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THE ACTIVITY OF LUNG IRRITANT RECEPTORS DURING PNEUMOTHORAX, HYPERPNOEA AND PULMONARY VASCULAR CONGESTION

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SUMMARY

1. The activity of lung irritant receptors during pneumothorax, hyperpnoea and pulmonary congestion has been studied by recording from single vagal nerve fibres from the receptors in rabbits.

2. The receptors were stimulated during induction and during removal of pneumothorax.

3. Pneumothorax caused a greater depression of minute volume in bilaterally vagotomized rabbits, compared with those with intact vagus nerves.

4. Hyperpnoea due to breathing through an added dead space increased the discharge of the receptors. Experiments on paralysed and artificially ventilated rabbits showed that this was not a direct action of the asphyxial changes in blood gas tensions.

5. Pulmonary congestion, induced by inflating a balloon in the left atrium, stimulated the receptors in paralysed artificially ventilated rabbits.

6. The evidence that the receptors cause vagal reflex hyperpnoea and bronchoconstriction is discussed, together with their role in the reflex ventilatory and bronchomotor changes in the conditions studied.

INTRODUCTION

Lung irritant receptors are stimulated by intravenous injections of histamine and phenyl diguanide, and by pulmonary micro-embolism, anaphylaxis, and the inhalation of irritant gases (Mills, Sellick & Widdicombe, 1969). They are involved in the reflex bronchoconstriction and hyperpnoea of these conditions. The afferent fibres from the receptors are in the vagus nerves.

In addition to these chemically induced excitations, the receptors are stimulated by lung volume changes. It therefore seemed likely that their discharges would be affected by hyperpnoea and pneumothorax. If this

were so, two components of the respiratory changes in these conditions would be the reflex bronchoconstriction and the stimulation of breathing caused by irritant receptor discharge.

Pneumothorax causes hyperventilation (hyperpnoea with a decrease in arterial P_{CO_2}) probably by a vagal reflex (Binet, Strumza & Leobardy, 1948; Simmons & Hemingway, 1957; Hemingway & Simmons, 1958). The hyperpnoea due to severe hypercapnia or asphyxia can be decreased by vagotomy in experimental animals (Scott, 1908; Sasaki, 1927; Wiemer & Kiwull, 1965; Richardson & Widdicombe, 1965, 1969) and by vagal blockade in man (Guz, Noble, Widdicombe, Trenchard & Mushin, 1966). A third condition which induces reflex hyperpnoea by a vagal afferent pathway is pulmonary vascular congestion (Schwiegk, 1935; Daly, Ludány, Todd & Verney, 1937; Aviado, Li, Kalow, Schmidt, Turnbull, Peskin, Hess & Weiss, 1951; Downing, 1957). Several types of lung receptor will change their activity in these conditions. One example is the pulmonary stretch receptor which mediates the Hering–Breuer inflation reflex, but this group of endings is unlikely to account for the respiratory responses (see Discussion). Deflation receptors, with vagal C fibres, are stimulated by pulmonary congestion (Paintal, 1955). Rapidly adapting receptors, probably in the walls of the larger airways, increase their discharge in hyperpnoea (Knowlton & Larrabee, 1946) and in pneumothorax (Homberger, 1968). This paper describes a study of activity in vagal afferent fibres from irritant receptors within the lungs, during pneumothorax, hyperpnoea due to asphyxial blood gas changes, and pulmonary congestion.

METHODS

The methods were as described in the previous paper (Mills *et al.* 1969) with the following additions.

Pneumothorax was induced by injecting air from a 50 ml. syringe through a three-way tap into the right pleural cavity, through the cannula otherwise used to measure intrapleural pressure. Between each stepwise injection of air the tap was closed to the syringe so that transpulmonary pressure was recorded for several (usually three–five breaths. Apart from preliminary experiments, all pneumothoraces were induced and removed in steps of 10 ml. with a maximum of 50 ml. A femoral arterial blood sample was taken 30–60 sec after the 50 ml. pneumothorax had been completed, as well as control samples before induction and after removal of the pneumothorax. There was no attempt to allow the rabbits to reach respiratory equilibrium during the pneumothorax, and the 50 ml. maximum was maintained only for 1–2 min.

Hyperpnoea due to asphyxial blood gas tensions was produced in spontaneously breathing rabbits by the addition of a dead space of 80 ml. An arterial blood sample was taken when breathing had been clearly stimulated, usually after 30–60 sec. Blood samples were also taken before asphyxia and after recovery from the asphyxia. To test the effect of asphyxial gas tensions on irritant receptors in rabbits without hyperpnoea, the animals were first paralysed with gallamine triethiodide (10 mg) and artificially ventilated at a rate which maintained their end-tidal CO_2 % close to the pre-paralysis value. Asphyxia was induced by

connecting a Douglas bag containing an 8% O_2 and 5% CO_2 in N_2 mixture to the inlet of the ventilation pump. As well as control blood samples, a sample was taken after 1–2 min when end-tidal CO_2% had become constant.

Pulmonary congestion was produced by inflating a rubber balloon in the left atrium of paralysed and artificially ventilated rabbits. The chest was opened in the third or fourth left intercostal space and the balloon and catheter were tied into the left auricle. The chest was then closed around the catheter and the pneumothorax removed. The balloon was inflated with volumes of saline up to 1·5 ml., while femoral arterial pressure and end-tidal CO_2% were monitored. Inflations were maintained for 1–2 min. In some experiments the same inflations were then repeated while right atrial pressure was recorded in place of femoral arterial pressure.

During the experimental procedures vagal nerve impulses, arterial or right atrial pressure, tidal volume and transpulmonary pressure were displayed on a Tektronix 551 oscilloscope and photographed. The vagal discharge, transpulmonary pressure and tracheal airflow were also recorded on tape (Thermionix T1000) for subsequent analysis of impulse frequencies and of total lung resistance and lung compliance. End-tidal CO_2% was displayed on the meter of a Beckman LB1 infra-red CO_2 analyser, and the values were written down during experimental procedures. Arterial blood oxygen and carbon dioxide tensions were determined from 1–2 ml. samples by Radiometer gas-tension electrodes and galvanometer.

Impulse frequencies were measured over respiratory or ventilatory cycles, and expressed as impulses/sec. Values are usually means of 3–10 cycles.

RESULTS

The criteria for identification of lung irritant receptors are described in the previous paper (Mills *et al.* 1969). In the present study the criteria used were: rapid adaptation to maintained inflations and/or deflations of the lungs, irregular discharge patterns on excitation, stimulation by intravenous injections of histamine, and small diameter myelinated vagal fibres, judged by the size of the action potentials (relative to those from slowly adapting pulmonary stretch receptor fibres) and by some conduction velocity measurements. Stimulation by ammonia vapour or by contact with the tip of a polyethylene catheter deep to the tracheal bifurcation was regarded as supporting evidence. Receptors stimulated by the catheter at depths which suggested that they were in the walls of the airways outside the lungs were rejected.

Pneumothorax

Preliminary tests were made on seventeen lung irritant receptors. Pneumothoraces of up to 50 ml. were induced and removed. Fourteen of the receptors clearly increased their discharge during induction of the pneumothorax.

For the pneumothoraces which were induced, progressively increased and then reduced in steps of 10 ml., five receptors were studied in rabbits with the left vagus nerve intact, and six receptors in rabbits with both vagus nerves cut. Figure 1 shows experimental records, Fig. 2 a sequential plot of some of the variables measured in one experiment, and Fig. 3 mean

changes in impulse frequencies and ventilatory variables for the two groups of rabbits.

In the experiments with left vagus nerve intact, during induction of pneumothorax, impulse frequency increased from a control value of $1{\cdot}8 \pm 1{\cdot}12$ by $+2{\cdot}6 \pm 0{\cdot}79$ impulses/sec (means and S.E.; $n = 25$ measure ments, $P < 0{\cdot}01$ for the change in frequency). During removal of the pneumothorax the discharge increased by $+8{\cdot}6 \pm 2{\cdot}35$ impulses/sec com-

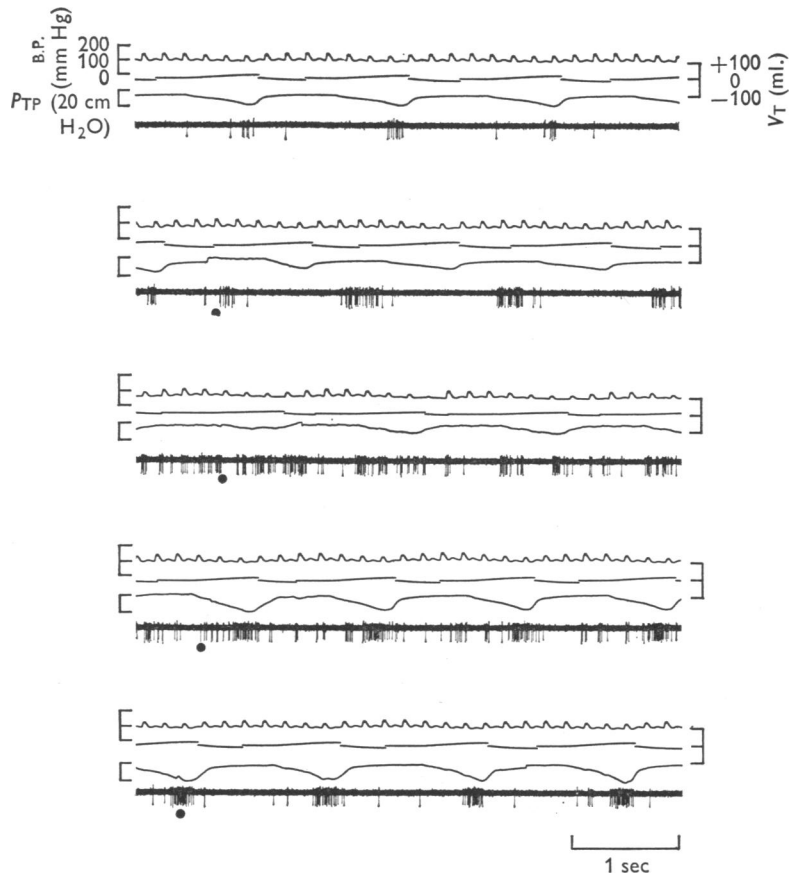

Fig. 1. Discharge of a single irritant receptor during induction and removal of pneumothorax. Traces from above down: systemic arterial blood pressure (B.P.); tidal volume (V_T) zeroing at times of zero airflow; transpulmonary pressure (P_{TP}); and action currents from a single vagal nerve fibre. Records from above down: control; injection of 10 ml. into the right intrapleural space; increase of pneumothorax from 40 to 50 ml.; decrease of pneumothorax from 40 to 30 ml.; decrease of pneumothroax from 10 to 0 ml. The records are examples of progressive induction and removal of pneumothorax in steps of 10 ml. Both vagi had been cut. The black dots indicate times of pneumothorax volume change. In this and some of the other Figures the action potentials have been retouched.

pared with the pre-pneumothorax control ($P < 0{\cdot}01$). The corresponding values for the group of bilaterally vagotomized rabbits are: control, $6{\cdot}2 \pm 0{\cdot}95$; during induction, $+11{\cdot}8 \pm 3{\cdot}02$ ($n = 30$, $P < 0{\cdot}01$); during removal of the pneumothorax, $+14{\cdot}2 \pm 3{\cdot}20$ impulses/sec ($P < 0{\cdot}01$). As Fig. 3 indicates, there was considerable variation in the size of the responses for individual receptors. However, for results with left vagus nerve intact, of twenty-five measured impulse frequencies during induction of pneumothorax twenty-two showed increases in discharge; during removal of the pneumothorax, of twenty-five measurements twenty-four were greater

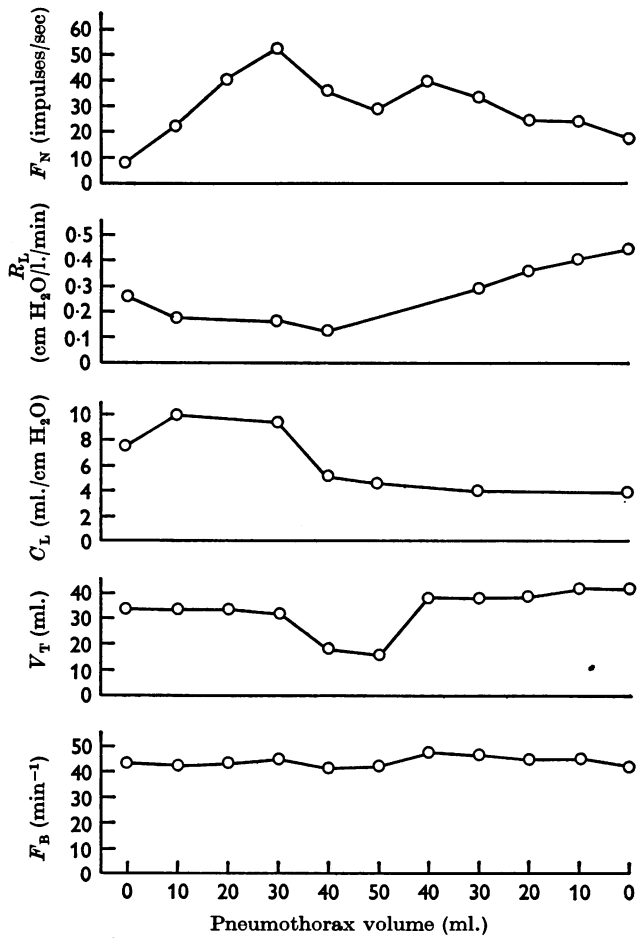

Fig. 2. Sequential plot of some of the variables measured in one experiment during induction and removal of pneumothorax. Abscissa, pneumothorax volume. Ordinates, from above down: impulse frequency (F_N) in a single vagal fibre from a lung irritant receptor; total lung resistance (R_L); lung compliance (C_L); tidal volume (V_T) and breathing frequency (F_B). The rabbit was bilaterally vagotomized.

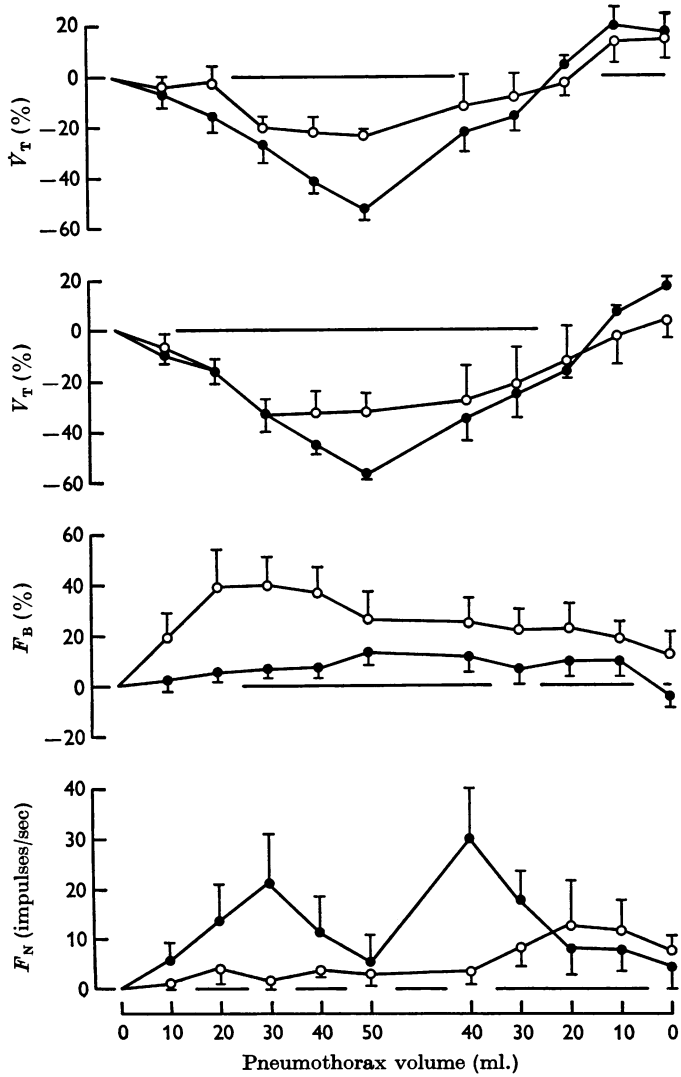

Fig. 3. Sequential plot of mean changes in variables during induction and removal of pneumothorax. — ○ — means of five experiments on rabbits with left vagus nerve intact. — ● — means of six experiments on rabbits with both vagi cut. Vertical lines give standard errors of the means. Values are expressed as changes from pre-pneumothorax controls, and are percentage changes except for impulse frequencies which are absolute changes. Abscissa: volume of pneumothorax. Ordinates, from above down: minute volume ($\dot{V}_T$); tidal volume (V_T); breathing frequency (F_B); and impulse frequency (F_N) in single vagal nerve fibres from lung irritant receptors.

than the control frequency. In the bilaterally vagotomized rabbits, of thirty measurements during induction of pneumothorax twenty-two showed increases in discharge; during removal of the pneumothorax, again twenty-two of thirty measurements were greater than the corresponding control values.

The mean receptor discharge values in Fig. 3 are typical of individual results in several other respects. There was considerable variation in discharge at different levels of pneumothorax (Fig. 2); after removal of all the pleural gas most receptors (ten out of eleven) had a discharge frequency greater than that for the pre-pneumothorax control; and impulse frequencies tended to be higher during removal of the pneumothorax than during its induction (all experiments with left vagus nerve intact, and four of the six experiments with both vagi cut). For the experiments on bilaterally vagotomized rabbits four out of six receptors showed conspicuous peaks of discharge at pneumothorax volumes of 30–40 ml. during induction and removal of pneumothorax. This pattern of response is reflected in the mean values in Fig. 3. During individual experiments there was no clear correlation between the change in receptor discharge frequency and the changes in any of the other variables measured (e.g. Figs. 1 and 2; see also Discussion).

Since pneumothorax and its removal increased the discharge frequency of lung irritant receptors, we tried to determine the effect of vagal integrity and bilateral section on the respiratory responses to pneumothorax in our particular experimental conditions. Figure 4 illustrates the results. For rabbits with both vagus nerves intact induction of pneumothorax caused an increase in breathing frequency and a decrease in tidal volume, with little change in minute volume. There was an increase in minute volume during removal of the pneumothorax. When both vagus nerves had been cut the increase in breathing frequency was much smaller, tidal volume decreased more and minute volume was considerably reduced. In these experiments blood gas samples were taken before and after the pneumothoraces, and during the maximum stage of pneumothorax. Figure 4 shows that vagal integrity lessened the asphyxial blood gas changes and the decrease in minute volume seen in the rabbits with both vagi cut.

The values for lung compliance and for total lung resistance in Fig. 4 indicate that vagal integrity abolished the fall in compliance and reduced the increase in resistance seen in the bilaterally vagotomized rabbits during induction of pneumothorax. During removal of the pneumothorax total lung resistance and compliance approached the same values for the two vagal conditions. The interpretation of these lung mechanical changes will be considered in the Discussion.

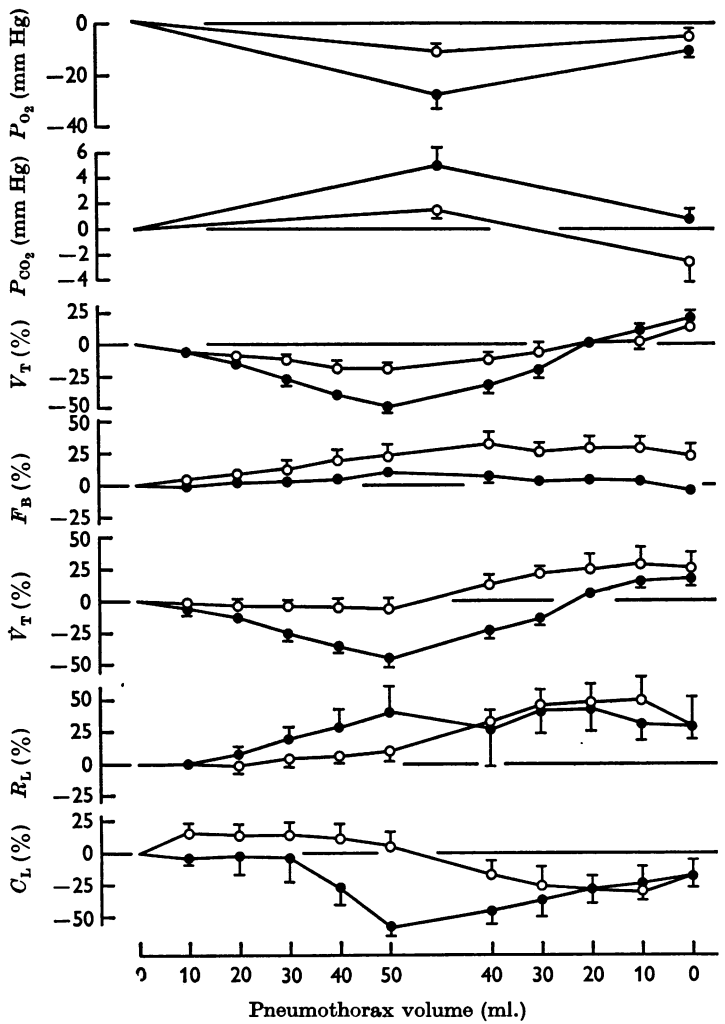

Fig. 4. Sequential plot of mean changes in variables during induction and removal of pneumothorax. — ○ — means of eight experiments on rabbits with both vagus nerves intact. — ● — means of six experiments on rabbits with both vagus nerves cut. Vertical lines give S.E. of the means (where not shown, the standard error was within the span of the symbol). Values are expressed as changes from pre-pneumothorax controls, and are percentage changes except for blood gas tensions which are absolute changes. Abscissa: volume of pneumothorax. Ordinates: arterial oxygen tension (P_{O_2}); arterial carbon dioxide tension (P_{CO_2}); tidal volume (V_T); breathing frequency (F_B); minute volume ($\dot{V}_T$); total lung resistance (R_L); and lung compliance (C_L). Control values for blood gas tensions were for oxygen $64{\cdot}8 \pm 4{\cdot}9$ and $67{\cdot}2 \pm 5{\cdot}8$ mm Hg, and for carbon dioxide $34{\cdot}7 \pm 1{\cdot}4$ and $32{\cdot}8 \pm 4{\cdot}0$ mm Hg for the vagus intact and vagotomized groups respectively.

TABLE 1. Effect of hyperpnoea due to asphyxia, and of ventilation with 'asphyxial' gases on irritant receptor impulse frequency and on other variables

	Breathing, left vagus intact			Breathing, both vagi cut			Paralysed		
Variable	N	Control	Change	N	Control	Change	N	Control	Change
Impulse frequency (per/sec)	10	6·8 ± 1·4	+7·41 ± 1·14**	5	6·6 ± 1·69	+13·2 ± 4·50*	8	7·9 ± 1·6	−1·5 ± 1·28
Tidal volume (ml.)	10	30·2 ± 7·30	+27 ± 3·5 %**	4	60·0 ± 7·68	+22·5 ± 7·8 %	—	—	—
Breathing frequency (min^{-1})	10	42·7 ± 4·03	+16 ± 4·5 %**	4	25·8 ± 6·6	+17·8 ± 5·0 %*	—	—	—
Minute volume (l./min)	10	1·25 ± 0·33	+50·5 ± 8·2 %**	4	1·64 ± 0·56	+45 ± 8·6 %*	—	—	—
Total lung resistance (cm H_2O/l./min)	7	0·74 ± 0·17	+38 ± 12·4 %*	3	1·14 ± 0·13	+17 ± 16·9 %	4	0·48 ± 0·10	+4·1 ± 2·49%
Lung compliance (ml./cm H_2O)	7	3·3 ± 0·50	−17 ± 5·5 %*	3	3·1 ± 0·59	−8 ± 7·3 %	4	2·2 ± 0·11	+0·2 ± 3·76%
Arterial P_{O_2} (mm Hg)	7	76·4 ± 5·73	−39·5 ± 6·50**	4	73·2 ± 8·67	−34 ± 10·38*	8	65·2 ± 7·92	−22·8 ± 6·50**
Arterial P_{CO_2} (mm Hg)	7	35·4 ± 4·45	+14·0 ± 1·62**	4	37·7 ± 6·53	+12·9 ± 2·30*	8	40·3 ± 4·20	+13·7 ± 2·18**

Values are means ± S.E. * $P < 0·05$, ** $P < 0·01$. Changes are given as percentages apart from impulse frequencies and blood gas tensions. The 'breathing' columns refer to asphyxial hyperpnoea, and values correspond to measurements after about 1 min. The 'paralysed' columns refer to ventilation with an asphyxial gas mixture, and values correspond to measurements after 2 min. For the 'paralysed' values, three of the eight receptors were in rabbits with right vagus intact, the remainder in rabbits with both vagi cut.

Hyperpnoea due to asphyxia

Nerve impulse frequencies and other variables were measured during asphyxia-induced hyperpnoea in rabbits with the left vagus nerve intact (the right being cut for impulse recording) and in bilaterally vagotomized rabbits. Asphyxia was maintained for about 1 min. The results are summarized in Table 1; Fig. 5 illustrates an experiment, and Fig. 6 shows a sequential plot of some of the variables during one experiment. Fifteen tests on eleven receptors were made.

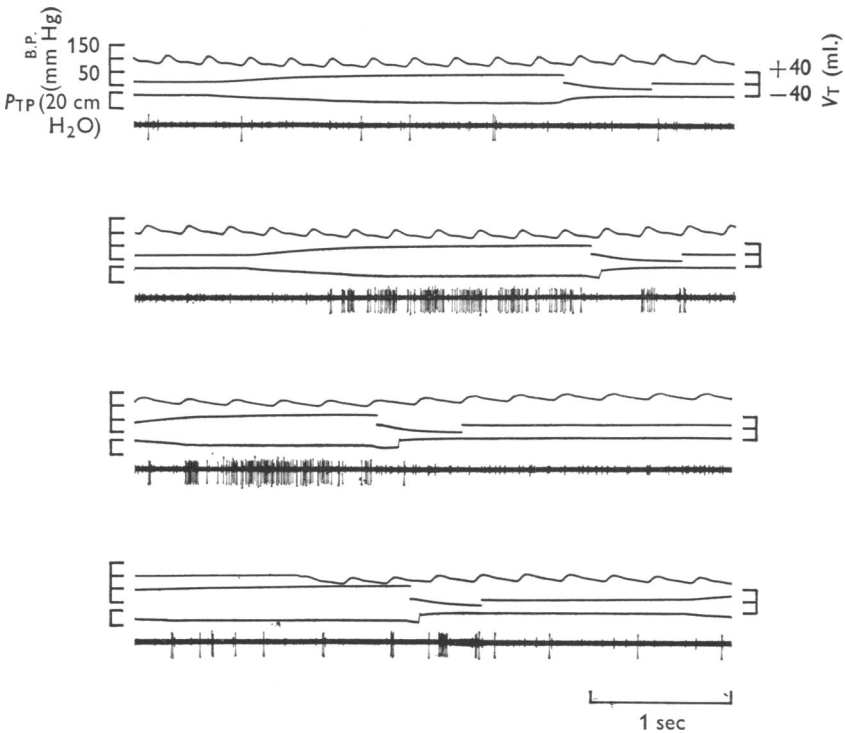

Fig. 5. Response of a lung irritant receptor to hyperpnoea caused by breathing through an added dead space. Traces as in Fig. 1. Records from above down: control before asphyxia; 22 sec after start of asphyxia; 65 sec; control after recovery from asphyxia. The rabbit was bilaterally vagotomized, and a single respiration is shown in each record. In the lowest record the blood pressure trace is interrupted while a blood sample was being taken.

In every instance impulse frequency increased during hyperpnoea and in both groups the mean increase in impulse frequency was statistically significant. The respiratory stimulations were of similar intensity in the two groups, as judged by the changes in blood gas tensions and in the patterns of breathing.

To see whether the increases in impulse frequency were secondary to the hyperpnoea or whether they were due to changes in blood gas tensions acting directly on the receptors, for eight receptors in six rabbits the animals were paralysed and artificially ventilated, and then asphyxiated with unchanged minute volume. Blood samples for gas analysis were taken after about 2 min. In five instances there was a decrease in impulse frequency, although the mean decrease for the eight receptors was small and

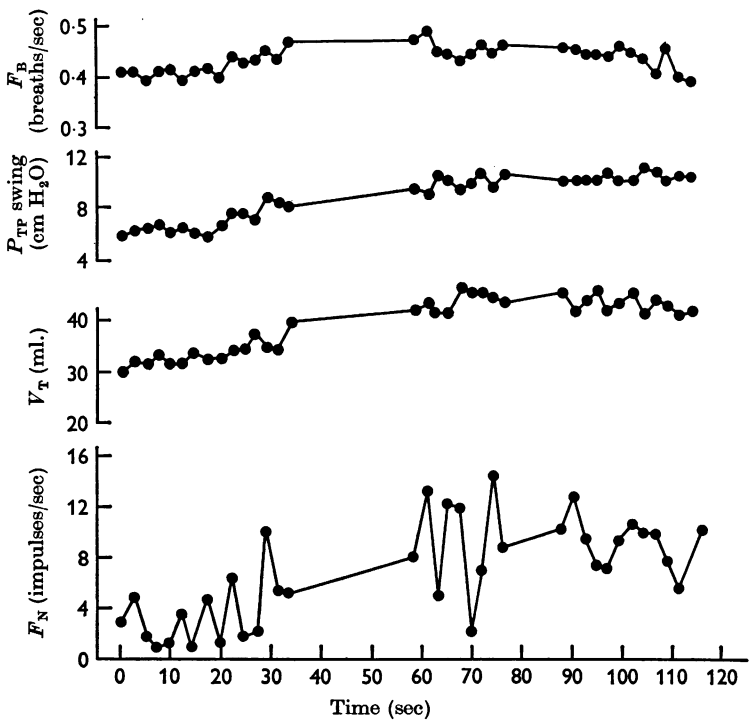

Fig. 6. Sequential plot of some of the variables measured in one experiment during hyperpnoea due to breathing through an added dead space. Abscissa: time. Ordinates from above down: frequency of breathing (F_B); transpulmonary pressure (P_{TP}) swing; tidal volume (V_T); and impulse frequency (F_N) in a single vagal fibre from a lung irritant receptor. Values are averaged over single breaths, and correspond to three periods of photographic recording. The rabbit was bilaterally vagotomized. Rebreathing started at 20 sec. Note the variation in control impulse frequency (time 0–20 sec) and frequency during asphyxia (time 58–76 sec).

not statistically significant (Table 1). The changes in blood gas tensions were not always as large as for the spontaneously breathing rabbits because the methods of inducing asphyxia were different. For both the breathing and the paralysed rabbits the blood samples were taken at the end of the periods of asphyxia, since frequently repeated blood sampling

was undesirable. In the spontaneously breathing animals the increase in receptor discharge was invariably progressive (e.g. Figs. 5 and 6) and present during the early hyperpnoea when it would be associated with lesser degrees of asphyxia than those measured in the paralysed and artificially ventilated rabbits. For three of the eight receptors we adjusted control ventilation to avoid asphyxia (P_{O_2} = 101, 89 and 90 mm Hg; P_{CO_2} = 23·5, 17·5 and 32·3 mm Hg), and gave stronger asphyxial gas mixtures to produce larger changes in gas tension (P_{O_2} = −55, −36 and −36 mm Hg; P_{CO_2} = +23·5, +29·0 and +18·3 mm Hg). The control receptor discharge frequencies were 12·6, 17·2 and 0·8 impulses/sec, and the changes in discharge were +4, −6 and +0·1 impulses/sec. We therefore believe that the increase in receptor discharge was caused by the hyperpnoea and not directly by the changes in blood gas tensions.

Pulmonary vascular congestion

Intravenous injections of isoprenaline into rabbits increase the discharge of lung irritant receptors, and this response is not secondary to respiratory changes since it is present in paralysed and artificially ventilated rabbits (Mills *et al.* 1969). Injections of adrenaline (10–50 μg, intravenously) have a similar action. In tests on ten receptors, the control and increase in discharge frequency were 4·1 + 1·96 and + 6·1 ± 2·73 impulses/sec (means and S.E., $P \simeq 0{\cdot}05$). Figure 7 illustrates a receptor response and Fig. 8 shows a sequential plot of some of the variables measured. The increase in receptor discharge did not follow the rise in blood pressure.

One possibility was that the receptors were being stimulated by lung vascular changes induced either by pulmonary venous constriction or by an imbalance between the outputs of the two sides of the heart. To test this we studied the responses of nine irritant receptors to inflation of a balloon in the left atrium. The rabbits were bilaterally vagotomized, paralysed and artificially ventilated to prevent possible receptor stimulation by the hyperpnoea associated with pulmonary congestion. Table 2 summarizes the results and Fig. 9 illustrates an experiment.

Out of twenty-nine tests, twenty-five showed increases in discharge. Two receptors each gave no change or small decreases in discharge on two out of three tests. Four of the seven tests with 1·5 ml. inflations, the largest balloon distension used, were with these two insensitive receptors, which therefore weight the corresponding mean values in Table 2. In three further experiments, not included in Table 2, balloon inflations of 0·25 and 0·5 ml. increased receptor discharges by 2·4, 7·5 and 0·9 impulses/sec, with no measurable change in total lung resistance and decreases in compliance of 2, 13 and 0%.

The pulmonary vascular congestion was relatively mild, judged by the

changes in blood pressure and lung mechanics (Table 2). The receptor discharges were almost doubled, but the changes in impulse frequency could not be correlated with changes in total lung resistance, compliance or vascular pressures (e.g. Figs. 9 and 10). In eight tests clear increases in impulse frequency were associated with unchanged total lung resistance, and in three instances with unchanged lung compliances. On emptying the left atrial balloon impulse frequency fell promptly to control values. Resistance and compliance were usually restored equally quickly, but occasionally there was a small residual decrease in compliance and increase in resistance. Figure 10 illustrates such an experiment, when seven balloon

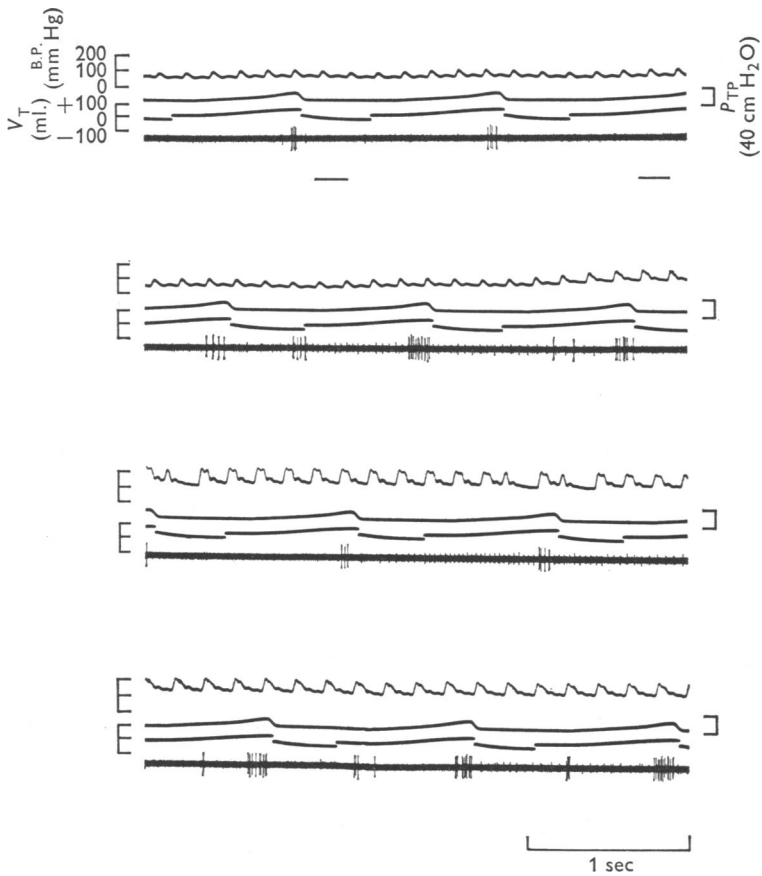

Fig. 7. Response of a lung irritant receptor to injection into the right atrium of 10 μg adrenaline (at first signal, washed in at second signal in uppermost record). Traces, from above downwards: systemic arterial blood pressure (B.P.): transpulmonary pressure (P_{TP}); tidal volume (V_T); and action potentials in a single vagal nerve fibre. Records from above down: control; at start of blood pressure rise; at maximum blood pressure response; and during subsequent fall in blood pressure. The rabbit was vagotomized, paralysed and artificially ventilated.

inflations were performed. There is a clear lack of correlation between impulse frequency and both resistance and compliance.

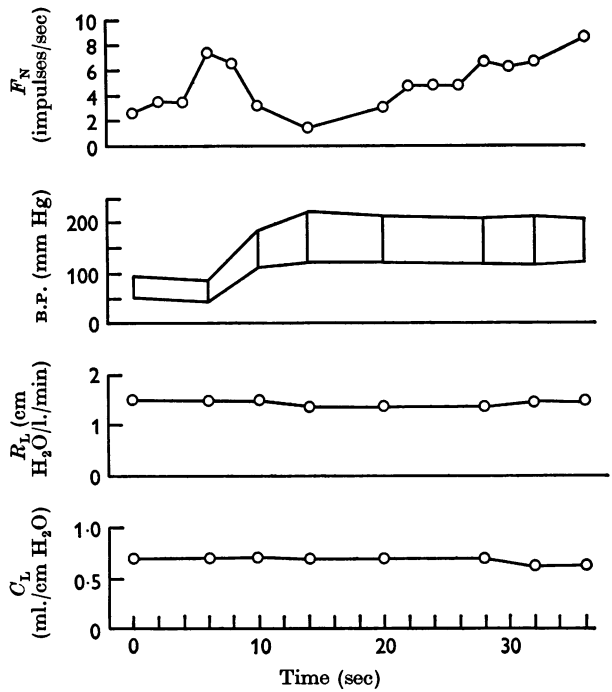

Fig. 8. Sequential plot of some of the variables measured in one experiment showing the response of a lung irritant receptor to injection of 10 μg adrenaline into the right atrium of a vagotomized, paralysed and artificially ventilated rabbit. Abscissa; time, with injection at zero time. Ordinates, from above down: impulse frequency (F_N) in a single vagal nerve fibre from a lung irritant receptor; systemic arterial blood pressure (B.P.), systolic and diastolic; total lung resistance (R_L); and lung compliance (C_L).

Spontaneous variation of discharge

There was often considerable variation in discharge frequency during stimulation which could not be correlated in size with changes in ventilatory or lung mechanical variables measured (Fig. 6) although the receptors often discharged with respiratory phase (e.g. Figs. 1 and 5). Similar variation was seen in 'control' impulse frequencies. For six receptors the coefficients of variation (ratio of standard deviation to the mean) for breath-to-breath average impulse frequencies during control conditions were 1·83, 1·07, 1·12, 0·81, 0·68 and 0·59. This variation in discharge frequency would tend to reduce the apparent statistical significance for changes in impulse frequency, although this tendency would be lessened by the averaging of impulse frequencies over seve ral ventilatory cycles.

TABLE 2. Effect of distension of a left atrial balloon on irritant receptor discharge frequency, total lung resistance, lung compliance, systemic arterial blood pressure, and right atrial blood pressure

Balloon distension volume (ml.)	Irritant receptor discharge: No. of Fibres	No. of Tests	Control frequency (impulses/sec)	Change in frequency (impulses/sec)	Lung mechanics: N	Change in resistance %	Change in compliance %	Vascular pressures: B.P. N	B.P. Change (mm Hg)	R.A.P. N	R.A.P. Change (cm H_2O)
0·75	4	9	5·2 ± 0·91	+4·8 ± 1·22**	7	+10·7 ± 2·35**	−7·6 ± 4·1	4	−20·2 ± 4·76*	3	+0·48 ± 0·30
1·0	9	13	5·6 ± 1·06	+4·3 ± 1·10**	9	+13·9 ± 5·82*	−8·8 ± 4·90	5	−9·4 ± 4·32	4	+1·29 ± 0·51
1·5	3	7	1·2 ± 0·39	+1·2 ± 0·60	5	+21·6 ± 5·71**	−24·6 ± 4·50**	4	−10·6 ± 2·33**	1	0
All	9	29	4·4 ± 0·62	+3·4 ± 0·62**	21	+15·4 ± 2·81**	−13·7 ± 2·67**	13	−13·4 ± 2·13**	8	+0·82 ± 0·27*

Values are means ± S.E. for the numbers of tests shown.

B.P. = systemic arterial blood pressure; R.A.P. = right atrial pressure. * $P < 0·05$, ** $P < 0·01$

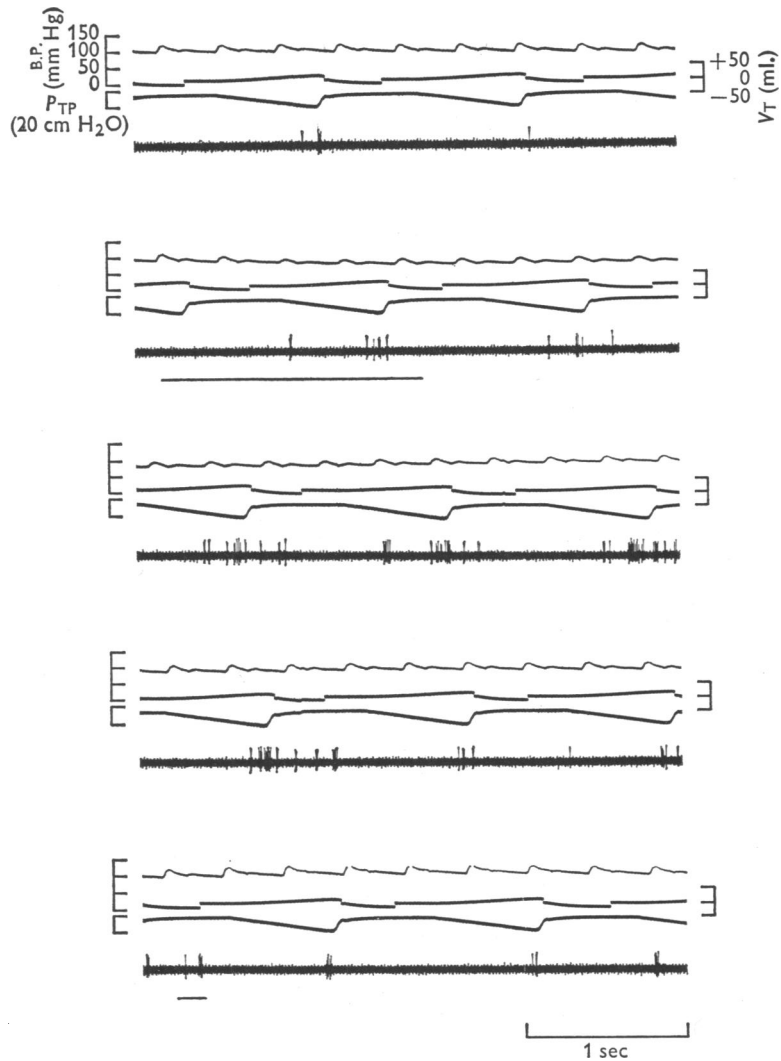

Fig. 9. Response of a lung irritant receptor to inflation of a balloon in the left atrium. Traces as in Fig. 1. Records, from above down: control; inflation of the balloon with 1 ml. at signal; continuation of previous record; 20 sec later; emptying of balloon at signal. The rabbit was vagotomized, paralysed and artificially ventilated.

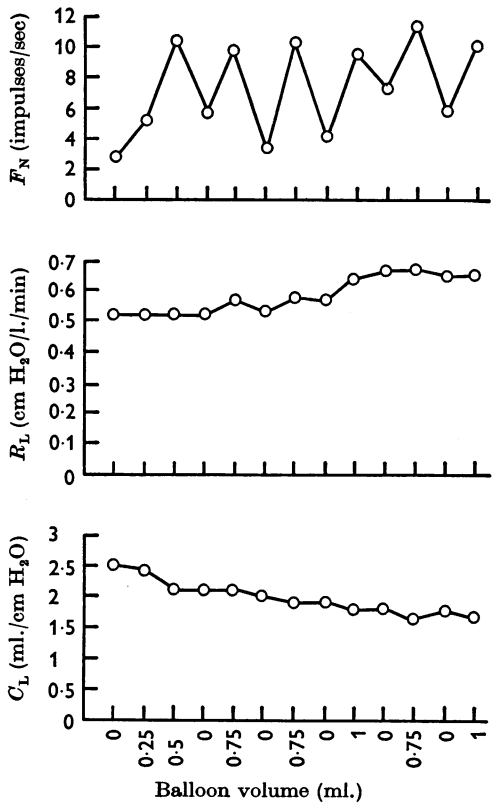

Fig. 10. Changes in impulse frequency (F_N) from a lung irritant receptor, total lung resistance (R_L) and lung compliance (C_L) during a series of inflations and deflations of a balloon in the left atrium. The abscissal values give balloon volumes, each volume change being maintained for about 30 sec. Note the lack of correlation between impulse frequency and either resistance or compliance. The rabbit was vagotomized, paralysed and artificially ventilated.

DISCUSSION

Pneumothorax. Homberger (1968) has shown that rapidly adapting lung mechanoreceptors with small diameter myelinated vagal fibres increase their discharge during pneumothorax. The receptors were not localized or identified, but if they are lung irritant receptors then our results confirm those of Homberger, and show in addition that the greatest stimulation is during removal of the pneumothorax. In animals with intact vagus nerves this discharge would contribute to the reflex effects of pneumothorax.

Pneumothorax in experimental animals can cause hyperventilation which is prevented by vagotomy (Binet *et al.* 1948; Simmons & Hemingway, 1957; Hemingway & Simmons, 1958). In man pneumothorax stimulates

breathing with a decrease in arterial P_{CO_2} (Richards, Riley & Hiscock, 1932) and, if the increased breathing is not due to chemical stimuli such as hypoxia, a reflex is probably involved. In our experiments induction of pneumothorax caused little change in minute volume when the vagus nerves were intact. Since the inspiratory muscles would be working at a mechanical disadvantage, the maintained minute volume would probably require an increased inspiratory neuromuscular drive. When the vagus nerves were cut there was hypoventilation with more asphyxial blood gas changes. These results suggest that a vagal reflex stimulates respiratory drive and minimizes blood gas changes. Removal of the pneumothorax stimulated breathing in rabbits with intact vagi, and this response corresponded with the increase in irritant receptor discharge during removal of the pleural gas.

Our results do not indicate if there are changes in nervously mediated bronchomuscular tone during pneumothorax, and there seems to be no published evidence on this. The changes in total lung resistance (Fig. 4) will reflect not only vagal efferent discharge, but also the pattern of breathing, the volume of gas in the lungs and changes in blood gas tensions. All may be affected by vagotomy. Similarly, the changes in lung compliance will be influenced by the pattern of breathing (since dynamic compliance was measured) and by the total lung gas volume (since the compliance curve is alinear over large tidal volumes). Thus although the results show that vagal integrity resulted in smaller increases in resistance and the absence of decrease in compliance during induction of pneumothorax, the mechaisms involved are not clear. There is similar uncertainty about the cause of the low compliance and high resistance recorded consistently during removal of the pneumothorax.

As well as lung irritant receptors, other afferent end-organs will be involved during pneumothorax. Most pulmonary stretch receptors (slowly adapting) decrease their discharge during collapse of the lungs (Adrian, 1933; Knowlton & Larrabee, 1946). The reflex effects of these receptors include depression of breathing (Ramos, 1956; Albers, Usinger & Pleschka, 1966) and their decreased discharge during induction of pneumothorax must therefore help to maintain minute volume. During removal of the pneumothorax the discharge from pulmonary stretch receptors would increase, and since they inhibit breathing they are unlikely to explain the increase in minute volume occurring at this time in rabbits with intact vagus nerves. Furthermore, Troelstra (1960) has shown by differential vagal cooling in rabbits that the main part of the respiratory reflex response in pneumothorax is mediated by a vagal pathway other than that from pulmonary stretch receptors. 'Deflation receptors' in the cat can be stimulated by pneumothorax, but the response is transient (0·5 sec)

and weak and only appears with extreme degrees of lung collapse (Paintal, 1955). In the rabbit 'deflation receptors' with non-myelinated vagal fibres are seldom stimulated by pneumothorax induced as described here (J. E. Mills, H. Sellick & J. G. Widdicombe, unpublished).

Hyperpnoea due to asphyxia. Hyperpnoea due to asphyxial blood gas tensions increased the discharge of lung irritant receptors. This is not surprising since they are stimulated by inflations and deflations of the lungs. The response was secondary to the respiratory changes and not primarily due to alterations in blood gas tensions, since with constant ventilation asphyxia caused no increase in discharge.

In rabbits, anaesthetized or unanaesthetized, bilateral vagotomy has little effect on hyperpnoea due to mild hypercapnia, but lessens the greater hyperpnoea due to more severe hypercapnia (P_{CO_2} increases of 15–30 mm Hg, and minute volume increases of 100–400 %) (Wiemer & Kiwull, 1965; Richardson & Widdicombe, 1965, 1969). In man bilateral anaesthetization of the vagus and glossopharyngeal nerves approximately halves the ventilatory response to breathing a CO_2 in O_2 mixture (Guz *et al.* 1966). Thus in both species there can be, in certain experimental conditions, a vagal reflex drive during hypercapnia which potentiates the hyperpnoeic response. This conclusion is based on the assumption that the degrees of hypercapnia in the presence of hyperoxia were not adequate to exert an appreciable respiratory drive by stimulation of aortic body chemoreceptors. This particular drive is unlikely to be due to pulmonary stretch receptors which, although they increase their firing in hyperpnoea, tend to depress breathing (see above); or to deflation receptors which, although they may stimulate breathing, are not activated by lung inflations or moderate lung deflations (Paintal, 1955). However, stimulation of lung irritant receptors could be the mechanism potentiating the hyperpnoea due to asphyxia and hypercapnia when the vagi are intact.

Table 1 shows that asphyxia did not cause a greater stimulation of breathing in rabbits with left vagus intact compared with bilaterally vagotomized rabbits. This may be because with mild hyperpnoea the increased inhibiting discharge of pulmonary stretch receptors may balance the potential excitatory action of irritant receptors; because one vagus was already cut; and because the depression of the hyperpnoeic response to hypercapnia caused by vagotomy is less in anaesthetized compared with unanaesthetized animals (Richardson & Widdicombe, 1965).

Our results give no indication about nervously mediated bronchomuscular tone during hyperpnoea since, as with pneumothorax, lung resistance and lung compliance measurements will be affected passively by changes in the pattern and level of breathing as much or more than by changes in efferent nervous discharge.

Pulmonary congestion. The lung irritant receptors were stimulated by pulmonary vascular congestion and, since the rabbits were paralysed, this response cannot be due to a change in the pattern of breathing. If in spontaneously breathing animals pulmonary congestion caused hyperpnoea, this would presumably lead to an even larger increase in discharge than we observed. The degree of congestion was not determined, but vascular pressure and lung compliance measurements indicate that it was not extreme.

Pulmonary vascular congestion causes an increase in minute volume which is prevented by bilateral vagotomy and is therefore probably due to a vagal reflex (Schwiegk, 1935; Daly *et al.* 1937; Aviado *et al.* 1951; Downing, 1957). Pulmonary stretch receptors increase their discharge during congestion of the lungs (Marshall & Widdicombe, 1958; Constantin, 1959), but the increase is small (about 20 %) and the reflex effect would be a depression of breathing (see above). Deflation receptors are stimulated by pulmonary vascular congestion (Paintal, 1955) and presumably they and the irritant receptors are jointly involved in the reflex hyperpnoea of this condition. There seems to be no evidence on possible changes in nervously mediated bronchomuscular tone during pulmonary congestion.

Modes of stimulation of lung irritant receptors. Evidence that the receptors studied are localized beneath or between the epithelial cells of the intrapulmonary airways has been considered previously (Mills *et al.* 1969). The endings are mechanoreceptors, as shown by their responses to inflation and deflation of the lungs, and to excitation by an intrabronchial catheter. Our results indicate that the discharge of lung irritant receptors in eupnoeic breathing rabbits with both vagus nerves intact would be small or absent, but there has been no means of testing this directly. Since the receptors are rapidly adapting their discharge depends not only on the size but also on the rate of change of the mechanical stimulus. In this respect they resemble rapidly adapting 'cough receptors' in the trachea and extrapulmonary bronchi (Knowlton & Larrabee, 1946; Widdicombe, 1954). Their stimulation during asphyxial hyperpnoea is therefore not surprising. Their response during pneumothorax and its removal is more complicated, since tidal volume decreased throughout most of the pneumothorax. However, breathing frequency increased. In addition the lungs were being ventilated in a more collapsed state, and the receptors may have been responding to this mechanical change. Collapsed lung is known to exert a greater pull on the bronchi and bronchioles (Severinghaus & Stupfel, 1955; Caro, Butler & Dubois, 1960) and this may play a part in the stimulation of discharge during induction and removal of the pneumothorax. The peaks of discharge seen in bilaterally vagotomized rabbits at pneumothorax

volumes of 30–40 ml. could be due to a maximum deformation of the bronchial wall at this volume, but there is no direct evidence on this.

The stimulation of irritant receptors during pulmonary vascular congestion was not due to changes in the pattern of breathing, since the rabbits were paralysed; or to generalized changes in lung mechanics, since these could not be correlated with receptor discharge and were sometimes absent. One possibility is that back pressure along the bronchial vessels might change the environment of receptors in the bronchial epithelium. This may be compared with the suggestion that the receptors can be stimulated by local contraction of adjacent airway smooth muscle (Mills *et al.* 1969). Other possibilities include chemical stimulation by release of active substances, the involvement of local nervous reflex pathways and excitation by intraluminal mucus.

The sensitivity of the receptors to several different types of mechanical changes in the lungs may explain the considerable variations in discharge of individual receptors during control conditions and during stimulation, variations which could not be correlated with ventilatory or lung mechanical measurements (e.g. Figs. 2, 6 and 8). It is probable, on the basis of histological evidence (Larsell, 1921; Elftman, 1943) that lung irritant receptors are found in the epithelium throughout the bronchi and bronchioles, and that receptors in different sites have different sensitivities to the various tests used.

The reflex action of lung irritant receptors. To establish the reflex action of the irritant receptors it is desirable to use a stimulus which has no effect on other types of end-organ. No such stimulus has been devised, and the reflex responses in the conditions we have studied undoubtedly include the effects of changes in discharge from other receptors, in particular pulmonary stretch endings, deflation receptors, and 'cough receptors' in the extrapulmonary airways. Activity of these different types of receptor would sometimes have a reinforcing and sometimes an opposing action, as indicated in the appropriate sections above. This makes it impossible to compare, for different conditions, the size of the increase in irritant receptor discharge with the size of the reflex changes in minute volume or of lung resistance. In addition, quantitative conclusions with respect to nervous changes in total lung resistance can only be made in animals with the pattern of breathing constant, and this excludes hyperpnoea and pneumothorax.

However, the responses in the various conditions point to the probable reflex action of the lung irritant receptors. Thus the vagally mediated increases in breathing in pulmonary congestion and in severe hyperpnoea cannot be due to pulmonary stretch receptors, the action of which is to decrease minute volume. Nor can pulmonary stretch receptors be chiefly

responsible for the reflexes described in the previous paper (Mills *et al.* 1969). Deflation receptors are unlikely to be the major factor in the experiments with hyperpnoea or pneumothorax. None of the conditions led to coughing (except for inhalation of ammonia), so 'cough receptors' in the extrapulmonary airways are unlikely to be involved. Of the known afferent end-organs in the lungs, only lung irritant receptors increase their discharge in all the following conditions: anaphylaxis, pulmonary microembolism, pneumothorax, pulmonary vascular congestion, asphyxial hyperpnoea and after injections of histamine and phenyl diguanide and inhalation of ammonia. All these conditions are associated with vagal reflex stimulation of breathing. There is also known to be a vagal reflex bronchoconstriction in five of the eight conditions, the exceptions being pneumothorax, pulmonary congestion and asphyxial hyperpnoea which have not been studied in this respect. Those of the conditions which have been observed in man are usually associated with the sensation of dyspnoea. These correlations lead, first, to the hypothesis that lung irritant receptors cause reflex hyperpnoea and bronchoconstriction; and, secondly, to the speculation that they may contribute to respiratory sensation in disease. It seems unequivocal that they are a component of the vagal reflex mechanisms involved in the experimental conditions we have investigated.

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REFERENCES

ADRIAN, E. D. (1933). Afferent impulses in the vagus and their effect on respiration. *J. Physiol.* **79**, 332–358.

ALBERS, C., USINGER, W. & PLESCHKA, K. (1966). Der Einfluss des Lungenvolumens auf die Spontanatmung und die ventilatorische CO_2-Reaktion beim Hund. *Pflügers Arch. ges. Physiol.* **291**, 221–235.

AVIADO, D. M., LI, T. H., KALOW, W., SCHMIDT, C. F., TURNBULL, G. L., PESKIN, G. W., HESS, M. E. & WEISS, A. J. (1951). Respiratory and circulatory reflexes from the perfused heart and pulmonary circulation of the dog. *Am. J. Physiol.* **165**, 267–277.

BINET, L., STRUMZA, M. V. & DE LEOBARDY, H. J. (1948). Méchanisme de l'hyperpnée par pneumothorax. *C. r. Séanc. Soc. Biol.* **142**, 876–877.

CARO, C. G., BUTLER, G. J. & DUBOIS, A. E. (1960). Some effects of chest cage expansion on pulmonary function in man: an experimental study. *J. clin. Invest.* **39**, 573–583.

CONSTANTIN, L. L. (1959). Effect of pulmonary congestion on vagal afferent activity. *Am. J. Physiol.* **196**, 49–53.

DALY, I. DE B., LUDÁNY, G., TODD, A. & VERNEY, E. B. (1937). Sensory receptors in the pulmonary vascular bed. *Q. Jl exp. Physiol.* **27**, 123–146.

DOWNING, S. E. (1957). Reflex effects of acute hypertension in the pulmonary vascular bed of the dog. *Yale J. Biol. Med.* **30**, 43–56.

ELFTMAN, A. G. (1943). The afferent and parasympathetic innervation of the lungs and trachea of the dog. *Am. J. Anat.* **72**, 1–28.

GUZ, A., NOBLE, M. M., WIDDICOMBE, J. G., TRENCHARD, D. & MUSHIN, W. W. (1966). The effect of bilateral block of vagus and glossopharyngeal nerves on the ventilatory response to CO_2 of conscious man. *Resp. Physiol.* **1**, 206–210.

HEMINGWAY, A. & SIMMONS, D. H. (1958). Respiratory response to acute progressive pneumothorax. *J. appl. Physiol.* **13**, 165–170.

HOMBERGER, A. C. (1968). Beitrag zum Nachweis von Kollapsafferenzen im Lungenvagus des Kaninchens. *Helv. physiol. pharm. Acta* **26**, 97–118.

KNOWLTON, G. C. & LARRABEE, M. G. (1946). A unitary analysis of pulmonary volume receptors. *Am. J. Physiol.* **147**, 100–114.

LARSELL, O. (1921). Nerve terminations in the lung of the rabbit. *J. comp. Neurol.* **33**, 105–131.

MARSHALL, R. & WIDDICOMBE, J. G. (1958). The activity of pulmonary stretch receptors during congestion of the lungs. *Q. Jl exp. Physiol.* **43**, 320–330.

MILLS, J. F., SELLICK, H. & WIDDICOMBE, J. G. (1969). Activity of lung irritant receptors in pulmonary micro-embolism, anaphylaxis and drug-induced bronchoconstrictions. *J. Physiol.* **203**, 337–357.

PAINTAL, A. S. (1955). Impulses in vagal afferent fibres from specific pulmonary deflation receptors. The response of these receptors to phenyl diguanide, potato starch, 5-hydroxytryptamine and nicotine, and their role in respiratory and cardiovascular reflexes. *Q. Jl exp. Physiol.* **40**, 89–111.

RAMOS, J. G. (1956). The reflex responses to inflation or deflation of the lungs and their importance in the regulation of respiration. *Am. Rev. Tuberc. pulm. Dis.* **73**, 519–528.

RICHARDS, D. W., RILEY, C. E. & HISCOCK, M. (1932). Cardiac output following artificial pneumothorax in man. *Archs intern. Med.* **49**, 996–1006.

RICHARDSON, P. S. & WIDDICOMBE, J. G. (1965). Ventilatory responses to carbon dioxide in rabbits before and after cervical vagotomy. *J. Physiol.* **181**, 43–45*P*.

RICHARDSON, P. S. & WIDDICOMBE, J. G. (1969). The role of the vagus nerves in the ventilatory responses to hypercapnia and hypoxia in anaesthetized and unanaesthetized rabbits. *Resp. Physiol.* (In the Press.)

SASAKI, S. (1927). The effect of an excess of CO_2 in the inspired air before and after section of the vagi. *J. Biophys., Tokyo* **2**, 197–213.

SCHWIEGK, H. (1935). Der Lungenentlastungreflex. *Pflügers Arch. ges. Physiol.* **236**, 206–219.

SCOTT, F. H. (1908). On the relative parts played by nervous and chemical factors in the regulation of respiration. *J. Physiol.* **37**, 301–326.

SEVERINGHAUS, J. W. & STUPFEL, M. (1955). Respiratory deadspace increase following atropine in man, and atropine, vagal or ganglionic blockade and hypothermia in dogs. *J. appl. Physiol.* **8**, 81–87.

SIMMONS, D. H. & HEMINGWAY, A. (1957). Acute respiratory effects of pneumothorax in normal and vagotomized dogs. *Am. Rev. Tuberc. pulm. Dis.* **76**, 195–214.

TROELSTRA, H. J. (1960). De invloed van afferente vagusimpulsen op ademniveau en-frequentie (University of Groningen Thesis). Groningen: Hoitsema.

WIDDICOMBE, J. G. (1954). Receptors in the trachea and bronchi of the cat. *J. Physiol.* **123**, 71–104.

WIEMER, W. & KIWULL, P. (1965). Die Bedeutung der Nn. Vagi für die chemische Atmungssteuerung des Kaninchens. *Pflügers Arch. ges. Physiol.* **283**, R 46.