J. Physiol. (1987), **394**, *pp.* 249–265
With 5 text-figures
Printed in Great Britain

BEHAVIOUR OF CANINE PULMONARY VAGAL AFFERENT RECEPTORS DURING SUSTAINED ACUTE PULMONARY VENOUS PRESSURE ELEVATION

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(*Received 17 April 1986*)

SUMMARY

1. The effects of an acute sustained increase in pulmonary venous pressure induced by partial obstruction of the mitral valve on the activity of the four types of pulmonary receptors, namely, slowly adapting, rapidly adapting, pulmonary C-fibre and bronchial C-fibre receptors, were studied in the dog.

2. Fifteen slowly adapting receptors, eleven rapidly adapting receptors and nine bronchial C-fibre receptors showed significant sustained increases in activity when stimulated by the elevated left atrial pressure by $9{\cdot}4 \pm 0{\cdot}2$ mmHg for 15 min. Nine pulmonary C-fibre receptors did not show a significant increase (six of these nine receptors increased their activity in response to the stimulus).

3. When the left atrial pressure was increased in graded steps of 5 mmHg for 5 min each up to 15 mmHg, a significant graded response was found in all of seven slowly adapting receptors, five rapidly adapting receptors and five bronchial C-fibre receptors. The five pulmonary C-fibre receptors examined also showed increases, but the changes were not statistically significant.

4. In response to stimulation by the elevated left atrial pressure, increases in activity occurred within 1 min of application of the stimulus in all the receptors and returned to control levels within 1 min of removal of this stimulus.

5. It is concluded that in the dog, pulmonary vagal receptors are influenced by small increases in pulmonary venous pressure induced by partial obstruction of the mitral valve. The changes appeared to be greatest in the case of rapidly adapting receptors. The physiological significance of these responses remains to be investigated.

INTRODUCTION

There are a variety of receptors in the lung which discharge into branches of the cervical vagus. These receptors are believed to convey afferent information which regulates reflexly many functions of the cardiovascular and respiratory systems (see Paintal, 1973; Sant'Ambrogio, 1982; Coleridge & Coleridge, 1984, for reviews). There is general agreement that there are four distinct types of receptor endings which can be identified electrophysiologically. These are: (1) slowly adapting pulmonary stretch receptors (s.a.r.) (Widdicombe, 1954, 1961; Marshall & Widdicombe, 1958); (2) rapidly adapting receptors (r.a.r.) (Knowlton & Larrabee, 1946; Widdicombe,

1954; Sellick & Widdicombe, 1969); (3) pulmonary C-fibre receptors (J receptors) (Paintal, 1969; Coleridge & Coleridge, 1977 *a*, *b*; Kaufman, Iwamoto, Ashton & Cassidy, 1982) and; (4) bronchial C-fibre receptors (Coleridge & Coleridge, 1977 *a*, *b*; Kaufman *et al.* 1982).

Over the past 40 years, many studies in experimental animals have been directed at elucidating both the natural stimuli to these different receptors and the reflex responses elicited by their activation. One aspect of their function relevant to clinical syndromes is their response to pulmonary venous congestion which is a common manifestation of early left ventricular dysfunction.

There are a few reports in the literature describing the responses of these receptors to increments in pulmonary venous pressure and their findings raise several issues which merit further investigation. First, there are no studies in which the relative sensitivities of all the receptor types to changes in pulmonary venous pressure have been examined in one species. Such species differences have been demonstrated in the responses of some pulmonary receptors to certain chemical stimuli (Widdicombe, 1961; Paintal, 1969; Coleridge & Coleridge, 1977*a*, *b*). Second, the intensity of the stimulus applied (i.e. the increase in pulmonary venous pressure) was usually in excess of changes observed in common physiological or pathophysiological settings (Marshall & Widdicombe, 1958). Third, the stimuli were applied for relatively short periods of time (usually 1–2 min) (Marshall & Widdicombe, 1958; Sellick & Widdicombe, 1969). The latter makes it difficult to speculate upon their possible involvement in reflex mechanisms of a 'compensatory' nature. For these reasons, the investigation described in this paper was undertaken in anaesthetized dogs to answer the following questions: (a) what is the effect of increasing the pressure in the left atrium by 10 mmHg for 15 min on the activity of the four types of pulmonary receptors described above, and (b) could such effects be graded? A preliminary report of this study has been presented to the American Physiological Society (Teo, Man & Kappagoda, 1985*a*).

METHODS

Fifty-three dogs weighing 18–32 kg were given an injection of morphine sulphate (0·5 mg/kg subcutaneously). Thirty minutes later the dogs were anaesthetized with an infusion of α-chloralose (5 g α-chloralose in 500 ml normal saline, 0·9% w/v; dose 0·1 g/kg; Fisher Scientific) given intravenously through a cannula (i.d. 1.65 mm polyethylene tubing; Clay Adams, Parsipanny, NJ, U.S.A.) inserted into the inferior vena cava via the left saphenous vein. Subsequently a state of light anaesthesia was maintained by a continuous infusion of α-chloralose (dose 0·5–0·75 ml/kg per 10 min). The animals were not paralysed.

After induction of anaesthesia, the animals were ventilated artificially using a Harvard ventilator (Model 607, Harvard Instruments, Millis, MA, U.S.A.) through a cuffed endotracheal tube (i.d. 10 mm, length 20 cm; National Catheter, Argyle, NY, U.S.A.) at the rate of 18 /min and with a tidal volume of 12–15 ml/kg. The ratio of the inspiration period/expiration period was 1:2. The open end of the expiratory tube of the ventilator was placed under 2 cm of water in order to prevent collapse of the lungs in the animal with the open chest. Supplemental oxygen was given to maintain an adequate level of arterial P_{O_2}. A cannula (i.d. 1·67 mm, Intramedic polyethylene tubing) with multiple side holes at one end, was positioned in the trachea so that this open end was at the level of the carina while the other end was connected to a pressure transducer for measuring airway pressure. The right femoral vein was cannulated (i.d. 1·67 mm, Intramedic polyethylene tubing) and used for administration of intravenous solutions. A second cannula (i.d. 1·67 mm, Intramedic polyethylene tubing) was inserted into the right femoral artery and advanced into the

aorta. This cannula was used for recording the pressure in the aorta and for obtaining samples of blood periodically for measuring the blood gases. An additional cannula was inserted through the right external jugular vein and advanced into the right atrium. This cannula was used for the injection of drugs and sodium bicarbonate (see below).

The chest was opened in the mid-line and a balloon attached to the end of a polyethylene cannula (i.d. 1·67 mm, Intramedic polyethylene tubing) was inserted into the left atrium through the left atrial appendage. Inflation of this balloon caused partial obstruction of the mitral valve and pulmonary venous hypertension. A second cannula (i.d. 1·67 mm, Intramedic polyethylene tubing) was inserted into the left atrium and used for recording the pressure in the left atrium.

The ventilatory parameters of the animals were maintained within normal limits by adjusting the ventilator and non-respiratory acidaemia was prevented by the administration of an infusion of sodium bicarbonate (7·5% w/v) at the rate of 0·1 ml/min. Once the blood gases of the animal were adjusted to the normal range, the ventilatory parameters were unchanged, so as not to distort the experimental recordings.

The cannulae for recording pressures were connected to transducers (Model P23 DB, Statham Instruments Ltd, Hato Rey, Puerto Rico) the output of which was amplified and recorded on light-sensitive paper (Model VR 12, Electronics for Medicine–Honeywell, Pleasantville, NY, U.S.A.). The zero values for the atrial pressures were obtained post-mortem after exposure of the tip of the cannula to the atmosphere. The frequency response of the system for recording pressure was flat to 50 Hz (±2%). The electrocardiogram was recorded for standard lead II using the same system. The temperature of the animal was monitored using a thermistor placed in the oesophagus (Yellow Springs Instruments Co., Yellow Springs, OH, U.S.A.). The temperature of the animal was maintained at 37±1 °C using heating lamps.

Clamping carotid arteries

Both common carotid arteries were dissected out in the neck and separated from the vagi. Two spring-loaded clamps (Crile Hemostat, size 5·5 in, Lawton GmbH & Co., Tuttlingen, F.R.G.) were positioned on the arteries. Both clamps were applied simultaneously without additional tension on the length of the arteries. When they were removed, care was taken to synchronize the removal on both sides. The times of application and removal of the clamps were defined on the recording by an event marker.

Recording action potentials

Action potentials were recorded from branches of the cervical vagus using techniques described previously (Kappogoda, Linden & Sivananthan, 1979; Man, Man & Kappagoda, 1983). Slips of the cervical vagus were placed on silver electrodes and the output from them was amplified and recorded. The action potentials were also displayed on an oscilloscope in addition to being fed into an audio amplifier (Model 32–2025, Tandy Electronics, Barrie, Ontario, Canada). In order to facilitate the calculation of the activity in the pulmonary receptors, the signals were fed into a discriminator and counted electronically. The fidelity of the recordings was established by recording simultaneously both the 'raw' and the 'processed' signals.

The conduction velocity in the fibres was measured by stimulating the vagus electrically at a point 4–5 cm caudal to the site of the recording with the cathode positioned proximal to the latter. In order to minimize the current spread during stimulation of the nerve, the vagus was grounded between the recording and stimulating electrodes at a point 1 cm from the latter. The site of stimulation was not de-sheathed. The action potential evoked in the nerve was monitored on an oscilloscope which was triggered by the stimulus. The stimulus parameters were as follows: myelinated fibres, duration 0·05–0·10 ms, strength 17·8±3·7 V (range 8–80 V); non-myelinated fibres, duration 0·5–1·0 ms, strength 58·0±2·2 V (range 50–80 V). The time taken for the impulse to traverse the length of the nerve from the site of stimulation to the recording site was obtained from the oscilloscope. The distance between the site of recording and the point of stimulation was measured also. These two values were used to calculate the conduction velocity. The identity of the unit stimulated was established in two ways. In the case of the s.a.r. which had a 'constant' rate of discharge, it was possible to demonstrate 'collision'. For the other types of fibres examined, the identification was based upon amplitude and shape of the action potential discharges (see below). The above procedures permitted the differentiation of myelinated vagal afferents from non-myelinated ones.

Identification of the receptors

The s.a.r. and r.a.r. were identified usually from the pattern of afferent activity recorded from slips of the cervical vagus nerve and from their conduction velocities. These units were differentiated further by their reponses to stepwise sustained inflation of the lungs in the manner of Knowlton & Larrabee (1946): the s.a.r. showed a sustained increase in the pattern of discharge while the r.a.r. exhibited evidence of rapid adaptation. An adaptation index was obtained by hyperinflating the lungs in a stepwise manner by occluding the expiratory line of the ventilator for three ventilatory cycles. The ventilator was switched off at peak inspiration and the inflation maintained for 5 s. Experimental recordings were obtained at a paper speed of 50 mm/s and the adaptation index was calculated using the following formula:

$$\text{adaptation index} = \frac{\text{Peak} - \text{average frequency during 2nd second}}{\text{peak frequency}} \times 100\,\%.$$

The peak frequency was calculated over 0·2 s and coincided with the peak of inflation. The 2nd second was defined as the period between 1·2 and 2·2 s following the start of the measurement.

The non-myelinated C fibres had conduction velocities less than 2 m/s. Pulmonary C-fibre (J) receptors were identified further by their rapid response (less than 3 s) following injection of capsaicin (capsaicine natural, Fluka AG, Chemische Fabrik, Switzerland, 10 μg/kg) into the right atrium (Coleridge, Coleridge & Luck, 1965; Coleridge & Coleridge, 1977*a*; Kaufman *et al.* 1982). Bronchial C-fibre receptors were identified by their response within 7–12 s to an injection of phenyldiguanide (1–phenyldiguanide hydrochloride, Fluka AG, Chemische Fabrik, Switzerland, 10 μg/kg) into the right atrium (Coleridge & Coleridge, 1977*a*; Kaufman *et al.* 1982).

Experimental protocols

After a unit having one of the above characteristics was identified, the preparation was left for 10 min for equilibration. Next, one of the following protocols was completed.

Protocol 1. Recordings were made for an initial control period of 10 min. At the end of this period, the balloon in the left atrium was distended to increase the left atrial pressure by approximately 10 mmHg. After 1 min, recordings were made for a period of 15 min. At the end of this period, the balloon was deflated and after a period of 1 min, recordings were made for a second control period of 10 min.

Protocol 2. In this protocol, the effect of a graded increment of left atrial pressure on the discharge of these receptors was examined. After obtaining records for an initial control period of 5 min, the balloon in the left atrium was distended to increase the left atrial pressure in steps of 5 mmHg up to a maximum increase of 15 mmHg. Each step was maintained for 5 min.

Protocol 3. Since the results in protocol 1 indicated that the r.a.r. were activated to the greatest degree by the increments in the left atrial pressure, the following additional procedures were carried out to establish the possible mechanisms involved in this phenomenon. (a) After demonstrating the reponse to an increase in left atrial pressure of 10 mmHg (5 min), the procedure was repeated after cooling both vagi to 8 °C. The vagi were rewarmed and the stimulus was applied once more. The vagi were then sectioned and the procedure repeated. On the nerve from which recordings were made, both procedures were done cranial to the site. (b) The activity of the r.a.r. was recorded over a 2 min control period. The common carotid arteries were occluded by clamps for 30 s and the effect of this manoeuvre on the activity in the units was noted. The clamps were then released and further control recordings made for 5 min.

Location of the receptors in the lung

After completion of the above protocols the location of the receptors was established by discrete palpation of the mediastinum and the lungs. In the case of the s.a.r. and the r.a.r. the localization was limited to a segment of the bronchus approxmiately 1 cm in length. In the case of the pulmonary C-fibre and bronchial C-fibre receptors, the process was continued until the receptor was localized to a segment of parenchymal tissue of the lung.

Statistical analysis

The activity in the fibres was expressed in terms of the numbers of action potentials/ventilatory cycle. The settings of the ventilator were unchanged (18 ventilatory cycles/min) after recording

commenced. In all protocols, the data obtained during the experimental periods was compared to those obtained during the control periods. Where required, a further analysis was done after dividing the activity into the inspiratory or expiratory phases of ventilation. This separation was done on the basis of the changes in tracheal pressure. In protocol 1, a factorial analysis was undertaken to establish the effects of the two phases of ventilation and elevation of left atrial pressure upon receptor activity. In protocols 2 and 3 the comparison was made by an analysis of variance. Where the analysis of variance was significant ($P < 0.05$), the difference between means was detected by a least significant difference test (Snedecor & Cochran, 1980).

Group data were expressed as means ± S.E.M.

RESULTS

Protocol 1

Protocol 1 was completed in thirty-two dogs. At the start of the recordings, the heart rate, arterial pressure and left atrial pressure were 150 ± 3 beats/min, 111 ± 3 mmHg and 7·5 ± 0·2 mmHg, respectively. The arterial blood pH, P_{CO_2} and P_{O_2} were 7·33 ± 0·01, 34·7 ± 1·4 mmHg and 212·6 ± 11·9 mmHg, respectively. The average increase in left atrial pressure was 9·4 ± 0·2 mmHg.

Activity in slowly adapting receptors. Fifteen s.a.r. were examined using protocol 1. The activity in the units was related to expansion of the lungs and in all these units the activity during inspiration was greater than that during expiration. All the units showed sustained activity when the airway pressure was increased i.e. had adaptation indices of < 20% (Fig. 1*A*). The average conduction velocity in these fibres was 22·2 ± 4·2 m/s (range 7·5–45·8 m/s).

During the control period the average activity was 69·3 ± 8·5 action potentials/ventilatory cycle (inspiratory phase, 46·6 ± 5·4 action potentials/cycle; expiratory phase, 22·5 ± 3·7 action potentials/cycle). When the left atrial pressure was elevated, the activity increased to 79·3 ± 8·6 action potentials/cycle (inspiratory phase, 49·2 ± 5·6 action potentials/cycle; expiratory phase, 30·2 ± 4·1 action potentials/cycle). The increase in activity per ventilatory cycle was statistically significant ($P < 0.005$) as was the increase in activity during the expiratory phase ($P < 0.01$). The change during the inspiratory phase was not significant. When the left atrial pressure was restored to control, the activity returned to values which were not significantly different from the initial control values. An example of one such unit is shown in Fig. 2*A*. The results of all the s.a.r. are summarized in Fig. 3*A*.

When the left atrial pressure was elevated, the activities during both phases of ventilation increased, but that during the expiratory phase showed a greater proportional increase, In two s.a.r., the activity during the expiratory phase became greater than that during inspiration. During the final control period, activity decreased during both phases of ventilation (as compared with the period of elevated left atrial pressure) but one s.a.r. continued to be more active during expiration than during inspiration.

Activity in rapidly adapting receptors. Eleven r.a.r. were examined under the same conditions as the s.a.r. The activity in these units had a respiratory rhythm but this relationship was less consistent than in the s.a.r. Of the eleven r.a.r. studied, nine showed predominantly inspiratory activity and two predominantly expiratory activity during the initial control period. All the units showed evidence of rapid adaptation (Fig. 1*B*) and the adaptation index in these r.a.r. was 82·6 ± 2·6% (range

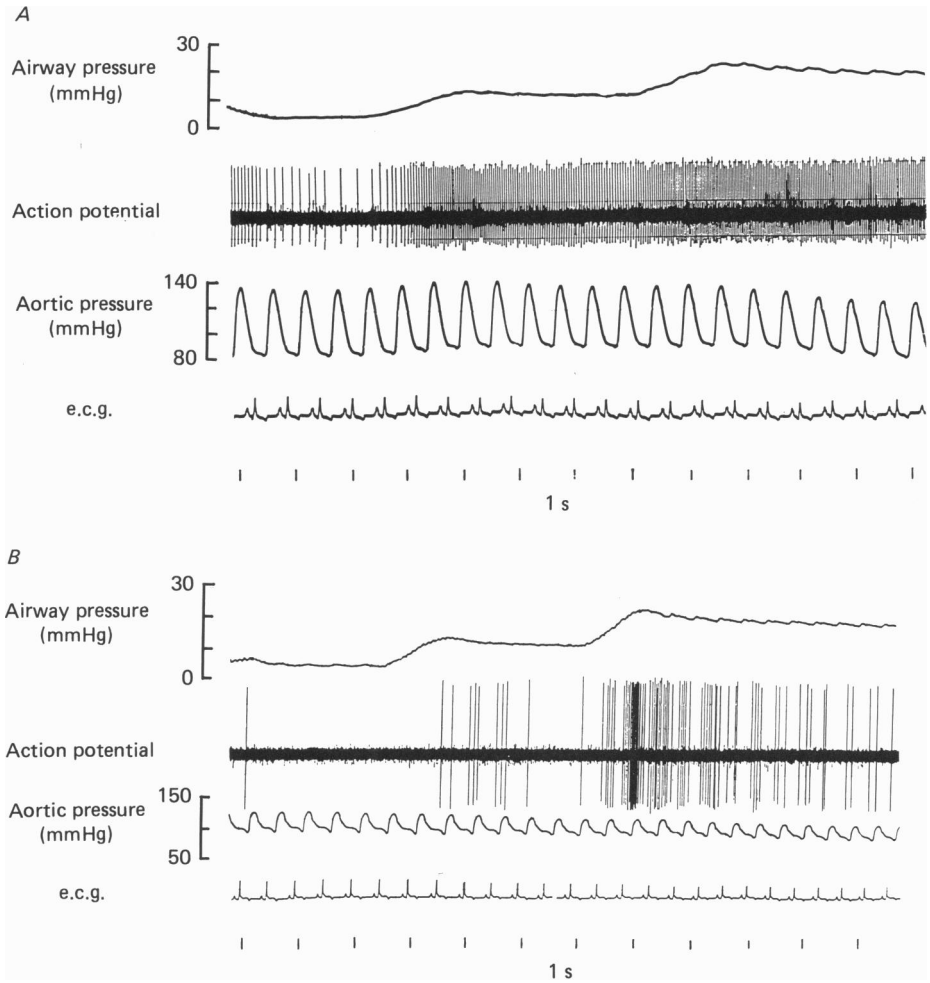

Fig. 1. Examples of s.a.r. (*A*) and r.a.r. (*B*) showing the effects of sustained inflation of the lungs. The adaptation index was calculated after three breaths (see text). The s.a.r. (conduction velocity 24·3 m/s) showed sustained activity and the r.a.r. (conduction velocity 20·0 m/s) showed adaptation. Each diagram shows, from above downwards: airway pressure (mmHg), nerve action potentials, aortic pressure (mmHg), electrocardiogram (e.c.g.) and times (s). Note that the expiratory line of the ventilator was clamped during the expiratory phase of the first cycle for the s.a.r. in *A* above.

73–91 %). The average conduction velocity in these fibres was $25{\cdot}6 \pm 10{\cdot}4$ m/s (range 13·6–40·0 m/s).

During the initial control period, the average activity was $12{\cdot}3 \pm 4{\cdot}2$ action potentials/ventilatory cycle (inspiratory phase, $8{\cdot}8 \pm 3{\cdot}0$ action potentials/cycle; expiratory phase, $3{\cdot}5 \pm 1{\cdot}3$ action potentials/cycle). When the left atrial pressure was elevated, the activity increased to $22{\cdot}7 \pm 5{\cdot}7$ action potentials/cycle (inspiratory phase, $14{\cdot}5 \pm 4{\cdot}5$ action potentials/cycle; expiratory phase, $8{\cdot}2 \pm 2{\cdot}0$ action potentials/cycle). These increases were statistically significant ($P < 0{\cdot}005$ for overall changes, $P < 0{\cdot}01$ for the change during the inspiratory phase and $P < 0{\cdot}05$ for the

change during the expiratory phase). After the left atrial pressure was restored to the control level, the activity returned to values which were not different from those during the initial control period. An example of an r.a.r. is shown in Fig. 2*B*. The results of all the r.a.r. are summarized in Fig. 3*B*.

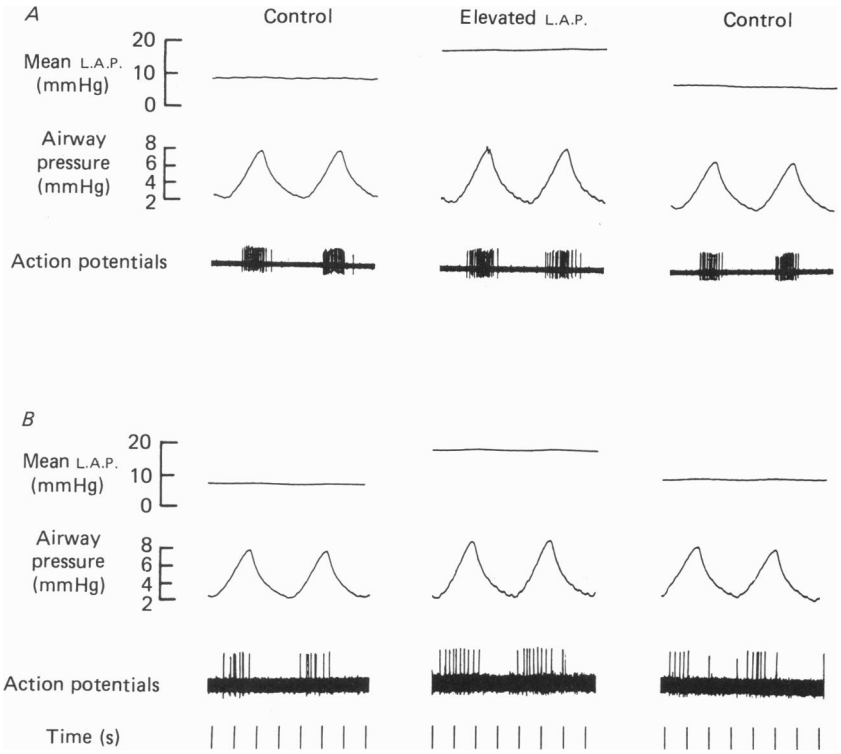

Fig. 2. Effect of an increase in mean left atrial pressure (L.A.P.) on the discharge from an s.a.r. (*A*) and from an r.a.r. (*B*). Tracings on the left were recorded before elevation of left atrial pressure, middle tracings 1 min after elevation and those on the right 1 min after return of left atrial pressure to control levels. The discharges from both receptors increased when the left atrial pressure was elevated.

During the period of elevated left atrial pressure, predominantly inspiratory activity was shown by four r.a.r., predominantly expiratory activity by four and equal activity during the two ventilatory phases by three. During the final control period, seven r.a.r. showed predominantly inspiratory activity, three predominantly expiratory activity and one equal activity.

Activity in bronchial C-fibre receptors. Nine bronchial C-fibre receptors having an average conduction velocity of $1{\cdot}3 \pm 0{\cdot}1$ m/s (range 0·5–1·7 m/s) were examined. All the units were activated $8{\cdot}8 \pm 0{\cdot}8$ s after injection of phenyldiguanide into the right atrium (Fig. 4*A*). The activity in the C-fibres was irregular with no apparent modulation caused by inflation and deflation of the lungs. Nevertheless, during the initial control period, their average activity was $8{\cdot}2 \pm 1{\cdot}6$ action potentials/ventilatory cycle (inspiratory phase, $3{\cdot}2 \pm 0{\cdot}6$ action potentials/cycle; expiratory phase, $5{\cdot}0 \pm 1{\cdot}2$ action potentials/cycle). When the left atrial pressure was increased,

the activity became 11·4±2·0 action potentials/cycle (inspiratory phase, 4·4±0·9 action potentials/cycle; expiratory phase, 6·7±0·9 action potentials/cycle). These increases were statistically significant ($P < 0.005$ for overall change, $P < 0.01$ for both phases of ventilation). When the left atrial pressure was restored to control values, the activities in these fibres were similar to those during the initial control period. An example of a response in a bronchial C-fibre is shown in Fig. 5*A*. The results of all the bronchial C-fibre receptors are summarized in Fig. 3*C*.

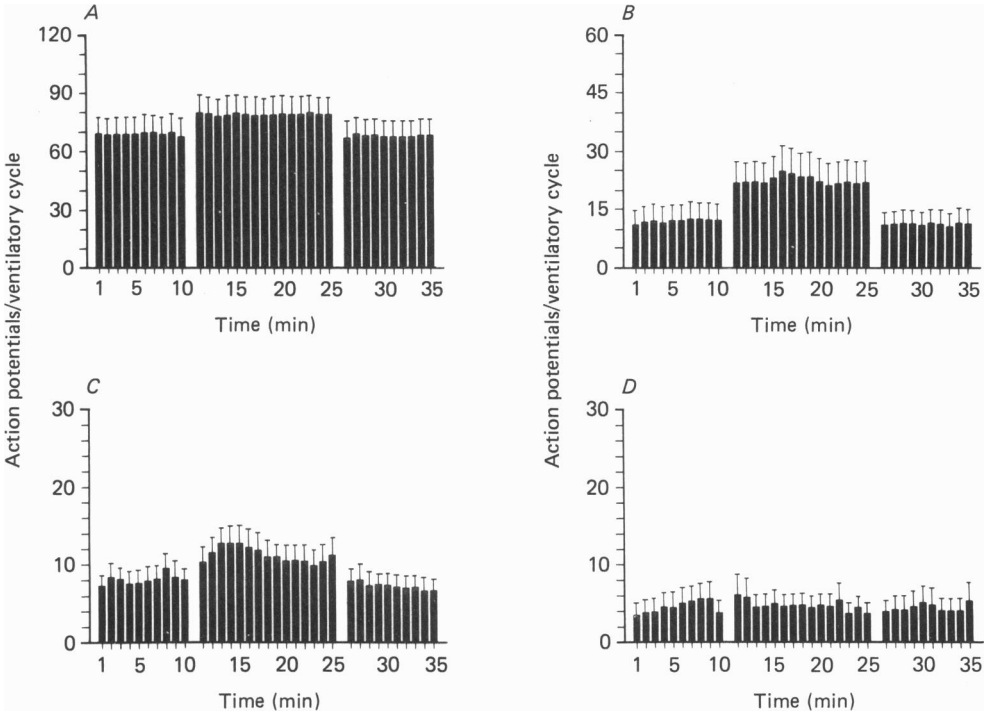

Fig. 3. Average receptor activity as action potentials per ventilatory cycle in each minute during 10 min of initial-control (1–10 min), 15 min of elevated left atrial pressure (11–25 min) and 10 min of final control (26–35 min). *A*, s.a.r. activity ($n = 15$); *B*, r.a.r. activity ($n = 11$); *C*, bronchial C-fibre receptor activity ($n = 9$); *D*, pulmonary C-fibre receptors activity ($n = 9$). Bars represent +S.E.M.

During the initial control period, three bronchial C-fibre receptors showed predominantly inspiratory activity and six predominantly expiratory activity. When the left atrial pressure was elevated, the proportion of receptors showing predominant activity in either phases of the ventilatory cycle remained unchanged, but one receptor changed from showing predominantly inspiratory activity to predominantly expiratory activity and another receptor showed the opposite change. During the final control period, two bronchial C-fibre receptors showed predominantly inspiratory activity and seven predominantly expiratory activity.

Activity in pulmonary C-fibres receptors. Nine pulmonary C-fibre receptors having an average conduction velocity of 1·0±0·05 m/s (range 0·8–1·2 m/s) were examined. They responded to capsaicin 1·8±0·2 s after injection of the drug into the right

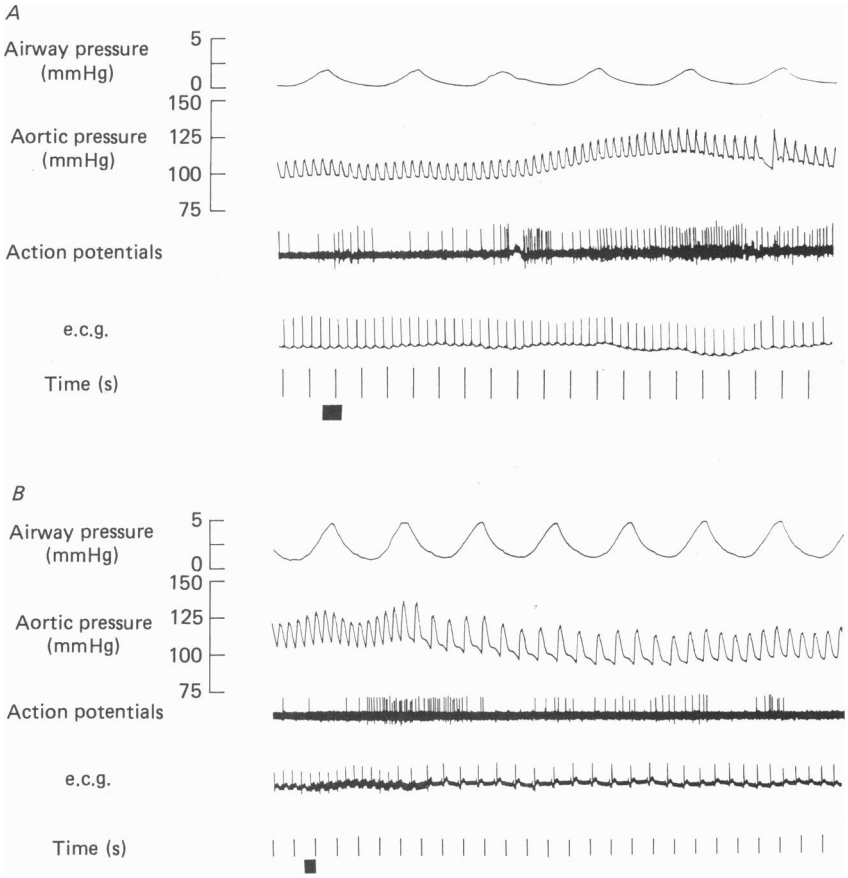

Fig. 4. Examples of a bronchial C-fibre receptor (*A*) and a pulmonary C-fibre receptor (*B*), showing the effects of administration of phenyldiguanide (*A*) and capsaicin (*B*) into the right atrium. The bronchial C-fibre receptor (conduction velocity 1·7 m/s) showed increased activity 7·5 s after phenyldiguanide and the pulmonary C-fibre receptor (conduction velocity 1·1 m/s) showed increased activity 2·0 s after capsaicin. Each diagram shows, from above downwards: airway pressure (mmHg), aortic pressure (mmHg), nerve action potentials, electrocardiogram (e.c.g.), time (s) and marker (filled bars) indicating administration of drugs.

atrium (Fig. 4*B*). Like the bronchial C-fibre receptors, these receptors were without any respiratory modulation.

During the initial control period, the average activity was $4{\cdot}6 \pm 1{\cdot}8$ action potentials/ventilatory cycle (inspiratory phase, $2{\cdot}0 \pm 0{\cdot}9$ action potentials/cycle; expiratory phase, $2{\cdot}6 \pm 0{\cdot}9$ action potentials/cycle). When the left atrial pressure was raised the corresponding activities were $4{\cdot}8 \pm 1{\cdot}6$, $2{\cdot}0 \pm 0{\cdot}8$ and $2{\cdot}8 \pm 0{\cdot}9$ action potentials/cycle, respectively. These changes were not statistically significant. After the atrial pressure was restored to control levels, the activities were $4{\cdot}5 \pm 1{\cdot}8$, $2{\cdot}1 \pm 1{\cdot}0$ and $2{\cdot}4 \pm 0{\cdot}8$ action potentials/cycle, respectively. An example of a response in a pulmonary C-fibre receptor is shown in Fig. 5*B*. The results of all the pulmonary C-fibre receptors are summarized in Fig. 3*D*.

Two pulmonary C-fibre receptors showed predominantly inspiratory activity, four predominantly expiratory activity and three equal activity in both phases of ventilation during the initial control period. When the left atrial pressure was increased, one receptor showed predominantly expiratory activity and eight predominantly inspiratory activity. During the final control period two showed

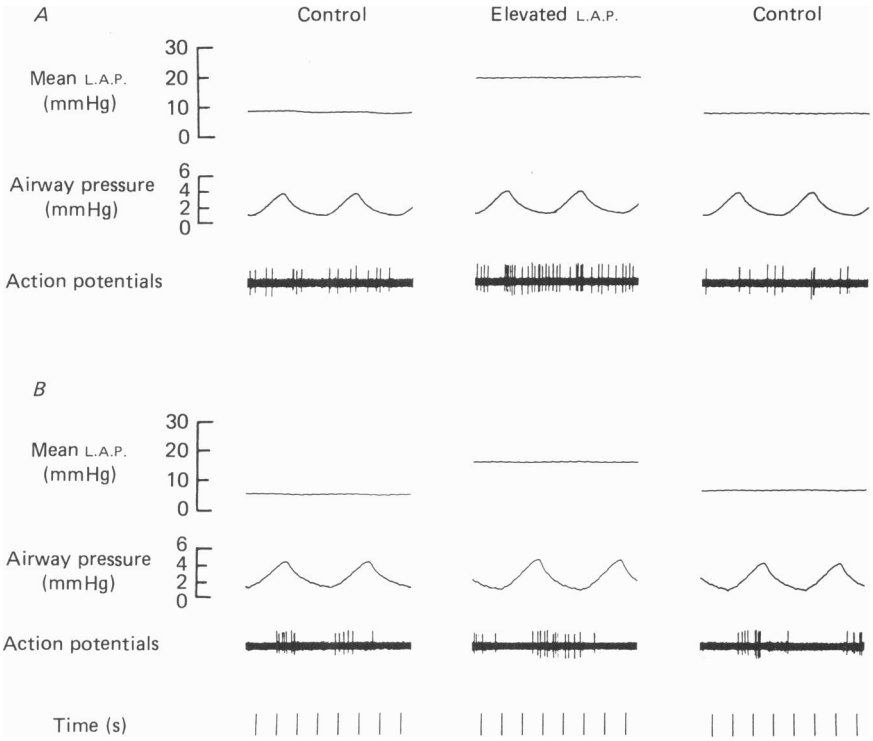

Fig. 5. Effect of an increase in mean left atrial pressure (L.A.P.) on the discharge from a bronchial C-fibre receptor (*A*) and a pulmonary C-fibre receptor (*B*). The receptor activity increased during stimulation in these two units. Tracings on the left were recorded before elevation of left atrial pressure, middle tracings 1 min after elevation and those on the right 1 min after return of left atrial pressure to control levels.

predominantly inspiratory activity, six predominantly expiratory activity and one equal activity.

In general, partial obstruction of the mitral valve was associated with a reduction in arterial pressure and an increase in heart rate. In addition, there were small but significant increases in mean and peak airway pressures ($P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively). These changes are summarized in Table 1.

Protocol 2

In this protocol, the changes in the activity of the pulmonary receptors brought about by graded increases in left atrial pressure were examined in seventeen dogs. It was found that the s.a.r., r.a.r. and bronchial C-fibre receptors showed graded

TABLE 1. Cardiovascular parameters, airway pressures and arterial blood gas measurements during control periods and during stimulation of pulmonary receptors

	Control*		Stimulation
Heart rate (beats/min)	150±3		157±6
Mean aortic pressure (mmHg)	111±3		97±4
Mean left atrial pressure (mmHg)	7·5±0·2		16·9±0·3
Mean airway pressure (mmHg)	2·44±0·16	$P < 0·01$	2·60±0·22
Peak airway pressure (mmHg)	5·13±0·20	$P < 0·001$	5·56±0·22
Arterial blood gases:			
pH	7·33±0·01		7·33±0·01
P_{CO_2} (mmHg)	34·7±1·4		34·7±0·7
P_{O_2} (mmHg)	212·6±11·9		197·3±14·3
$[HCO_3^-]$ (mmol/l)	17·2±0·7		17·5±0·2

* Average of the control observations during the 10 min before and after stimulation of the pulmonary receptors.

TABLE 2. Pulmonary receptor activity (action potentials/ventilatory cycle) during control periods and during graded increases in left atrial pressure

		Increases in left atrial pressure			
	Control	5 mmHg	10 mmHg	15 mmHg	Control
s.a.r. ($n = 7$)	34·0±11·2*	35·9±12·1*	38·8±13·6‡	41·8±13·7†	34·5±11·8*
r.a.r. ($n = 5$)	7·9±2·9*‡	12·3±5·4‡	16·3±7·4†	20·4±8·8†	7·1±1·7*
Bronchial C fibres ($n = 5$)	8·9±1·9*	11·6±2·4‡	16·2±3·0†‡	17·2±3·1†	9·8±2·0*
Pulmonary C fibres ($n = 5$)	8·8±4·0*	9·2±4·0*	9·3±4·0*	9·9±4·5*	9·1±4·3*

In each category of receptors, values with different suffixes are significantly different from each other ($P < 0·05$).

TABLE 3. Effects of blocking transmission in the cervical vagi on the responses of r.a.r. to increases in left atrial pressures (*A*) and, bilateral carotid artery occlusion on r.a.r. activity (action potentials/ventilatory cycle; $n = 4$) (*B*)

(*A*) Vagal block	At 37 °C	At 8 °C	After vagotomy (37 °C)‡
Control	9·1±2·2	8·7±0·9	9·4±1·4
Left atrial pressure elevation	18·3±1·8*	20·0±6·0*	19·7±5·2*
Control	10·6±1·5	7·3±0·7	8·2±1·0
(*B*) Carotid occlusion			
Control	11·3±1·0		
Carotid occlusion†	11·6±1·2		
Control	11·0±0·9		

* Significantly increased from controls ($P < 0·01$).
† During carotid occlusion left atrial pressure was not changed.
‡ Vagotomy performed on the side of recording above the site.

increases in activity when the left atrial pressure was raised in this manner. This trend was statistically significant ($P < 0·05$). The trend in the pulmonary C-fibre receptors was not significant. The findings are summarized in Table 2.

Protocol 3

In this protocol, specific attention was paid to the behaviour of the r.a.r in response to an increase in left atrial pressure. As observed in protocol 1, the r.a.r. appeared to have the most profound response to this stimulus.

In four units, the activity was examined after interrupting transmission in the cervical vagi above the point of recording. Cooling the vagi to 8 °C to block transmission in the myelinated afferent fibres did not influence either the control activity or the response to an increase in left atrial pressure (+10 mmHg). Cutting the vagi at the site of cooling was without effect also. In these four dogs, the small

TABLE 4. Localization of pulmonary receptors studied

Location	s.a.r.	r.a.r.	Bronchial C fibres	Pulmonary C fibres
Trachea–carina	6	3	—	—
Bronchi				
Right main	6	2	—	—
Left main	3	2	—	—
Right upper lobe	2	1	—	—
Right lower lobe	1	5	—	—
Left lower lobe	—	1	—	—
Lungs				
Right upper lobe	—	—	2	5
Right middle lobe	—	—	2	1
Right lower lobe	—	—	—	1
Left upper lobe	—	—	1	1
Left middle lobe	—	—	3	—
Left lower lobe	—	—	2	1
Total	18	14	10	9

changes in airway pressures noted in protocol 1 persisted even after the vagotomy (+0·35 mmHg for peak airway pressure and +0·23 mmHg for mean airway pressure).

In four units, the effects of bilateral carotid occlusion on the activity of r.a.r. was examined. The activity during carotid occlusion did not differ significantly from that during the control periods before and after the occlusion (Table 3).

Localization of receptors. Of the receptors investigated in this study, eighteen s.a.r., fourteen r.a.r., nine pulmonary C-fibre receptors and ten bronchial C-fibre receptors were localized to the lungs and large airways. All the s.a.r. and r.a.r. were found to be in the main bronchi while the bronchial C-fibre receptors and the pulmonary C-fibre receptors were in the lung parenchyma. The locations of the receptors are given in Table 4. Localization was not attempted in the other units to avoid collapsing the lungs mechanically, as it was felt that such disturbances could affect the behaviour of the receptors. As a consequence, in fourteen out of the fifty-three dogs, more than one receptor was examined. Of these, more than one type of receptor was examined in only two animals.

DISCUSSION

In this investigation an attempt has been made to examine the influence of relatively small degrees of pulmonary venous hypertension on the activity of pulmonary receptors in a single species, the dog. There are two aspects of this study which are novel. The first is that the degree of pulmonary venous hypertension was

relatively small (increments in the left atrial pressure up to 15 mmHg) and the second is that the increase in pressure was maintained for a period of 15 min. It is emphasized also that the findings of this study are derived from animals with an open chest, during intermittent positive-pressure ventilation.

Effect on slowly adapting receptors

One of the earliest studies on the effect of pulmonary venous congestion was that reported by Bulbring & Whitteridge (1945). They observed that in perfused preparations of the cat lung, pulmonary venous congestion (up to 20 cmH_2O) was without any significant effect upon the activity of vagal stretch receptors. Later, Marshall & Widdicombe (1958) re-examined the problem in anaesthetized cats (closed chest) and found that increments of left atrial pressure of 20–40 cmH_2O produced a significant increase in activity in the stretch receptors. In these studies, the period of stimulation was less than 2 min. There are no other investigations of a comparable nature in the dog. In the present study we observed a small but sustained increase in the discharge of these receptors during periods of elevated left atrial pressure which lasted 15 min. In addition, it was possible also to grade the response over a range of pressures up to 15 mmHg.

Effect on rapidly adapting receptors

The effects on irritant receptors was examined by Sellick & Widdicombe (1969) in anaesthetized cats. Again, the left atrial pressure was increased by inflating a balloon in the lumen of the left atrium but the actual pressures in the left atrium were not recorded. The period of stimulation was 1–2 min and the responses were not consistent in all the fibres examined. In the present study in the dog, it was found that an increase in left atrial pressure produced a large sustained increase in activity in the discharge from the receptors. In contrast to the effect of increments in airway pressure (Widdicome, 1954) the effect of an increase in left atrial pressure did not show evidence of adaptation. During graded increases in left atrial pressure, the r.a.r. showed a graded response in receptor activity.

Effects on C-fibre afferents

There has been considerable debate on the effect of pulmonary congestion upon the discharge from the C-fibre receptors originating from the lung. In the case of the pulmonary (J) receptors, Anand & Paintal (1980) have demonstrated, in the cat, that pulmonary congestion caused by augmenting the inflow of blood into one lung activated the receptors in that lung. This phenomenon also was not a consistent finding in all the receptors examined. The bronchial C-fibre receptors and the pulmonary C-fibre receptors were examined in the dog by Coleridge & Coleridge (1977*b*). In the case of the bronchial C-fibre receptors, partial obstruction of the mitral valve was found to activate these receptors but the phenomenon could not be demonstrated in all receptors examined (Coleridge & Coleridge, 1975, 1977*b*). In general, the majority of the receptors examined were activated when the pressures were increased to levels exceeding 20 mmHg. Having reviewed the available information, Coleridge & Coleridge (1984) concluded that the pulmonary C-fibre receptors were more consistent in their responses to the changes in left atrial

pressure. In the present study, it was found that in protocol 1, six out of nine pulmonary C-fibre receptors and nine out of nine bronchial C-fibre receptors responded to an increase in left atrial pressure of 10 mmHg. Thus in general, the behaviour of these receptors over a period of stimulation of 15 min was comparable to that described by Coleridge & Coleridge (1977*b*). There was no significant adaptation in the activity of these receptors to increases in left atrial pressure.

Time course of responses

The responses in receptor activity were obtained within 1 min of increasing left atrial pressure and returned to control levels within 1 min of deflation of the left atrial balloon. This response was maintained, with little fluctuation, especially in the case of s.a.r. and r.a.r., during the period for which the left atrial pressure was raised (15 min in protocol 1 and 5 min each in protocol 2). Guyton & Lindsey (1959), employed a model similar to that used in the present study, to examine changes in pulmonary fluid volumes. They observed that at normal plasma protein concentrations, pulmonary oedema occurred after 30 min only when the left atrial pressure exceeded 25 mmHg (see Fig. 1, Guyton & Lindsey, 1959). Since the pressures employed in the present study (Table 1) were lower than this critical value, it would seem unlikely that oedema of the lung was the cause of the increase in activity of the receptors observed in the present study (see below).

Speculation on mechanisms

Pulmonary venous congestion caused by partial obstruction of the mitral valve has been shown to increase the activity in s.a.r., r.a.r. and bronchial C-fibre receptors. In view of the nature of the stimulus used, it is of interest to speculate upon the nature of the change transduced by these receptors.

Effect of a change in bronchomotor tone. Since the s.a.r. and r.a.r. are located in the large airways (both extra- and intra-pulmonary) (Barlett, Jeffrey, Sant'Ambrogio & Wise, 1976; Sant'Ambrogio, Remmers, De Groot, Callas & Mortola, 1978), it is possible that the changes observed in the activity in these receptors were mediated by a concurrent change in tone of the smooth muscle of the airways. In the dog, partial obstruction of the mitral valve produces a change in total pulmonary airway resistance. Hogg, Agarawal, Gardiner, Palmer & Macklem (1972) showed that this increase in resistance resided primarily in the smaller airways. However, recent studies from this laboratory using a stimulus similar to that described in the present paper, have shown that increments in the left atrial pressure of 10 mmHg caused a reflex increase in tone in the trachea at a site immediately below the larynx (Teo, Man & Kappagoda, 1985*b*). Thus, secondary influences of changes in bronchomotor tone upon receptor activity should be considered.

One approach to the problem is to examine the influence of agents which alter bronchmotor tone by a direct effect upon the smooth muscle of the airways. In the dog, acetylcholine increases bronchial tone by such a direct effect (Dixon, Jackson & Richards, 1979). The studies of Davenport, Lee, Lee, Yu, Miller & Frazier (1981) suggest that, in the dog, the changes in bronchomotor tone necessary to bring about increases in the activity of s.a.r., result in increments in peak airway pressure in excess of 10 cmH_2O (7·4 mmHg). The effect of changes in bronchomotor tone upon

the activity of r.a.r. in the dog was examined by Vidruk, Hahn, Nadel & Sampson (1977). They found that the activity of the r.a.r. was almost unchanged even after the peak airway pressure had increased by more than 20 cmH_2O (14·8 mmHg) following the injection of acetylcholine. There are no comparable studies on the bronchial C-fibre receptors in the dog. The changes in peak airway pressure which resulted from the stimulus employed in the present study were very small in comparison to those observed in the above investigations. Thus a change in bronchomotor tone, *per se*, is unlikely to have produced the findings of the present investigation.

Further, bilateral carotid occlusion, which is a procedure which produces an enhancement in sympathetic activity, failed to influence both the discharge of the r.a.r. in the control state and the effect of mitral valve obstruction.

Effect of a change in compliance of the lung. In the present study, both the mean airway pressure and the peak airway pressure, as measured at the carina, increased when the left atrial pressure was raised. Since these changes in airway pressures persisted after sectioning the vagi (+0·35 mmHg before and +0·23 mmHg after vagotomy), it is likely that they were secondary to a change in the (dynamic) compliance of the lungs. It is not possible to establish with any certainty the influence of changes in dynamic compliance signified by such small changes in airway pressures (+0·16 mmHg for mean, +0·43 mmHg for peak pressure protocol 1) upon the activity of the pulmonary receptors. In the cat, it was found that injection of micro-emboli into the pulmonary arteries increased the peak intra-tracheal pressure and the activities of r.a.r., s.a.r. and pulmonary C-fibre receptors (Armstrong, Luck & Martin, 1976). Since the increase in peak intra-tracheal pressure persisted even after vagotomy, Armstrong *et al.* (1976) attributed it to a decrease in dynamic compliance of the lung. In their study, it was found that the activity of these receptors was not elevated in a persistent manner, until the peak airway pressure increased by more than 1 cmH_2O (0·74 mmHg). If these findings could be extrapolated to the dog, a reduction in compliance, as signified by the changes in airway pressure observed in the present study, is unlikely to influence the activity of the receptors.

Pulmonary venous congestion and oedema. This stimulus of partial obstruction of the mitral valve interferes with venous return from the lung, increases capillary filtration in the lung and eventually enhances the production of lymph. The studies of Guyton & Lindsey (1959) suggest that there is no significant oedema following exposure to an increase in left atrial pressure of 10 mmHg for a period of 15 min. Further, if the changes observed in the present study were the consequence of interstitial oedema, it is likely that the effect upon receptor activity would be progressive when a constant stimulus was applied as in protocol 1.

Apart from the issue of pulmonary oedema, it is conceivable that obstruction of the venous return from the lung could have a bearing upon the activity in the receptors. In addition to obstruction to flow from the lung parenchyma it is likely that the venous return from the bronchial mucosa is interfered with also. The proximal and distal bronchial mucosa derives its blood supply from the bronchial circulation but the venous return occurs via the pulmonary veins (Coleridge *et al.* 1965; Coleridge & Coleridge, 1977*a*). Within the bronchi, the veins are located

transversely and obstruction to flow in them could engorge the mucosal cell lining which contains the majority of the r.a.r. (Pietra & Fishman, 1978). Such speculation is supported by the time course of the 'on' and 'off' effects of the stimulus on the r.a.r. (Fig. 3) with both effects being observed within 1 min of the change.

In summary, the present study demonstrated that all the pulmonary vagal receptors are influenced by small changes in pulmonary venous pressure induced by partial obstruction of the mitral valve. Even though the magnitude of these changes appears to be greatest in the case of the r.a.r., the physiological significance of these responses remains to be investigated.

The authors thank Alvin Todd and Jacob Ahrend for their technical assistance. The authors acknowledge the financial support of the Medical Council of Canada. K. K. Teo is supported by the Alberta Heritage Foundation for Medical Research.

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