rapidly adapting receptors remained active to the end of the experiment, and all were located in edematous lung. Firing invariably decreased when pulmonary vascular pressures were reduced (Figs. 4C and 5C), remaining above control in five fibers, but falling below it in nine. Hence, average activity of rapidly adapting receptors during edema was not significantly different from control (Table 2).

**Slowly Adapting Pulmonary Stretch Receptors**

Eleven slowly adapting receptors remained active to the end of the experiment, 17 of them being located in edematous lung. Firing of most receptors decreased promptly when pressures were reduced
(Fig. 4C), decreasing to below control in more than half of them. On average, activity in inflation and deflation at the end of the experiment was not significantly different from control (Table 2).

**Unnamed Pulmonary Afferents**

The unnamed pulmonary afferents continued to fire after congestion was relieved. Owing to the multifiber nature of the discharge and the generally low amplitude of the potentials, we were unable to compare quantitatively their firing frequency in stage 2 with that in stage 1.

**Assessment of Pulmonary Edema**

When action potential studies were completed, lung tissue was removed for estimation of extravascular lung water and morphological examination of frozen and stained specimens.

**Extravascular Lung Water**

In 28 of the 31 dogs, extravascular lung water ranged between 4.0 and 13.7 g/g dry lung (6.3 ± 0.5 g/g dry lung). In the remaining three dogs, extravascular lung water was between 3.2 and 3.8 g/g dry lung, in spite of an infusion of Krebs-Henseleit solution equivalent to 19.2-22.1% of body weight that increased lung microvascular pressure by 34.7-43.2 cm H₂O. Thus, in these three dogs, lung water remained within normal limits.

**Lung Morphology**

Examination of frozen and stained specimens of lung tissue provided morphological evidence of lung edema in all 28 dogs whose extravascular lung water was 4.0 g/g dry weight or more. Perivascular cuffing was present in all frozen specimens from these dogs, being most pronounced, and accompanied by conspicuous peribronchial cuffing, in dogs whose lung water exceeded 5.9 g/g dry lung. Cuffing was graded in severity (Table 3). Grade + cuffing (Fig. 6B) denotes the presence of clear (nonhemorrhagic), small, or crescentic perivascular cuffs that did not encircle the vessel completely; the cuffs were present on small and large arteries and veins. Distended lymphatics were present. Peribronchial cuffs were absent, and the lung was well aerated. Grade ++ (Fig. 6C) denotes the presence of clear, thick, and often complete perivascular cuffs; occasional thin, incomplete peribronchial cuffs were also present in some specimens. Grade +++ (Fig. 6D) denotes the presence of thick, complete perivascular cuffs and conspicuous peribronchial cuffs. Perivascular and peribronchial cuffs were often hemorrhagic. Patchy collapse of lung tissue was often present in grades ++ and +++.

We found evidence of alveolar edema in only one of the 20 dogs in which extravascular lung water was less than 6.0 g/g dry weight, but in eight of 11 dogs in which lung water was higher. However, we never observed gross signs of alveolar flooding, i.e., froth in the tracheal cannula or expiratory line during the experiment or in the larger airways during postmortem dissection.

Neither cuffing nor alveolar edema was observed in the three dogs in which lung water was 3.2-3.8 g/g dry lung (Fig. 6A; Table 3).

**Grade of Edema and Pulmonary Afferent Stimulation**

Seventeen pulmonary C-fiber endings were located in lungs in which edema was confirmed. The incidence of stimulation appeared to be related to the grade of edema. Thus six of 10 endings were stimulated in moderately edematous lung with interstitial, but not alveolar, edema, and seven of seven endings were stimulated in severely edematous lung with both interstitial and alveolar edema. However, the intensity of stimulation was not related significantly to the grade of edema. Indeed, the most vigorously stimulated pulmonary C-fiber, whose firing increased from 0.3 impulses/sec in the control period to 7.9 impulses/sec in edema, originated in lung with an extravascular water of 5.3 g/g dry lung and grade + perivascular cuffing.

Although, overall, the increase in bronchial C-fiber activity in the edematous lung was not statistically significant (Table 2), the incidence of C-fiber stimulation increased with the grade of edema (Table 3). Moreover, when the grade of edema was

<table>
<thead>
<tr>
<th>Grade of edema</th>
<th>Extravascular lung water (g/g dry)</th>
<th>Cuffing</th>
<th>Pulmonary</th>
<th>Bronchial</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.2-3.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>4.0-5.9</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Severe</td>
<td>6.0-13.7</td>
<td>4</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Edema was graded on the basis of extravascular lung water, the degree of perivascular and peribronchial cuffing (+, ++, +++: see text) and the presence of alveolar edema (Alv. ed.). Numbers in parentheses indicate number of dogs. A total of 31 pulmonary and bronchial C-fibers were examined (Exam.) after vascular pressures were restored to baseline level (stage 2); 21 were stimulated (Stim.).
taken into account, the eight bronchial C-fibers arising from severely edematous lung were found to be stimulated significantly, their discharge frequency increasing from $0.6 \pm 0.1$ impulses/sec (control) to $1.6 \pm 0.4$ impulses/sec (stage 2) ($P < 0.02$).

Although the incidence of stimulation of C-fibers appeared to increase with the severity of edema, this was not so in the case of rapidly adapting receptors. Only one of nine rapidly adapting receptors was stimulated in the severely edematous lung. Indeed, the activity of these nine receptors was reduced significantly by severe edema, from $1.7 \pm 0.6$ impulses/sec (control) to $0.8 \pm 0.5$ impulses/sec (stage 2) ($P < 0.05$).

The decrease in impulse frequency of slowly adapting receptors in stage 2 was unrelated to the severity of edema.

**Discussion**

Our aim was to increase lung extravascular water rapidly, by infusing large volumes of isotonic fluid with the dual effect of diluting plasma proteins and increasing lung microvascular pressure. In previous studies of infusion-induced edema, pulmonary vascular pressures were allowed to revert spontaneously to control, a process requiring up to 90 minutes, before lung water was measured (Snashall et al., 1977). Because of the limited recording life of our single fiber preparations, we shortened this final stage, and relieved congestion by withdrawing blood from a femoral artery. The disappearance of water from the edematous lung is a relatively slow process (Matthay et al., 1982). Hence, it seems reasonable to assume that the overall decrease in pulmonary afferent activity between the end of infusion, when the lung was both congested and edematous, and the restoration of vascular pressures to control some few minutes later was attributable to relief of congestion, rather than to reduction of interstitial edema. Although our protocol allowed us to examine the effects of edema alone at the end of the experiment, the combination of plasma protein dilution with increasing microvascular pressures made it impossible to assess accurately the effects of congestion alone in the early stages of the experiment.
Infusion of isotonic protein-free solution equivalent to 20% body weight causes widely different increases in extravascular lung water in different dogs (Snashall et al., 1977; Chung et al., 1983; present experiments). In several of our dogs, lung water was greatly increased, and there was morphological evidence of a severe degree of cuffing and of alveolar edema. However, in three dogs, lung water was in the normal range, and there was no morphological evidence of interstitial edema. These wide variations probably resulted from multiple factors, including variations in the amount of water infused relative to lean body weight and in the renal handling of the water load.

Activity in each of the four types of afferent increased significantly when pulmonary vascular pressures were high, but, on average, only pulmonary C-fiber activity remained significantly above control when congestion was relieved (Table 2). Our results support Paintal’s suggestion that pulmonary edema, independent of other factors, is an effective stimulus to pulmonary C-fibers (J-receptors) (Paintal, 1969, 1970). Paintal’s hypothesis was based on his observation that, in cats given alloxan intravenously, or chlorine by inhalation, a dramatic increase in pulmonary C-fiber activity coincided with the agonal upwelling of edema fluid in the trachea (Paintal, 1969). Paintal held that the stimulus to C-fibers in his experiments was the increase in interstitial pressure or volume secondary to edema formation. He postulated that J-receptors were surrounded by collagen fibers, the collagen providing an ideal matrix that, in the presence of the increased interstitial fluid of edema, would swell and distort afferent nerve endings. Morphological studies confirmed that nonmyelinated fibers with sensory enlargements are present in the interstitium of the alveolar walls (Hung et al., 1973) and are surrounded by collagen fibers (Fox et al., 1980). Nevertheless, doubts about Paintal’s hypothesis still remained. Thus, Coleridge and Coleridge (1977a) found in dogs that the increase in pulmonary C-fiber activity after alloxan was not related consistently to the appearance of edema fluid. Moreover, their results did not exclude the possibility that afferent C-fibers were stimulated directly by alloxan, as a result of its irritant properties. In the present experiments, we attempted to determine the effects of lung edema per se, avoiding the use of noxious chemicals. It was clear that vascular congestion was the major factor in stimulating some pulmonary C-fibers, because the activity of approximately one-third of the fibers reverted to control when congestion was relieved; two of these C-fibers were in experiments in which lung water remained within normal limits. In the majority of pulmonary C-fibers, however, activity remained elevated at the final “edema-only” stage, and several were stimulated by interstitial edema in the absence of alveolar edema. We think that Paintal’s hypothesis provides the most reasonable explanation for the residual stimulation of pulmonary C-fibers.

Firing rates in individual pulmonary C-fibers varied widely, and there was no significant difference between the afferent responses in moderate and severe edema. If one accepts Paintal’s hypothesis that pulmonary C-fibers function as interstitial pressure receptors (Paintal, 1969), the absence of a direct relationship between afferent response and degree of edema could be explained by the observation that pulmonary interstitial pressure soon reaches a plateau as extravascular water accumulates (Bhattacharya et al., 1984). As edema becomes more severe, however, it becomes more widespread throughout the lung lobe, and therefore, will stimulate more C-fiber endings. This was born out by our observation that, of 10 pulmonary C-fiber endings in lungs with moderate edema, only six were stimulated, whereas all seven endings in lungs with severe edema were stimulated.

Bronchial C-fibers were invariably stimulated during the prolonged and severe congestion caused by the infusion, possibly in part because high pulmonary venous pressures led to a severe degree of bronchial venous congestion. In a third of the fibers, stimulation was due to congestion alone, for it ceased when congestion was relieved. Although bronchial C-fibers were not stimulated by moderate (interstitial) edema, they were stimulated by severe edema. Such stimulation may have been related to the formation of cuffs around the bronchi.

Although it seems reasonable to suggest that the mechanical consequences of vascular engorgement and extravascular water accumulation were largely responsible for C-fiber stimulation, we cannot exclude the possibility that chemicals released in the edematous lung may have played a part. Prostacyclin is known to be released in pulmonary edema (Grondelle et al., 1984), and both pulmonary and bronchial C-fibers are stimulated by prostacyclin (Roberts et al., 1985).

Pulmonary congestion played a central role in the pronounced stimulation of rapidly adapting receptors during infusion, and activity usually decreased below control when vascular pressures were finally reduced. Our observation that rapidly adapting receptors are stimulated more consistently and more vigorously by congestion than slowly adapting receptors agrees with the results of studies in which congestion was induced by inflating a balloon in the left atrium (Marshall and Widdicombe, 1958; Sellick and Widdicombe, 1969; Teo et al., 1985). The augmented discharge of rapidly adapting receptors had a pronounced ventilatory rhythm, firing being maximal during inflation. Since the ventilatory discharge was abolished, and the overall frequency markedly reduced, when we switched off the ventilator briefly, we conclude that changes in lung mechanics played a major role. Activity during congestion often had a pronounced cardiac modulation, suggesting...
that the receptors were stimulated also by the movement of the beating heart or the pulsation of adjacent blood vessels. We found little evidence that rapidly adapting receptors were stimulated by edema alone; indeed, the activity of receptors in severely edematous lung was significantly less than before the infusion.

Slowly adapting receptors had the most varied response to infusion of any of the afferents studied. Many receptors were stimulated by the infusion, but the response was small, and significant only during deflation. Others have described an augmentation of discharge during acute congestion that varied from the trivial (Bulbring and Whitteridge, 1945), to the modest but significant (Marshall and Widdicombe, 1958; Costantin, 1959), and also an increase in activity in deflation, which may have been an expression of a decrease in firing threshold (Marshall and Widdicombe, 1958; Costantin, 1959). As in these earlier studies, stretch receptor activity in our experiments usually reverted to control when congestion was relieved. In some cases, activity fell below control, possibly as a result of the accumulation of extravascular lung water, leading to patchy increases in airflow resistance and underventilation of parts of the lung. Receptors whose discharge decreased during infusion were probably located peripherally, in small airways distal to the site of airflow resistance.

Our results suggest that vagal respiratory reflexes initiated during the development of congestive pulmonary edema are likely to involve all four types of pulmonary afferent. Thus, although certain features of the respiratory responses are typical of the reflex respiratory effects of stimulating pulmonary and bronchial C-fibers (tachypnea, bronchoconstriction, and increased airway secretion) (Coleridge and Coleridge, 1984), the marked increase in rapidly adapting receptor input is likely to make an important contribution. There is good evidence that rapidly adapting receptor input is excitatory to the respiratory centers, and is capable of generating deep breaths or sighs (Glogowska et al., 1972; Coleridge and Coleridge, 1986). Rapidly adapting receptors may also be responsible for a breath-by-breath augmentation of inspiratory effort and reflex bronchoconstriction (Coleridge and Coleridge, 1986). As to the role of slowly adapting receptors during the congestive stage of pulmonary edema, an increased discharge at the peak of inflation is likely to contribute to rapid breathing by shortening inspiratory time, but an increased discharge in deflation is unlikely to contribute to rapid breathing—indeed, by itself, it would tend to increase expiratory time (Bradley, 1977). The reflex function of the unnamed pulmonary afferents activated in stages 1 and 2 of our experiments remains to be determined.

Our results suggest that vagal reflex effects evoked by pulmonary edema in the absence of congestion result largely from stimulation of lung C-fibers. Vagally mediated bronchoconstriction and tachypnea have been demonstrated at a late stage of infusion-induced edema in dogs, when vascular pressures had reverted to control, and significant blood gas changes were absent (Chung et al., 1983).

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