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IDENTIFICATION OF VAGAL SENSORY RECEPTORS IN THE RAT LUNG: ARE THERE SUBTYPES OF SLOWLY ADAPTING RECEPTORS?

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SUMMARY

1. We studied the characteristics of pulmonary sensory receptors whose afferent fibres are in the left vagus nerve of opened-chest rats. The activity of these receptors was recorded during mechanical ventilation approximating eupnoea, as well as during deflation, stepwise inflations and constant-pressure inflations of the lungs. Data were also collected from closed-chest rats and analysed separately.

2. Ninety-four per cent of receptors were located in the ipsilateral lung or airways with the remainder in the contralateral lung.

3. Not only were slowly adapting receptors (SARs) the most abundant pulmonary receptors but 21% of them were either exclusively or predominantly active during the deflationary phase of the ventilatory cycle. Deflationary units were found in opened- and closed-chest rats. The average conduction velocity for all fibres innervating SARs averaged 29·7 m s^{-1}.

4. We found rapidly adapting receptors (RARs) to be extremely rare in the rat. Their activity was sparse and irregular. The conduction velocities of fibres innervating RARs averaged 12·3 m s^{-1}.

5. Far more abundant than RARs in the remaining population of pulmonary fibres were C fibres. They were observed to have an average conduction velocity of 2·1 m s^{-1}, base-level activity which was irregular and a high pressure threshold of activation and were stimulated by intravenous capsaicin injection.

6. Notable differences exist between pulmonary receptors in rats and those reported in other species. The variations include the abundant existence of intrapulmonary SARs with exclusively deflationary modulation and the rarity of RARs. We also encountered C fibres which have not previously been described systematically in the rat.

INTRODUCTION

There has been a growing interest in the rat as a model for the study of respiratory function and its control, as well as a model for disease states such as human allergic asthma. However, little is known about the afferent properties of pulmonary receptors in this species. Studies of pulmonary afferent endings in dogs (Sampson &

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Vidruk, 1975; Coleridge & Coleridge, 1977), cats (Knowlton & Larrabee, 1946; Widdicombe, 1954; Paintal, 1957) and rabbits (Mills, Sellick & Widdicombe, 1969) have been largely responsible for the establishment of the categories of pulmonary receptors. Presently there are but three accepted categories of pulmonary sensory receptors; slowly adapting receptors (SARs), rapidly adapting receptors (RARs) and C fibre endings, both pulmonary and bronchial (Paintal, 1973; Sampson, 1977; Coleridge & Coleridge, 1986; Widdicombe, 1986). These three categories of receptors are thought to mediate all pulmonary reflexes.

Pulmonary sensory receptors found within the lungs of rats were studied by Tsubone (1986) who reported three categories of endings: pulmonary stretch receptors (inflationary), deflation-sensitive receptors and 'irritant-like' receptors. No C fibres were reported in that study, nor were rapidly adapting receptors reported. Although three categories of sensory receptors were reported in that study they were quite different from those reported in other species. Neither conduction velocities of the fibres nor locations of their receptive endings were reported. Specific information concerning the response to changes in insufflation pressure or chemical challenge was also not obtained.

Because of the increased interest in the rat as a model to study pulmonary mechanics and respiratory control, we decided to investigate more thoroughly the receptor profile in the rat in so far as it appears to be different from that in other species. Our study, therefore, was designed to answer the following questions: in the rat what is the nature of slowly adapting receptors and do rapidly adapting receptors or C fibres exist in the lungs?

METHODS

Nineteen Sprague–Dawley rats weighing 413–523 g (mean $\pm$ S.E.M. = 464 ± 8) were anaesthetized with either 35–50 mg kg^{-1} sodium pentobarbitone or with chloralose (50–100 mg kg^{-1}) and urethane (500–1000 mg kg^{-1}) by intraperitoneal injection as recommended by Flecknell (1987). A surgical plane of anaesthesia was maintained throughout the experiment with periodic intraperitoneal injections of one-quarter of the original dosage of the anaesthetic agent (see below). The level of anaesthesia was determined by testing spinal and corneal reflexes. A mid-line incision was made above the trachea and also above the sternum from the xiphoid process to the manubrium. A right carotid cannula was connected to a pressure transducer attached to a polygraph (Grass 7D, Quincy. MA, USA) for the purposes of monitoring arterial blood pressure and heart rate. A right jugular vein cannula was used for drug injection. The trachea was cannulated below the larynx. The cannula extended to the point where the trachea enters the thoracic cavity. The position of the cannula eliminated extrathoracic tracheal receptors from the study.

Gallamine (5–10 mg kg^{-1}) was infused into the jugular vein which eliminated spontaneous respiratory movement for about 30–60 min. The level of anaesthesia was assessed prior to each subsequent administration of gallamine. The tracheal cannula was then connected to a rodent ventilator (Harvard Apparatus, South Natick, MA, USA). The ventilatory rate and chest excursion were set to approximate those observed during anaesthesia prior to the gallamine infusion. Ventilatory rates and tidal volume were consistent with parameters recommended by Flecknell (1987).

In twelve rats the chest was opened in the mid-line to provide access to the lung parenchyma so that we could determine the receptive field of the fibre being studied. The expiratory line of the ventilator was placed under about 3–5 cmH_2O to approximate functional residual capacity (FRC). The lungs were frequently inflated to as much as 3 times their tidal volume in order to maintain a consistent compliance and volume history. In seven rats the chest was left intact in order to determine if the thoracotomy altered general characteristics of either inspiratory or expiratory SARs. Positive end-expiratory pressure was not applied in these experiments. A pressure transducer connected to the expiratory line from the trachea to the ventilator monitored

intratracheal (insufflation) pressure in all animals. Insufflation pressure in the opened-chest rats was a measure of transpulmonary pressure while in the closed-chest rats it was a measure of transrespiratory pressure.

The left vagus nerve was exposed in the cervical region for about 30 mm and was cut near the nodose ganglion. The distal end was freed from surrounding tissue for about 5 mm and placed on a dissection platform. The surrounding skin had previously been elevated creating a trough which

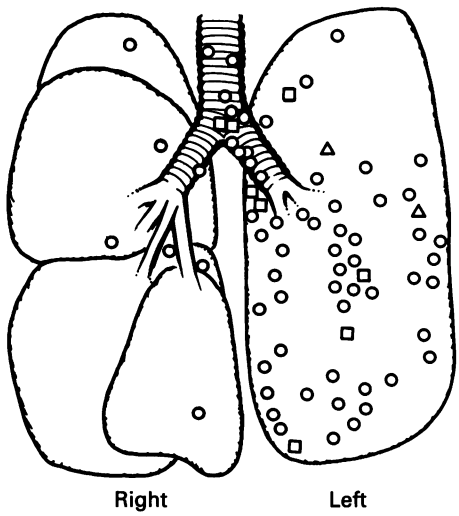

Fig. 1. General location of slowly adapting receptors (SARs) studied in the opened-chest rat lung. ○, SAR; △, RAR; □, C fibre. Note that the rat ordinarily has 4 lobes on the right side but only a single lobe on the left (Green, 1963).

was then filled with mineral oil to prevent drying of the nerve. Nerve fibres were dissected from the main nerve bundle and placed on bipolar, platinum recording electrodes held by a micro-manipulator. The signal from the electrodes was relayed to a preamplifier (Tektronix AM502, Beaverton, OR, USA), and then to an oscilloscope (Tektronix 5228, Beaverton, OR, USA). The output from the oscilloscope was separately relayed to a window discriminator (Frederick Haer Co, FMC, Brunswick, ME, USA), tape recorder (Hewlett-Packard, Model 3968A, San Diego, CA, USA) and an audiomonitor. The signal from the window discriminator was relayed to a rate–interval analyser (FHC), polygraph and an A/D converter-computer (Issac 91A, Newton, MA, USA, Apple IIe, Cupertino, CA, USA).

The right vagus was left intact. Since we performed a unilateral vagotomy efferent innervation was largely eliminated to the left lung in which the majority of receptors we studied were located. Because the rats were paralysed and artificially ventilated, reflexes influencing tidal volume, breathing frequency, inspiratory and expiratory time were eliminated. Furthermore, vagal efferent outflow appears to have little effect upon lung compliance (Sant'Ambrogio, Sant'Ambrogio, Matthew & Tsubone, 1988).

Pulmonary receptor activity was recorded during mechanical ventilation which approximated eupnoeic breathing, as already described, and during stepwise inflations of the lungs. Stepwise inflations of the lungs were accomplished by occluding the expiratory line of the ventilator for two, three and sometimes four tidal strokes of the ventilator. With the ventilator turned off, intrapulmonary pressure fell to 0 cmH_2O. The lungs were then subjected to constant-pressure increases of 5, 10, 20 and occasionally 30 cmH_2O for 5 s while pulmonary receptor activity was recorded. The lungs were allowed to deflate to 0 cmH_2O after each inflation. In the majority of cases eupnoeic ventilation was not resumed until the entire sequence of three or four inflations was completed. Generally it took about 5 s to adjust the pressure to the next level before that pressure was introduced into the lungs. The pressure for these inflations was generated from the exhaust

port of a vacuum motor attached to a voltage-variable transformer and was administered through the expiratory line of the ventilator. Receptor activity at or near FRC was determined when the ventilator was turned off during the deflation phase. A deflationary pressure of approximately 10 cmH_2O was then administered through the expiratory line to determine its effects on nervous activity. The lungs were then inflated in a stepwise manner to reverse atelectasis and artificial ventilation was resumed.

The lungs were gently probed with a cotton pledget to determine the general location of the receptive field of the fibre being studied. To determine the fibre's conduction velocity, a pair of stimulating electrodes were placed on the vagus nerve near its emergence from the thorax. The distance between the stimulating and recording electrodes was measured during each experiment and was usually about 20 mm. The stimulator was used to trigger the oscilloscope. The conduction velocity was then determined as the conduction distance divided by the time between the peak of the stimulus artifact and the onset of the action potential of the receptor being studied.

To further characterize the sensory receptors in the rat lung, suspected C fibre endings were challenged with an intravenous bolus of capsaicin (0·1–4 μg, I.V. bolus of 100 μl 0·9% NaCl) and RARs with histamine (100 μg to 10 mg, I.V. bolus of 100 μl 0·9% NaCl). SARs were challenged with 100% dimethyl ether administered through the air intake of the ventilator for 5–20 s at the normal tidal volume and respiratory rate. The administration of 20 s of dimethyl ether silenced all categories of SARs.

Data analysis included receptor base-level activity, activity during constant-pressure inflation and deflation, and activity after administration of capsaicin, histamine or dimethyl ether vapour. We determined the adaptation index of pulmonary receptors to constant-pressure inflation and deflation of the lungs as the peak frequency of the receptor during the first second of the constant-pressure inflation minus the average final frequency during the fifth and final second of the constant-pressure inflation and then divided by the peak frequency. This number was multiplied by 100 to obtain a percentage adaptation. This adaptation index is similar to that described by Knowlton & Larrabee (1946) and later by Widdicombe (1954). Statistical analyses included Student's t test and one-way analysis of variance when appropriate (Zar, 1974). Student–Newman–Keuls test was used for pairwise comparisons after separation had been determined by analysis of variance.

RESULTS

A total of 111 receptors were identified and characterized in this study. The general location of seventy-four receptors was determined in the opened-chest rats by gentle probing and the distribution within the lungs and airways is illustrated in Fig. 1. Seven SARs were located in the contralateral lung. Four of these were identified in a single rat. Other studies have reported contralateral innervation of the lungs both in the dog (Bergren, Myers & Mohrman, 1985) and cat (Kubin & Davies, 1988). All other receptors were located on the ipsilateral side.

Slowly adapting receptors (SARs)

We found that SARs dominate the activity of pulmonary origin in the cervical vagus. This was not surprising. However, unlike other species in which SARs have been described, we found that a large number of intrapulmonary receptors have activity predominantly or exclusively within the deflationary phase of the ventilatory cycle.

We subdivided the eighty-five SARs into four subgroups according to their activity in the ventilatory cycle (Fig. 2). The twenty-four inflationary (I) SARs discharged predominantly during inflation and only during the phase of the ventilatory cycle which there was lung movement. Biphasic SARs, either mostly inflationary (MI, 42 units) or mostly deflationary (MD, 3 units), discharged throughout the ventilatory cycle. The fifteen deflationary (D) SARs discharged almost exclusively within the static phase of deflation in the absence of lung

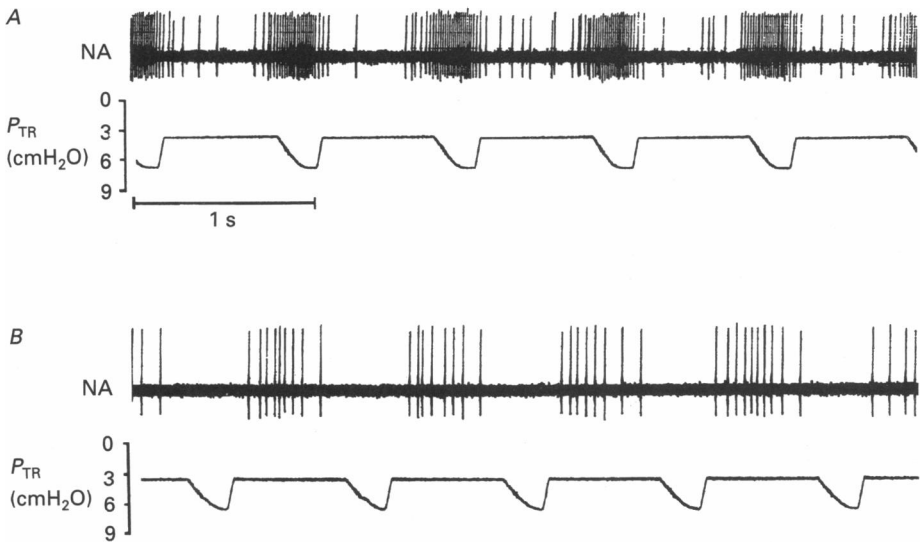

Fig. 2. Slowly adapting pulmonary stretch receptors (SARs) in the opened-chest rat lung having primarily inflationary (*A*) and completely deflationary (*B*) activity. Ventilatory cycle for each is shown below the neurogram. We identified SARs as inflationary, mostly inflationary, mostly deflationary and deflationary with respect to the activity of the unit during conditions simulating eupnoeic breathing. NA, nerve activity; P_{TR}, intra-tracheal pressure.

TABLE 1. Characteristics of SARs in the opened-chest rat lung

Units	Type	I–D	Impulses cycle^{-1}	CV (m s)$^{-1}$
17	I	29·25 ± 35·51*	20·4 ± 12·3†	34·8 ± 17·9
35	MI	1·36 ± 0·77	42·4 ± 22·4	29·5 ± 15·9
3	MD	0·37 ± 0·21	29·3 ± 6·4	19·8 ± 3·9
12	D	0·04 ± 0·09	23·7 ± 9·6	28·9 ± 12·2
	$P <$ ...	0·0001	0·0004	0·44

Units identified include inflationary (I), mostly inflationary (MI), most deflationary (MD) and deflationary (D). I–D represents the ratio of activity between inflation and deflation. All rats were ventilated at 1·5 ml (300 g)$^{-1}$ by a small animal ventilator. CV is conduction velocity. Significant differences were identified using one-way ANOVA with the Newman–Keuls subtest. * Value for I different from the other three types of units. † Value for I different than that for MI. All results given as means ± S.D.

movement (Fig. 2). The mean conduction velocity, base-level activity and inflationary-to-deflationary activity ratio in opened-chest rats are presented in Table 1. From the table it is clear that there is no difference in conduction velocities among groups. On the other hand those units whose activity was confined primarily to inflation had a significantly lower rate of spontaneous discharge than those mostly inflationary units active throughout the cycle.

In the closed-chest rats sixteen SARs were studied; seven I, six MI, three D and no MD units. The activity of these categories of SARs were similar to those in the opened-chest rats. The inspiratory–deflationary ratios for the I, MI and D units were 28·14 ± 33·46, 1·76 ± 0·98 and 0·07 ± 0·98 respectively (means ± S.D. here and through-

out test). No differences were demonstrable when each group was compared to its respective group in the opened-chest rats. Impulses per cycle for I, MI and D units were $13{\cdot}6 \pm 8{\cdot}1$, $50{\cdot}2 \pm 20{\cdot}5$ and $33{\cdot}0 \pm 13{\cdot}0$, respectively. There was a difference between I units in the opened *versus* the closed-chest rats ($P = 0{\cdot}034$).

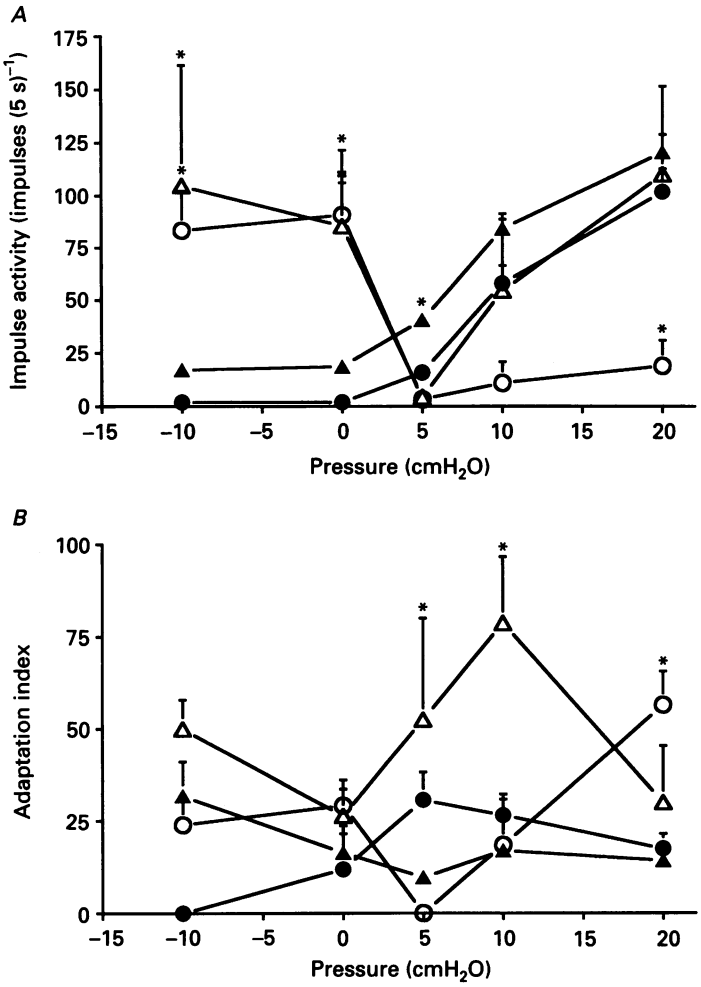

Fig. 3. *A*, response of SARs to 5 s constant-pressure inflation or deflation of the lungs in opened-chest rats. Symbols are: inflationary SARs, ●; mostly inflationary SARs, ▲; mostly deflationary SARs, △; deflationary SAR, ○. * represents statistical separation by ANOVA from the inflationary SARs ($P < 0{\cdot}05$). *B*, adaptation index of SARs to constant pressure inflation and deflation of the lungs in opened-chest rats. Symbols are: inflationary SARs, ●; mostly inflationary SARs, ▲; mostly deflationary SARs, △; deflationary SAR, ○. * represents statistical separation by ANOVA from the inflationary SARs ($P < 0{\cdot}05$).

In addition there were two units that underwent transformation from being deflation sensitive to inflation sensitive during continuous recording over several minutes. Other deflationary units were activated by hyperinflation but none of them

showed the clear shift in sensitivity from one phase to another. These units were within the category we considered mostly deflationary (MD).

The response of SARs to constant pressure inflation and deflation of the lungs in opened-chest rats is presented in Fig. 3*A*. Inflationary and mostly inflationary SARs

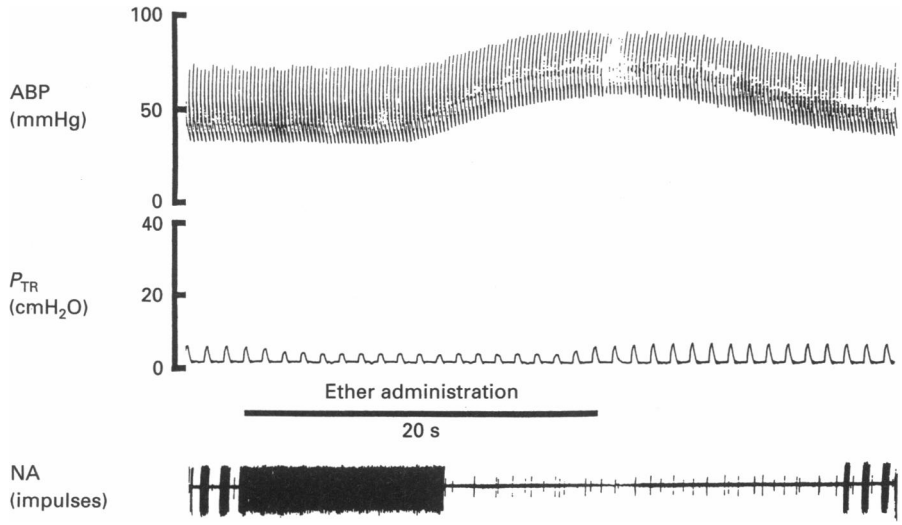

Fig. 4. The effect of dimethyl ether flow on an inflationary slowly adapting receptor. From top to bottom are arterial blood pressure (ABP), intratracheal pressure (P_{TR}), nerve activity (NA) of the SAR unit. Event bar represents duration of exposure (20 s). Several smaller spikes of receptors of unknown origin continue to be active during the exposure.

increase their activity linearly as intratracheal pressures increase above atmospheric pressure with the mostly inflationary SARs having a lower threshold. These receptors were not stimulated by deflation to 0 cmH_2O or by exposure to subatmospheric pressure of -10 cmH_2O. Deflationary and mostly deflationary SARs were activated by lung deflation and subatmospheric pressure exposure. The activity of deflationary SARs was silenced or suppressed by positive pressure of 5 cmH_2O and greater, whereas, the mostly deflationary SARs, although suppressed at positive 5 cmH_2O, were activated by 10 and 20 cmH_2O pressure.

The adaptation indices of the SARs are presented in Fig. 3*B*. The adaptation indices of the inflationary and mostly inflationary SARs indicated little or no adaptation. The deflationary SARs and mostly deflationary SARs tended to show adaptation to constant pressure inflation of the lungs. The adaptation index for deflationary SARs was elevated above inflationary SARs at 20 cmH_2O and the adaptation index for mostly deflationary SARs was elevated above inflationary SARs at 5 and 10 cmH_2O.

Dimethyl ether was administered while recording from three inspiratory, five mostly inspiratory, one deflationary and one mostly deflationary units. All of these units responded similarly to the administration. SARs initially tended to become tonically active without respect to the phase of the ventilatory cycle. With continued administration of dimethyl ether, SARs became silent and unresponsive to changes

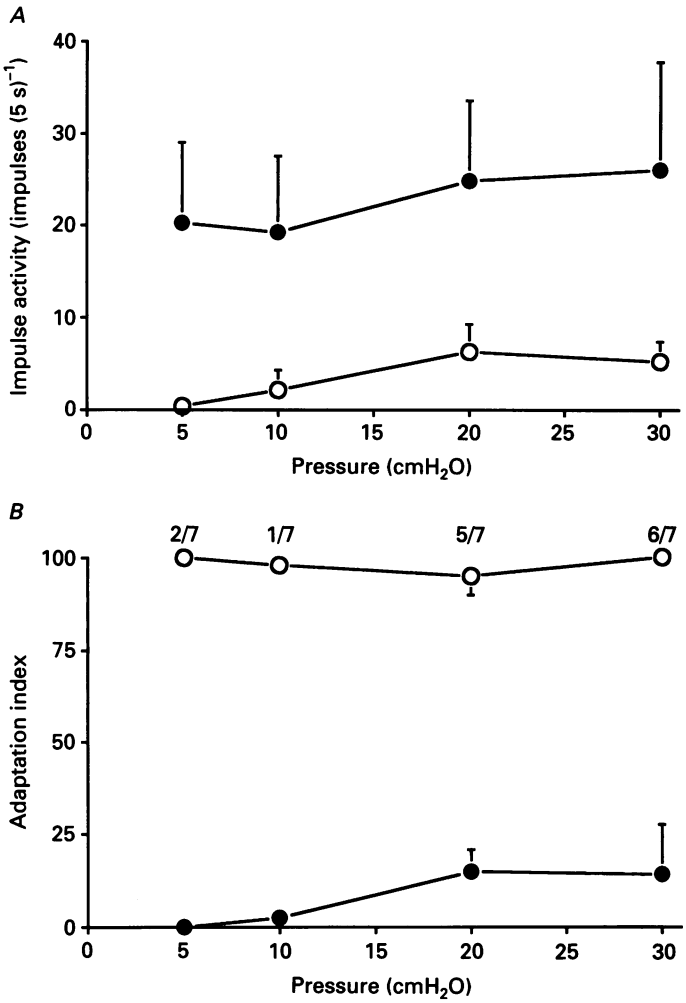

Fig. 5. *A*, response of rapidly adapting receptors (RARs, ○) and C fibres (●) to 5 s constant-pressure inflation of the lungs in opened-chest rats; * represents statistical separation by Student's *t* test from response of RARs ($P < 0{\cdot}05$). *B*, adaptation index of RARs (○) and C fibres (●) to constant pressure inflations of the lungs 5 s in duration in opened-chest rats. The numbers above the RAR adaptation index represent the number of units responding at each pressure. The last unit responded at 40 cmH_2O. $n = 13$ for C fibres at 5, 10 and 20 cmH_2O, and $n = 10$ for 30 cmH_2O.

in intratracheal pressure within 10–20 s after initiation of the challenge. Within 10–20 s after withdrawal of the dimethyl ether administration, SAR activity spontaneously returned at nearly full sensitivity (Fig. 4). From Fig. 4 it is apparent that the influence of dimethyl ether on the SAR pictured precedes changes in both arterial blood pressure and intratracheal pressure and, therefore, cannot be a result of those changes.

Rapidly adapting receptors (RARs)

Fibres originating from RARs in the lungs were identified by their rapidly adapting responses to stepwise hyperinflation of the lungs and then to constant-pressure inflation of the lungs of 5, 10, 20 and 30 cmH_2O. We identified only eight RARs out of the total 111 units studied (7%) in nineteen rats. The conduction velocity of the RARs of the rat ranged from 7·6 to 23·0 m s^{-1} (mean ± S.D., 12·3 ± 5·5). Base-level activity of the RARs during the prescribed eupnoeic ventilatory pattern of the respirator was 17 ± 14 impulses min^{-1} which is not similar to the 5–45 impulses $cycle^{-1}$ reported for 'irritant-like' receptors (Tsubone, 1986). The responses of seven RARs in opened-chest rats to constant-pressure inflation of the lungs and their adaptation index are shown in Fig. 5*A* and *B*. Lung inflation stimulated all RARs while negative pressure stimulated only one of six RARs. This is markedly different from the effects of negative pressure on RARs in guinea-pigs (Bergren & Sampson, 1982) and the cat (Knowlton & Larabee, 1946).

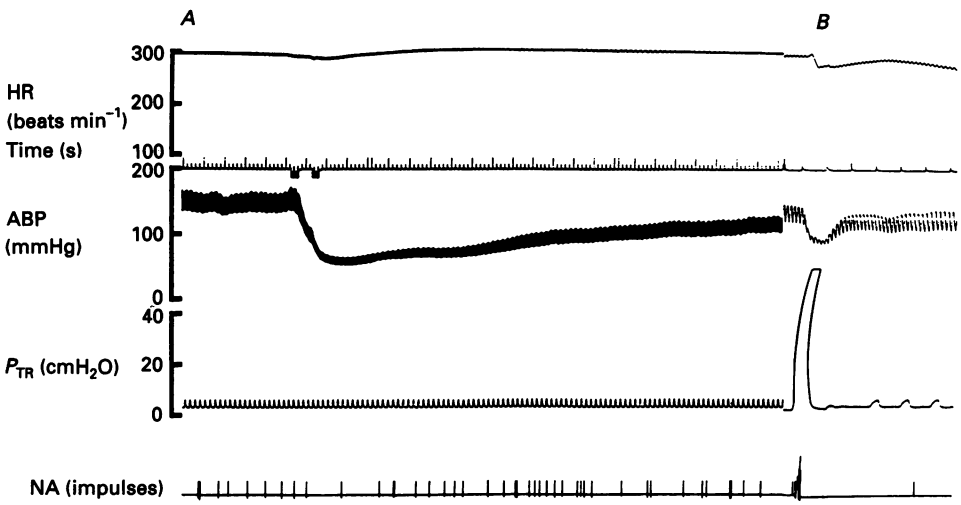

Fig. 6. *A*, effect of histamine injection (100 μg, I.V.) (first event marker, ■) and saline flush (second event marker) on heart rate (HR), arterial blood pressure (ABP), intracheal pressure (P_{TR}) and nerve activity (NA) of an RAR. *B*, effect of the response of hyperinflation of the lungs on this receptor.

Histamine had little effect on RAR activity or intratracheal pressure while at the same time it induced dramatic hypotension. We challenged three RARs with intravenously injected histamine as a bolus. Representative responses to injection of 100 μg histamine are illustrated in Fig. 6. For this RAR nerve activity was 18 impulses min^{-1} prior to histamine injection and 20 impulses min^{-1} afterward. Blood pressure decreased from 82/68 mmHg to a low of 32/26 mmHg. Intratracheal pressure was 3 cmH_2O before its injection and 3·5 cmH_2O after its injection. Similar responses were seen in the other two trials. Blood pressure decreased dramatically while little or no changes were observed in nerve activity or intratracheal pressure.

C fibres

C fibres were identified initially by the low tone heard through the audiomonitor caused by the slower conduction velocity of its fibre compared to either SARs or RARs, low voltage and relatively long duration action potential. The average conduction velocity of the eighteen fibres studied was $2{\cdot}1 \pm 1{\cdot}5$ m s^{-1}. This is within

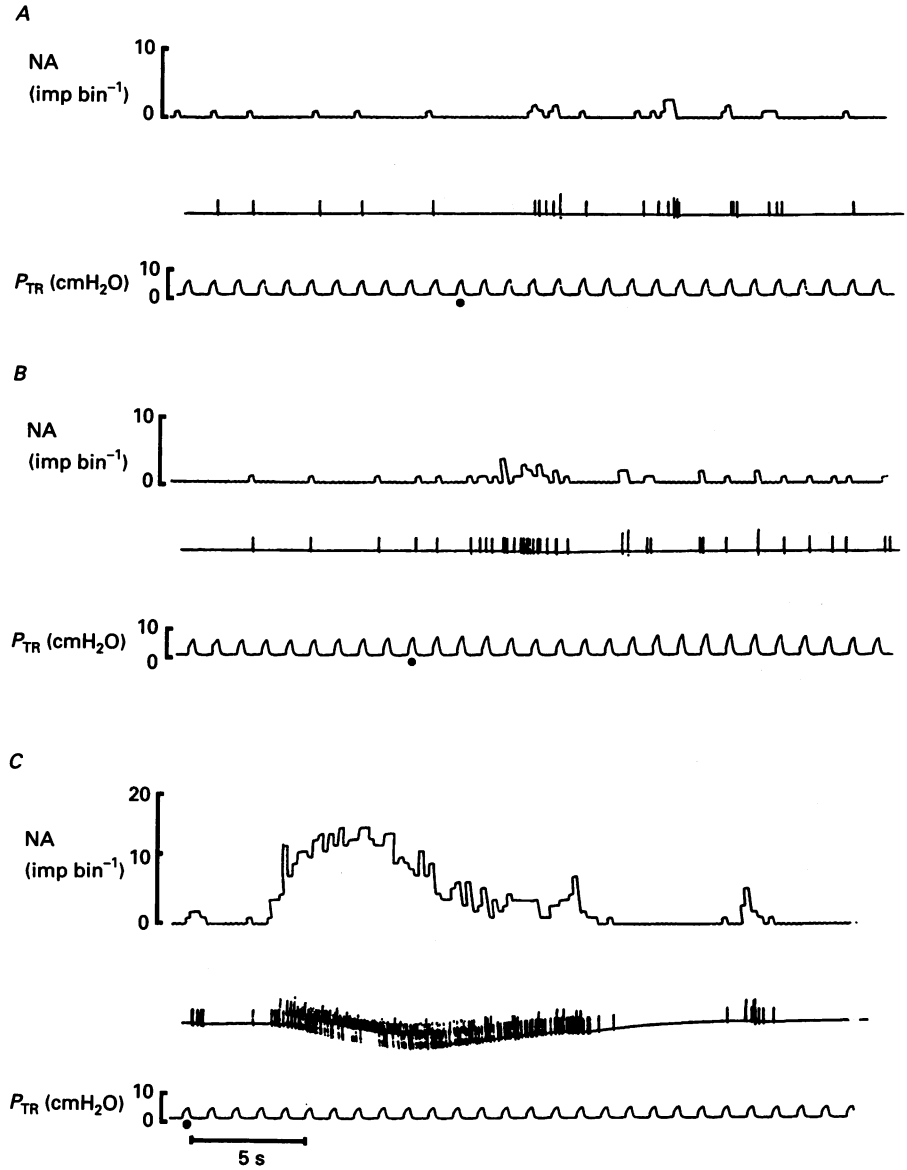

Fig. 7. Response of a C fibre ending to 1 μg (*A*), 2 μg (*B*) and 4 μg (*C*) capsaicin (I.V. bolus). Injection is represented by the event marker below tracheal pressure. Nerve activity (NA) is expressed both as individual spikes and impulses per 0·1 second bin (imp bin^{-1}) in each panel. P_{TR} represents intratracheal pressure.

the range reported for C fibres in the cat (Paintal, 1957, 1973) and dog (Coleridge, Coleridge & Luck, 1965; Coleridge & Coleridge, 1977). The base-level activity of C fibres during the ventilation approximating eupnoeic conditions was 90 ± 84 impulses min^{-1} and is similar to that reported for those in the dog (Kappagoda, Mah & Teo, 1987; Jonzon, Pisarri, Roberts, Coleridge & Coleridge, 1988). C fibres

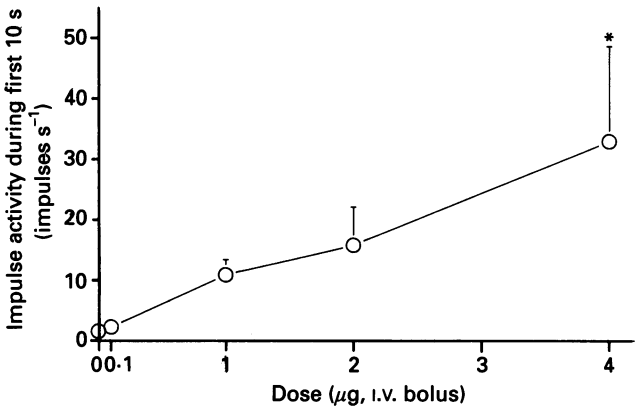

Fig. 8. Dose–response relationship of capsaicin injection (I.V. bolus) during the first 10 s after the injection. At 0 μg $n = 19$, $n = 3$ at 0·1 μg, $n = 15$ at 1 μg, $n = 9$ at 2 μg and $n = 5$ at 4 μg.

responded to stepwise and constant-pressure inflations of the lungs, but the response was weak even at high intratracheal pressures (3–4 × tidal volume or 30–40 cmH_2O). The pattern of response to the high pressure inflation of the lungs was not rapidly adapting. The results of constant pressure inflation of the lungs from the opened-chest rats are shown in Fig. 5*A* and *B*.

The activity of the C fibre endings in response to capsaicin, injected as an intravenous bolus, increased in a dose-related manner despite no detectable concurrent increase in intratracheal pressure (Figs 7 and 8). C fibre stimulation by capsaicin characteristically began and ended abruptly (Fig. 7). C fibre activity returned to base level $7·7 \pm 3·8$ s after 1 μg, $13·7 \pm 7·4$ s after 2 μg and $14·6 \pm 6·9$ s after 4 μg ($P = 0·0196$). In one case, C fibre activity remained elevated above base level for 85 s after 1 μg capsaicin challenge. No other C fibre unit acted in such a manner and this result was omitted from the foregoing data. Because of the limited accuracy of the location probe and the presence of anastomosis of the pulmonary vasculature between the pulmonary and bronchial circulation in the rat, C fibres were not categorized as either pulmonary or bronchial in this study.

DISCUSSION

We have recorded from pulmonary sensory receptors in the rats which fall into the three recognized categories established in other species, i.e. slowly adapting receptors, rapidly adapting receptors and C fibre endings (Sampson, 1977; Coleridge & Coleridge, 1986; Widdicombe, 1986). The notion that there are but three categories

of receptors in the lungs is largely based upon work done in the dog (Sampson & Vidruk, 1975; Coleridge & Coleridge, 1977), cat (Knowlton & Larabee, 1946; Widdicombe, 1954; Paintal, 1957) and rabbit (Mills *et al.* 1969). Other categories of sensory receptors in the lungs have been proposed such as the deflation or collapse receptors of the guinea-pig (Koller & Ferrer, 1973), cat (Wei & Shen, 1985), rabbit (Luck, 1970; Roumy & Leitner, 1980; Wei & Shen, 1985) and monkey (Wei & Shen, 1985). The receptors described in those studies are widely believed to be identical to irritant or rapidly adapting receptors (Wei & Shen, 1985; Coleridge & Coleridge, 1986). Our results suggest that these receptors may be a subcategory of slowly adapting pulmonary stretch receptors in rats. Therefore, as the sensory receptors in the lungs of other species are characterized, the notion that there are but three types of sensory receptors in the lungs may, by necessity, have to be reconsidered.

Slowly adapting receptors

Our results show that the afferent pulmonary activity of the vagus nerve in the rat is dominated by SARs (77% of all units). Tsubone (1986) described three different types of afferent units in the rat phasic with respiration and originating in the lungs. He termed them pulmonary stretch receptors, 'irritant-like' receptors and deflation-sensitive receptors. We have observed similar types of units but have found them all to be slowly adapting receptors. We found 21% of the SARs in the rat are active primarily or exclusively in the deflationary phase of the ventilatory cycle. Tsubone (1986) found 35% of sixty-nine units described were deflationary. From our results we formulated four subtypes of SARs: inflationary, mostly inflationary, mostly deflationary and deflationary. These subtypes seem to include the three types of units described by Tsubone (1986) and are consistent with four categories of receptors described by Wei & Shen (1985) while studying rabbits, cats and monkeys.

Wei & Shen (1985) called their four fibre types: phasic and tonic inspiratory units and phasic and tonic expiratory units. In rabbits phasic expiratory units were 9% of those studied while tonic expiratory units were 2% of those studied. In cats and monkeys only 2% of the units were phasic expiratory units and no tonic expiratory units were reported. The receptors were not located. Conduction velocities of only four of twenty-three units were reported. Widdicombe (1954) found about 2% of 400 units were deflationary receptors in cats. Wei & Shen (1985) also reported that all expiratory units in their study became inactive when subjected to positive pressure. On the other hand, two deflationary SARs reported by Luck (1970) had high activity at both -10 and $+15$ cmH_2O but low activity at $+5$ cmH_2O. The differences between the two studies may be attributable to the small number of units studied. We have observed expiratory units in rats which behave as described in both studies. Our deflationary units had very little or no activity when subjected to positive pressure whereas our mostly deflationary did respond to positive pressure. A probable continuum exists between these two classifications of deflationary receptors.

Our analysis of conduction velocity, base-level activity, response to constant-pressure inflation of the lungs and computation of the adaptation index supports the possible existence of subpopulations of SARs which have differing transduction properties in the rat. The obvious difference in activity of receptors during the

inflationary and deflationary phases of the ventilatory cycle justifies the existence of at least two subclassifications of SARs. Further subclassifications of SARs becomes difficult for SARs active during the inflationary phase of the ventilatory cycle. Although statistical separation was demonstrable between the inflationary and mostly inflationary SARs for base-level activity and the response to 5 cmH_2O constant-pressure inflation of the lungs, these results most probably represent differences only in the threshold level of pressure activation. This interpretation is in agreement with that of Tsubone (1986) who observed SARs with both low and high thresholds of activation.

Separation of deflationary SARs may be justified based upon both conduction velocity and response to constant-pressure inflation of the lungs. Deflationary SARs had higher mean conduction velocity than the mostly deflationary SARs. However, more important is the differing response of these two groups to constant-pressure inflation of the lungs. The excitability of the mostly deflationary SARs to both forced inflation and deflation was unique to this classification of SARs (Fig. 2*A*). This suggests transduction properties differing from other SARs; especially those of the deflationary SARs; however the low number of mostly deflationary SARs observed makes any interpretation of the data difficult.

What stimulates SARs? Different variables have been proposed such as lung volume, airway pressure, wall tension or perhaps wall distortion (Bradley & Scheurmier, 1977; Sant'Ambrogio, 1982, & 1987; Sant'Ambrogio, Fisher & Sant'Ambrogio, 1983; Davenport & Wozniak, 1986). Changes in circumferential force has also been proposed as the stimulus of SARs (Bartlett, Jeffrey, Sant'Ambrogio & Wise, 1976; Mortola & Sant'Ambrogio, 1977; Davenport, Sant'Ambrogio & Sant'Ambrogio, 1981). As yet, however, such a measurement has not been recorded. Intratracheal pressure is routinely reported as the variable monitoring pulmonary resistance and compliance during *in vivo* nerve recording of pulmonary sensory receptors (Mills *et al.* 1969; Armstrong & Luck, 1974; Coleridge & Coleridge, 1977, Bergren & Sampson, 1982; Tsubone, 1986). Intratracheal pressure does reflect circumferential force. Therefore, our measure of intratracheal pressure or insufflation pressure has been the most monitored variable used to assess pulmonary mechanics during nerve recordings of pulmonary sensory receptors.

It is generally believed that SARs are oriented in series with the airway smooth muscle (Widdicombe, 1954; Jeffery & Reid, 1973; Bartlett *et al.* 1976; Bradley & Scheurmier, 1977; Roumy & Leitner, 1980) and that other supporting tissues such as the tunica fibrosa are in parallel with SARs (Davenport *et al.* 1981). Few studies describe the histology of suspected SARs in any species (Jeffery & Reid, 1973; von During, Andres & Iravani, 1974; Das, Jeffrey & Widdicombe, 1978; Krauhs, 1984). From these studies it appears that SARs have typical features of mechanoreceptors with polymodal endings. Thus far, there are no reports of obvious transverse or longitudinal orientation of these receptors, although, in the dog, there appear to be three different branching patterns of SARs (Krauhs, 1984).

It is possible that receptor orientation may help to explain the adequate stimulus for inspiratory and expiratory activity of SARs. As discussed above the classical inspiratory SAR may be oriented in series with the airway smooth muscle. Therefore, with stretch or distension of the airway, the activity of the receptor increases. If an

SAR were oriented perpendicular to the axis of the airway smooth muscle, the stretch or lengthening of the muscle could reduce receptor activity. On the other hand with deflation or airway collapse the airway smooth muscle thickens and as a result the receptive field of the SAR oriented in a perpendicular manner lengthens and receptor activity might increase as a function of the thickening.

It has been reported that the same receptive ending can be made to vary in its transduction properties under different efferent influences (Matthews, 1962) or due to changes in the mechanical environment in which the receptor is suspended (Smith, 1966). Either mechanism may explain the change in the pattern of discharge we saw in two SARs after hyperinflations of the lungs. We were able to repeat the phasic change in the receptor's activity several times by repeating the inflation or inducing a deflation. The deflation manoeuvre which altered the phasic activity of the two SARs may have collapsed the airway which contained the receptive ending of the SAR. If the mechanics of the airways were altered this then could have altered receptor behaviour.

The function of differing SARs in the rat is not readily apparent. While inflationary SARs are widely believed to mediate the Hering–Breuer stretch reflex and reflex bronchodilatation, Knowlton & Larabee (1946), Luck (1970) & Tsubone (1986) have proposed that deflationary SARs mediate the Hering–Breuer deflation reflex. Another possible function of these deflationary and mostly deflationary SARs may be to monitor the compliance of the respiratory system. In other species this function appears to be mediated by RARs (Mills *et al.* 1969; Pisarri, Yu, Coleridge & Coleridge, 1986; Yu, Coleridge & Coleridge, 1987). However, we found RARs in the rat to be extremely few in number compared to other species in which this information is available. It is conceivable that the intrapulmonary deflationary SARs serve as a signal to monitor pulmonary compliance in the rat.

After characterizing a number of inflationary and deflationary SARs, we wished to determine if by opening the chest cavity we influenced the characteristics of either inflationary or deflationary units. In seven rats the chest was left intact. The inflationary and deflationary units indeed had a similar activity profile in the closed-chest preparation as that observed in the opened-chest preparation. Both inspiratory and deflationary units were found in opened- and closed-chest rats. The receptor activity was qualitatively similar to that recorded when the thorax was opened and positive end-expiratory pressure of 3–5 cmH_2O was applied in series with the expiratory line. Other investigators have compared SAR activity in the open *versus* the closed-chest preparation (Davenport *et al.* 1981; Wei & Shen, 1985; Tsubone, 1986) and studies all report that SAR activity is qualitatively similar although quantitative changes in activity were reported. Luck (1970) and Tsubone (1986) also indicate that activity of expiratory units is accentuated with artificial respiration.

Rapidly adapting receptors

In the studies of Tsubone (1986) 'irritant-like' receptors are described. In other species the term irritant receptor and rapidly adapting receptor is used to describe the same population of receptors. However, the 'irritant-like' receptors apparently do not meet the accepted criterion of an adaptation index of 80% or greater established by Knowlton & Larabee, 1946 (average adaption index = 59%, ranging

as low as 39%). The receptors classified as 'irritant-like' receptors as described by Tsubone (1986) may primarily represent what we classify as mostly inspiratory and mostly deflationary receptors, although this group probably contained what we considered to be rapidly adapting receptors. The pattern of the 'irritant-like' receptor presented in Fig. 1 of that study appears to be a low-threshold SAR with possible cardiac modulation. Furthermore, in no species in which RARs are described does the base-level activity appear so active during conditions simulating eupnoeic ventilation (i.e. up to 45 impulses per respiratory cycle). Those receptors we classified as RARs in the rat had high adaptation indices and relatively low base-level activity, both of which are similar to the characteristics ascribed to the RARs of other species (Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Bergren & Sampson, 1982).

It is believed that the 'irritant' receptors or RARs mediate reflex broncho-constriction across species line (Hegardt, Lowhagen & Svedmyr, 1980; Ganong, 1989). In the dog, mediators such as histamine are believed to act on RARs directly to initiate this reflex (Vidruk, Hahn, Nadel & Sampson, 1977). Direct chemical stimulation of RARs by histamine was neither dramatic nor obvious in the rat in spite of being a much higher dose than that used in other species. Intratracheal pressure was not affected at any dose used in this study although dramatic cardiovascular effects occurred. It does not appear that RARs of the rat, like those of the guinea-pig (Bergren & Sampson, 1982), are directly stimulated by histamine and; therefore probably do not mediate reflex bronchoconstriction in the rat.

C fibres

The conduction velocity (Coleridge *et al.* 1965; Paintal, 1973; Coleridge & Coleridge, 1977), base-level activity (Kappagoda *et al.* 1987; Jonzon *et al.* 1988) and mechanical sensitivity (Coleridge *et al.* 1965; Armstrong & Luck, 1974; Kappagoda *et al.* 1987) of C fibres of other species compare similarly with C fibre endings in the rat lung. Rat C fibre endings have a high pressure threshold and adaptation characteristics that are not considered rapidly adapting to mechanical stimuli. We observed that base-level activity of C fibre endings increases when lung compliance decreases and decreases when lung compliance increases, such as after stepwise inflation of the lungs. C fibre endings in rats may serve to monitor lung compliance. However in dogs, C fibre endings were actually relatively insensitive to change in compliance (Jonzon *et al.* 1988).

C fibre endings of the rat are stimulated by capsaicin and it appears to be a direct action because intratracheal pressure was not altered by the doses of capsaicin used in this study. This appears to be the case in dogs as well (Coleridge *et al.* 1965, 1968; Coleridge & Coleridge, 1977). C fibre endings demonstrated a dose-dependent response to capsaicin injection. In other species the reflexes associated with C fibre ending stimulation have included apnoea followed by rapid and shallow breathing, bronchoconstriction, increased airway secretions, bradycardia and hypotension (Coleridge & Coleridge, 1986) which Dawes & Comroe (1954) call the chemoreflex. These defence reflexes may be mediated by C fibres endings in the rat lung as well (Hamel & Ford-Hutchinson, 1985).

Capsaicin can increase airway resistance, decrease lung compliance or increase

airway secretions through both direct and reflex action (Lundberg, Lundbland, Martling, Saria, St Jarne & Anggard, 1987; Bergren, 1988) and therefore can mechanically influence C fibre ending activity. However, no changes occurred in intratracheal pressure with the injection of capsaicin in these experiments. Therefore, we expect centrally mediated reflexes or local action on airway smooth muscle to have had little effect on C fibre ending activity as the result of the injections. It appears that any stimulation caused by capsaicin we observed was likely to be a direct one.

In conclusion, the three classical categories of sensory receptors of the lungs exist in the lungs of rats: SARs, RARs and C fibre endings. However, these three categories are more in agreement with those described in other species rather than the three categories of sensory receptors described in the only other study reported characterizing sensory receptors in the rat lung (Tsubone, 1986). There are obvious differences in the transduction properties of SARs in the rat compared with other species studied thus far, especially noteworthy is the high percentage of intrathoracic deflationary units. The function and reflex effects of the deflationary SARs are unknown, however it is possible that they monitor lung compliance and mediate the Hering–Breuer deflation reflex. RARs in the rat are rare, not sensitive to histamine challenge at doses that has dramatic effects on the cardiovascular system, and thus may have little function in the rat. As appears to be the case in guinea-pigs, RARs in the rat do not seem to function as irritant receptors. The characteristics of C fibre endings such as conduction velocity, base-level activity, mechanical and capsaicin sensitivity are similar to those reported in the dog. As with other species this category of receptor probably mediates defence reflexes.

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