ROLE OF PULMONARY VAGAL AFFERENT NERVE FIBRES IN THE DEVELOPMENT OF RAPID SHALLOW BREATHING IN LUNG INFLAMMATION

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SUMMARY

1. The administration of the polysaccharide carageenin through a catheter into the lungs of cats and rabbits has produced an inflammatory lesion confined to one lobe of a lung. The lesion consisted of an alveolar and interstitial infiltration with polymorphonuclear leucocytes and, subsequently, macrophages. There was no apparent damage to alveolar walls and no pleurisy. The rest of the lung remained normal.

2. In both conscious cats and anaesthetized rabbits there was an increased frequency of breathing dependent on an intact vagus nerve on the same side as the lesion. It was independent of changes in body temperature and was not due to hypoxaemia.

3. By using a direct current to the right cervical vagus nerve in the rabbits (with the left vagus nerve sectioned), it has been possible to block conduction in myelinated fibres; the non-myelinated fibres conduct normally. Studies with this differentially blocked nerve have shown that the increased frequency of breathing is dependent on activity in the non-myelinated vagal afferent fibres.

Key words: lung inflammation, vagus nerve, tachypnoea, rabbit, cat.

The tachypnoea of pneumonia is a well-known clinical phenomenon (Wood, 1968) and occurs even in the absence of pleuritic pain. It may be present when only a small area of one lobe is involved. The increased frequency of breathing in these patients is generally unaffected by correction of any accompanying arterial hypoxaemia. It is not uncommon to find a decreased $P_{a}CO_2$ as evidence of alveolar hyperventilation. The tachypnoea is unlikely to result from any increase in body temperature because, first, it often continues when fever has subsided and, secondly, tachypnoea is not a striking clinical feature in other diseases with moderate elevation of body temperature.

The mechanism underlying the tachypnoea remained unstudied until Porter & Newburgh (1917) showed that the tachypnoea of a dog with an induced Friedlander's bacillus pneumonia...
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was entirely dependent on intact vagi. This observation has never been confirmed. More recently, Frankstein & Sergeeva (1966) using boiling water have induced an acute necrotic lung lesion in the cat and have obtained evidence of an afferent discharge from lung non-myelinated fibres within the vagi. There was no evidence of any increase in discharge in myelinated fibres from the pulmonary stretch receptors that mediate the Hering–Breuer inflation reflex. These authors postulated that the discharge in these non-myelinated pulmonary afferent fibres was responsible for the tachypnoea of their experimental pneumonia.

We have studied this problem by using a model of lung inflammation produced by the intratracheal administration into one lung of a polysaccharide, carageenin, obtained from a species of red algae, Chondrus crispis (Smith & Cook, 1953). Carageenin produces an inflammation in many tissues and the evolution of the pathological change resembles the acute inflammatory reaction that is seen clinically in response to both microbial and non-microbial agents (Williams, 1957; Benitz & Hall, 1959). The pathological changes in the lung are described in detail by Gardner, Marriott, Trenchard & Guz (1972).

The plan of study was firstly to show whether any tachypnoea resulting from the induction of a unilateral lesion depended on the integrity of the ipsilateral vagus nerve. Since this proved to be the case, we then investigated whether the tachypnoea depended on the integrity of the myelinated fibres, the non-myelinated fibres or a contribution from each set of fibres.

METHODS

Studies in conscious cats

Adult cats (3–5 kg) were used in these studies. Serial measurements in duplicate of respiratory frequency (counted over at least 1 min) and rectal temperature were made two to four times a day throughout the study. One of the vagus nerves was cut in the neck under halothane anaesthesia before the beginning of the study. The cats were resting quietly on the lap of the observer while the measurements were being made. Any cat that remained restless or that failed to give good duplicate measurements of respiratory frequency was not included in the experimental results. In these cats, and a further two in which the vagi remained intact, control measurements were then made for at least 3 days. The cats were then anaesthetized with 0·5–1% halothane in 50% N₂O in O₂ and the trachea was exposed through a mid-line neck incision. A small transverse hole about 4 mm in length was made in the trachea and a firm flexible catheter (outer diam. 1·5 mm) was inserted until it wedged in a lower lobe of one of the lungs; it was then withdrawn approximately 1 cm. It was not known into which of the lungs the catheter had passed. A 10 ml sample of 1% carageenin in saline warmed to 40°C was injected and the catheter withdrawn. The overlying muscle in the neck was sewn across the trachea, the skin sutured and the animals allowed to recover. The serial measurements of respiratory frequency and rectal temperature were continued for a further 4–7 days. At the end of this time the cats were killed by intravenous Nembutal injection, the lung removed and fixed by the intratracheal infusion of 10% formal-saline. This solution was given until it reached a vertical height of 20 cm above the lung hila, with the lungs held vertically by the trachea.

It was only after post-mortem examination that the cats could be assigned to a ‘control’ or ‘experimental’ group depending on whether or not there was an intact vagus nerve connecting the brain with the ‘carageenin’ lung. Thus, the experimental group consisted of those cats with
an intact vagus nerve on the side of the lung which had received the carageenin. The measurements of respiratory frequency and rectal temperature were therefore made ‘blind’.

To ensure that any changes in respiratory frequency were not due to hypoxia or hypercapnia, two cats were studied in which a catheter was implanted into the left carotid artery before any measurements were made. The catheter was exposed to the exterior at the back of the neck; access to it was via a rubber cap over the end of the catheter, just exposed at the surface of the skin (Hamilton, 1965). The rubber could be pierced with a syringe needle, and after clearing the catheter it was possible to obtain a sample of arterial blood without any disturbance to the conscious cat. This blood sample was analysed for PO₂ and PCO₂ by using electrodes kept at 38°; no correction was made for the body temperature of the animal. It was very important to ensure that the animal was undisturbed during the taking of the blood as any change in the pattern of breathing, for example a breath-hold or tachypnoea, could seriously affect the values obtained. The left vagus nerve in these two cats was sectioned at the time of insertion of the arterial catheter, and the carageenin was administered only after 5 days of control measurements had been made.

Studies in anaesthetized rabbits

Carageenin was administered to rabbits (2–3.3 kg) in exactly the same way as described for the cats, and the animals were allowed to recover. After 1–5 days they were re-anaesthetized for the differential block studies. The surgical procedures, anaesthesia (chloralose 40 μg/kg, Merck), method of differential block and the recording equipment have all been described elsewhere (Guz & Trenchard, 1971). A tracheal cannula was inserted and oxygen added to the inspired air to maintain a PaO₂ > 120 mmHg and thus minimize chemoreceptor stimulation. The body temperature of the animals was maintained at 38 ± 0.5°. The basis of the differential-block technique is that a direct current is applied to the right cervical vagus nerve and adjusted so that the ‘A–B’ complex of the monitored electroneurogram is abolished, while the ‘C’ wave remains intact. This indicates a block of conduction in all the myelinated fibres, with preservation of normal conduction in the non-myelinated fibres. Since the left vagus nerve is always sectioned before the studies, any information reaching the brain can only be mediated by these non-myelinated fibres.

Since all studies were made on the right vagus nerve, the rabbits shown at post-mortem to have received the carageenin in their right lung (R-Car) were grouped as experimental rabbits; those which had received carageenin in their left lungs (L-Car) were classed as experimental rabbits in studies with both vagi intact, but as control rabbits after the left vagus nerve had been sectioned. These differential block studies were therefore done ‘blind’ since it was not known if the carageenin had entered the right or left lung. A further control group comprised rabbits with normal lungs on which the studies were performed under similar conditions, but without prior administration of carageenin.

Four series of observation on breathing patterns were made in these rabbits; first, a comparison in all groups of rabbits with both vagus nerves intact; secondly, the effects of sectioning the left vagus nerve; thirdly, the effects of producing a differential block of conduction in the right vagus nerve; fourthly, the effect of cutting the differentially blocked, i.e. ‘non-myelinated’ nerve. At the end of the experiments the lungs were fixed by the same procedure of intratracheal formalin as described above.
The fixed lungs of both cats and rabbits were photographed, and then examined by light microscopy.

Statistics

Cats. All the results on breathing frequency and body temperature in each animal before the administration of the carageenin were pooled into either the control or experimental group. The significance of any difference between the two groups was tested by using the Mann–Whitney ‘U’ test (Siegal, 1956). The test was then repeated for both groups of pooled results obtained after carageenin had been given.

Rabbits. The significance of any differences in the pattern of breathing between the rabbits that had received carageenin and the control group, all with both vagi intact, was assessed by the unpaired t test (Snedecor & Cochran, 1970). The significance of changes in the pattern of breathing as a result of an experimental procedure has been assessed by using the Wilcoxon Matched-Pairs Signed-Rank test (Siegal, 1956). The significance of differences between small groups of animals has been examined by using the Mann–Whitney ‘U’ test.

The level of significance throughout has been taken as \( P<0.05 \) in a two-tailed test. In many of the studies it would have been justifiable to use a one-tailed test, but this was not done for the sake of uniformity.

RESULTS

Pathological findings

Similar lesions were found in cats and rabbits. There was an obvious ‘solidification’ of usually less than two-thirds of a lower-lobe (Figs. 1 and 2). Occasionally a right-sided lesion included the middle lobe and a left-sided lesion included the lingula. It was rare to find bilateral lesions; the other lung usually appeared normal. There was no abnormality of the bronchi which were free of secretions. There was no evidence of pleurisy over the ‘solid’ area as judged by a preservation of the shiny surface of the visceral pleura and the absence of any reddening, surface exudates or adhesions. Histological examination confirmed that there was no inflammatory reaction involving the pleura (Fig. 3). Histological examination also showed no evidence of necrosis of lung tissue and the alveoli were free from fibrin. There was an infiltration of the interstitial and alveolar spaces, predominantly with polymorphonuclear leucocytes in the first 24 h; these cells were slowly replaced by macrophages over the next 48 h. After these three days the lesion became infiltrated with fibroblasts. Free carageenin (stained with Alcian Blue) could not be seen within alveolar spaces after 24 h. A detailed report of these pathological findings has been presented elsewhere (Gardner et al., 1972).

Studies in conscious cats

A total of eight control and seven experimental cats have been studied. In the experimental group were two cats with carageenin in the left lung and a right vagotomy; three cats with carageenin in the right lung and a left vagotomy, and two cats with both vagi intact, one with the carageenin in the left lung and one in the right lung. Of the control group, three cats had a left vagotomy with the carageenin in the left lung and five cats a right vagotomy with the carageenin in the right lung. In addition, two further cats had received carageenin: both had left vagotomies and catheters implanted in their left carotid arteries for blood sampling; one received carageenin in the left lung and one in the right lung.
FIG. 1. Lungs of rabbit. Carageenin instilled into right lower lobe 5 days previously. Magnification \( \times 1.2 \). (a) Ventral view of horizontal section of right lung showing solidification of right lower lobe. (b) Dorsal view of same rabbit showing absence of fibrin over affected lobe, the pleura of which appears smoother than over the other lobes.

FIG. 2. X-ray of the lungs of a rabbit 2 days after the administration of carageenin showing a 'pneumonia' in the right lower lobe. N.B. A 1% solution of carageenin is not radiopaque.

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Fig. 3. Four histological fields to show lung and pleura of rabbits at various times after the intrabronchial instillation of carageenin. Magnification × 320. (a) 30 h; (b) 3 days; (c) 4 days; (d) 21 days. There is a cellular infiltration into the lung alveoli and interstitium (see text). There is no pleurisy.
Vagal afferent fibres in lung inflammation

The results in the control and experimental groups have been averaged for each day of the study and are shown in Fig. 4. Respiratory frequency increases when the ipsilateral vagus nerve linking the inflamed lung with the brain remains intact. This increased rate of breathing does not depend on any change in body temperature. The variance of both respiratory frequency and temperature measurements is large but this reflects the wide range of starting values. Before lung inflammation was induced, there was no significant difference for the average respiratory rate and rectal temperature between the control and experimental groups (pooled results before carageenin administration: 'U' test for frequency $P = 0.2$, for temperature $P = 0.13$). After the induction of lung inflammation there was a highly significant difference between the average respiratory rates of the two groups, but not for the rectal temperatures (pooled results after carageenin administration: 'U' test for frequency $P = 0.006$, for temperature $P = 0.2$).

Serial observations in the two cats (one control and one experimental) in which blood gas values were obtained are shown in Figs. 5(a) and 5(b). The experimental cat shows an increase in respiratory frequency after the induction of the lung inflammation which was not due to any change in body temperature and not due to arterial hypoxaemia. There was an accom-
panying increase in alveolar ventilation as shown by the fall in \( Pa_{CO_2} \). These changes are conspicuously absent in the control experiment.

**Studies in anaesthetized rabbits**

Of the sixteen rabbits used in these studies in whom lung inflammation was induced, twelve had received carageenin in the right lung and four in the left. Of the twelve experimental animals four were studied after 1 day and two on each of the subsequent days. Of the control group of four animals, one was studied on each of the 1st, 2nd, 3rd and 5th days. Similar results were obtained on any day of study, in spite of the differences in the underlying histological appearance.

*Ventilation with both vagi intact.* The results are shown in Fig. 6. The measurements were made when the rabbits were in a steady state about 30 min after the halothane anaesthetic had been replaced by the standard chloralose anaesthetic and before the electrodes had been applied to the right vagus nerve. The animals with lung inflammation breathed faster and more shallowly \( (P<0.001) \) than the control group: minute ventilation was similar in the two
groups. By contrast, the $P_a,CO_2$ was significantly lower in the experimental group. The values of $P_a,CO_2$ merely reflect the amount of oxygen added to the inspired air; this could not be accurately adjusted with the flowmeter available (Boyle's Anaesthetic apparatus).

**Effect on breathing of section of the left vagus nerve.** The results are shown in Fig. 7. In each group there was a slowing and deepening of breathing with a small increase in minute ventilation. Tests of statistical significance have not been performed on the L-Car group because of the very small number ($n = 4$).

Frequency and tidal volume changes in the control and R-Car groups were all significant ($P<0.01$) and these changes were not significantly different from each other ($P>0.1$). The changes in minute ventilation just reached significance in the control group ($P = 0.05$) but not in the R-Car group ($0.10>P>0.05$). The changes were not significantly different from each other ($P>0.2$).

**Effect on breathing of block of conduction in the myelinated fibres.** These observations (Fig. 8) were made during application of the direct current to the right vagus nerve for at least 2 min when a stable pattern of breathing had been established (Guz & Trenchard, 1971).

There was a significant slowing of breathing in both the control and R-Car groups ($P<0.005$); the effect was less marked in the R-Car group ($P<0.02$).

The depth of breathing increased significantly in both groups ($P<0.005$), but again with less of an effect in the R-Car group ($P = 0.02$). The changes in minute ventilation were not significant either within each group ($P>0.1$) or between groups ($P>0.2$).
FIG. 7. Effect of sectioning the left cervical vagus nerve in anaesthetized rabbits on the frequency of breathing (f), tidal volume (VT) and minute ventilation (VE). In each set of results the left-hand observations were made in animals with both vagi intact immediately before left vagal section, and the right-hand observations were made within 1 min of section of the left vagus nerve, as soon as a steady state had been reached. The mean ± 1 SD are indicated at the side of each group of observations in the control group and in the rabbits with carageenin in their right lungs. This has not been done in the rabbits who had received carageenin in the left lung because of the small number of observations.
Effect on breathing of cutting the non-myelinated fibres of the right vagus nerve. The results (Fig. 9) show that the respiratory frequency of the control group did not fall significantly ($P > 0.1$), whereas that of the R-Car group did ($P = 0.01$). These two groups behaved differently.

![Graph showing respiratory frequency, tidal volume, and minute ventilation](image)

**Fig. 8.** Effect of producing a block of conduction in the myelinated fibres of the right cervical vagus nerve. The left vagus nerve had previously been sectioned. The left-hand measurements in each set of results were obtained immediately before the block and the right-hand measurements were made as soon as a steady state had been reached after the differential block had been established. Description of the figure as for Fig. 7.

(0.05 > $P > 0.02$). Neither tidal volume nor minute ventilation changed significantly in either group ($P > 0.1$). The L-Car group appeared to behave as the control group. Tidal volume was strikingly decreased in the bilaterally vagotomized rabbits (right-hand observations in Fig. 9).
with carageenin inflammation, when compared with the control rabbits. The same phenomenon of decrease in tidal volume can also be seen in Figs. 6–8.

![Diagram](image)

**Fig. 9.** Effect of cutting the differentially blocked right cervical vagus nerve when only the non-myelinated fibres are conducting. In each set of results the left-hand measurements were made when there was a block of conduction in the myelinated fibres. The right-hand observations were made after section of this differentially blocked nerve, as soon as a steady state had been reached. Description of the figure as for Fig. 7.

**DISCUSSION**

The method of producing an inflammatory lesion of the lung by the intratracheal administration of carageenin has proved very successful in the present studies. The experimental situation
of a lesion confined to one lung has enabled control studies to be made on a nerve from a normal lung in an animal with inflammation in the other lung. It was only post mortem that the animal could be designated as 'control' or 'experimental'; this eliminated any bias on the part of the experimenter, a situation that was especially important in the measurements on conscious cats. The lesion resembled human lobar pneumonia with preservation of alveolar-wall structure. Important differences from human pneumonia were the absence of fibrin within the alveoli and the absence of pleurisy. Pleural inflammation in man causes pain which affects the respiratory frequency by limiting inspiration. The presence of pleurisy in our animal model would therefore have been a disadvantage.

The studies in conscious animals were done in the cat and not in the rabbit, because the respiratory frequency in the rabbit is strikingly variable from moment to moment; this is not true in the quiescent cat. There was no evidence that the cats were in pain. The studies in the anaesthetized rabbits have given results comparable to those in the cats. Both series of studies suggested that a vagus nerve ipsilateral to the side of the lesion must be intact for the tachypnoea to occur, and this clearly implied that an afferent discharge, excitatory to breathing, must be occurring within these ipsilateral vagal afferents. It is relevant that there is physiological evidence that vagal afferent innervation of a lung is ipsilateral without cross-over to the other side (Klassen, Morton & Curtis, 1951; Troelstra, 1960; Guz, Noble, Trenchard, Smith & Makey, 1966).

Our experiments with differential block suggest that the lung receptors that are excited are those innervated by non-myelinated vagal afferents. These have been termed 'J' receptors (Paintal, 1969). There is both physiological (Paintal, 1970) and anatomical (Meyrick & Reid, 1971) evidence that the receptors lie in the interstitial space of the lung at alveolar level. The lesion produced in the animals would be well sited to stimulate these receptors. It is possible that some of the chemical substances that are released in an inflammation due to carageenin (Spector & Willoughby, 1968; Di Rosa & Willoughby, 1971) may depolarize non-myelinated nerve endings that are sensitive to chemical stimulation (Paintal, 1971).

In a previous investigation on the role of non-myelinated fibres, Guz & Trenchard (1971) found that the activity in these fibres played little or no part in the control of normal breathing pattern, but that the tachypnoea occurring in certain pathological diseases of the lung (patchy areas of collapse, haemorrhage, oedema, infarction and microembolism) was dependent on the integrity of these fibres. The present studies extend these findings to experimental 'pneumonia'. No tests were made in these studies to determine whether reflex activity from lung irritant receptors was abolished by anaesthesia. If this had occurred, then it is possible that part of the respiratory changes in conscious animals may have been due to alteration in reflex activity of the lung irritant receptors (Mills, Sellick & Widdicombe, 1970). This seems highly unlikely for under exactly similar experimental conditions the reflex activity of lung irritant receptors has been shown to be intact (D. Trenchard & S. Jain, unpublished work).

A point of note was the effect on breathing of the block of conduction in myelinated fibres. There was significantly less slowing and deepening of breathing in the rabbits with the lesion in the ipsilateral lung than in the control animals with no carageenin. In a different approach to this subject Frankstein (1970) studied the effects on the inflation reflex (mediated by large myelinated fibres from pulmonary stretch receptors) in cats with local experimental pneumonia produced by the injection of hot water into the lungs. This author showed that the strength
of this reflex was markedly decreased; in addition it was demonstrated that the inhibitory inspiratory reflex evoked by threshold electrical stimulation of the central cut end of the vagus nerve was also decreased. Frankstein (1970) concluded that the activity in the non-myelinated fibres stimulated by the pulmonary damage was changing the excitability of the respiratory centre, and this resulted in a decrease in the strength of the inflation reflex. This observation is entirely compatible with our findings that block of the myelinated fibres, mostly consisting of fibres from pulmonary stretch receptors (Paintal, 1963) had a smaller effect on the pattern of breathing when there was an inflammatory lesion, than when the lungs were normal.

Another point of note is the evidence of alveolar hyperventilation seen from the fall in $P_aC_{O_2}$ in both cat and rabbit when an intact ipsilateral vagus innervates a lung with an inflammatory lesion. This is entirely in keeping with our findings in patients with lobar pneumonia even when arterial hypoxaemia has been eliminated (A. Guz, unpublished work). The alveolar hyperventilation (as judged by the $P_aC_{O_2}$) in anaesthetized rabbits with carageenin inflammation, occurred in spite of a decrease in tidal volume sufficient to decrease minute ventilation. This decrease in tidal volume had nothing to do with any vagal afferent mechanism since it was also a feature of the rabbits with bilateral vagotomy. We have no information about tidal volume in the conscious cat.

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