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AFFERENT VAGAL FIBRES WITH AN EXPIRATORY DISCHARGE IN THE RABBIT

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SUMMARY

1. An investigation was undertaken in the spontaneously breathing, anaesthetized rabbit to observe the response of vagal sensory endings to inflation and deflation stimuli which were sufficient to produce Hering–Breuer reflexes.

2. When artificial changes in lung volume were produced by applying step changes in intratracheal pressure, activity in pulmonary stretch receptors increased with inflation and decreased with deflation.

3. A small number of vagal afferent fibres were encountered which exhibited a discharge with a respiratory modulation consisting of an increase in activity during the phase of expiration. This pattern of discharge was accentuated when the animal was artificially ventilated with the chest either open or closed.

4. These expiratory receptors were stimulated by step changes in intratracheal pressure of -5 and $+15$ mm Hg to produce a high frequency, slowly adapting discharge which persisted for the 10 sec period of the pressure application. The endings were localized in the extrapulmonary bronchi and lung parenchyma and the afferent fibres were myelinated with conduction velocities of 10·6–29·5 m/sec.

5. The pronounced effect of deflation stimuli on the endings indicates that they probably act as one of the afferent mechanisms in the Hering–Breuer deflation reflex.

INTRODUCTION

Although much is known about pulmonary stretch receptors and their role in the Hering–Breuer inflation reflex, little information is available concerning the role of receptors in the deflation reflex. In the cat, Paintal (1955, 1957, 1969) described pulmonary 'deflation' or type J fibres, but the receptors were excited minimally by deflation unless the endings had been previously stimulated by phenyl diguanide. Homberger (1968), Mills,

Sellick & Widdicombe (1969) and Sellick & Widdicombe (1969, 1970) have recently described receptors in the rabbit that were stimulated by deflation, but the evoked discharge was usually rapidly adapting whereas the deflation reflex may be produced for relatively long periods (Widdicombe, 1964).

The present experiments were carried out to search for vagal afferent activity from the lungs which might show activity during expiration, rather than the usual inspiratory discharge displayed by pulmonary stretch fibres. Fibres with such expiratory activity were encountered and the response of the receptors to artificial airway pressure changes indicated that they could play some part in the Hering–Breuer deflation reflex.

METHODS

Experiments were carried out on adult rabbits anaesthetized with a solution of urethane and pentobarbitone (dose of urethane 0·5 g/kg, and pentobarbitone sodium 15 mg/kg) injected into an ear vein. A glass endotracheal tube was inserted into the trachea below the cricoid cartilage and positioned so that its tip lay at the carina. The trachea was effectively isolated from the lower respiratory tree by inflating a rubber cuff around the tip of the endotracheal cannula; thus movement of air during respiration was restricted to the tracheal cannula, extrapulmonary bronchi and lungs. Pressure within the tracheal cannula (hereinafter termed intratracheal pressure) was measured with a transducer (Ether UP 3) connected to a side arm of the cannula. Artificial inflation and deflation of the lungs was produced by occluding tubing on the end of the tracheal cannula and then connecting the side arm, via a three-way tap, to a 25 l. drum containing room air. Pressure in the reservoir was changed by 5 or 10 mm Hg steps and a given pressure was applied to the lungs for a set period of 10 sec. In some animals the chest was opened during the course of the experiment and ventilation was maintained with a Palmer respiratory pump.

One of the roots of the left or right phrenic nerve was cut low in the neck and activity was recorded by placing the central end of the whole nerve strand on a pair of platinum electrodes. The right vagus nerve was cut high in the neck and activity was recorded from fine filaments dissected from the distal cut end of the nerve. Action potentials from the nerves were amplified by Tektronix 122 preamplifiers and displayed on two Tektronix oscilloscopes and a Siemens Oscillomink ink writer (frequency response linear to 500 Hz), together with the intratracheal pressure. The peak frequency of discharge was calculated from measurements of the least interval between impulses during a period of maximum impulse activity. Conduction velocity determinations were made by stimulating the right vagus nerve low in the neck and recording the evoked discharge in the fine filament. In some experiments the animal was paralysed by an intravenous injection of gallamine triethiodide (1 mg/kg) and ventilation was maintained artificially. Injections of phenyl diguanide (40 μg/kg) were administered via a catheter in a jugular vein.

RESULTS

Experiments were performed on twenty-seven animals. In all cases the Hering–Breuer reflexes were produced by constant pressures applied to the tracheal cannula.

Inflation and deflation reflexes

With both cervical vagi intact, positive or negative intratracheal pressures of 5 mm Hg were sufficient to produce marked Hering–Breuer reflexes as evidenced by changes in the phrenic efferent discharge, e.g. inflation produced an inhibition of phrenic activity, whereas deflation led to an increase in the rate of breathing of from 25 to 150% (mean 76%) in different animals compared to the control period. The reflex effects were reduced after right-sided vagotomy and abolished after bilateral vagal section. When action potentials were recorded from the cut right vagus nerve, the left vagus was always intact to allow reflex changes to occur.

Response of pulmonary stretch receptors

Although it is believed that pulmonary stretch receptors are involved in the Hering–Breuer inflation reflex, their role in the deflation reflex is

TABLE 1. Response of pulmonary stretch receptors and expiratory receptors to changes in intratracheal pressure

Intratracheal pressure (mm Hg)	Peak frequency (impulses/sec), mean ± S.D.	
	Pulmonary stretch receptors $n = 68$	Expiratory receptors $n = 12$
0 (atmospheric)	80·1 ± 29·5	31·1 ± 10·7
+5	99·9 ± 30·7	15·2 ± 12·8
−5	43·8 ± 29·4	63·4 ± 40·5

much less certain (Widdicombe, 1964). Therefore it seemed important to observe the response of pulmonary stretch receptors under the present experimental conditions.

A total of sixty-eight pulmonary stretch receptors which exhibited a slowly adapting discharge to lung volume changes were studied during inflation and deflation. The results, shown in Table 1, confirm previous observations in other species (Paintal, 1963).

Response of receptors producing a discharge during expiration

Since the discharge in the pulmonary stretch fibres was not increased by artificial deflation, a search was made for receptors which might be spontaneously active during the phase of expiration in normal respiration and which might therefore be expected to be markedly affected by forced deflation.

A total of eighteen fibres were encountered which exhibited a discharge that increased during expiration and decreased during inspiration.

Of these fibres, twelve showed a similar pattern of activity in that the discharge was markedly increased by a deflation pressure of -5 mm Hg and decreased by an inflation pressure of $+5$ mm Hg (Figs. 1, 2; Table 1). When the animal was breathing spontaneously, the discharge produced by artificial deflation was in the form of high frequency bursts of activity as the animal laboured against the applied negative pressure. To determine whether the discharge would be continuous under more controlled con-

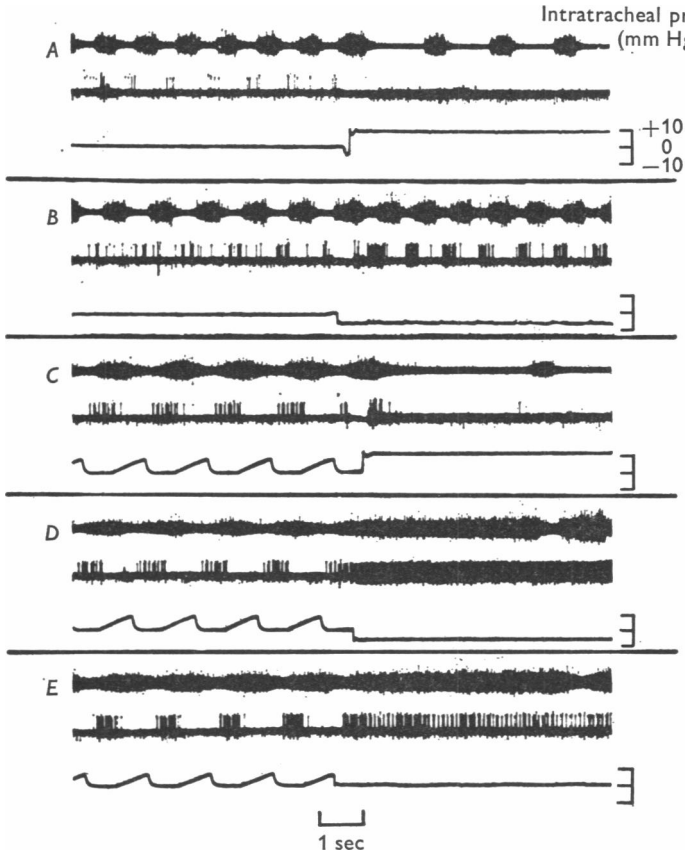

Fig. 1. Effect of inflation and deflation on the activity of a vagal afferent fibre which showed a predominantly expiratory discharge. *A*, *B*, chest closed and animal breathing spontaneously. Animal paralysed with gallamine between *B* and *C*, and artificially ventilated in traces *C*, *D* and *E*. Step inflation pressure applied in *A* and *C* and deflation pressure in *B* and *D*. Chest opened between *D* and *E*. Side tube of tracheal cannula opened to atmospheric pressure in the middle of *E*; oscillations in the intratracheal pressure indicate that the lungs continued to be partially inflated with each pump stroke. From above down: efferent activity in branch of phrenic nerve; discharge recorded from vagal afferent fibre; intratracheal pressure (mm Hg).

ditions, the animal was paralysed with gallamine and artificially ventilated. Under these conditions the rhythmic pattern of discharge remained pronounced and the application of a negative intratracheal pressure produced a continuous discharge of high frequency which persisted for the duration of the 10 sec deflation (Fig. 1*D*); this discharge was slowly adapting to the constant pressure stimulus (adaption indices of 8–63 %,

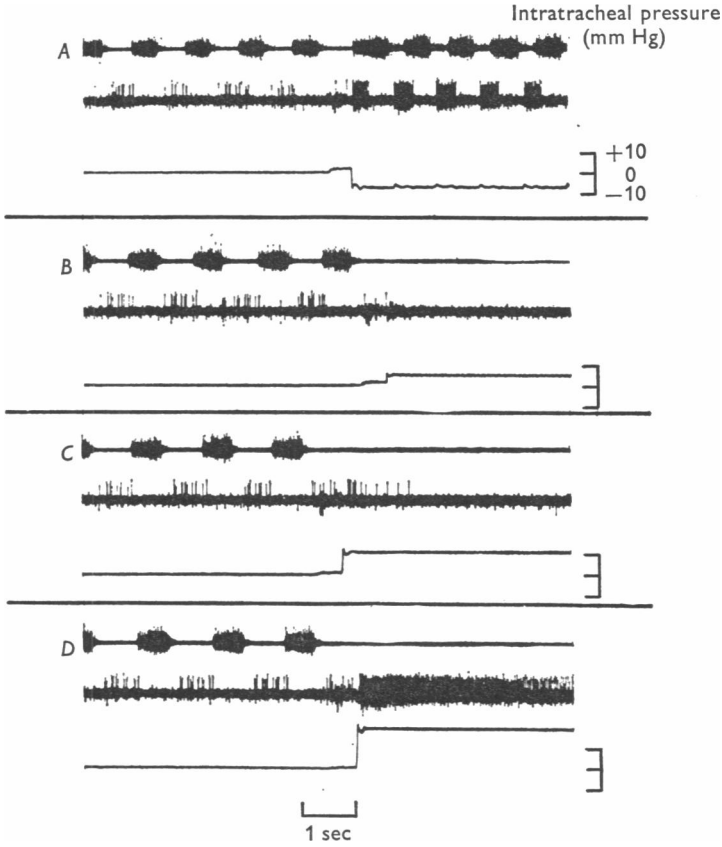

Fig. 2. Effect of increasing step changes in intratracheal pressure on the discharge from a receptor that normally was active during expiration. Closed chest, spontaneous respiration. Intratracheal pressure changed from atmospheric to −5, +5, +10 and +20 mm Hg in *A*, *B*, *C* and *D* respectively. From above down as in Fig. 1.

mean 32 %) when calculated with the formula of Widdicombe (1954). When the rhythmical inflation of the lungs was abolished by stopping the respiratory pump or reduced by turning the three-way tap and opening the tracheal cannula to atmospheric pressure (Fig. 1*E*), a continuous discharge was produced.

Since there was some indication that the endings responded to inflation (Fig. 1*C*) as well as to deflation, the effect of a graded range of inflations was investigated. With an inflation pressure of 10 mm Hg only a few spikes were produced at the beginning of the manoeuvre (Fig. 2*C*). However, with higher pressures of 15 or 20 mm Hg a high frequency discharge was evoked comparable to that produced by deflation. The response of the receptors to changes in intratracheal pressure is shown in Fig. 3 and Table 1 and is compared to the behaviour of pulmonary stretch receptors under similar conditions.

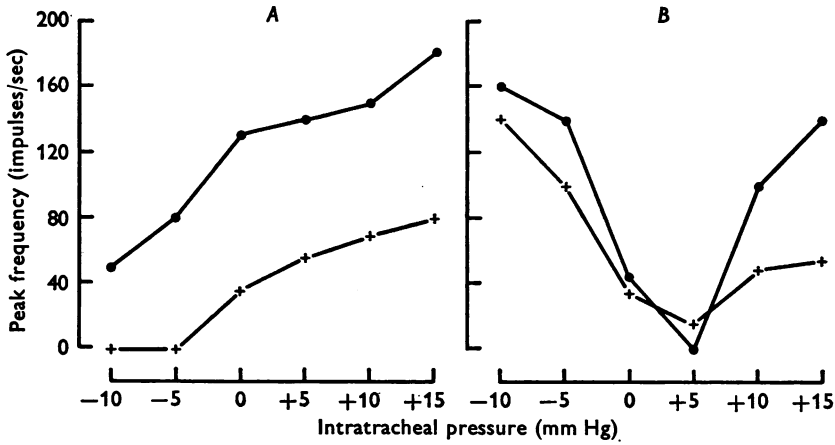

Fig. 3. Graph showing the response of pulmonary stretch receptors and expiratory receptors to changes in intratracheal pressure. Two pulmonary stretch fibres in *A* and two fibres with a spontaneous expiratory discharge in *B*: closed chest. Abscissa, intratracheal pressure (mm Hg); ordinate, peak frequency of discharge (impulses/sec).

Localization of the endings was established by pushing a stiff plastic catheter down the tracheal cannula into the lung, and also by eliciting bursts of action potentials from localized regions of tissue by external stimulation with a glass rod after the chest had been opened. Using these procedures, five receptors were localized to the extrapulmonary bronchi and seven receptors were located within the lung parenchyma.

The conduction velocity of four expiratory fibres varied from 10·6 to 29·5 m/sec (mean 19 m/sec) and in two fibres conduction was blocked when the vagus was cooled to 4–5° C. The effect of phenyl diguanide (40 μg/kg) was tested on six expiratory receptors in animals that were paralysed and artificially ventilated. None of the endings was stimulated by the drug, although the dose produced an increase in efferent phrenic activity.

The remaining six fibres out of the eighteen with an expiratory discharge

responded in a different manner to those just described in that the discharge was increased by inflation stimuli and reduced by deflation pressures. When the animal was artificially ventilated the pattern of activity in these fibres did not exhibit the pronounced expiratory modulation seen in Fig. 1, but instead, showed a prominent discharge during the inflation phase. Also, when the chest was opened any respiratory modulation in the discharge disappeared. These six endings could be classified as mediastinal receptors since movement of the thoracic viscera by manoeuvres such as lifting the chest produced a slowly adapting discharge in the fibres, as seen by Adrian (1933) and Widdicombe (1954); further confirmation for this classification was the localization of the endings in tissue around the trachea and extrapulmonary bronchi.

DISCUSSION

Pulmonary stretch receptors have been studied by many workers and the pattern of activity in the afferent fibres has generally been agreed upon as that of a discharge occurring during a part or the whole of the respiratory cycle, with a pronounced increase in activity occurring during the phase of inspiration or inflation (Paintal, 1963). By way of contrast, there is a relative paucity of information concerning receptors which may fire primarily during the phase of expiration in spontaneous respiration. There have been scattered reports of fibres with a prominent expiratory discharge in the mammal (Partridge, 1932; Adrian, 1933; Dawes, Mott & Widdicombe, 1951; Bein & Helmich, 1949) and the most valuable information has come from the work of Paintal (1955, 1957), Homberger (1968), Mills *et al.* (1969) and Sellick & Widdicombe (1969, 1970).

The 'pulmonary deflation receptors' described by Paintal (1955, 1957) exhibited little or no spontaneous discharge and their response to deflation stimuli was minimal in the absence of prior stimulation by phenyl diguanide. Paintal (1969) has recently reclassified these endings as 'type J receptors' and has suggested that they are more sensitive to vascular changes such as congestion than to respiratory changes. The response of the receptors described by Homberger (1968), Mills *et al.* (1969) and Sellick & Widdicombe (1969, 1970) bear similarities to the receptors described in this paper in regard to their irregular discharge, the responses to inflation and deflation, stimulation by an intrabronchial catheter and conduction velocities of the afferent fibres. They differ from the present expiratory fibres in their response to phenyl diguanide, rates of adaption and the respiratory modulation of the spontaneous discharge. The differences in response to phenyl diguanide may have been due to different doses (40 μg/kg, present experiments; 100–150 μg, Mills *et al.* 1969), and those of the

adaption rates to the fact that constant pressure inflations and deflations were used in the present experiments, whereas Mills *et al.* (1969) and Sellick & Widdicombe (1970) used transient constant volume stimuli. Therefore it would seem that the present expiratory receptors are similar to the 'irritant' receptors described by Mills *et al.* (1969) and just as pulmonary stretch receptors have a wide range of thresholds to inflation stimuli (Widdicombe, 1964), so irritant fibres display a variety of patterns of discharge during spontaneous respiration, ranging from a discharge with no apparent respiratory modulation to that with an expiratory or inspiratory pattern.

A further possible correlation of the present findings with previous work is that the endings could be similar to the slowly adapting tracheobronchial receptors described by Widdicombe (1954) in the cat, except for the fact that the present endings were located in more distal airways. Evidence to support this hypothesis is the fact that Widdicombe (1954) found that the density of the tracheo-bronchial receptors increased from the trachea towards the major bronchi, and also that they responded to both positive and negative intratracheal pressures in a manner similar to that shown in Fig. 3. The investigation on tracheo-bronchial receptors was carried out on an isolated portion of the respiratory tree, and therefore it is not possible to correlate the spontaneous discharge of these endings with that of the present expiratory receptors which were affected by rhythmic changes in airway calibre as the animal breathed spontaneously or was artificially ventilated.

When considering the role of receptors in the Hering–Breuer deflation reflex, it seems likely that the present expiratory fibres could play an important role in mediating the deflation reflex because they were stimulated continuously for the duration of an applied deflation stimulus which was sufficient to produce a marked increase in reflex respiratory activity. In contrast, the irritant receptors (Mills *et al.* 1969) and the type J receptors (Paintal, 1955, 1957) responded in a rapidly adapting manner to deflation stimuli, and therefore would only possibly be involved in the initiation of the reflex effects and not in any prolonged response.

As type J receptors (Paintal, 1969), and irritant receptors, including the present expiratory endings, are stimulated by large inflations as well as by deflation stimuli, it would appear that so far no truly deflation or expiratory fibres have been described, if such receptors are to be defined specifically as those which respond only during normal expiration or artificial deflation.

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