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IDENTIFICATION OF NEURONS RECEIVING INPUT FROM PULMONARY RAPIDLY ADAPTING RECEPTORS IN THE CAT

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SUMMARY

1. Extracellular and intracellular recordings were made in the caudal subdivisions of the nucleus tractus solitarii (NTS) to locate and characterize neurons excited by afferents from pulmonary rapidly adapting receptors (RARs) in Nembutal-anaesthetized cats.

2. Neurons identified as second-order cells activated by RARs (RAR-cells) were activated by electrical stimulation of myelinated afferents in the cervical portion of the vagus nerve(s) and by at least two of the following 'physiological' stimuli: (a) collapse of the lungs to atmospheric pressure; (b) hyperinflation of the lungs by either increasing tidal volume or maintained lung inflations; and (c) brief inhalation of ammonia vapour.

3. Of the ninety-nine RAR-cells identified and studied extracellularly, eighty-four were localized within the commissural nucleus of the NTS. Seventy-four cells responded monosynaptically to electrical stimulation of both ipsi- and contralateral vagal stimulation. The remaining ten RAR-cells located in the commissural nucleus and the fifteen located in the caudal portion of the medial subnucleus of the NTS rostral to the obex, responded to the ipsilateral vagus only.

4. Under control ventilatory conditions (bilateral pneumothorax, positive end-expiratory pressure of approx. 2 cmH_2O), forty-eight of the ninety-nine RAR-cells showed spontaneous ventilator-related activity, occurring primarily during ventilator-induced deflations (thirty of forty-eight). 'Reversal' of this ventilator-related modulation, from firing predominantly during lung deflations to lung inflations, and vice versa, could be induced in eleven of the RAR-cells by changing the lung volume or by the inhalation of ammonia vapour.

5. Modulation of firing in synchrony with the central respiratory rhythm was observed in the activity of fourteen of the ninety-nine RAR-cells.

6. Intracellular recordings were made from twenty-two NTS neurons caudal to the obex that received monosynaptic excitatory postsynaptic potentials (EPSPs) from both ipsi- and contralateral vagus nerves. Two were positively identified as RAR-cells.

7. It is concluded that the commissural nucleus of the NTS is the major location

of RAR-cells, and that the response characteristics of these neurons largely, but not entirely, correspond to the known properties of RARs.

INTRODUCTION

Since the original observations by Keller & Loeser (1929) and Knowlton & Larrabee (1946), many studies have focused on the properties of pulmonary rapidly adapting receptors (RARs) and have demonstrated specific differences in their response characteristics in comparison to the other major class of airway mechanoreceptors, the pulmonary stretch receptors (PSRs) (for reviews see Pack, 1981; Coleridge & Coleridge, 1986; Sant'Ambrogio, 1987). Also referred to as lung 'irritant' receptors (Mills, Sellick & Widdicombe, 1969; Armstrong & Luck, 1974), RARs are active in a variety of physiological and pathological conditions, including hyperinflations of the lungs (Knowlton & Larrabee, 1946), decreases in lung compliance (Mills *et al.* 1969; Jonzon, Pisarri, Coleridge & Coleridge, 1986; Yu, Coleridge & Coleridge, 1987), deflation of the lungs (Knowlton & Larrabee, 1946; Armstrong & Luck, 1974; Yu *et al.* 1987) and, in some species (including cats), the inhalation of irritant gases (Widdicombe, 1954). Intrapulmonary RARs have been implicated in a number of respiratory reflexes that result in both bronchoconstriction (Widdicombe, 1954; Mills *et al.* 1969) and pronounced changes in ventilation. These changes include an increase in respiratory frequency and inspiratory activity (Mills *et al.* 1969; Sellick & Widdicombe, 1969), induction of augmented breaths or 'sighs' (Sellick & Widdicombe, 1970; Cherniack, von Euler, Glogowska & Homma, 1981), shortening of expiratory duration (Davies & Roumy, 1982) and initiation of inspiratory efforts (Davies, Sant'Ambrogio & Sant'Ambrogio, 1981).

The central pathways through which these reflex responses are mediated are largely unknown. Several recent studies have identified the areas of central projection of RAR afferent fibres to different subdivisions of the nucleus of the solitary tract (NTS) by using antidromic mapping (Davies & Kubin, 1986), spike-triggered averaging (Kubin & Davies, 1988) and intra-axonal labelling (Kalia & Richter, 1988*a*). Although some discrepancies exist between the results of these studies (see Discussion), they indicate a predominant projection to the commissural nucleus and to the caudal portions of the medial subnucleus of the NTS (Davies & Kubin, 1986). This is supported by recordings of focal synaptic potentials (Kubin & Davies, 1988) which indicate the presence of second-order neurons.

The aim of this study was to locate and characterize these second-order neurons in the pathway originating from RARs by using conventional extracellular and intracellular recording techniques and a combination of stimuli known to activate this group of lung receptors. Identification of these neurons is critical to the understanding of the neuronal organization of respiratory reflexes originating from the lungs.

METHODS

General procedures

Experiments were conducted on seventeen adult cats, initially anaesthetized with sodium pentobarbitone (Nembutal, 35–45 mg kg^{-1} I.P.) and subsequently maintained with supplementary doses of 5–10 mg kg^{-1} h^{-1}. The depth of anaesthesia was assessed by monitoring the central

respiratory rhythm (slow and regular phrenic discharge) and arterial blood pressure (mean pressure less than 150 mmHg, with no fluctuations related to nociceptive stimuli). The trachea and the femoral artery and vein were cannulated. The animals were placed in a stereotaxic frame and the dorsal surface of the medulla was exposed by occipital craniotomy and cerebellectomy. Large bilateral parietal craniotomies were made to reduce the movement of the brain stem. The C5 branch of the phrenic nerve was cut distally and mounted on a bipolar recording electrode. Cervical portions of both vagus nerves were dissected free for approximately 3 cm and mounted intact on bipolar stimulating/recording electrodes. The animals were paralysed with pancuronium bromide (Pavulon, 0·5 mg kg^{-1} I.V., supplemented with 0·2 mg kg^{-1} h^{-1}) and artificially ventilated with oxygen-enriched air. A bilateral pneumothorax was made and a positive end-expiratory pressure (PEEP) of approximately 2–3 cmH_2O was applied. To further reduce recording artifacts, low tidal volumes (< 20 ml) and high-frequency ventilation (50–65 min^{-1}) were often used. Rectal temperature (36 ± 1 °C), arterial blood pressure, end-tidal CO_2 (4–5%), mean-expiratory O_2 (30–40%) and tracheal pressure were monitored. Atropine (0·1 mg kg^{-1} I.M.) was administered to reduce mucosal secretion in the airways and to abolish bronchoconstriction evoked by the inhalation of ammonia vapour.

Recording and stimulation

The activity of the C5 branch of the phrenic nerve was amplified, filtered (60 Hz–3 kHz), full-wave rectified and integrated (time constant, 10 or 100 ms). The vagus nerves were stimulated at a frequency of 2 Hz with single 0·1 ms pulses at an intensity of around 2 times (max. 3 times) the threshold for PSR afferents. The threshold was defined as the strength above which repetitive stimulation of the vagus at 100 Hz inhibited central respiratory activity (as assessed by phrenic nerve discharge). In twelve animals the activity of the whole cervical vagus was recorded and integrated (time constant, 100 ms) in the same way as the phrenic signal, to assess the response of vagal afferents to the application of 'physiological' stimuli applied to the lungs (see below). The afferent nature of this activity was verified when signals persisted despite cutting the proximal part of the cervical trunk.

Extracellular recordings from single units were made with glass microelectrodes (tip sizes between 1·0 and 1·5 μm) filled with 3 M-NaCl, or (in five animals) 3 M-NaCl saturated with Fast Green FCF dye to allow direct marking of the recording sites. The signals were amplified and filtered (usually 100 Hz–6 kHz). Extracellular spikes were discriminated using a time–amplitude window discriminator. Standard output pulses were fed into a digital frequency meter, or used to generate 'cycle-triggered histograms' (CTHs) in order to relate the firing of the units to variables such as changes in tracheal pressure or arterial blood pressure. Recordings from afferent axons were distinguished from cellular recordings by the shape of their action potentials, fixed response latency to electrical stimulation of the vagus, and by their ability to follow paired stimuli separated by a short interval. In addition, somatic recordings were usually confirmed by the observation that the majority of tested units responded to the stimulation of both the ipsi- and contralateral vagus nerves. Recordings of 'field potentials' were made with low-impedance microelectrodes (tip size approx. 3 μm) filled with 3 M-NaCl. Intracellular recordings were made with glass microelectrodes filled with 3 M-KCl, having DC resistances of 6–10 MΩ. The intracellular signals were displayed in both DC mode (0–10 kHz) and higher-gain AC mode following AC filtering (10 Hz–6 kHz). To further improve recording stability, the majority of extracellular trackings, and all the intracellular recordings, were made with the microelectrodes angled rostrally (45 deg to the dorsal surface of the medulla).

All recorded signals were monitored on a multi-channel pen-recorder and selected signals (microelectrode potentials, tracheal pressure, phrenic nerve discharge and blood pressure) were stored on digital tape (Vetter, Model 3000, rise time for microelectrode channel, 25 μs; or FM Data Recorder, bandwidth: DC–10 kHz, Sony A-69) for subsequent off-line analysis.

Activation of rapidly adapting receptors ('physiological' stimuli)

The following procedures that are known to activate RARs (for reviews see Pack, 1981; Coleridge & Coleridge, 1986; Sant'Ambrogio, 1987) were used:

(1) *Collapse of the lungs to atmospheric pressure.* This was done by manually opening a side valve interposed between the inspiratory outlet of the ventilator and the lungs for a period of 2–15 s.

(2) *Changes in tidal volume.* These were achieved by adjusting the stroke volume of the ventilator. Tidal volume was increased progressively from the control value (approx. 15–20 ml) to

approximately twice that (max. 40 ml) and maintained for two or three cycles of the ventilator before restoration to the control value. This manoeuvre lasted for approximately twenty to thirty cycles of the ventilator and resulted in an increase in both the peak and the rate of rise of intratracheal pressure. The effects resulting from activation of RARs were distinguished from those of high-threshold PSRs by the pattern of activity recorded from NTS cells. Cells activated by RARs had an irregular frequency of action potentials within each burst of discharge associated with each inflation, which contrasted with the regular bursts evoked in P cells and R_β cells (e.g. Berger, 1977).

(3) *Maintained lung inflations.* These were produced by a constant-pressure system which was independent of the ventilator. A custom-made switching valve allowed for virtually instantaneous switching between the two. The constant-pressure system consisted of a 30 l pressure reservoir fed by a continuous flow of air delivered through a pressure regulator. The pre-set inflation pressure was controlled by a 'bleed-off' pipe that was immersed under water. Varying the depth of immersion (9–15 cmH_2O) changed the pressure stored in the reservoir and consequently the volume to which the lungs were expanded when the reservoir was connected to the lungs. The rate of the inflation could be adjusted by a variable-resistance valve attached to the outlet of the reservoir. After a maintained inflation of 5–15 s, the ventilator was reconnected through the switching valve.

(4) *Administration of ammonia vapour.* This was achieved by placing the inlet tube of the ventilator into the neck of a bottle which was half-filled with a solution of ammonium hydroxide. As the inlet for the oxygen enrichment was downstream of the ventilator inlet, dilution of the vapour inevitably occurred, and thus the exact concentration reaching the animal was unknown. The level of ammonia stimulation was ascertained indirectly by the magnitude of the responses, as assessed by the activity recorded in the vagus nerve, the changes in the phrenic nerve discharge and the increase in tracheal pressure observed before the administration of atropine. The degree of ammonia stimulation could be varied by using different concentrations of ammonia (5–35% solutions) and by varying the duration of inhalation (one or two strokes of the ventilator). The time delay between administration of the vapour and the onset of increased activity in the vagus nerve depended upon the ventilation system's 'dead-space' and the 'minute' ventilation. In general, the time delay was around 6–10 s.

Experimental protocol

After the threshold for PSRs had been established (see above), the vagus nerves were stimulated at an intensity of 2·0–3·0 times threshold. Initial trackings in each experiment were made with a low-impedance microelectrode and once the areas with the largest orthodromic field potentials evoked by both ipsi- and contralateral vagal nerve stimulation were located, a higher impedance extracellular microelectrode was substituted. Only those units that were excited by electrical stimulation of the vagus nerve(s), at an intensity below 3·0 times threshold for the activation of PSRs, were characterized further.

Allowing the lungs to collapse to atmospheric pressure was usually the first 'physiological' test. This manoeuvre evoked smaller changes in blood pressure than subsequent inflation tests and therefore resulted in better recording stability. It also decreased lung compliance, resulting in an increase in RAR activity (Jonzon *et al.* 1986; Yu *et al.* 1987). Increases in tidal volume and administration of ammonia vapour were then performed. Maintained lung inflation was usually performed last as it caused large changes in blood pressure and increased lung compliance. Before and after each 'physiological' test, the vagus was stimulated to ensure that the unit was still present. Responses to a test were only accepted for further analysis if the unit fulfilled this criterion. A unit was designated as a RAR-cell if it responded to at least two of the 'physiological' stimuli in addition to activation following vagal stimulation.

When an intracellular microelectrode was used, only those neurons that showed a stable membrane potential and received synaptic inputs from the vagus were tested for their responses to 'physiological' stimuli. The order in which the stimuli were presented was the same as for the extracellular units.

In twelve experiments, the response of the entire cervical vagus trunk was tested with the 'physiological' stimuli prior to central recording. The initial dose of atropine given earlier in the experiment had usually worn off by this stage, and following these tests a supplementary dose of atropine (0·1 mg kg^{-1}, I.M.) was administered.

At the end of the experiment, the brain stem of animals in which Fast Green FCF dye had been deposited was removed for histological analysis.

RESULTS

Recordings were made from neurons located in the NTS between 1 mm rostral and 2 mm caudal to the obex. Those that received excitatory inputs from RARs were referred to as RAR-cells (pulmonary rapidly adapting receptor-activated cells). The database for this study consists of ninety-nine extracellularly recorded RAR-cells and twenty-two intracellularly recorded cells which fulfilled only some of the criteria set for RAR-cells. Extracellular recordings were made principally in the area caudal to the obex (between 0·5 and 2·0 mm), as initial recordings demonstrated a larger population of RAR-cells in that area. All intracellular recordings were made from neurons located caudal to the obex.

As none of the stimuli used in the present study can be regarded as being specific for RARs (see Discussion), a combination of stimuli was used. Due to the generally unsatisfactory recording stability of the small neurons in the caudal subdivisions of the NTS it was often not possible to perform all the tests. Therefore, a unit was designated as a RAR-cell if it was excited by electrical stimulation of the vagus nerve(s) at an intensity below 3·0 times threshold for the activation of PSRs and by at least two of the following 'physiological' stimuli (see Methods): (1) allowing the lungs to collapse to atmospheric pressure; (2) increasing tidal volume; (3) producing maintained lung inflations; and (4) administering ammonia vapour.

Responses to electrical stimulation of the vagus

Cells which fulfilled the criteria of RAR-cells were always sampled in the NTS area where short-latency (< 5 ms) orthodromic field potentials could be evoked by vagal nerve stimulation. Caudal to the obex, the existence of fields from both ipsi- and contralateral vagus nerves was characteristic. These fields developed after the recording microelectrode passed through the more superficially located nucleus gracilis, identified through gentle manipulation of the animal's body. Subsequently, the area of recording was confirmed histologically to correspond to the commissural nucleus of the NTS (see below). Orthodromic fields evoked from the contralateral vagus diminished greatly in amplitude as the microelectrode trackings came closer to the obex level, and were virtually non-existent rostral to the obex, particularly when recordings were made with fine-tip (approx. 1·0 μm) microelectrodes.

The majority (seventy-four of eighty-four) of the RAR-cells recorded both extracellularly and intracellularly, caudal to the obex, could be orthodromically activated following electrical stimulation of both ipsi- and contralateral vagus nerves (Figs 1*A*, 2*A*, 6*A* and 8*A*). The remaining RAR-cells ($n = 10$) recorded caudal to the obex, and all of the RAR-cells ($n = 15$) recorded at, or rostral to, the obex, were activated from the ipsilateral vagus only (Fig. 3*A*). At threshold stimulation, the latencies of the extracellular spikes fluctuated (Fig. 2*A*), but became more stable as the intensity of stimulation was increased above 1·5 times threshold (Figs 1*A*, 6A and 8*A*). This small degree of fluctuation in response latency (generally less than 0·8 ms) was consistent with monosynaptic excitation (see Discussion). The distribution of response latencies is shown in Fig. 7*A*.

Spontaneous activity and response to 'physiological' stimulation

Under control ventilatory conditions around half of the RAR-cells ($n = 51$) were either silent, or exhibited some irregular activity not clearly related to the ventilator. The other RAR-cells showed ventilator-related activity that was primarily related to

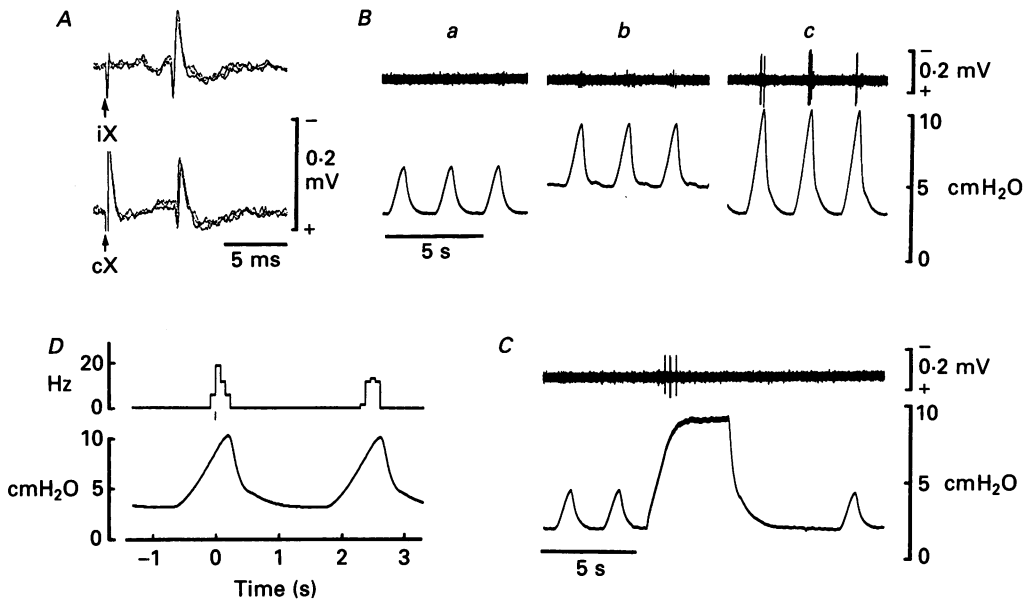

Fig. 1. RAR-cell silent under control ventilatory conditions. *A*, response to ipsi- (iX) and contralateral (cX) vagal stimulation at an intensity of 1·5 times threshold (three superimposed sweeps). *B* and *C*, responses to 'physiological' stimuli (upper traces extracellular recording; lower traces, tracheal pressure). *D*, CTH (computed from nine cycles) showing the relationship between unit discharge and tracheal pressure during the manoeuvre illustrated in *Bc* (upper trace, firing frequency; lower trace, tracheal pressure). Short vertical bar below histogram marks 'zero' time of the histogram.

either ventilator-induced deflations (thirty of forty-eight) or inflations (eighteen of forty-eight).

Figure 1 shows an example of a unit that was silent under control ventilatory conditions (Fig. 1*Ba*), but fired with irregular bursts when the tidal volume was increased (Fig. 1*Bc*). During maintained lung inflation, this unit was activated towards the end of the rising phase of the inflation but the firing quickly adapted during the static phase (Fig. 1*C*). The high degree of dynamic sensitivity was also evident during the increased tidal volume test, and was emphasized after computation of the CTH (Fig. 1*D*, cf. Fig. 2*E*), which shows that the peak firing frequency occurs before the peak of tracheal pressure. Increasing PEEP from 3 to 5·5 cmH_2O did not excite this unit, despite the fact that this manoeuvre also increased the amplitude of the ventilator-related pressure changes (attributable to decreased lung compliance at higher PEEPs) (Fig. 1*Bb*). The increase in 'background noise' associated with the inflations indicated that other units were recruited by this stimulus. Although the responses of this unit to collapse of the lungs and to the

inhalation of ammonia vapour were not tested, the unit illustrated in Fig. 1 was classified as a RAR-cell on the basis of its response to both increased tidal volume and maintained lung inflation.

Figure 2 shows an example of a spontaneously active unit that fired at, or just before, the peak of inflation during control ventilatory conditions (Fig. 2*Ba*). This

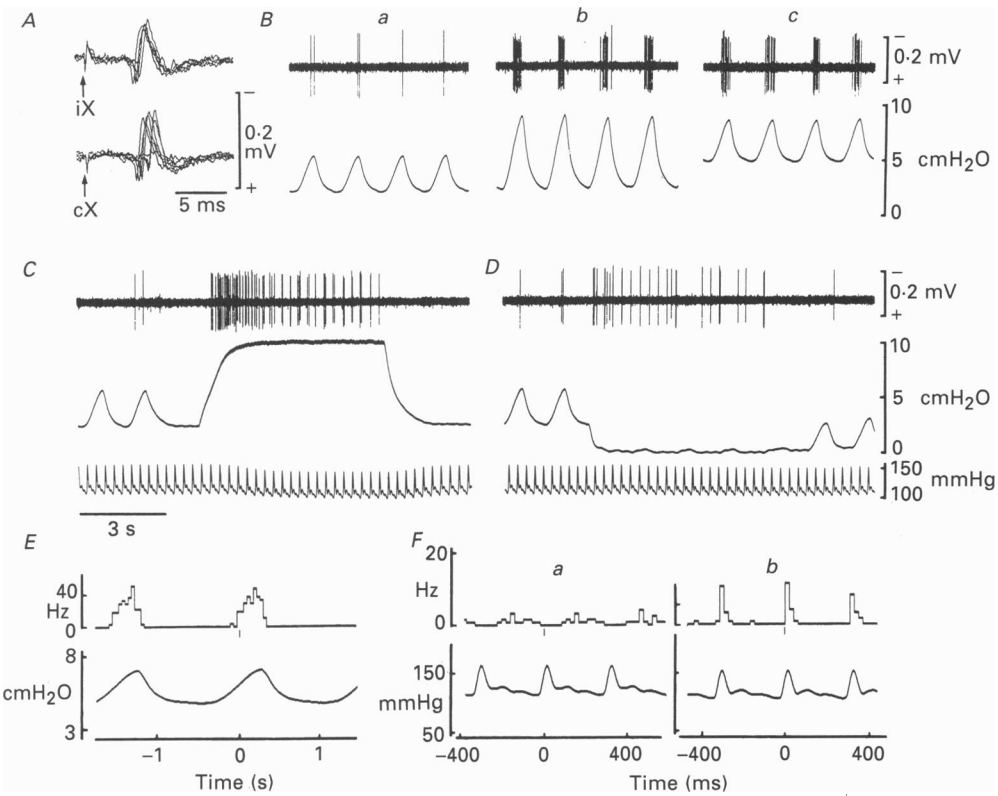

Fig. 2. 'Inflation-sensitive' RAR-cell also activated by collapse of the lungs to atmospheric pressure. *A*, response to ipsi- (iX) and contralateral (cX) vagal stimulation at an intensity slightly greater than threshold (seven superimposed sweeps). *B*, *C* and *D*, responses to 'physiological' stimuli (upper traces, extracellular recordings; lower traces, tracheal pressure). Note rapid adaptation and residual firing with cardiac rhythm following excitation evoked by maintained lung inflation (*C*) and collapse of the lungs to atmospheric pressure (*D*). *E*, CTH computed during the increase in PEEP (*Bc*, seven cycles) showing firing associated with the rising phase of inflation (upper trace, firing frequency; lower trace, tracheal pressure). *F*, CTHs showing the relationship between unit discharge and cardiac cycle (upper traces, firing frequency; lower traces, arterial pressure). Weak cardiac modulation under control ventilatory conditions (*Fa*, sixty-three cycles) becomes clearer following collapse of the lungs to atmospheric pressure (*Fb*, seventeen cycles). Also note the phase change between the systolic pressure and the maximum firing probability of the unit.

relation between the unit's activity and the ventilator became clearer after the tidal volume (Fig. 2*Bb*) or PEEP (Fig. 2*Bc*) was increased. This unit showed increases in activity in response to both maintained inflation (Fig. 2*C*) and collapse of the

lungs to atmospheric pressure (Fig. 2*D*) which rapidly adapted during the static phases of these manoeuvres. The residual firing associated with maintained inflation was shown to have a clear cardiac modulation (Fig. 2*C*). This modulation also became obvious after allowing the lungs to collapse to atmospheric pressure (Fig. 2*D* and *Fb*). In this unit, cardiac modulation could also be revealed under control ventilatory conditions after the firing with respect to the cardiac rhythm (CTH, Fig. 2*Fa*). Interestingly, RAR-cells did not show a fixed phase relationship between their peak firing (as assessed by CTHs) and the cardiac cycle. For the unit illustrated in Fig. 2 there was a higher probability of firing during diastole (Fig. 2*Fa*) under control ventilatory conditions. Following collapse of the lungs to atmospheric pressure, there was a phase shift to systole. Eighteen RAR-cells showed clear cardiac modulation, although a systematic examination for this modulation was not performed on all RAR-cells.

Some RAR-cells fired during ventilator-induced decreases in tracheal pressure (Fig. 3*Ba*). The firing of these units was suppressed by inflation of the lungs produced by increased PEEP or maintained inflation (Fig. 3*Bd* and *Da*), but strongly facilitated by reducing PEEP (Fig. 3*Bb*) or allowing the lungs to collapse to atmospheric pressure (Fig. 3*Db*). This excitation was not simply proportional to the absolute level of the tracheal pressure, but was also dependent upon the dynamic component of the deflation. Firing was more pronounced in response to deflations from higher peak tracheal pressures, such as during the increased tidal volume test (Fig. 3*Bc*) and release from maintained inflation (Fig. 3*Da*), even if the minimum tracheal pressure was not lower than that during control ventilatory conditions. This dynamic sensitivity is emphasized in the CTH which shows that the peak firing frequency corresponded to the decay phase rather than to the minimum level of tracheal pressure (Fig. 3*C*).

Inhalation of ammonia vapour resulted in: (1) an increase in the overall activity recorded from the cervical vagus nerves (Fig. 4*A*), which persisted after the proximal part of the cervical trunk was cut, confirming the afferent nature of the increased activity; (2) an increase in the amplitude and/or duration of the phrenic nerve bursts in most cases (e.g. Figs 4*C* and 8*B*); (3) acceleration of the frequency of central respiratory rhythm (tachypnoea) in most cases (e.g. Figs 4*A* and 8*B*); (4) the induction of bronchoconstriction, which could be abolished by atropine (0·1 mg kg^{-1} I.M.); and (5) occasional small decreases in blood pressure (e.g. Fig. 4*A*). Before atropine administration, changes in both tracheal and blood pressures usually occurred around 1·5–3 s after the onset of changes in vagal and phrenic activity, which were induced almost simultaneously.

Thirty-eight units, classified as RAR-cells on the basis of their responses to manipulations of the lung volume, were tested for their responses to short periods of ammonia vapour inhalation. These units were recorded rostral and caudal to the obex. The majority (twenty-four of thirty-eight, 63%) of these RAR-cells were excited by the inhalation of ammonia vapour, and a typical response of excitation following inhalation of ammonia vapour is illustrated in Fig. 4*C*. This unit was classified as a RAR-cell by its irregular firing during the control period and its response to increased tidal volume and maintained lung inflation (Fig. 4*Da–c*). There was no response to collapse of the lungs to atmospheric pressure (Fig. 4*Dd*). (This

unit's responses to vagal stimulation are shown in Fig. 6*Aa*.) The increase in firing frequency induced by the inhalation of ammonia vapour (Fig. 4*C*) started before the change in the phrenic nerve activity occurred. Another example of a RAR-cell's response to ammonia inhalation is illustrated in Fig. 8*B*. Some RAR-cells ($n = 14$,

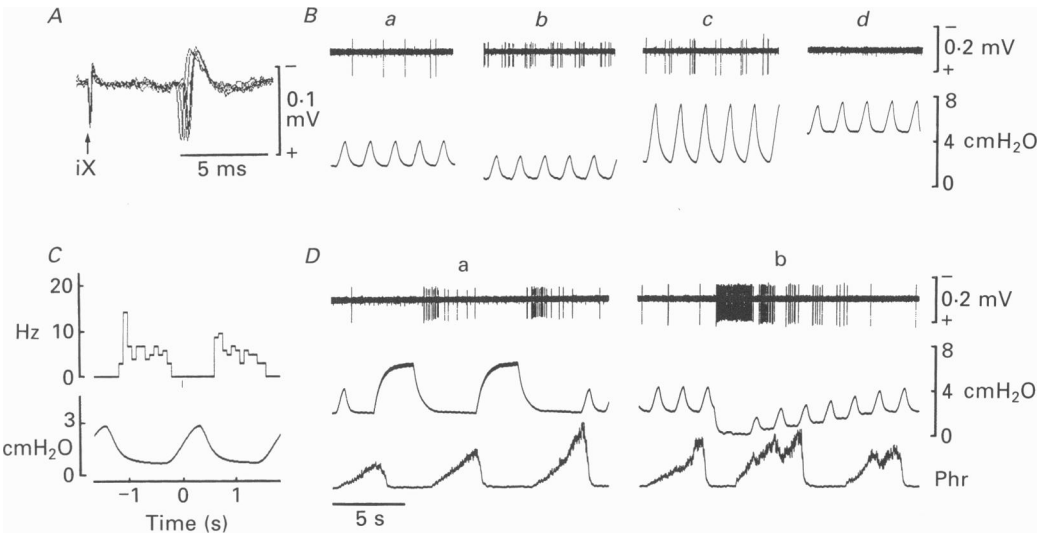

Fig. 3. 'Deflation-sensitive' RAR-cell. *A*, response to ipsilateral vagal stimulation (iX) at an intensity of approximately 1·5 times threshold (five superimposed sweeps). The unit did not respond with a 'short' latency following contralateral vagal stimulation. *B*, (upper traces, extracellular recording; lower traces, tracheal pressure) and *D* (top traces, unit recording; middle traces, tracheal pressure; bottom traces, integrated phrenic discharge (Phr) (time constant = 10 ms)), responses to 'physiological' stimuli. *C*, CTH (computed from thirteen cycles during *Bb*) showing maximal firing during the decay phase of tracheal pressure (upper trace, firing frequency; lower trace, tracheal pressure).

37 %) tested were not clearly affected by ammonia. In addition, ammonia evoked excitation in ten neurons which responded to electrical stimulation of the vagus and to one 'physiological' stimulus involving manipulation of the lung volume, in a manner indicative of RAR input. Consequently, these units were classified as RAR-cells. Thus, of the forty-eight RAR-cells tested, thirty-four were excited (71 %). The remaining fourteen RAR-cells were neither excited nor inhibited by the inhalation of ammonia vapour.

As described above, many RAR-cells showed firing clearly synchronized to either ventilator-induced inflations or deflations. This synchronization was apparent during control ventilatory conditions, or became apparent after an increase of the tidal volume or when PEEP was decreased. In a few cases, however, the precise phase relationship to lung volume was found to be changeable and dependent upon experimental conditions. Figure 5 shows two such examples. A RAR-cell which was normally excited during ventilator-induced deflations (Fig. 5*Aa*) fired during both deflation and inflation after increasing PEEP (Fig. 5*Ab*), and predominantly during inflations after a further increase of PEEP (Fig. 5*Ac*). Another RAR-cell which was initially excited mainly during ventilator-induced deflations (Fig. 5*Ba*) also fired

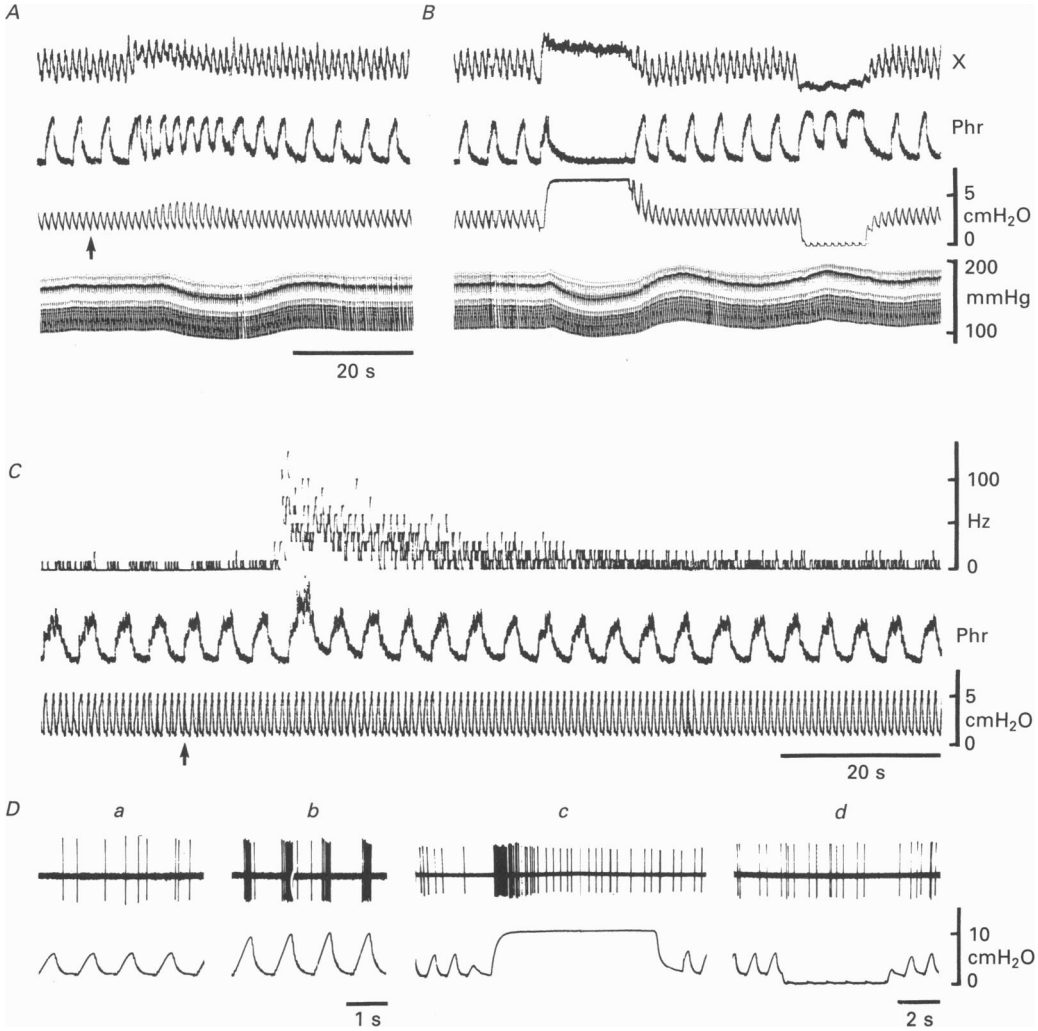

Fig. 4. Responses of the whole vagus (*A*) and of a RAR-cell (*C*) to the inhalation of ammonia vapour and other 'physiological' stimuli (*B* and *D* respectively). Traces in *A* and *B* (from top down): integrated discharge recorded from an intact cervical vagus (X) (time constant = 100 ms), integrated phrenic nerve discharge (Phr) (time constant = 100 ms), tracheal pressure, and blood pressure. Note in *A* the increase in the afferent vagal activity and reflex responses evoked by inhalation of ammonia administered at the arrow. Also note the premature onset of inspiration triggered by the rising phase of the maintained lung inflation (*B*) and the increase in phrenic discharge during collapse of the lungs to atmospheric pressure. Both responses are likely to be associated (in part) with activation of RARs. *C*, response of a RAR-cell to ammonia vapour (top trace, firing frequency; middle trace, integrated phrenic discharge (Phr) (time constant = 100 ms), bottom trace, tracheal pressure). *D*, responses of the unit recorded in *C* to 'physiological' stimuli (upper traces, extracellular recording; lower traces, tracheal pressure). Atropine was administered in the interval between recording the whole vagus (*A* and *B*) and the individual RAR-cell (*C* and *D*), which abolished the airway pressure changes induced by ammonia vapour inhalation seen in *A*.

during ventilator-induced inflations following a combination of increased tidal volume and PEEP (Fig. 5*Bb*), and after inhalation of ammonia vapour (Fig. 5*Bc*). Similar phase 'reversals' were observed in another nine RAR-cells.

Intracellular recordings

A total of twenty-five neurons were recorded intracellularly. The majority ($n = 22$) responded with EPSPs following stimulation of the vagus nerve(s) (membrane

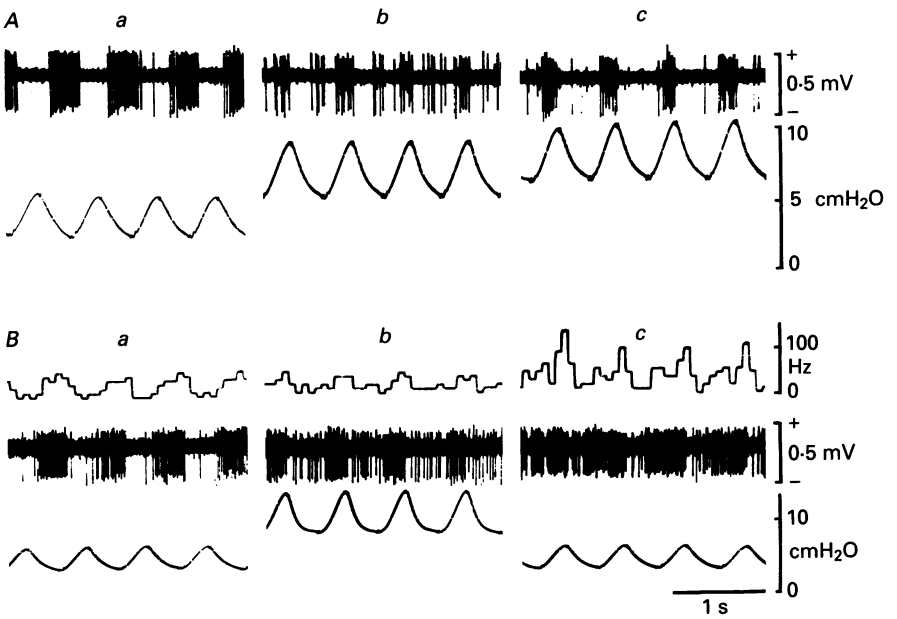

Fig. 5. Variability of ventilator-related activity in two RAR-cells. *A*, 'deflation-sensitive' RAR-cell becomes sensitized to inflation following increasing PEEP (*Ab* and *Ac*) (upper traces, extracellular recording; lower traces, tracheal pressure). *B*, another RAR-cell showing alteration of phase relationship following both lung volume manipulation (*Bb*) and inhalation of ammonia (*Bc*) (top traces, firing frequency; middle traces, extracellular recording; bottom traces, tracheal pressure).

potentials ranging from 10 to 60 mV (mean 37 ± 14 mV)). It is unlikely that these responses represented 'reversed' inhibitory postsynaptic potentials (IPSPs) as the depolarizing potentials were observed immediately following impalement. Hyperpolarizing potentials, indicative of IPSPs, were observed in three other neurons following electrical stimulation of the ipsilateral vagus (latencies were 4·3, 4·5 and 5·6 ms). Most of the intracellular recordings (twenty-three of twenty-five) were not stable enough to allow 'physiological' tests to positively verify these neurons as RAR-cells. On two occasions, however, successful identification of a penetrated neuron as a RAR-cell was possible. A RAR-cell was penetrated just after electrical stimulation of the vagus nerves (Fig. 6*Aa*) and complete characterization with 'physiological' stimuli (Fig. 4*C* and *Da–d*). This neuron received excitatory postsynaptic potentials (EPSPs) from both vagus nerves (Fig. 6*Ab*) with fixed

latencies (ipsilateral, 4·3 ms; contralateral, 5·1 ms) and fast rise times, indicating that the EPSPs were monosynaptic. Another RAR-cell was also presumed to be monosynaptically activated from both vagus nerves (Fig. 6*Ba*). It exhibited no synaptic activity related to lung volume during control ventilatory conditions (Fig.

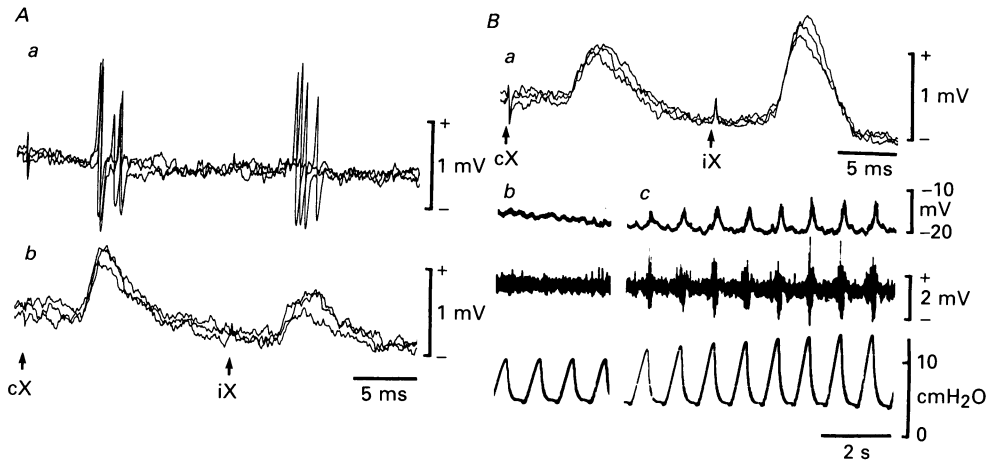

Fig. 6. Intracellular recordings from two RAR-cells. *Aa*, a RAR-cell (characterization of this cell illustrated in Fig. 4*C* and *Da–d*) was excited by stimulation of both ipsi- (iX) and contralateral (cX) vagus at an intensity of approximately 1·5 times threshold (four superimposed sweeps). *Ab*, EPSPs recorded following penetration of RAR-cell shown in *Aa* (three superimposed sweeps). Arrows mark stimulus artifacts for vagal stimulation. *B*, another RAR-cell also responding with EPSPs following vagal stimulation (*Ba*). In *Bb* and *Bc*: top traces, membrane potential; middle traces, high-gain intracellular AC recording; bottom traces, tracheal pressure. Note increased synaptic noise evoked by increasing tidal volume (*Bc*).

6*Bb*), but after increasing the tidal volume, depolarizing membrane shifts locked to lung inflations became evident (Fig. 6*Bc*, upper trace). These ventilator-induced depolarizing shifts of the membrane potential were associated with increased levels of synaptic noise as shown in a high-gain AC recording (Fig. 6*Bc*, middle trace). Furthermore, inhalation of ammonia vapour induced depolarization in this neuron in conjunction with an increase in phrenic nerve activity (not illustrated).

Figure 7 shows a comparison between the orthodromic latencies following electrical stimulation of the vagus nerves in twenty-two impaled neurons and eighty-one of the eighty-four extracellular RAR-cells. (The latencies for three extracellularly recorded RAR-cells were not measured.) Only those RAR-cells recorded caudal to the obex are included in Fig. 7*A*, and only those impaled neurons that responded with EPSPs (including the two neurons verified as RAR-cells) are included in Fig. 7*B*. Ipsilateral latencies were significantly shorter than contralateral latencies in extracellular recordings ($P < 0{\cdot}0001$) but less so for intracellular recordings ($P < 0{\cdot}03$). The majority of extracellularly recorded RAR-cells caudal to the obex (seventy-one of eighty-one) were excited following stimulation of both ipsi- and contralateral vagus nerves, with latencies ranging from 3·7 to 7 ms (mean $4{\cdot}8 \pm 0{\cdot}8$ ms) following ipsilateral, and 4 to 10 ms (mean $5{\cdot}9 \pm 1{\cdot}0$ ms) following

contralateral vagal stimulation. By comparison, RAR-cells recorded extracellularly at, and rostral to, the obex showed a similar distribution of latencies (3·5–5·5 ms; mean 4·5 ± 0·7 ms), although they were activated from the ipsilateral vagus only.

Respiratory modulation of RAR-cells

Some RAR-cells showed firing that was modulated synchronously with central respiratory rhythm. An example is shown in Fig. 8. This RAR-cell, firing

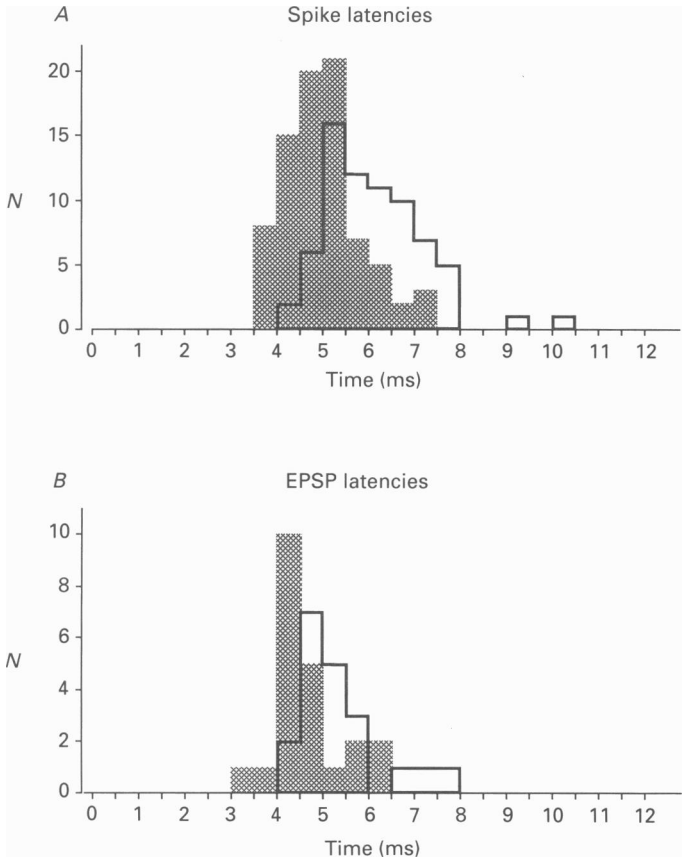

Fig. 7. Distribution of response latencies following vagal stimulation for extracellular spikes (*A*) and intracellular EPSPs (*B*). Latencies to extracellular spikes were measured following stimulation at 1·5–3·0 times threshold, whereas latencies to the onset of EPSPs were measured following stimulation at lower intensities (usually 1–1·5 times threshold) in order to avoid action potentials by evoked EPSPs. ▨, ipsilateral vagus; □, contralateral vagus.

synchronously with ventilator-induced lung deflations, was excited by vagal stimulation from both sides, ammonia and collapse of the lungs to atmospheric pressure (Fig. 8*A*, *B* and *D*). Under control ventilatory conditions, the respiratory modulation in its firing was weak, but it became more evident when the PEEP was

decreased (Fig. 8*C*) and especially obvious during the maintained collapse of the lungs to atmospheric pressure (Fig. 8*D*). This manoeuvre also induced strong continuous firing in this RAR-cell.

The respiratory modulation was superimposed upon the RAR-cell's ventilatory modulation, and manifested as a ramp-like increase in firing frequency. This was

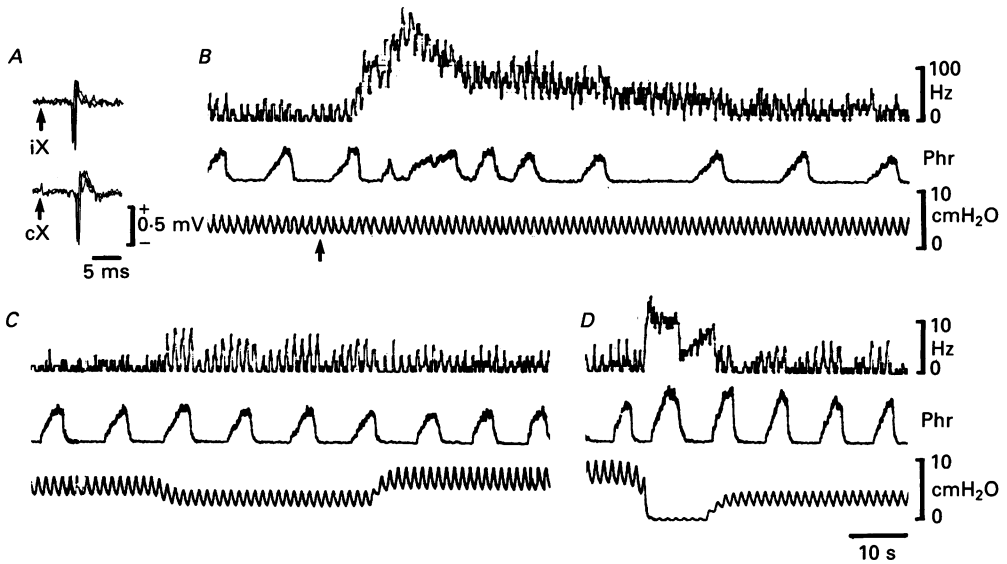

Fig. 8. Respiratory modulation in RAR-cell activity. *A*, response to both ipsi- (iX) and contralateral (cX) vagal stimulation at an intensity of approximately 1·5 times threshold (three superimposed sweeps). *B*, response to inhalation of ammonia vapour applied at the arrow (in *B*, *C* and *D*: top trace, firing frequency; middle trace, integrated phrenic discharge (Phr) (time constant = 20 ms); bottom trace, tracheal pressure). Respiratory modulation was exaggerated by decreasing PEEP (*C*) and by allowing the lungs to collapse to atmospheric pressure (*D*). Note the clear reduction of firing associated with the inspiration–expiration transition in *D*, and the ventilatory pattern of this 'deflation-sensitive' RAR-cell superimposed upon its respiratory pattern.

maximal during late inspiration before it was suddenly inhibited at the inspiration-to-expiration transition (see Fig. 8*D*). The firing frequency then gradually increased through the remaining period of expiration and the subsequent inspiration. The magnitude of the respiratory modulation tended to parallel central respiratory activity. Respiratory modulation of this type could be observed in fourteen RAR-cells, with the majority ($n = 11$) being spontaneously active and 'deflation sensitive'.

Location of RAR-cells

The location of forty-three RAR-cells (from eleven experiments) are illustrated in Fig. 9*A*. Fifteen of the forty-three were histologically verified based upon the deposits of Fast Green FCF dye from five experiments. Fourteen 'marked' RAR-cells were located caudal to the obex, and twelve of these (located 0·75–1·75 mm caudal to the obex) have been projected onto the transverse plane (Fig. 9*B*). Inspiratory neurons recorded in the course of these experiments are also plotted on the same horizontal

plane in order to give an indication of the ventral and ventrolateral subnuclei of the NTS, which are known to include a large concentration of inspiratory neurons (e.g. von Baumgarten & Kanzow, 1958).

All of the RAR-cells recorded caudal to the obex and marked by dye (except for one which was dorsomedial to the solitary tract) were located in the commissural

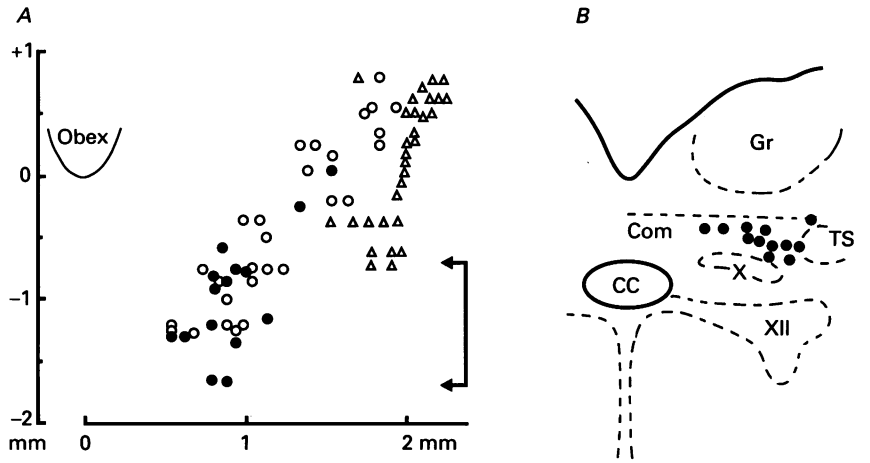

Fig. 9. Location of RAR-cells. *A*, horizontal projections of RAR-cells 'marked' by Fast Green deposits (●) and those reconstructed from co-ordinate readings relative to the obex(○). △ indicate inspiratory neurons of the ventrolateral subnucleus of the NTS. *B*, projections of 'marked' RAR-cells (located between the arrows in *A*) onto a transverse section corresponding to the level approximately 1·0 mm caudal to the obex. In *B* the locations were reconstructed relative to the solitary tract (TS) and the dorsal motor nucleus of the vagus (X). CC, central canal; Com, commissural nucleus; Gr, gracile nucleus; XII, hypoglossal nucleus.

nucleus (Fig. 9*B*). Many RAR-cells (more than sixty-five) were sampled using oblique electrode trackings (see Methods), and their positions could not be reliably reconstructed. Nevertheless, using the existence of orthodromic fields from both vagus nerves as being indicative of the commissural nucleus, the location of the RAR-cells (caudal to the obex) within this nucleus was confirmed by the existence of these fields at the sites of the unit recordings.

RAR-cells recorded rostral to the obex were recorded in the areas medial and dorsomedial to the site of inspiratory neurons as assessed from co-ordinate readings. A RAR-cell which was marked at the level of the obex (see Fig. 9*A*) was situated in the NTS just medial to the solitary tract.

Other units encountered in the nucleus of the solitary tract caudal to the obex

In addition to the RAR-cells, smaller numbers of the following classes of neurons were also found in the caudal subdivisions of the NTS:

(1) Twelve inspiratory neurons were found in the ventral and ventrolateral subnuclei of the NTS, in a column extending 0·7 mm caudal to the obex (cf. Fig. 9*A*, △).

(2) Thirteen units were found that responded to vagal stimulation but not to any of the 'physiological' stimuli used to characterize RAR-cells. All the units responded to the ipsilateral vagus (latency, $5{\cdot}0 \pm 1{\cdot}0$ ms), and some ($n = 9$) also responded to the contralateral vagus ($6{\cdot}7 \pm 1{\cdot}6$ ms).

(3) Eight P cells (Berger, 1977) and three PSR afferents.

(4) Six cells and two vagal afferents were found that fired with a strong cardiac modulation. One (out of five tested) of these units was clearly excited by the ipsilateral vagus (latency, 4 ms) while another showed a very high threshold to ipsilateral vagal stimulation (latency not recorded). Two showed unclear responses and one was not affected by ipsilateral vagal stimulation. None responded to the contralateral vagus.

DISCUSSION

Although the terminal projection sites for RAR afferents have been described previously (Davies & Kubin, 1986; Kubin & Davies, 1988; Kalia & Richter, 1988*a*, *b*), to our knowledge this is the first report describing the location and response characteristics of NTS neurons receiving this input in the cat. The existence of cells excited by RARs has recently been briefly described in the caudal NTS in the rat by Bonham & McCrimmon (1990). Several factors have hindered the identification of these neurons (cf. Kubin & Davies, 1988): (1) the small size of the neurons in the caudal and medial subdivisions of the NTS (8–15 μm; King, 1980; Maley & Panneton, 1988); (2) the fact that large changes in lung volume are necessary to activate RARs and thus identify RAR-cells, resulting in unstable recording conditions; and (3) the difficulty in selectively activating RARs without concomitant activation of other types of lung receptors (PSRs and J receptors). The latter two factors will be discussed first.

Criteria for the identification of RAR-cells

Although RARs are known to have little or no activity during normal, eupneic breathing in the intact cat (Paintal, 1973), many show pronounced discharge in animals with a pneumothorax (Sellick & Widdicombe, 1969; Armstrong & Luck, 1974; Yu *et al.* 1987). To reduce ventilatory movement artifacts, in addition to a pneumothorax, low tidal volumes (< 20 ml) and high-frequency (50–65 min^{-1}) ventilation were often used in the present study. While deflation was passive under our ventilatory conditions, the high frequency of ventilation would have increased the rate of change in lung volume for inflations. This may have offset the decrease in tidal volume, and thus maintained the activity of many RARs, as they are known to have a marked dynamic sensitivity (Pack, 1981) in addition to their generally high volume thresholds. Baseline activity of RARs was inferred from the fact that under our control ventilatory conditions, around half of the RAR-cells showed spontaneous activity, either irregular or with a rhythmic component linked to the ventilator. In cases when such rhythmic activity was present, it was observed either during ventilator-induced inflations or deflations, suggesting that under our experimental conditions the activation of at least some RARs occurred in both phases (cf. Armstrong & Luck, 1974; Yu *et al.* 1987)

Responses to low-intensity electrical stimulation of the vagus nerves were used as an initial stimulus to identify neurons excited by vagal afferents. The vagal stimuli

used in the present study were subthreshold for the activation of unmyelinated afferents, and thus prevented the recruitment of neurons activated solely by afferents from pulmonary (i.e. J receptors) and bronchial C fibre receptors (Coleridge & Coleridge, 1986). The possibility of an additional input from unmyelinated afferents onto RAR-cells cannot be excluded, but this concept is not supported by a previous finding (Bennett, Goodchild, Kidd & McWilliam, 1985) that showed no convergence from both myelinated and unmyelinated vagal afferents onto NTS neurons. Although electrical stimulation cannot be used to selectively activate one of the two main groups of myelinated vagal pulmonary afferents (RARs and PSRs), thresholds to excite RAR-cells were usually higher than those needed to evoke the reflex inhibition of inspiratory activity. The threshold difference could be explained by a generally smaller diameter and lower conduction velocity of afferent fibres from RARs (Paintal, 1973; Armstrong & Luck, 1974; Davies & Kubin, 1986) compared with PSRs, which are known to mediate the Hering–Breuer reflex.

Increasing tidal volume was used to induce or increase RAR activity. Despite the fact that this stimulus also recruited PSRs, and thus could not be used as a single criterion for the identification of RAR-cells, it was, nevertheless, useful for several reasons. First, it triggered activity in a large number of RAR-cells which showed no spontaneous activity under control ventilatory conditions. Secondly, the frequency of action potentials within each burst of discharge evoked in RAR-cells by large tidal volume inflations was generally irregular in comparison to the activity induced by PSRs in P cells and R_β cells (e.g. Berger, 1977). This probably reflected the variability of RAR discharge (cf. Armstrong & Luck, 1974) and, as such, was indicative of this input. Thirdly, increased tidal volume provided a means of producing strong activation of lung mechanoreceptors without evoking large changes in blood pressure, observed during maintained inflations or following collapse of the lungs to atmospheric pressure, that led to recording instability. Finally, this stimulus turned out to be useful in demonstrating inflation sensitivity in some RAR-cells which were otherwise preferentially activated by lung deflations (see below).

Assessing the rate of discharge adaptation during maintained lung inflations has been used by many investigators to distinguish between afferents from PSRs and RARs (Knowlton & Larrabee, 1946; Widdicombe, 1954; Mills *et al.* 1969; Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Pack & DeLaney, 1983; Yu *et al.* 1987; Yu & Roberts, 1990). In our study, adaptation of firing in RAR-cells after the initial burst evoked during the rising phase of inflation was observed, but was not used systematically as a criterion due to three complicating factors. First, in some RAR-cells, the initial burst of activity in response to the rising phase of the maintained inflation was followed by a rhythmic discharge associated with the cardiac cycle during the static phase of the inflation, and thus these RAR-cells showed less evidence of adaptation in response to maintained inflation. This type of cardiac cycle-related activity (also observed in some RAR-cells during collapse of the lungs at atmospheric pressure) was likely to be a 'physiological artifact' induced in the firing of RARs following increased mechanical coupling of the lungs with the heart and large blood vessels in the thorax. This interpretation is supported by previous reports describing cardiac rhythmicity in the firing of a large proportion of RAR afferents (Knowlton & Larrabee, 1946). The possibility that a component of this phenomenon was due to

the input from baroreceptor afferents was excluded on the basis of our observation that the phase relationship between the cardiac-related activity of RAR-cells and the cardiac rhythm could be changed when the degree of lung inflation was altered. The significance of this heart-related discharge is twofold: (1) during maintained lung inflations, the 'adaptation index' could not be easily measured in RAR-cells; and (2) due to this apparent lack of adaptation, cells receiving a RAR input can be mistakenly identified as receiving PSR input.

The second complicating factor is that some RARs genuinely show little evidence of adaptation during maintained lung inflations, particularly when the dynamic lung compliance is reduced and 'moderate' inflations are used (Yu *et al.* 1987). The third factor relates to the mechanism of adaptation. Adaptation of firing can result from accommodative properties of neurons (e.g. due to the summation of after-hyperpolarizations; cf. Gustafsson, 1984), or as a result of increasing inhibition (cf. Davies, Metzler, Silage & Pack, 1986) as opposed to a decrease in excitation from RAR afferents. Activation of RAR-cells by the inhalation of ammonia vapour, however, did not result in clear adaptation despite the often high frequencies of firing attained in response to this stimulus.

Collapse of the lungs to atmospheric pressure increased (or induced) activity in many RAR-cells, particularly those which showed spontaneous firing associated with ventilator-induced deflations under control ventilatory conditions. This manoeuvre is known to result in strong activation of RARs in animals with pneumothorax (e.g. Sellick & Widdicombe, 1969; Armstrong & Luck, 1974). Such deflations were used as an important differentiating stimulus in that they did not activate J receptors, which also have an irregular pattern of discharge and are activated by inflations (Armstrong & Luck, 1974).

Exposure of the lungs to irritant gasses has been shown to be an effective stimulus in activating RARs in some species, including cats, in which a large proportion of RARs are excited by this stimulus. For example, nine out of eleven RAR afferents tested in the study by Armstrong & Luck (1974) were stimulated. Short periods of ammonia vapour inhalation activated the majority of our commissural RAR-cells that responded to electrical stimulation of the ipsi- and contralateral vagus, and to at least one additional test involving change of the lung volume. Excitatory responses of RAR-cells after ammonia inhalation paralleled the onset of both the increased afferent activity recorded from the whole cervical vagus nerve and the increased inspiratory activity, followed by bronchoconstriction and a decrease in blood pressure. Although these responses confirmed the usefulness of ammonia vapour in differentiating neurons receiving RAR inputs from those receiving PSR inputs (the latter group was shown to be unaffected), the specificity of the stimulus is limited in light of a previous finding showing the concomitant excitation of J receptors with this stimulus (Armstrong & Luck, 1974).

Some neurons examined in this study responded to low-threshold stimulation of the vagus, but were not affected by any of the above-described 'physiological' stimuli. It is not clear whether such neurons belonged to a 'high-threshold' group of RAR-cells (i.e. they would require levels of RAR activation beyond that used in this study), or represent another, functionally unidentified, category.

Although there remains the possibility that some of the identified RAR-cells could

have represented a neuronal population activated by both P cells and J receptors, this is considered unlikely in view of the multiple criteria used to identify RAR-cells.

Deflation-sensitive RAR-cells

Two previous studies (Shannon, 1980; Pantaleo & Corda, 1986) identified NTS units which were activated by lung deflations, but inhibited by inflations. The firing pattern of these units, designated by Pantaleo & Corda (1986) as 'inverse P', appeared to be similar to the 'deflation-sensitive' RAR-cells in our study which were also activated by lung deflations but usually stopped firing during inflations. Despite these similarities, the neurons described by Pantaleo & Corda (1986) were inhibited by vagal stimulation (no vagal stimulation was used in the experiments by Shannon (1980)) whereas the units recorded in our study were excited. For that reason, and given that our units showed less regular firing than the 'inverse P' neurons of Pantaleo & Corda (1986), it is unlikely that they belong to the same class.

Another group of lung volume-sensitive NTS neurons called P cells (Berger, 1977) is known to be excited by the input from PSRs. A subclass of these project to the contralateral commissural nucleus (Davies *et al.* 1987). As it is not known whether these neurons are excitatory or inhibitory, the significance of their commissural projection is unclear. If the P cells exert an inhibitory synaptic action, then it could be suggested that the deflation-sensitive units observed in our experiments form a separate group inhibited by P cells. This hypothesis is not compatible with the findings which, for the following reasons, indicate that the 'deflation-sensitive' units are excited by RARs: (1) administration of ammonia vapour invariably excited deflation-sensitive units; (2) when recordings were made from units showing spontaneous activity during lung deflations, changing the level of excitation of RARs (by increasing PEEP, or during the decay phase of excitation evoked by ammonia) often resulted in the 'reversal' of the firing pattern, i.e. stronger firing was observed during lung inflations. A similar 'reversal' in RAR afferent activity was observed in comparable experimental conditions by others (Yu *et al.* 1987, Fig. 6). (3) During lung deflations associated with the control ventilatory condition, or during deflations following release from maintained lung inflations, the peak firing frequency of the 'deflation-sensitive' units often occurred during the decay phase of the tracheal pressure rather than at the lowest pressure values, when the activity of P cells would be minimal. (4) RARs with activity present during lung deflations were observed by other investigators (Mills *et al.* 1969). However, these results do not exclude the possibility that the hypothesized inhibitory input from P cells contributes, at least in part, to the firing pattern of the deflation-sensitive neurons. The units active during lung deflations receive stronger excitatory input from RARs during that phase.

Is the vagal RAR input monosynaptic?

Assessment of the number of synapses interposed in the pathway from RARs to the examined RAR-cells could be done only on the basis of their responses following electrical stimulation of the vagus. Several lines of evidence indicated that the RAR input was monosynaptic. Firstly, RAR-cells were found in the NTS region known to

contain dense terminal branching of RAR afferents (Davies & Kubin, 1986). Secondly, unit responses were evoked by single-pulse electrical stimuli, and there was little latency variability when the stimuli were at 1·5–3·0 times threshold. Thirdly, the range of response latencies after vagal stimulation was comparable to the latencies of antidromic responses reported from RAR-activated neurons in the nodose ganglion following electrical stimulation within the ipsi- and contralateral commissural nucleus (Davies & Kubin, 1986, and personal communication). In the study by Davies & Kubin (1986), the antidromic latency measured for six nodose ganglion cells ranged from 2·3 to 9·4 ms following ipsilateral stimulation and from 2·8 to 7·8 ms following contralateral stimulation. The somewhat longer orthodromic latencies observed in the present study could be due to the longer conduction distance since the cervical portion of the vagus was stimulated in our experiments, while in the study by Davies & Kubin (1986) recordings were made from the nodose ganglion, approximately 2·0 cm rostral. The conduction distance of 2·0 cm would account for the latency difference of 1·2 ms, assuming that the mean conduction velocity of RAR fibres is 16·1 m s^{-1} (Armstrong & Luck, 1974; Davies & Kubin, 1986). An additional delay for orthodromic responses was caused by the synaptic delay and the time for the EPSPs to reach threshold.

The majority of RAR-cells localized caudal to the obex responded to bilateral stimulation of the vagus nerves with latencies indicating monosynaptic inputs from both sides. This conclusion is also supported by a previous study (Davies & Kubin, 1986) showing the bilateral projections of RAR afferents. This pattern of vagal afferent connections contrasts with the organization of PSR input to both R_β and P cells, known to receive monosynaptic excitation from the ipsilateral side only (for discussion see Davies, Kubin & Pack, 1987), corresponding to previous findings demonstrating PSR afferents projecting to the ipsilateral subdivisions of the NTS only (e.g. Donoghue, Garcia, Jordan & Spyer, 1982; Kalia & Richter, 1985; Davies & Kubin, 1986).

Intracellular recordings from the commissural neurons revealed fast-rising, clearly monosynaptic (latency jitter $< 0{\cdot}1$ ms) EPSPs that showed latencies similar to those of extracellularly recorded spikes. Although testing for RAR input could not be done in most of these cells due to the poor recording stability, identification of this input in two neurons indicated that the group belonged to the same category of RAR-cells.

Location of RAR-cells

Conflicting results were obtained in previous studies mapping the areas of terminal arborizations of vagal RAR afferent fibres. In the antidromic mapping study by Davies & Kubin (1986) the densest branching of these fibres was found in the ipsilateral commissural nucleus, with some crossing the mid-line to the contralateral nucleus. In parts of the NTS rostral to the obex, a smaller number of branches were found. Similar projection areas, as well as sites of focal synaptic potentials, were found in another study utilizing the technique of spike-triggered averaging (Kubin & Davies, 1988). On the other hand, intra-axonal labelling experiments (Kalia & Richter, 1988*a*) revealed the largest concentrations of terminal boutons to be rostral to the obex, in the dorsal, dorsolateral and intermediate subnuclei of the NTS. The reason for the discrepancy between the electrophysiological and anatomical findings is not clear. One contributing factor could be the difficulty in tracing stained

branches several millimetres away from the axonal injection sites in darkly stained (osmicated) sections prepared for electron microscopic analysis (Kalia & Richter, 1988*a*, *b*).

The location of RAR-cells described here corresponds to the sites of RAR afferent terminations identified in previous electrophysiological studies (Davies & Kubin, 1986; Kubin & Davies, 1988). The largest concentration of RAR-cells was found in the commissural nucleus while fewer RAR-cells were identified between the obex level and 0·8 mm rostral to the obex. This group could be receiving input from RAR afferents terminating rostral to the obex, as described by Kalia & Richter (1988*a*, *b*), or from rostral branches of the caudally projecting RAR afferents as they coursed to the commissural nucleus. Davies & Kubin (1986) found that each RAR afferent gave a few branches off to the medial NTS, rostral to the obex.

Although RAR-cells appeared to be the most easily identifiable neuronal group in the commissural nucleus, other types of units were also encountered, including cells driven by afferents from arterial baroreceptors and PSRs. This observation is consistent with the view that there are few, if any, specific subnuclei of the NTS dedicated to receiving afferent input from any particular visceral organ, although differences exist in terms of the relative importance of different subnuclei (cf. Kalia & Mesulam, 1980; Jordan & Spyer, 1986; Kalia & Richter, 1988*b*).

Connections of RARs and RAR-cells with respiratory neurons

Stimulation of RARs evokes bronchoconstriction and a complex pattern of responses in the respiratory motor output which, in deeply anaesthetized animals, includes an increase in the frequency of inspiratory efforts and an increase in the rate of rise and amplitude of the inspiratory activity (Mills, Sellick & Widdicombe, 1970). At present it is not clear whether all these reflex responses are mediated through the RAR-cells described in this study. It has been suggested that RARs can contribute to the excitation of at least two other groups of NTS neurons: P cells (Anders & Richter, 1987) and some R_{β} cells (Bianchi & Barillot, 1975; Berger, 1977; Marino, Davies & Pack, 1981; Davies & Kubin, 1986). In addition, increased activity from RARs was shown to excite some early expiratory neurons in the ventrolateral medulla, with excitation preceded by a short period of inhibition (Richter, Ballantyne & Remmers, 1987).

Several anatomical studies describe a strong projection from the commissural and medial NTS to the dorsolateral region of the rostral pons (e.g. Kalia, 1977; Loewy & Burton, 1978; King, 1980), the area often associated with the so-called 'pneumotaxic centre' (e.g. Feldman, 1986). It remains to be established whether RAR-cells project to this or other regions of the brain stem. It is also not clear as to what degree the activity of RAR-cells is influenced from brain stem respiratory neurons. Some of the deflation-sensitive RAR-cells described in the present study showed respiratory modulation, typically a decrease in the firing frequency following the peak of the phrenic nerve discharge.

Undoubtedly further studies are needed to investigate connections between RAR-cells and other groups of brain stem respiratory neurons in order to elucidate the neural organization of respiratory reflexes evoked by RARs.

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