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PLASMAPHERESIS AFFECTS RESPONSES OF SLOWLY AND RAPIDLY ADAPTING AIRWAY RECEPTORS TO PULMONARY VENOUS CONGESTION IN DOGS

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SUMMARY

1. The effects of plasmapheresis on the responses of rapidly adapting receptors (RARs) and slowly adapting receptors (SARs) of the airways to pulmonary venous congestion were examined in dogs anaesthetized with α-chloralose. Pulmonary venous congestion was produced in a graded manner by partial obstruction of the mitral valve sufficient to raise the mean left atrial pressure by 5, 10 and 15 mmHg. Plasmapheresis was performed by withdrawing 10% of blood volume twice.

2. Both RARs ($n = 11$) and SARs ($n = 5$) responded to pulmonary venous congestion by increasing their activities. The responses of the former were proportionately greater.

3. After plasmapheresis which reduced the concentration of plasma proteins by $12{\cdot}3 \pm 1{\cdot}0\%$, the responses of the RARs to pulmonary venous congestion were enhanced significantly. There was no significant change in the responses of SARs.

4. In another set of six RARs, the effects of graded pulmonary venous congestion were investigated twice with an interval of 45 min between the two observations. No significant differences were noted between the two responses.

5. Collection of lymph from the tracheobronchial lymph duct ($n = 6$) showed that after plasmapheresis, there was an increase in the control lymph flow. In addition, the lymph flow was enhanced during pulmonary venous congestion (mean left atrial pressure increased by 10 mmHg).

6. It is suggested that a natural stimulus for the excitation of the RAR is a function of the fluid fluxes in the pulmonary extravascular space.

INTRODUCTION

One of the earliest effects of acute left ventricular dysfunction is pulmonary venous congestion (Braunwald, 1980). If uncorrected, there is a progressive loss of fluid from the vascular compartment. This fluid is believed to accumulate first in the bronchial perivascular space, then in the interstitial space between capillaries and alveoli and finally in the alveoli (Staub, Nagano & Pearce, 1967). Several investigators have attempted to define the sensory mechanisms which are activated during this process (e.g. Paintal, 1969; Roberts, Bhattacharya, Schultz, Coleridge & Coleridge, 1986).

It has been shown that the J receptors (pulmonary C fibre receptors) of the lungs are activated in pulmonary interstitial and alveolar oedema (Paintal, 1973; Roberts

et al. 1986). However, in dogs, the J receptors are not activated in a consistent manner during acute pulmonary venous congestion (i.e. before the appearance of alveolar oedema). For instance, mild degrees of pulmonary venous congestion produced by partial obstruction of the mitral valve (mean left atrial pressure increased by 5–15 mmHg), produced no significant change in J receptor activity (Kappagoda, Man & Teo, 1987). It was noted also that under similar conditions, there was a significant increase in the activity of the rapidly adapting receptors (RARs) of the airways (Kappagoda *et al.* 1987; Ravi, Teo & Kappagoda, 1988). These findings suggested that RARs are one of the sensory mechanisms activated in the early stages of left ventricular dysfunction. If such is the case, it should be possible to activate RARs by altering the components of the Starling equation (see Discussion).

The present investigation was undertaken to determine whether the effect of pulmonary venous congestion on the activity of RARs was modified by changes in the concentration of plasma proteins. The study was conducted on anaesthetized dogs and the concentration of plasma proteins was altered by plasmapheresis. As a subsidiary investigation, the influence of these procedures upon the activity of SARs was examined also. Portions of this study have been published as preliminary reports (Kappagoda & Ravi, 1988; Ravi & Kappagoda, 1988).

METHODS

Twenty-five dogs (body weight 19–24 kg) were anaesthetized with an intravenous infusion of α-chloralose (0·1 g/kg) after premedication with morphine sulphate. After induction of anaesthesia, the animals were ventilated artificially with 40% O_2 (v/v). A catheter passed through an endotracheal tube was positioned at or near the carina for measuring airway pressure. The details of these procedures have been described previously (Kappagoda *et al.* 1987).

The chest was opened in the mid-line. The expiratory outlet from the ventilator was kept immersed in 2–3 cm water. Catheters were introduced into the descending aorta through the femoral artery and into the left atrium through the atrial appendage. The aortic blood pressure and the left atrial pressure were measured by connecting these catheters to strain-gauge manometers (Model P23 DB, Statham Instruments Ltd, Puerto Rico), the outputs of which were amplified and recorded on light-sensitive paper (Model VR 12, Electronics for Medicine/Honeywell, Pleasantville, New York, USA). The electrocardiogram (lead 11) was also recorded in the same system. A second balloon-tipped catheter was introduced into the left atrium and positioned at the mitral valve. Inflation of this balloon with small volumes of saline obstructed the flow of blood through the mitral valve resulting in pulmonary venous congestion.

The temperature of the animals was measured using a thermistor inserted into the oesophagus and was maintained at 37 ± 1 °C. The arterial blood gases were monitored periodically and maintained in the normal range by adjusting ventilation and by infusing sodium bicarbonate (8·4%, w/v).

Plasmapheresis

Approximately 10% of the blood volume (estimated as 80 ml/kg) was withdrawn and centrifuged at the rate of 15000 revolutions/min for a period of 10 min in a refrigerated centrifuge (Beckman, Model TJ-6). The supernatant plasma was removed. The red blood cells were resuspended in a volume of lactated Ringer solution (Travenol Canada Inc., Ontario, Canada) equal to that of plasma removed and infused back into the animal. This procedure was performed twice. The syringes and the containers used were sterile. The plasma protein concentration was determined using a Microcentrifugal Analyzer (Multistat 111 Instrumentation Laboratory, Spokane, WA, USA) and the osmolarity was estimated by an osmometer (Osmette S, Precision

Systems, Massachusetts). The haematocrit was determined by using a microcentrifuge (Readacrit, Clay Adams, Parsipany, NJ, USA).

Recording of action potentials from the vagus

Single afferent fibre activity originating from SAR and RAR of the airways was recorded from the right cervical vagus using bipolar silver electrodes. The criteria used for the identification of the SARs and RARs have been described previously (Kappagoda *et al.* 1987). The neural activity was recorded continuously and counted electronically (Man, Man & Kappagoda, 1983). The conduction velocities of the afferent fibres examined were measured using procedures described previously (Ravi *et al.* 1988). At the end of each experiment, the receptors were located by gently probing the external surface of the trachea and the bronchi with a glass rod 3 mm in diameter.

Collection of pulmonary lymph

In the dog, pulmonary lymph is drained by the right lymph duct (Uhley, Leeds, Sampson & Friedman, 1961) and by the left tracheobronchial lymph duct (Staub, 1974). The latter drains into the thoracic duct which in turn opens into the left external jugular vein (Staub, 1974). The left tracheobronchial lymph duct was identified and catheterized. Since opalescence in the lymph draining from this duct is considered an indication of contamination with thoracic duct lymph (Turino, Pine, Beech & Cottrell, 1977), the experiment was continued only when the lymph collected was clear and had a haematocrit less than 1%. An increase in lymph flow resulting from pulmonary venous congestion in the control phase was adopted as an additional criterion in these experiments. The latter ensured that a significant portion of the lymph examined was derived from the lung. Lymph was collected in glass vials and was analysed for its protein concentration using the Microcentrifugal Analyzer (Multistat III).

Experimental protocols

Protocol 1: effect of pulmonary venous congestion and plasmapheresis on RARs. After identifying a RAR, the preparation was allowed to stabilize for 10 min. At the end of this period, the neural activity was recorded for an initial control period of 5 min. Then the mean left atrial pressure was increased by 5, 10 and 15 mmHg. Each pressure was maintained for a period of 5 min and RAR activity was recorded throughout. Next, the congestion was relieved and the activity recorded for a final control period of 5 min.

After completing the above steps, the concentration of plasma proteins was altered by plasmapheresis (see above). After an interval of 15 min, the stimulus–response curve relating left atrial pressure to receptor activity was repeated.

Protocol 2: effect of pulmonary venous congestion and plasmapheresis on SARs. After identifying a SAR, the procedures described in Protocol 1 were completed.

Protocol 3: variation in the responses of RARs to pulmonary venous congestion with time. This protocol was designed to determine whether the stimulus–response relationship between left atrial pressure and RAR activity varied over a period which corresponded to that taken for plasmapheresis (45 min). Two stimulus–response curves were elicited with an interval of 45 min between them. During this period, the unit was left undisturbed in the control state. This protocol was examined on five RARs not included in protocol 1.

Protocol 4: pulmonary venous congestion and lymph flow before and after plasmapheresis. Lymph flow was measured at intervals of 15 min. Lymph was collected during an initial control period of 30 min and the collection was continued into a 30 min period of pulmonary venous congestion (see above). Next, the congestion was relieved and lymph collected for a final control period of 30 min.

The above procedure was repeated after plasmapheresis.

Statistical analysis

Group data were expressed as means ± S.E.M. For each unit, a stimulus–response curve relating receptor activity to mean left atrial pressure (control, +5, +10 and +15 mmHg) before and after plasmapheresis was established. In each instance, the control activity was taken as the average of the activities during the initial and final control periods (see above). The average stimulus–response

curve obtained before plasmapheresis was compared with that obtained after plasmapheresis using an analysis of covariance.

The lymph flow was analysed using an analysis of variance (ANOVA). The flow during pulmonary congestion was compared to those during the initial and final control periods. The differences between means were detected by the least significant difference.

The intratracheal pressures were analysed using an analysis of variance as above. However, in this instance, the initial and final control values were averaged before analysis since there was no significant difference between them. The regression lines between the peak intratracheal pressures and the mean left atrial pressures before and after plasmapheresis were compared using an analysis of covariance.

A P value $< 0{\cdot}05$ was accepted as indicative of a significant difference.

RESULTS

At the commencement of the recordings in the dogs, the heart rate, mean arterial blood pressure, mean left atrial pressure and peak intratracheal pressure were $112{\cdot}9 \pm 7{\cdot}8$ beats/min, $102{\cdot}6 \pm 3{\cdot}6$ mmHg, $5{\cdot}7 \pm 0{\cdot}4$ mmHg and $3{\cdot}3 \pm 0{\cdot}4$ mmHg respectively. The arterial P_{CO_2}, P_{O_2} and pH values were $34{\cdot}5 \pm 0{\cdot}9$ mmHg, 191 ± 12 mmHg and $7{\cdot}37 \pm 0{\cdot}02$ respectively.

Protocol 1: effect of pulmonary venous congestion and plasmapheresis on RARs

This protocol was carried out on eleven RARs in eleven dogs. The conduction velocity of these fibres was 20 ± 2 m/s. Five RARs were found to be located in the lobar bronchus near the hilum of the lungs and four, in the lobar bronchus within 1·0 cm from the hilum. Two RARs were located in the right principal bronchus.

The average activity of the RARs during the control period was 40 ± 10 impulses/min. The mean left atrial pressure during the control period was $6{\cdot}3 \pm 0{\cdot}2$ mmHg. During graded increments in left atrial pressure, there was a graded increase in activity in all RARs examined. An example of one unit is shown in Fig. 1*A*. Overall, a significant positive correlation was seen in the activity of RARs ($r = 0{\cdot}4$, $P < 0{\cdot}01$). As the mean left atrial pressure was raised by $5{\cdot}4 \pm 0{\cdot}2$, $10{\cdot}0 \pm 0{\cdot}2$ and $16{\cdot}2 \pm 0{\cdot}7$ mmHg, the RAR activity increased to 95 ± 30, 165 ± 66 and 189 ± 53 impulses/min respectively (Fig. 2).

The total plasma protein concentration during the control period was $4{\cdot}5 \pm 0{\cdot}2$ g/100 ml. After plasmapheresis, the concentration was $3{\cdot}9 \pm 0{\cdot}2$ g/100 ml. The average decrease was $12{\cdot}3 \pm 1{\cdot}0\%$. This decrease was significant statistically ($P < 0{\cdot}01$). The haematocrit before and after plasmapheresis was $40{\cdot}6 \pm 1{\cdot}5\%$ and $40{\cdot}5 \pm 1{\cdot}3\%$ respectively. These values were not significantly different ($P > 0{\cdot}05$). The corresponding values for the osmolarity of plasma were 293 ± 3 and 294 ± 3 mosmol/l ($P > 0{\cdot}05$).

After plasmapheresis, the mean left atrial pressure during the control period was $6{\cdot}1 \pm 0{\cdot}3$ mmHg. The control activity in RARs was increased in nine units and decreased in two units. The average activity during the control period in all the eleven units studied was 54 ± 16 impulses/min. Overall, after plasmapheresis, the stimulus–response relationship between receptor activity and mean left atrial pressure was found to be enhanced. An example is shown in Fig. 1*B*. On average, when the mean left atrial pressure was raised by $5{\cdot}0 \pm 0{\cdot}2$, $10{\cdot}0 \pm 0{\cdot}2$ and $15{\cdot}8 \pm 0{\cdot}7$ mmHg, the RAR activity increased to 136 ± 40, 267 ± 83 and 352 ± 108 impulses/min respectively. These results are presented in Fig. 2. The regression lines relating

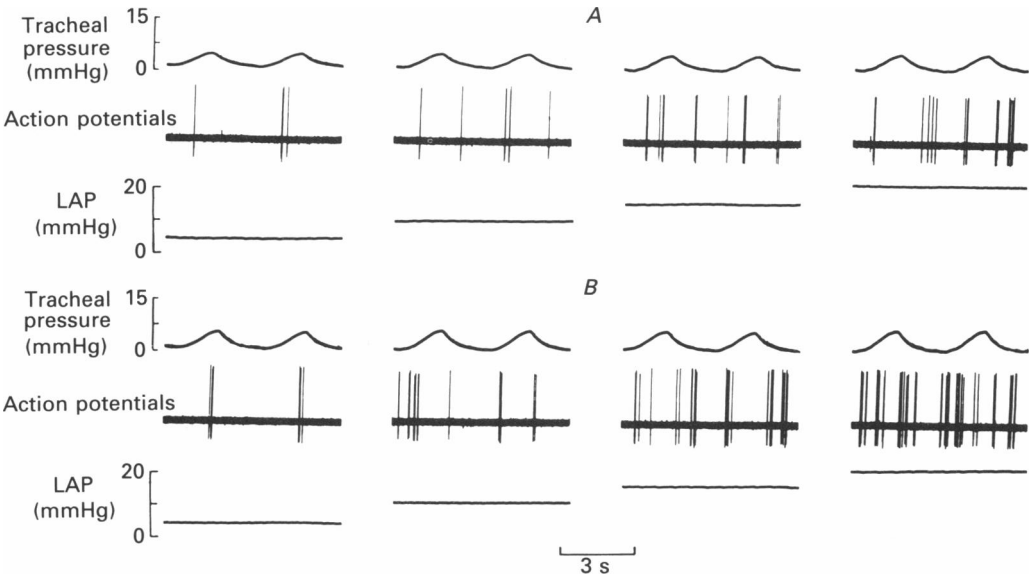

Fig. 1. Responses of an RAR to pulmonary venous congestion before (*A*) and after (*B*) plasmapheresis. In *A* and *B*, in each column, the upper trace is the tracheal pressure, the middle trace, the action potentials and the lower trace, mean left atrial pressure (LAP). The panels from left to right represent: control, +5, +10 and +15 mmHg increase in mean left atrial pressure. Note that the response in the receptor is enhanced in *B*.

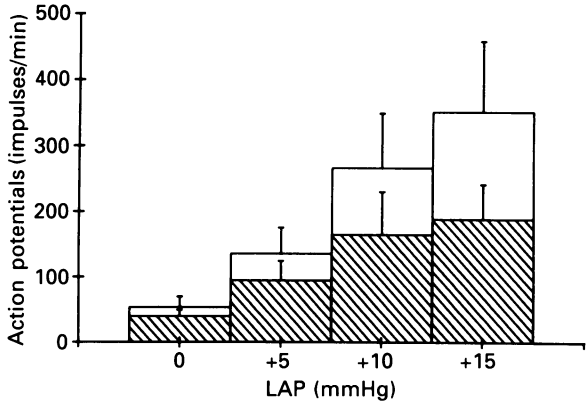

Fig. 2. Histograms showing the relationship between increments in mean left atrial pressure (LAP) and activity of RARs (action potentials) recorded before (shaded bars) and after (open bars) plasmapheresis. The RAR activity is expressed as impulses/min (means ± S.E.M.). The mean left atrial pressure in the control period is given as 0 mmHg. Note that there is an enhanced response after plasmapheresis.

impulse activity to left atrial pressure under the two circumstances were compared. With plasmapheresis, there was a significant elevation of the regression line ($F = 4{\cdot}4$, y intercept before plasmapheresis = 34·6, y intercept after plasmapheresis = 48·3, $P < 0{\cdot}05$).

The corresponding changes observed in peak intratracheal pressure before and

after plasmapheresis are shown in Table 1. Before plasmapheresis, the increments in peak intratracheal pressure observed when the mean left atrial pressure was raised by 10 and 15 mmHg were significant ($P < 0.05$). After plasmapheresis, the peak intratracheal pressure was elevated significantly in the control state (3·4±0·4

TABLE 1. Peak intratracheal pressures (mmHg) during pulmonary venous congestion produced by partial obstruction of the mitral valve. Venous congestion is expressed in terms of the increase in mean left atrial pressure (LAP)

	LAP (mmHg)			
	Control	+5	+10	+15
Protocol 1, RARs ($n = 11$)				
Pre-plasmapheresis	3·4±0·4	3·4±0·4	3·7±0·4*	4·0±0·4*
Post-plasmapheresis	3·8±0·4	3·9±0·4	4·1±0·4*	4·3±0·4*
Protocol 2, SARs ($n = 5$)				
Pre-plasmapheresis	3·8±0·5	3·9±0·6	4·1±0·4	4·7±0·8*
Post-plasmapheresis	4·2±0·7	4·3±0·7	4·5±0·7	5·0±0·8*

* Significant difference from other values in row ($P < 0.05$).

to 3·8±0·4 mmHg; $P < 0.01$, paired t test). The regression lines relating peak intratracheal pressure to mean left atrial pressure obtained before and after plasmapheresis were compared. It was found that the slopes and intercepts were not significantly different in the two regression lines. ($F = 1.85$, y intercept before plasmapheresis = 3·3 mmHg, y intercept after plasmapheresis = 3·8 mmHg, $P > 0.05$).

Protocol 2: effect of pulmonary venous congestion and plasmapheresis on SARs

This protocol was carried out on five SARs in five dogs. The conduction velocity of these fibres was 40±3 m/s. Of these five SARs, four were located in the lobar bronchus near the hilum of the lung and one in the right principal bronchus.

The average activity of these SARs during the control period was 1627±207 impulses/min. When the mean left atrial pressure was increased by 5, 10 and 15 mmHg, the SAR activity increased to 1753±212, 1916±211 and 2166±243 impulses/min respectively. Even though there was an apparent response in SARs, there was no significant correlation between increments in left atrial pressure and receptor activity ($r = 0.41$, $P > 0.05$). In addition, the activity was analysed in terms of the inspiratory and expiratory phases of ventilation as defined by the intratracheal pressure tracing. During the inspiratory phase, there was a significant increase in activity compared to the control state, when the mean left atrial pressure was increased by 10 and 15 mmHg. A significant increase in activity during the expiratory phase occurred only when the mean left atrial pressure was increased by 15 mmHg. These results are presented in Table 2.

The total plasma protein concentration before plasmapheresis was 4·8±0·2 g/100 ml. After plasmapheresis, the concentration was 4·1±0·2 g/100 ml. This decrease was significant statistically ($P < 0.01$). The haematocrit before and after plasmapheresis was 40·4±2·5% and 40·0±2·5% respectively. These values were not significantly different ($P > 0.05$).

Following plasmapheresis, the correlation between receptor activity and mean left atrial pressure remained non-significant ($r = 0{\cdot}23$, $P > 0{\cdot}05$). The average activity during the control period was 1679 ± 262 impulses/min. When the mean left atrial pressure was increased by 5, 10 and 15 mmHg, the SAR activity increased to 1743 ± 258, 1813 ± 261 and 2022 ± 264 impulses/min respectively. The changes in the activity during the inspiratory and expiratory phases of ventilation are presented in Table 2. Overall, this stimulus–response relationship was not significantly different from that obtained before plasmapheresis ($F = 0{\cdot}1$, $P > 0{\cdot}05$).

TABLE 2. Responses of SARs ($n = 5$) to pulmonary venous congestion produced by partial obstruction of the mitral valve. Venous congestion is expressed in terms of the increase in mean left atrial pressure (LAP). SAR activity is expressed as action potentials/ventilatory cycle during the inspiratory and expiratory phases

	LAP (mmHg)			
	Control	+5	+10	+15
Pre-plasmapheresis				
Inspiratory phase	45 ± 5	49 ± 7	55 ± 8*	63 ± 8**
Expiratory phase	45 ± 6	48 ± 6	52 ± 5	57 ± 5**
Post-plasmapheresis				
Inspiratory phase	46 ± 7	44 ± 7	50 ± 6	57 ± 8**
Expiratory phase	48 ± 8	53 ± 10	51 ± 10	55 ± 12

*$P < 0{\cdot}05$, compared to control. **$P < 0{\cdot}05$, compared to control and +5 mmHg LAP. Comparisons were made using ANOVA and least significant difference.

The corresponding changes observed in peak intratracheal pressure before and after plasmapheresis are shown in Table 1. Before plasmapheresis, the increment observed when the mean left atrial pressure was raised by 15 mmHg was significant ($P < 0{\cdot}05$). After plasmapheresis, the peak intratracheal pressure was elevated in the control state. However, the magnitude of the changes in intratracheal pressure caused by increasing the mean left atrial pressure was not enhanced.

Protocol 3: variation in the responses of RARs to pulmonary venous congestion with time

The spontaneous variation in the stimulus–response curve relating mean left atrial pressure and RAR activity was examined on five units in three dogs. There were no significant changes in the responses over a period of 45 min ($P > 0{\cdot}05$). The results are presented in Table 3.

Protocol 4: pulmonary venous congestion and lymph flow before and after plasmapheresis

This protocol was completed in six dogs. Plasmapheresis resulted in a reduction in the concentration of plasma proteins from $4{\cdot}7 \pm 0{\cdot}3$ to $4{\cdot}2 \pm 0{\cdot}3$ g/100 ml. This reduction of $11{\cdot}8 \pm 2{\cdot}0\%$ was significant ($P < 0{\cdot}05$). There was a concomitant significant fall in the concentration of proteins in lymph from $3{\cdot}2 \pm 0{\cdot}3$ to $2{\cdot}3 \pm 0{\cdot}3$ g/100 ml ($P < 0{\cdot}01$).

Before plasmapheresis, the average lymph flow during the initial control period

TABLE 3. Variation in the responses of RARs ($n = 5$) to pulmonary venous congestion with time. Venous congestion is expressed in terms of the increase in mean left atrial pressure (LAP)

	LAP (mmHg)			
	Control	+5	+10	+15
RAR activity (AP/min) at commencement of study	50±9	125±42	168±39	268±78
RAR activity (AP/min) after an interval of 45 min	58±6	90±14	136±32	199±54

The stimulus–response relationship between left atrial pressure and RAR activity did not change significantly after 45 min ($P > 0.05$). AP, action potential.

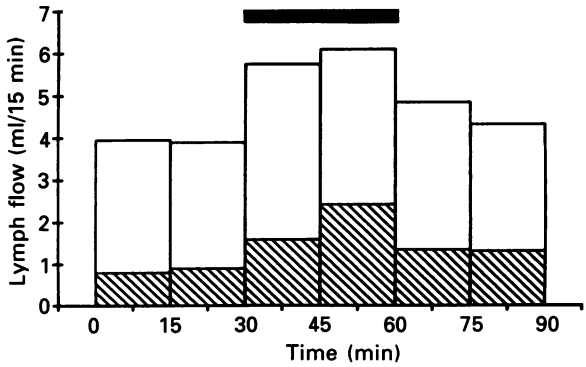

Fig. 3. Lymph flows during pulmonary venous congestion (mean left atrial pressure increased by 10 mmHg) before (shaded area) and after (open area) plasmapheresis in one dog. In both, the lymph flows in the initial and final control periods are shown between 0–30 and 60–90 min respectively. Pulmonary venous congestion was produced during the period 30–60 min, indicated by the horizontal bar.

TABLE 4. Lymph flow (ml/15 min, $n = 6$) and lymph/plasma protein concentration ratio (L/P ratio) during pulmonary venous congestion produced by partial obstruction of the mitral valve. Venous congestion is expressed in terms of the change in mean left atrial pressure (LAP)

	Initial control	LAP (+10 mmHg)	Final control
Pre-plasmapheresis			
Lymph flow	1·5±0·5	3·2±0·9*	2·5±0·7
L/P ratio	0·68±0·03	0·67±0·04	0·64±0·04
Post-plasmapheresis			
Lymph flow	4·9±1·1	8·5±2·5**	4·6±1·0
L/P ratio	0·55±0·06	0·54±0·05	0·48±0·2

*$P < 0.05$, compared to initial control only. **$P < 0.05$, compared to both controls.

was $1{\cdot}5 \pm 0{\cdot}5$ ml/15 min. The control mean left atrial pressure was $5{\cdot}8 \pm 0{\cdot}3$ mmHg. During pulmonary venous congestion (mean left atrial pressure increased by $10{\cdot}2 \pm 0{\cdot}2$ mmHg), the lymph flow increased significantly to $3{\cdot}2 \pm 0{\cdot}9$ ml/15 min ($P < 0{\cdot}05$). An example is shown in Fig. 3 and the results are summarized in Table 4.

After plasmapheresis, the lymph flow during the initial control period increased to $4{\cdot}9 \pm 1{\cdot}1$ ml/15 min. The control mean left pressure during this period was $5{\cdot}5 \pm 0{\cdot}2$ mmHg. Pulmonary venous congestion (mean left atrial pressure increased by $10{\cdot}2 \pm 0{\cdot}2$ mmHg) produced a further increase in lymph flow (Table 4).

DISCUSSION

It is generally accepted that there are four major types of vagal sensory receptors in the airways. These are the SARs, RARs, bronchial C fibre receptors and the J receptors (Sant'Ambrogio, 1982). Recent investigations demonstrated that pulmonary venous congestion produced by small elevations of mean left atrial pressure (< 10 mmHg) increased the RAR activity significantly (Kappagoda *et al.* 1987; Ravi *et al.* 1988) as did obstruction of the lymphatic drainage from the lung (Ravi *et al.* 1988). These results suggested that the RARs are exquisitely sensitive to changes in the transfer of fluid from the pulmonary vasculature into the extravascular fluid space and thus may play a role in detecting the early stages of pulmonary oedema.

Further evidence in support of this proposition is provided in this paper. There was a graded increase in RAR activity when the mean left atrial pressure was elevated and this relationship was potentiated by a 12% reduction in the plasma protein concentration. This observation is not attributable to a spontaneous variation in activity of RARs since the stimulus–response relationship was not altered when pulmonary congestion was repeated after an interval of 45 min (Table 3). A reduction in the concentration of plasma proteins failed to produce a consistent change in the overall sensitivity of SARs to pulmonary congestion. Thus, it appears that the increased sensitivity of RARs to pulmonary congestion after plasmapheresis is a feature of RARs.

Nature of the stimulus

The forces which regulate the exchange of fluid between the microvasculature and extravascular fluid compartment of the lungs have been reviewed extensively (for references see Staub, 1974, 1984). The net transfer of fluid into the pulmonary extravascular space is defined by the Starling equation,

$$Q_f = k_f[(P_{mv} - P_{pmv}) - \sigma(\pi_{mv} - \pi_{pmv})],$$

where Q_f is the net transvascular fluid flow, k_f is the fluid filtration coefficient, P_{mv} and P_{pmv} are the microvascular and perimicrovascular hydrostatic pressures respectively, σ is the solute reflection coefficient, and π_{mv} and π_{pmv} are the microvascular and perimicrovascular fluid protein osmotic pressures respectively (Staub, 1977).

An imbalance in these factors, e.g. an increase in the effective filtration (hydrostatic) pressure or a decrease in the protein osmotic pressure, leads to the

accumulation of fluid in the extravascular space. Studies in baboons (Zarins, Rice, Peters & Virgilio, 1978) and sheep (Kramer, Harms, Gunther, Renkin & Demling, 1981) have demonstrated that a reduction in the protein osmotic pressure by 60–70% caused an increase in pulmonary lymph flow without producing pulmonary oedema. In the experiments in baboons, the pulmonary artery wedge pressure was maintained constant at the control value. Thus, when these changes are coupled with a defect in the mechanisms which remove fluid from the extravascular space, there will be a tendency for fluid to accumulate. Hence, lymphatic obstruction would predispose to the formation of oedema (Low, 1978).

In experimental animals, pulmonary oedema is produced either by raising the effective filtration pressure or by rupturing the anatomical integrity of the pulmonary microvasculature (Staub, 1984). The former could be achieved by infusing large volumes of fluid intravenously (Roberts *et al.* 1986) and by obstructing the flow of blood in the left side of the heart (Guyton & Lindsey, 1959; Uhley *et al.* 1961). Disruption of the pulmonary microvasculature can be caused by chemicals such as alloxan (Paintal, 1969; Coleridge & Coleridge, 1977) and caprylic and capric acids in olive oil (Glogowska & Widdicombe, 1973). In anaesthetized dogs, it has been shown that the critical pressure in the left atrium above which alveolar oedema occurs is 23 mmHg (Guyton & Lindsey, 1959). Thus at pressures below 23 mmHg in the left atrium, the pulmonary vasculature is congested without any associated oedema. Therefore, oedema should be viewed as the end-stage of a continuum from the control state, through enhanced leakage of fluid at a rate which could be transported by lymphatics, to a stage of accumulation of fluid in the extravascular space. In this final stage, lymphatics are unable to cope with the excessive fluid leading to peribronchial cuffing and eventually to alveolar flooding (Staub *et al.* 1967).

The present study has focused upon the early stages of the evolution of this process, i.e. at a stage where there is only an enhanced transfer of fluid into the extravascular space. Pulmonary venous congestion was produced by obstruction of the mitral valve so as to increase mean left atrial pressures by 5, 10 or 15 mmHg. This stimulus, which does not result in alveolar oedema (Guyton & Lindsey, 1959), increases pulmonary lymph flow (Ravi *et al.* 1988; present results) indicating an expansion of the extravascular space. Guyton & Lindsey (1959) reported also that the critical pressure in the left atrium for producing pulmonary (i.e. alveolar) oedema was reduced to half when the total plasma protein concentration was reduced by 50%. In the present study, the concentration of plasma proteins was reduced by only $12{\cdot}3 \pm 1{\cdot}0$%. It is likely that the combination of a reduction in the concentration of plasma proteins and pulmonary congestion employed in the present study would have enhanced the transfer of fluid into the extravascular space without producing overt alveolar oedema. The data from the studies of the lymph flow support this proposition (Table 4).

Lymph flow

One aim of the present study was to demonstrate that a decrease in the total plasma concentration by approximately 12% was sufficient to increase lymph flow. The left tracheobronchial lymph duct was cannulated for this purpose. In the dog, it has been reported that 80% of the pulmonary lymph is drained by the right lymph

duct and the remaining 20% by the left tracheobronchial duct (Uhley *et al.* 1961; Staub, 1974). The lymph flow in the initial control period (before plasmapheresis) was $1{\cdot}5 \pm 0{\cdot}5$ ml/15 min. It increased to $4{\cdot}9 \pm 1{\cdot}1$ ml/15 min after plasmapheresis. Pulmonary venous congestion increased lymph flow under both conditions. It is likely that the lymph drained from the left tracheobronchial duct is contaminated by lymph from extrapulmonary sources. Nevertheless, the findings suggest that after plasmapheresis, there is an expansion of the pulmonary extravascular space, an effect which is augmented by pulmonary venous congestion.

There are two related issues which merit comment. The first is the potential influence of plasmapheresis upon pressure in the airways. It is known that during pulmonary congestion, there is an expansion of the extravascular space of the airways (Mills, Sellick & Widdicombe, 1970; Ravi *et al.* 1988) and an increase in the peak intratracheal pressure (Kappagoda *et al.* 1987). In the present study, one vagus was intact and the other, from which recordings were made, was partly so. The increase observed in peak intratracheal pressure during congestion could be due to (a) a passive stiffening of the lung due to vascular congestion, (b) a reflex bronchoconstriction (Kappagoda, Man, Ravi & Teo, 1988) and (c) a combination of the above. An earlier study examined the effect of bilateral vagotomy on the peak intratracheal pressure changes observed during pulmonary vascular congestion (Kappagoda *et al.* 1987). It was found that the increase in peak intratracheal pressure persisted, in an attenuated form, even after bilateral vagotomy. Thus, it is likely that the rise in peak intratracheal pressure reported in Table 1 was, at least in part, secondary to stiffening of the lung caused by vascular congestion.

These was an increase in peak intratracheal pressure after plasmapheresis. It is possible that this increase was due to stiffening resulting from leakage of fluid from the pulmonary vasculature. The observation of an increase in lymph flow in the initial control period after plasmapheresis is consistent with this suggestion.

Further examination of the data in Table 1 shows that in the control state, increasing the mean left atrial pressure by 10 mmHg raised the peak intratracheal pressure from $3{\cdot}4 \pm 0{\cdot}4$ to $3{\cdot}7 \pm 0{\cdot}4$ mmHg. After plasmapheresis, the peak intratracheal pressure in the *control period* was $3{\cdot}8 \pm 0{\cdot}4$ mmHg. Also of interest is the information relating to lymph flow contained in Table 4. The flow during pulmonary venous congestion in the control state was $3{\cdot}2 \pm 0{\cdot}9$ ml/15 min and that during the control period after plasmapheresis was $4{\cdot}9 \pm 1{\cdot}1$ ml/15 min. Nevertheless, the increase in the activity of RARs resulting from pulmonary congestion was considerably greater than that resulting from plasmapheresis *per se* (Fig. 2). These findings indicate that the stimulus for fluid transfer which results from congestion may have an additional stimulatory influence upon RARs. The mechanism underlying this phenomenon remains to be elucidated.

The second issue is the influence of the method of plasmapheresis employed in the present investigation, i.e. batch plasmapheresis as opposed to continuous flow plasmapheresis. Dodek, Rice, Bonsignore, Yamada & Staub (1986) demonstrated that batch plasmapheresis resulted in a transient increase in the concentration of proteins in lymph which was attributed to a change in the filtration coefficient of the vascular bed. This effect was considered to be caused by handling of blood *in vitro*. In their studies, the plasma protein concentration was reduced by approximately

50%. In the present study, plasmapheresis did not change the mean left atrial pressure significantly. There was a reduction in the concentration of protein in lymph and a reduction of approximately 12% in the concentration of plasma proteins. Thus, there was a small reduction in the lymph to plasma protein ratio and it is unlikely that the transient changes referred to above occurred. One explanation for this apparent discrepancy is that the volume of blood removed for plasmapheresis, and hence handled *in vitro*, was small. Nevertheless, regardless of the mechanism of the increase in lymph flow, the present finding that batch plasmapheresis increased pulmonary lymph flow is consistent with the observations of Dodek *et al.* (1986).

In summary, the present results show that a decrease in the plasma protein concentration enhances the sensitivity of RARs to pulmonary venous congestion. Such a response was not evident in the activity of SARs. It is suggested that the enhanced response seen in the activity of RARs could be due to an increased fluid flux into the extravascular space of the airways as a consequence of an increase in the net filtration pressure in the pulmonary microvasculature.

Speculation on the functional significance of the findings

It is recognized that pulmonary venous congestion is an early manifestation of acute left ventricular failure. In this condition, the RARs are more sensitive to congestion than the other pulmonary vagal afferents (Kappagoda *et al.* 1987). Previous studies from this laboratory have indicated that the reflex increases in respiratory rate (Kappagoda, Ravi & Teo, 1989) and tracheal tone (Kappagoda *et al.* 1988) associated with pulmonary venous congestion could be due to activation of RARs. These findings provide a physiological basis for the suggestion that the unpleasant sensation of dyspnoea which accompanies left ventricular failure may in part be due to activation of RARs (see Widdicombe, 1974).

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