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RESPONSES OF SLOWLY AND RAPIDLY ADAPTING RECEPTORS IN THE AIRWAYS OF RABBITS TO CHANGES IN THE STARLING FORCES

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SUMMARY

1. The responses of the rapidly adapting receptors (RARs) and the slowly adapting receptors (SARs) of the airways to changes in the Starling forces regulating fluid exchange in the pulmonary extravascular space were investigated in anaesthetized rabbits. The hydrostatic pressure in the pulmonary microvasculature was raised by partial obstruction of the mitral valve (mean left atrial pressure increased by approximately 5 and 10 mmHg above the control values) and the concentration of plasma proteins was reduced by plasmapheresis (the total plasma protein concentration reduced by 18%).

2. There was a significant correlation between the action potentials generated by RARs and mean left atrial pressure ($n = 12$). A similar response was not observed in SARs ($n = 12$).

3. After plasmapheresis, there was an increase in the resting activity of the RARs ($n = 5$). In addition, the stimulus–response curve relating mean left atrial pressure and RAR activity was significantly shifted to the left compared to the one elicited before plasmapheresis. Plasmapheresis failed to influence the activity of SARs ($n = 5$).

4. Obstruction of the pulmonary lymph flow by raising the afterload in the right external jugular vein caused a significant increase in the activity of RARs ($n = 6$). This response was also maintained during the entire period of lymphatic obstruction.

5. The results show that manipulation of the Starling forces within the lung influences the RAR activity profoundly. It is suggested that the stimulus for the RARs may be a function of the fluid fluxes in the pulmonary extravascular space.

INTRODUCTION

Pulmonary venous congestion, which is a feature of left ventricular dysfunction, is characterized by an increase in the movement of fluid across the pulmonary microvasculature into the extravascular space (Braunwald, 1988). The fluid accumulates first in the peribronchial region and later within the alveoli, eventually leading to alveolar flooding and pulmonary oedema (Staub, Nagano & Pearce, 1967).

The sensory mechanism(s) which transduce the earliest changes in pulmonary

venous congestion probably involve the vagal afferents of the airways. It is generally accepted that there are four major types of vagal sensory receptors in the airways: the slowly adapting pulmonary stretch receptors (SARs), the rapidly adapting receptors (RARs), the bronchial C fibre receptors and the type JU (pulmonary C fibre) receptors (Sant'Ambrogio, 1982). It has been shown that of these four, the RARs were the most sensitive to pulmonary venous congestion (Kappagoda, Man & Teo, 1987). It was suggested that the RARs were stimulated by fluid fluxes within the pulmonary extravascular space. If such is the case, selective manipulation of other factors which influence the rate of transvascular fluid flux across the pulmonary microvasculature (as defined by the Starling equation) may be expected to influence RAR activity also. Indeed, changes in oncotic pressure (Kappagoda & Ravi, 1989) and obstruction of the lymphatic drainage from the lung (Ravi, Teo & Kappagoda, 1988) were found to activate the RARs. Until now, the dog is the only species in which the influences of the factors which regulate fluid flux across the pulmonary vasculature have been examined in a systematic manner.

The present investigation was undertaken to establish the effect of manipulating Starling forces upon the activity of RARs in the rabbit. Specifically, the responses to the following were examined: (i) acute, sustained pulmonary venous congestion produced by partial mitral valve obstruction; (ii) a reduction in the concentration of proteins produced by plasmapheresis and (iii) an obstruction of the lymphatic drainage from the lung. The responses of the pulmonary SARs to these stimuli were examined as a subsidiary investigation.

METHODS

General

Rabbits weighing 2·5–4·5 kg were anaesthetized with sodium pentobarbitone (Somnolol; MTC Pharmaceuticals, Cambridge, Ontario; dose 30 mg kg^{-1} I.V. followed by 12 mg kg^{-1} h^{-1}, I.V.). After induction of anaesthesia, a tracheostomy was performed and the animals ventilated artificially with a Harvard ventilator (Model 607, Harvard Instruments, Millis, MA, USA) through an uncuffed endotracheal tube (i.d. 3 mm, length 10 cm; National Catheter, Argyle, NY, USA) at a rate of 22 breaths min^{-1} and a tidal volume of approximately 12 ml kg^{-1}. The inspired gas was supplemented with oxygen and the arterial P_{O_2} was maintained above 100 mmHg. A cannula (i.d. 0·86 mm, Intramedic polyethylene tubing) was introduced into the endotracheal tube through a side arm and used for recording the airway pressure.

Cannulae (i.d. 0·86 mm, Intramedic polyethylene tubing) were inserted also into the left femoral vein and artery. The former was used for the administration of drugs and other solutions as required and the latter was used to record aortic pressure and to obtain samples periodically for analysis of blood gases. The tip of the arterial cannula was positioned in the abdominal aorta.

The chest was opened in the mid-line after placing the tip of the expiratory tube of the ventilator under 1–2 cmH_2O to prevent collapse of the lungs. The pericardium was opened and a cannula (i.d. 1·67 mm, Intramedic polyethylene tubing) with a balloon attached to its distal end was inserted into the left atrium through the left atrial appendage. The mitral valve was obstructed partially by inflation of this balloon. A second cannula (i.d. 1·67 mm, Intramedic polyethylene tubing), also inserted into the left atrium through the appendage, was used to measure the left atrial pressure.

Cardiovascular pressures, electrocardiogram and action potentials were recorded on light sensitive paper as described previously (Kappagoda *et al.* 1987). The arterial P_{CO_2} and pH of the animals were maintained within physiological limits (see Kappagoda, Linden & Snow, 1970). The temperature of the animal was monitored by a thermistor placed in the rectum (Yellow Springs Instruments Co., Yellow Springs, OH, USA) and maintained at 37 ± 1 °C using heating lamps.

Recording action potentials and measurement of conduction velocity

These procedures have been described in detail previously (Kappagoda *et al.* 1987). Afferent impulses were recorded from slips of the left or right cervical vagus using silver chloride electrodes, the outputs of which were amplified and recorded. Receptor activity was counted electronically by means of an amplitude discriminator. The conduction velocity in the fibres was measured by stimulating the vagus electrically (strength, 2–6 V; duration, 1·5 ms) 2–3 cm caudal to the recording site.

Identification of the receptors

The SARs and RARs were usually identified by their characteristic patterns of discharge and their conduction velocities. They were further differentiated by their responses to stepwise sustained inflation (tidal volume × 3) of the lungs (Widdicombe, 1954; Vidruk, Hahn, Nadel & Sampson, 1977). At the peak of the third cycle, the ventilator was switched off and the inflation maintained by occluding the endotracheal tube approximately 1–2 in away from the animal. The SARs showed a slow rate of adaptation to the maintained inflation while the RARs adapted rapidly. Additionally, an adaptation index was also calculated as described previously (Kappagoda *et al.* 1987). Receptors with an adaptation index > 70% were accepted as RARs (Widdicombe, 1954; Vidruk *et al.* 1977). SAR had an index < 20%.

Localization of the receptors

Location of the receptors was established at the end of each experiment by careful probing of the lungs and airways using a blunt glass rod 3 mm in diameter.

Plasmapheresis

The concentration of plasma proteins was reduced by batch plasmapheresis using procedures described previously (Kappagoda & Ravi, 1989). Approximately 10% of the circulating blood volume (estimated in the rabbit as 80 ml kg^{-1}) was withdrawn for this purpose. Total protein, albumin and globulin concentrations in plasma were determined using a microcentrifugal analyser (Multistat 111 Instrumentation Laboratory, Spokane, WA, USA) and the osmolarity was estimated by an osmometer (Osmette S, Precision Systems, MA, USA). The haematocrit was determined by using a microcentrifuge (Readacrit, Clay Adams, Parsipany, NJ, USA).

Lymphatic obstruction

The lymphatic drainage from the rabbit lung is mainly via the right lymphatic duct (Courtice & Simmonds, 1954). The right lymphatic duct(s) open usually into the venous system in the region where the right internal jugular vein joins the right external jugular vein (Walker, 1965). Preliminary studies were undertaken in our laboratory to confirm this observation by injecting Evan's Blue into the lungs to delineate the lymphatic ducts.

A vascularly isolated pouch (length approximately 3 cm) was created in the right external jugular vein by occluding the veins surrounding the point(s) of entry of the lymphatic ducts in the manner described by Uhley, Leeds, Sampson & Friedman (1961) and Ravi *et al.* (1988).

A polyethylene cannula (i.d. 2 mm, Intramedic polyethylene tubing) was inserted into the pouch and its proximal end was attached to a reservoir containing heparinized saline. The reservoir was secured by a sliding clamp to allow vertical movement. To prevent coagulation within the isolated pouch during the experiment, the animal was heparinized (Heparin Sodium Inj, USP; 1000 u ml^{-1}; 100 u kg^{-1} h^{-1}.

Experimental protocols

After a unit was identified, the preparation was left for a period of 15 min before one of the following protocols was carried out.

Protocol 1: *the effect of pulmonary venous congestion on the activity of RARs and SARs.* After a unit (either RAR or SAR) was identified, the following recordings were made, each for a period of 5 min: (a) an initial control record, (b) two experimental records during which the left atrial pressure was

increased by approximately 5 and 10 mmHg above control respectively and (c) a final control record.

Protocol 2: the effect of pulmonary venous congestion and plasmapheresis on the activity of RARs and SARs. After a RAR or SAR was identified, the steps described in protocol 1 were performed. The plasma protein concentration was then reduced by plasmapheresis. Twenty minutes later, a second stimulus–response relationship between receptor activity and mean left atrial pressure was obtained. The total protein, albumin and globulin concentrations, the osmolarity of plasma and the hematocrit were determined before and after plasmapheresis.

In a separate group of RARs, the stimulus–response curves as described above were repeated after an interval of 20 min without plasmapheresis. During this period, the unit was left undisturbed. This part of the protocol was undertaken to establish the natural variation in the stimulus–response curves.

Protocol 3: the effect of pulmonary lymphatic obstruction on the activity of RARs. After a unit was identified, control recordings were made for a period of 20 min during which time the reservoir was maintained at the level of the right atrium. The reservoir was then elevated to 30 cm above the right atrium and recordings made for a period of 20 min. The reservoir was then returned to the level of the right atrium and the recordings continued for a final 20 min control period. This protocol was carried out only on RARs. In a separate group of RARs, the spontaneous variation in RAR activity with time was assessed by recording the activity in the control state for a period of 60 min.

Statistical analysis

Receptor activity was expressed as action potentials min^{-1}. Where no significant difference existed between initial and final control values, the two were averaged. Where appropriate, the activity was also expressed as action potentials per ventilatory cycle or per inspiratory and expiratory phase. The latter was derived from the airway pressure tracings.

Group data were expressed as mean $\pm$ standard error of the mean. The means of grouped data were compared by an analysis of variance and the difference between means detected by a least significant difference test (Snedecor & Cochran, 1980).

In protocol 2, the stimulus–response curves before and after plasmapheresis were compared using an analysis of covariance. A similar comparison was made for the curves which were obtained as 'time' control. A P value < 0.05 was accepted as indicative of significance.

RESULTS

A total of thirty-three RARs and seventeen SARs were examined in forty-five rabbits. Their respective conduction velocities were 14.2 ± 0.3 and 19.6 ± 0.4 m s^{-1}. At the time of recording, the heart rate, mean arterial blood pressure, mean left atrial pressure and peak airway pressure were, 192 ± 4 beats min^{-1}, 79.4 ± 2.2 mmHg, 5.2 ± 0.2 mmHg and 8.7 ± 0.3 mmHg respectively. The arterial blood pH, P_{CO_2} and P_{O_2} values were 7.39 ± 0.1, 37.2 ± 0.2 mmHg and 168 ± 2 mmHg respectively.

Protocol 1: the effect of pulmonary venous congestion on the activity of RARs

Twelve RARs were examined in twelve rabbits. All units examined demonstrated a respiratory rhythm with short bursts coinciding with peak airway pressure. In five units, the resting activity was limited to an inspiratory burst with no activity during expiration. In the remaining seven units, an inconsistent expiratory discharge was observed. All the receptors were located in a bronchus. Of the twelve RARs examined, eight were located in the lobar bronchi and four in bronchi within 1 cm from the hilum of the lung.

The average activity of the RARs during the initial control period was 146 ± 30

impulses min^{-1}. In all units examined, a stimulus–response relationship between RAR activity and mean left atrial pressure was observed during graded increments of mean left atrial pressure ($r = 0{\cdot}351$, $P < 0{\cdot}05$). A representative example is shown in Fig. 2. As the mean left atrial pressure was elevated by $5{\cdot}3 \pm 0{\cdot}4$ and $10{\cdot}3 \pm 0{\cdot}4$ mmHg,

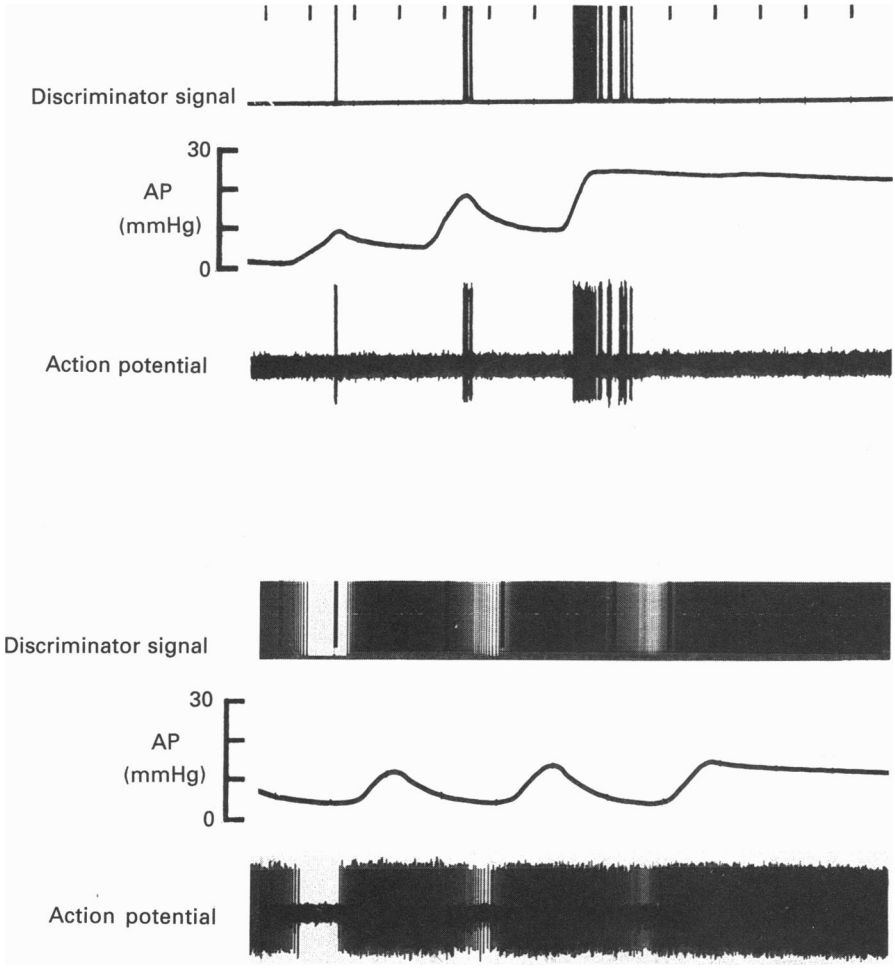

Fig. 1. Examples of a RAR (top) and SAR showing response to a sustained inflation. The RAR alone shows rapid adaptation. From above downwards, the tracings shown are: time marker (s), the processed signal from discriminator, airway pressure (AP) and action potentials. The lungs were inflated for three consecutive breaths by occluding the expiratory port of the ventilator. At the peak of the third breath, the inspiratory port of the ventilator was also occluded.

the RAR activity increased to 215 ± 42 and 296 ± 54 impulses min^{-1} respectively ($F = 10{\cdot}3$; $P < 0{\cdot}01$). The control activity following relief of mitral valve obstruction was 169 ± 36 impulses min^{-1}. The results are summarized in Fig. 3*A*. Receptor

activity was further analysed in terms of the inspiratory and expiratory phases of ventilation. During graded increments in mean left atrial pressure, the activity increased exclusively during inspiration in seven units and during expiration in two. In three units, both inspiratory and expiratory discharges increased (Table 1).

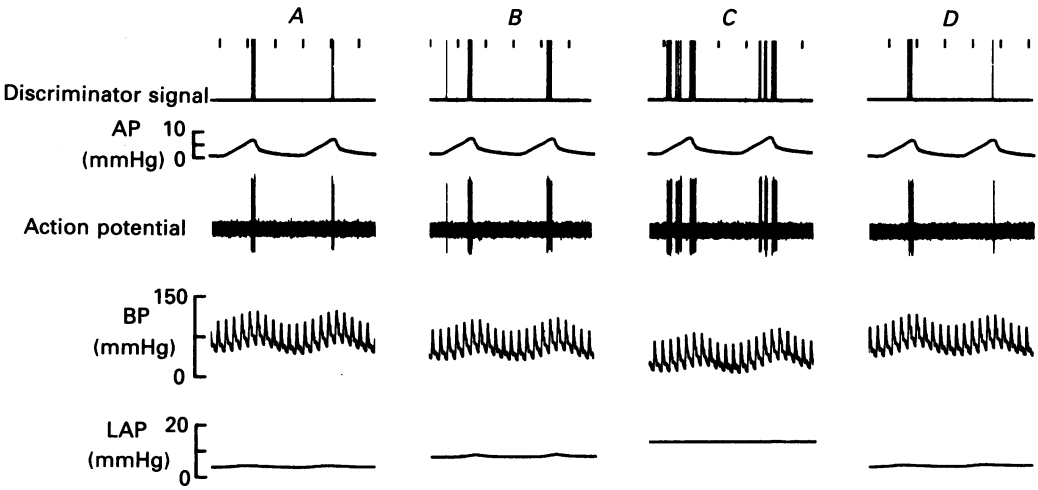

Fig. 2. Responses of a RAR to pulmonary venous congestion produced by partial obstruction of the mitral valve. Activities during the initial and final control periods are shown in *A* and *D* respectively. The responses of the unit to elevation of the mean left atrial pressure by 5 and 10 mmHg above the control value are shown in *B* and *C* respectively. Discriminator signal, the processed neural activity from the discriminator; AP, airway pressure; BP, arterial pressure; LAP, mean left atrial pressure. A time marker (s) is shown in each panel at the top.

There were no changes in peak airway pressure during graded increments in mean left atrial pressure ($P > 0{\cdot}05$). These values and those of the other haemodynamic variables are shown in Table 2.

Slowly adapting receptors

Twelve SARs were examined in nine rabbits. All twelve units examined demonstrated a characteristic respiratory rhythm with the peak activity coinciding with peak inspiratory pressure. Of the twelve SARs, seven were located in the lobar bronchus, three in the principal bronchus and two in the trachea just above the carina.

The average activity of the SARs during the initial control period was 1794 ± 475 impulses min^{-1}. During graded increments in mean left atrial pressure, there was a small but statistically significant increase in SAR activity. In contrast to the RAR, a significant correlation between receptor activity and mean left atrial pressure was not seen ($r = 0{\cdot}05$, $P > 0{\cdot}05$). As the mean left atrial pressure was raised by $4{\cdot}8 \pm 0{\cdot}2$ and $9{\cdot}4 \pm 0{\cdot}2$ mmHg, the SAR activity increased to 1870 ± 484 and 2003 ± 523 impulses min^{-1} respectively ($F = 3{\cdot}15$; $P < 0{\cdot}05$). The activity in the final control

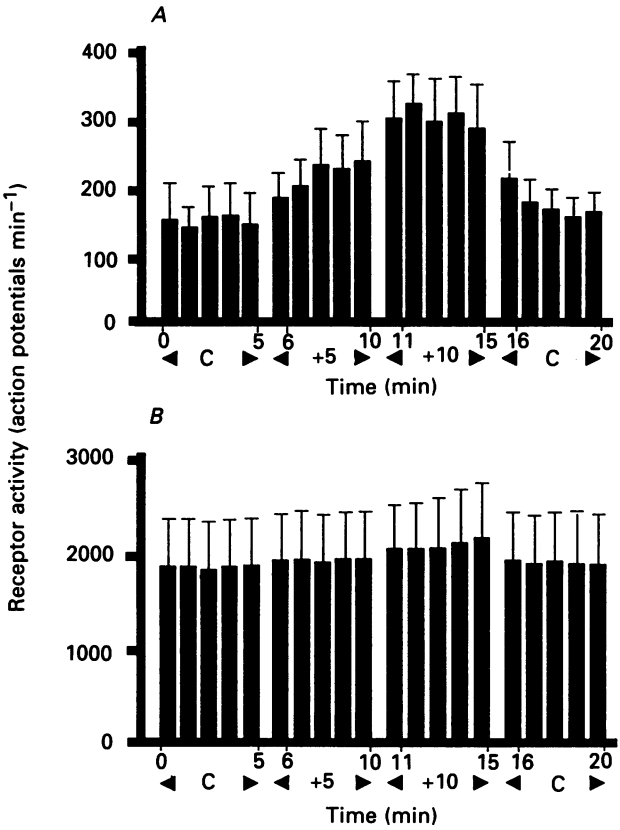

Fig. 3. Receptor activity (mean + S.E.M.) expressed as action potentials min^{-1} during: initial control period (C), elevation of mean left atrial pressure by 5 mmHg (+5), elevation of mean left atrial pressure by 10 mmHg (+10) and final control period (C). *A*, activity of RARs ($n = 12$); the values during elevation of left atrial pressure (+5 and +10 mmHg) were significantly different from each other and from the control values ($P < 0.05$). The control values were similar to each other ($P > 0.05$). *B*, activity of SARs ($n = 12$); the values during elevation of left atrial pressure by 10 mmHg were significantly different from the other values ($P < 0.05$).

period following relief of mitral valve obstruction was 1850 ± 489 impulses min^{-1}. The results are summarized in Fig. 3*B*. The SAR activity was further analysed in terms of the inspiratory and expiratory phases of ventilation. During graded increments in mean left atrial pressure, the activity increased during inspiration in all the units examined. There was an increase in activity during expiration also in two of these units (see Table 1).

A small but significant increase in peak airway pressure was seen during graded increments of mean left atrial pressure. These values and those of the other haemodynamic variables are given in Table 2.

TABLE 1. Changes in inspiratory (I) and expiratory (E) activity in RARs and SARs

Protocol 1: pulmonary venous congestion

	Control		+5		+10		Control	
LAP (mmHg)	I	E	I	E	I	E	I	E
RAR ($n = 12$)	5·3 ± 1·1	1·5 ± 0·7	7·4 ± 1·4	2·2 ± 1·2	8·7 ± 1·5†	3·5 ± 1·4†	6·4 ± 1·3	1·5 ± 0·7
SAR ($n = 12$)	70·5 ± 17·0	12·3 ± 3·2	71·6 ± 18·0	13·0 ± 3·8	75·3 ± 19·0*	13·5 ± 4·2	71·2 ± 17·0	12·4 ± 3·4

Protocol 2: pulmonary venous congestion and plasmapheresis

	Control		+5		+10		Control	
LAP (mmHg)	I	E	I	E	I	E	I	E
RAR ($n = 5$)								
Before plsis	7·0 ± 1·0	2·4 ± 0·8	8·3 ± 1·8	4·6 ± 1·8	9·0 ± 2·3†	5·6 ± 1·2†	7·4 ± 1·7	2·5 ± 0·9
After plsis	12·6 ± 2·2	3·1 ± 0·5	14·4 ± 2·0	4·5 ± 1·3	18·6 ± 3·8†	6·0 ± 1·2*	13·2 ± 3·0	3·5 ± 0·7
SAR ($n = 5$)								
Before plsis	107 ± 34	16·6 ± 5·9	110 ± 37	17·2 ± 6·0	119 ± 42	18·7 ± 6·6	112 ± 36	18·1 ± 6·3
After plsis	107 ± 34	15·9 ± 5·8	109 ± 36	16·9 ± 5·8	111 ± 36	17·8 ± 6·1	108 ± 34	16·7 ± 5·6

Protocol 3: lymphatic obstruction

	Control		Lymphatic obstruction		Control	
	I	E	I	E	I	E
RAR ($n = 6$)	7·3 ± 2·1	2·9 ± 1·8	11·4 ± 2·8	4·8 ± 1·9	9·6 ± 2·5	3·2 ± 1·8

Data expressed as action potentials per respiratory cycle (means ± S.E.M.). LAP, mean left atrial pressure; plsis, plasmapheresis.
†Significant difference from both controls; *significant difference from initial control; $P < 0·05$, ANOVA.

TABLE 2. The changes in heart rate (HR), mean arterial blood pressure (BP) and peak airway pressure (PAP) during pulmonary venous congestion (Protocol 1), pulmonary venous congestion with plasmapheresis (Protocol 2) and lymphatic obstruction (Protocol 3)

		Protocol 1		
LAP (mmHg)	Control	+5	+10	Control
RAR ($n = 12$)				
HR (beats min^{-1})	201 ± 8·8†	190 ± 8·9	190 ± 9·7	190 ± 9·2
BP (mmHg)	78·2 ± 4·2	68·1 ± 5·7†	60·1 ± 6·2†	74·6 ± 4·5
PAP (mmHg)	9·4 ± 0·6	9·6 ± 0·6	10·1 ± 0·7	9·8 ± 0·8
SAR ($n = 12$)				
HR	196 ± 7·9†	186 ± 6·5	182 ± 6·6	187 ± 8·0
BP	82 ± 3·5	76 ± 2·9	61 ± 4·0†	83 ± 3·0
PAP	9·0 ± 0·7	9·3 ± 0·7	9·9 ± 0·8*	9·2 ± 0·7
		Protocol 2		
LAP (mmHg)	Control	+5	+10	Control
RAR ($n = 5$)				
Before plasmapheresis				
HR	178 ± 10·0	155 ± 11·4	141 ± 8·2	160 ± 5·3
BP	79 ± 5·5	69 ± 5·4**	59 ± 8·9†	70 ± 5·6
PAP	8·7 ± 1·3	9·0 ± 1·1	9·6 ± 1·1†	8·8 ± 1·1
After plasmapheresis				
HR	184 ± 3·8	174 ± 7·1	167 ± 9·7	173 ± 4·4
BP	73 ± 4·3	61 ± 4·4**	47 ± 7·4†	68 ± 4·9
PAP	9·0 ± 1·3	9·6 ± 1·1**	10·4 ± 1·1†	9·2 ± 1·0
SAR ($n = 5$)				
Before plasmapheresis				
HR	208 ± 12·1	188 ± 11·6	188 ± 11·6	193 ± 12·6
BP	80 ± 6·2	79 ± 4·1	70 ± 4·7	82 ± 4·0
PAP	8·0 ± 1·5	8·4 ± 1·4	8·9 ± 1·5‡	8·0 ± 1·3
After plasmapheresis				
HR	186 ± 9·7	186 ± 10·0	184 ± 8·4	187 ± 11·2
BP	77 ± 5·6	67 ± 9·8	58 ± 8·1‡	73 ± 7·2
PAP	8·6 ± 1·6	9·2 ± 1·6	9·8 ± 1·6‡	8·8 ± 1·5

	Protocol 3		
RAR ($n = 6$)	Control	Lymphatic obstruction	Control
HR	198 ± 8·7	196 ± 7·9	196 ± 8·0
BP	79 ± 4·0	81 ± 4·1	81 ± 4·4
PAP	9·1 ± 1·2	9·1 ± 1·3	9·1 ± 1·3

†Significantly different from other values in row; *significantly different from initial control; **significantly different from initial control and +10; ‡significantly different from both controls; $P < 0·05$, ANOVA.

LAP, mean left atrial pressure. Data are means ± S.E.M.

Protocol 2: the effect of pulmonary venous congestion and plasmapheresis on the activity of RARs

Five RARs were examined in five rabbits. The average activity of the RARs during the control period was 220 ± 34 impulses min^{-1}. When the mean left atrial pressure was increased by 4·8 ± 0·6 and 11·0 ± 0·5 mmHg, the RAR activity increased significantly to 278 ± 26 and 313 ± 22 impulses min^{-1} respectively ($F = 11·6$;

$P < 0{\cdot}01$). The activity of each unit increased when the mean left atrial pressure was elevated. Overall, a significant positive correlation between mean left atrial pressure and receptor activity was seen ($r = 0{\cdot}50$, $P < 0{\cdot}05$).

The total plasma protein concentrations before and after plasmapheresis were $4{\cdot}0 \pm 0{\cdot}2$ g (100 ml)$^{-1}$ and $3{\cdot}3 \pm 0{\cdot}2$ g (100 ml)$^{-1}$ respectively (a decrease of $17{\cdot}5 \pm 1\%$;

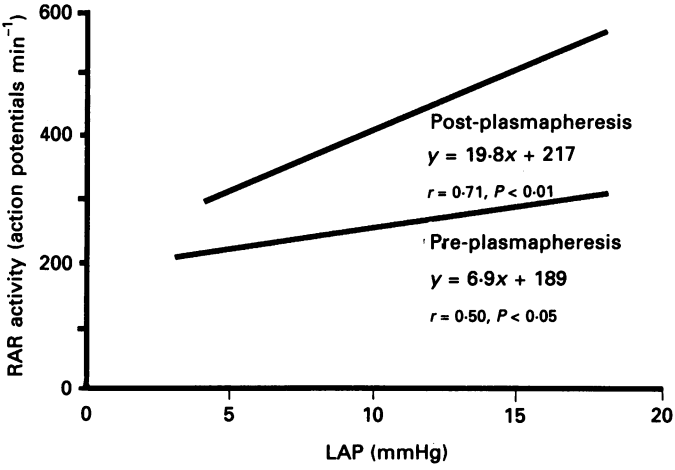

Fig. 4. Regression lines relating mean left atrial pressure (LAP) and RAR activity (expressed as action potentials min^{-1}) elicited before plasmapheresis (lower line) and after plasmapheresis (upper line). The abscissa represents the actual values of mean left atrial pressures (see text for details). The slope and intercept of the upper line were significantly different from the lower one.

$P < 0{\cdot}01$). The corresponding plasma albumin concentrations were $2{\cdot}5 \pm 0{\cdot}1$ and $2{\cdot}1 \pm 0{\cdot}1$ g (100 ml)$^{-1}$ ($P < 0{\cdot}05$) and globulin concentrations were $1{\cdot}5 \pm 0{\cdot}2$ and $1{\cdot}2 \pm 0{\cdot}1$ g (100 ml)$^{-1}$ ($P < 0{\cdot}05$). The osmolarity ($290 \pm 0{\cdot}9$ *vs.* $296 \pm 3{\cdot}2$ osmol l^{-1} and haematocrit values ($32{\cdot}0 \pm 2{\cdot}3$ *vs.* $32{\cdot}0 \pm 2{\cdot}3\%$) were not altered significantly by plasmapheresis ($P > 0{\cdot}05$) for both).

Following plasmapheresis, the sensitivity of all RAR to increases in left atrial pressure was increased. The average activity during the control period was 339 ± 25 impulses min^{-1}. When the mean left atrial pressure was increased by $5{\cdot}4 \pm 0{\cdot}6$ and $11{\cdot}2 \pm 0{\cdot}7$ mmHg, the RAR activity increased to 438 ± 45 and 579 ± 62 impulses min^{-1} respectively ($F = 12{\cdot}8$; $P < 0{\cdot}01$). After plasmapheresis, there was a significant increase in both the slope and the intercept of the regression line (Fig. 4) relating receptor activity to left atrial pressure (slope, $F = 5{\cdot}0$, $P < 0{\cdot}05$; intercept, $F = 32{\cdot}7$, $P < 0{\cdot}01$).

The data was analysed also in terms of the phases of the ventilatory cycle. It was found that the activities of the units were enhanced during both phases of respiration after plasmapheresis. These data are summarized in Table 1.

After plasmapheresis, there was a significant increase in the peak airway pressure ($P < 0{\cdot}05$, paired t test). However, for a given change in mean left atrial pressure, the increase in airway pressure was not significantly different from that observed before plasmapheresis (slope, $F = 0{\cdot}02$, $P > 0{\cdot}05$). These results are presented in Table 2.

The spontaneous variation in the stimulus–response curves relating mean left atrial pressure to RAR activity was examined in a separate group of five units. The activity during the initial control period was 252 ± 57 impulses min^{-1}. When the mean left atrial pressure was increased by $4{\cdot}8 \pm 0{\cdot}5$ and $10{\cdot}2 \pm 0{\cdot}6$ mmHg, the RAR activity increased significantly to 316 ± 87 and 388 ± 91 impulses min^{-1} respectively ($F = 8{\cdot}9$, $P < 0{\cdot}01$). After a period of 20 min, the control RAR activity was 240 ± 49 impulses min^{-1}. When the mean left atrial pressure was increased by $4{\cdot}8 \pm 0{\cdot}5$ and $10{\cdot}8 \pm 0{\cdot}6$ mmHg, the RAR activity increased significantly to 291 ± 67 and 362 ± 73 impulses min^{-1} respectively ($F = 6{\cdot}6$, $P < 0{\cdot}01$). In each instance, the relationship between receptor activity and mean left atrial pressure was not significantly altered after an interval of 20 min (slope, $F = 0{\cdot}002$, $P > 0{\cdot}05$; intercept, $F = 0{\cdot}001$, $P > 0{\cdot}05$).

Slowly adapting receptors

Five SARs were examined. The average activity of the SARs during the control period was 2720 ± 951 impulses min^{-1}. When the mean left atrial pressure was increased by $4{\cdot}8 \pm 0{\cdot}4$ and $9{\cdot}4 \pm 0{\cdot}4$ mmHg, the receptor activity increased to 2821 ± 957 and 3022 ± 1074 impulses min^{-1} respectively. These changes were not significant ($P > 0{\cdot}05$). There was no correlation between mean left atrial pressure and SAR activity ($r = 0{\cdot}044$, $P > 0{\cdot}05$).

After plasmapheresis, the total plasma protein concentration decreased from a control value of $3{\cdot}9 \pm 0{\cdot}1$ to $3{\cdot}2 \pm 0{\cdot}2$ g $(100 \text{ ml})^{-1}$ (a decrease of $18{\cdot}8 \pm 3\%$, $P < 0{\cdot}01$). Unlike the RARs, the stimulus–response curves for SAR were not altered significantly after plasmapheresis (slope, $F = 0{\cdot}008$, $P > 0{\cdot}05$; intercept, $F = 0{\cdot}007$, $P > 0{\cdot}05$). The details of the activities of SAR and the changes in airway pressures are given in Tables 1 and 2.

Protocol 3: the effect of pulmonary lymphatic obstruction on the activity of RARs

Six RARs were examined in six rabbits. Lymphatic obstruction increased the activity of all RARs examined. The average activity of the RARs during the initial control period was 208 ± 85 impulses min^{-1}. During the period of lymphatic obstruction, the average activity increased significantly to 298 ± 57 impulses min^{-1} ($P < 0{\cdot}05$). During the final 20 min control period following relief of lymphatic obstruction, the activity fell to 247 ± 56 impulses min^{-1}. This value was not significantly different from the initial control value ($P > 0{\cdot}05$).

The time course of the changes in receptor activity throughout the experiment is presented in Fig. 5*A*. The activity began to increase within 1 min following lymphatic obstruction and continued to increase progressively. Following relief of lymphatic obstruction, the activity slowly declined and after 10 min had returned to the initial control values. The data were analysed also in terms of the phases of the ventilatory cycle. It was found that the activity of the units was enhanced during both phases of respiration when the lymphatic drainage was obstructed. These data are summarized in Table 1.

Lymphatic obstruction produced no significant change in heart rate, mean arterial blood pressure, mean left atrial pressure and peak airway pressure. These results are presented in Table 2.

Spontaneous variation in the activity of RARs

Five RARs were examined in three rabbits to assess the spontaneous variation in neural activity over a period of 60 min. The data were analysed in three consecutive 20 min periods. The average RAR activity was 281 ± 78 impulses min^{-1} in the first

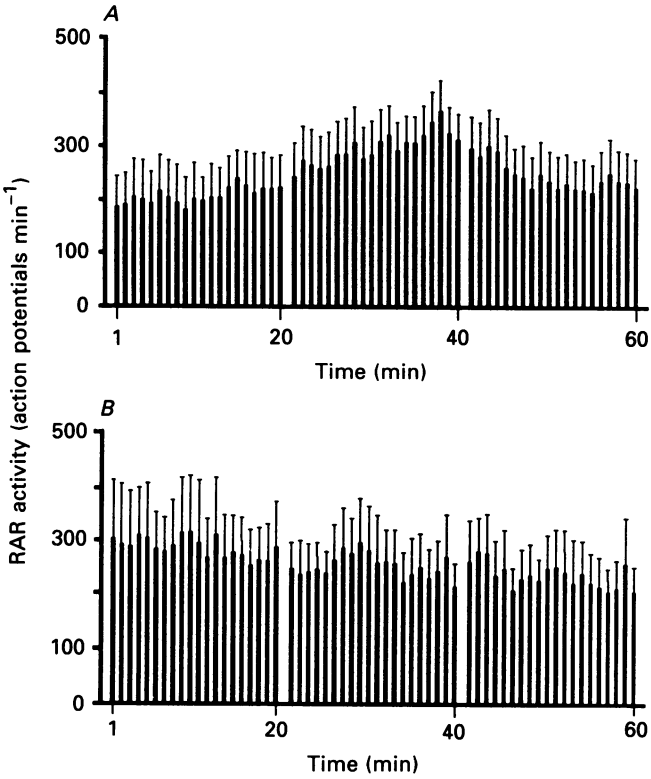

Fig. 5. *A*, effect of pulmonary lymphatic obstruction on the activity of RARs ($n = 6$). Activities during the initial control period (1–20 min), during lymphatic obstruction (21–40 min) and the final control period (41–60 min) are shown. *B*, spontaneous variation in the activity of RARs ($n = 5$). Activities were recorded continuously for 60 min. Note that the increase in RAR activity observed during lymphatic obstruction in *A* is different from the changes attributable to spontaneous variation. Bars represent + S.E.M.

20 min period, 255 ± 58 impulses min^{-1} in the second 20 min period and 227 ± 50 impulses min^{-1} in the final 20 min period. No significant difference was found between the three 20 min periods ($P > 0{\cdot}05$; Fig. 5*B*).

DISCUSSION

Left ventricular dysfunction is usually associated with an element of obstruction to venous return from the lung. Pressures in the left atrium and the pulmonary veins are elevated resulting in pulmonary venous congestion (Braunwald, 1988). If the

condition is permitted to progress, pulmonary oedema ensues, which is characterized by the accumulation of fluid, first in the peribronchial tissues and later in the alveoli (Staub, 1984). It is very likely that these events represent successive stages of a progressive phenomenon which is initiated by expansion of the extravascular fluid space in the lung. The investigation reported in this paper was designed to define the role of the RARs in sensing the early stages of this process, i.e. before the development of pulmonary oedema.

The Starling forces and vagal afferents

The net transfer of fluid into the extravascular space is given by the Starling equation:

$$Q_f = k_f[(P_{mv} - P_{pmv}) - \rho(\Pi_{mv} - \Pi_{pmv})]$$

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, ρ is the solute reflection coefficient and Π_{mv} and Π_{pmv} are the microvascular and perimicrovascular fluid protein osmotic pressures respectively (Staub, 1984). Previous investigations in anaesthetized dogs have shown that manoeuvres designed to increase Q_f, i.e. pulmonary venous congestion and a reduction in plasma oncotic pressure, activated the RARs (Kappagoda & Ravi, 1989). Obstruction of the lymphatic drainage from the lung also activated these receptors (Ravi *et al.* 1988). These findings suggested that in the dog, the RARs are sensitive to fluid fluxes in the extravascular space of the airways. The present investigation was undertaken to support this proposition in another species namely the rabbit, by examining the behaviour of RARs during manipulation of the Starling forces regulating fluid exchange in the airways.

Pulmonary venous congestion and vagal afferent activity

Pulmonary venous congestion produced by small increases in mean left atrial pressure (approximately +5 and +10 mmHg above control values) caused a significant graded increase in the activity of RARs (Figs 2 and 3*A*). The SARs did not respond in this manner. The degree of obstruction of the venous drainage from the lung applied in the present study was unlikely to produce overt pulmonary oedema (see Guyton & Lindsey, 1959). However, a small increase in peak airway pressure was observed during the period of congestion (Table 2). It is recognized that during partial obstruction of the mitral valve, there will be an increase in the total pulmonary resistance (Sellick & Widdicombe, 1969; Hogg, Agarawal, Gardiner, Palmer & Macklem, 1972) and a decrease in pulmonary compliance (Sellick & Widdicombe, 1969). This response can be attributed in part to a reflex mechanism mediated by the vagi and in part to trapping of blood in the pulmonary circulation. An earlier study in rabbits examined the responses of RARs to pulmonary venous congestion produced by partial obstruction of the mitral valve (Sellick & Widdicombe, 1969). Even though the left atrial pressures were not measured, the study showed that the RARs were stimulated during relatively short periods of pulmonary venous congestion, it was also observed that there was no correlation between changes in airway mechanics associated with pulmonary congestion and

RAR activity. The present study has demonstrated that pulmonary venous congestion activated RAR in a sustained manner and that the activity was correlated with the degree of congestion.

The receptors examined were located in the major bronchi which are supplied by the bronchial arteries. The apparent similarity between the anatomical locations of RARs and SARs and the discrepancy of their responses to pulmonary venous congestion may be explained by the venous drainage of the proximal airways. Significant communications have been demonstrated between the bronchial and pulmonary veins in several species. For instance in the rat, when Batson's casting resin was injected into the left atrium, the resin was identified in the bronchial vanules (Skepper, Kappagoda, McNaughton & Navaratnam, 1990). It is very likely that similar communications exist in the rabbit also. It is possible that the RARs were located in areas from where they could transduce such changes in P_{mv} of the bronchial veins.

Influence of changes in Π_{mv} *on vagal afferent activity*

In the present study, batch plasmapheresis reduced the total protein concentration by approximately 18% in the rabbits examined. This change was accompanied by a reduction of $16{\cdot}6 \pm 3\%$ and $20{\cdot}2 \pm 3\%$ in the albumin and globulin concentrations respectively. The data reported by Nitta, Ohnuki, Ohkuda, Nakada & Staub (1981) suggest that these changes in the concentrations of plasma proteins could reduce the protein osmotic pressure by approximately 30%. Previous studies in the dog have demonstrated that a reduction in plasma protein concentration of similar magnitude increases pulmonary lymph flow and that elevation of mean left atrial pressure magnifies this increase (Kappagoda & Ravi, 1989). Thus, it is suggested that batch plasmapheresis in the rabbit also resulted in an increase in fluid flux into the pulmonary extravascular space and the enhanced sensitivity of RARs to changes in left atrial pressure (after plasmapheresis) was due to localized expansion of the extravascular space.

Vagal afferent activity during pulmonary lymphatic obstruction

As in the dog, most of the lymph from the rabbit lung also drains via the right lymphatic ducts into the right external jugular vein (Courtice & Simmonds, 1954). Thus, by creating a similar pouch near the points of entry of the right lymphatic ducts into the right external jugular vein and increasing the pressure within it, it was possible to obstruct the flow of lymph from the lung. A pressure of 30 cmH_2O was selected because it has been reported that the 'pumping pressure' in the lymphatic vessels close to the external jugular vein is approximately 17 cmH_2O (Uhley *et al.* 1961). It was found that activity of RAR increased progressively from the first minute after lymphatic obstruction. Similarly, when lymphatic obstruction was relieved, receptor activity decreased from the first minute onwards and reached the control values in 10 min. The responses observed during the period of lymphatic obstruction cannot be explained by any spontaneous variation in the receptor activity with time (Fig. 5).

In general, the findings in the rabbit are similar to those in the dog, in which the behaviour of the RARs to pulmonary lymphatic obstruction was examined (Ravi *et*

al. 1988). In the dog, after relief of lymphatic obstruction, even though there was a tendency for the activity of RARs to return to control values, it remained significantly elevated compared to the initial control values. One explanation for this difference could lie in the overall compliance of the pulmonary extravascular space in the two species. For instance, the dog lung could be more compliant than that of the rabbit and hence retain fluid.

Mechanisms for the stimulation of RARs

In the present study, the stimuli applied were primarily aimed to increase the fluid flux across the pulmonary vasculature. Regardless of whether the increase in fluid flux was achieved by an increase in the hydrostatic pressure in the vasculature or by a decrease in the plasma oncotic pressure, the activity of RARs was enhanced significantly. The SARs were not found to be sensitive to these stimuli. Thus, it is likely that the RARs may be located in areas from where they could transduce such fluid fluxes. This claim is strengthened by the observations obtained following lymphatic obstruction. In the rabbit, the lymph flow in the right lymphatic ducts is approximately 0·35 ml h^{-1} (Hughes, May & Widdicombe, 1956). Since the increase in this activity of RAR commences during the first minute after lymphatic obstruction, it appears that the RARs are exquisitely sensitive to fluid fluxes in the pulmonary extravascular space.

It has been suggested that the decrease in lung compliance occurring during pulmonary venous congestion may be responsible for stimulation of the RARs (Sellick & Widdicombe, 1969; Armstrong, Luck & Martin, 1976; Yu, Coleridge & Coleridge, 1987). Support for this proposition is evident in the airway pressure measurements during pulmonary venous congestion particularly when the mean left atrial pressure was increased by 10 mmHg. However, plasmapheresis sensitized the RAR significantly while the relationship between left atrial pressure and peak airway pressure remained unchanged. Also, lymphatic obstruction activated RAR with no change in peak airway pressure. Thus, a change in pulmonary mechanics is unlikely to be a major determinant of the increase in activity of RAR. Nevertheless it is recognized that there may be a localized change in the compliance of the tissues surrounding the receptors which does not reflect upon the airway pressure (present study) or the compliance measurements (Ravi *et al.* 1988).

Speculation on the location of the receptors

It has been proposed recently that the RAR may be located in the extravascular space, close to the bronchial venules (Ravi & Kappagoda, 1990). A significant proportion of the bronchial veins drain directly into the pulmonary veins (Pietra & Fishman, 1978). Therefore partial obstruction of the mitral valve would cause an increase in hydrostatic pressure in the bronchial veins of the proximal airways. Such a location would enable the RARs to respond to an expansion of the extravascular space of the proximal airways during pulmonary venous congestion, lymphatic obstruction and reduction of oncotic pressure of plasma.

There is evidence in the rat that there are structures adjacent to the bronchial venules which have the features of sensory nerve endings. These structures are linked with the vagi (Skepper *et al.* 1990). Such a location though compatible with the

physiological findings relating to the RARs, appear to conflict with the conventional view that the RARs are located in the epithelial and subepithelial layers of the airways (Widdicombe, 1974). However, it is conceivable that the RARs may have a bimodal distribution (Ravi & Kappagoda, 1990). It would be of interest to establish in the rabbit whether similar sensory endings exist in the vicinity of the bronchial venules.

In summary, these findings indicate that the RARs may be a major afferent input to the central nervous system in conditions which promote the accumulation of fluid into the extravascular space of the airways as in pulmonary venous congestion. The stimulation of these receptors may account for some of the respiratory symptoms seen under these conditions.

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