Effect of cardiopulmonary C fibre activation on the firing activity of ventral respiratory group neurones in the rat

Christopher G. Wilson and Ann C. Bonham*

Departments of Internal Medicine and Pharmacology, University of California at Davis, Davis, CA 95616, USA

1. Cardiopulmonary C fibre receptor stimulation elicits apnoea and rapid shallow breathing, but the effects on the firing activity of central respiratory neurones are not well understood. This study examined the responses of ventral respiratory group neurones: decrementing expiratory (E_{dec}), augmenting expiratory (E_{aug}), and inspiratory (I) neurones during cardiopulmonary C fibre receptor-evoked apnoea and rapid shallow breathing.

2. Extracellular neuronal activity, phrenic nerve activity and arterial pressure were recorded in urethane-anaesthetized rats. Cardiopulmonary C fibre receptors were stimulated by right atrial injections of phenylbiguanide. Neurones were tested for antidromic activation from the contra- and ipsilateral ventral respiratory group (VRG), spinal cord and cervical vagus nerve.

3. E_{dec} neurones discharged tonically during cardiopulmonary C fibre-evoked apnoea and rapid shallow breathing, displaying increased burst durations, number of impulses per burst, and mean impulse frequencies. E_{dec} neurones recovered either with the phrenic nerve activity (25 s) or much later (3 min).

4. By contrast, the firing activity of E_{aug} and most I neurones was decreased, featuring decreased burst durations and number of impulses per burst and increased interburst intervals. E_{aug} activity recovered in ~3 min and inspiratory activity in ~1 min.

5. The results indicate that cardiopulmonary C fibre receptor stimulation causes tonic firing of E_{dec} neurones and decreases in E_{aug} and I neuronal activity coincident with apnoea or rapid shallow breathing.

Respiratory rhythm is generated by a central respiratory network, but it may be modified by synaptic input from peripheral sensory receptors, notably from those in the lungs and airways. The bronchopulmonary C fibre receptors are activated by various stimuli: the environmental toxicants ozone and cigarette smoke; changes in airway surface osmolarity; and autacoids and mechanical perturbations associated with acute lung oedema (Coleridge & Coleridge, 1986, 1994). When activated, the receptors elicit well-known defensive reflexes that include apnoea and/or rapid shallow breathing, bronchoconstriction, bradycardia, hypotension, and increased airway secretion (Coleridge & Coleridge, 1994). Vagal afferent C fibre receptors are also located in the myocardium, largely in the ventricles but also in the atria (Thorén, 1979). Like the bronchopulmonary C fibre receptors, cardiac C fibre receptors also elicit rapid shallow breathing, apnoea, bronchoconstriction, hypotension and bradycardia. The C fibre receptors in the lungs and heart are often collectively referred to as cardiopulmonary C fibre receptors (Thames & Schmid, 1979; Verberne & Guyenet, 1992; Vardhan, Kachroo & Sapru, 1993), the term we will use here.

The reflex ventilatory responses have been extensively studied (Coleridge & Coleridge, 1986, 1994), but there is very little information on the changes in firing activity of central respiratory neurones during cardiopulmonary C fibre receptor activation. Koepchen and colleagues originally suggested that cardiopulmonary C fibre receptor stimulation produced apnoea by dually inhibiting inspiratory and expiratory medullary unit activity (Koepchen, Kalia, Sommer & Klussendorf, 1977). More recent modelling of the respiratory network divides respiration into three phases: an inspiratory phase, an early expiratory (post-inspiratory) phase and a late expiratory phase (Richter, 1982; Richter, Ballantyne & Remmers, 1986). Simulations have generally confirmed that this triphasic network model is sufficient to explain the generation of respiratory rhythm and also the changes in respiratory rhythm in response to perturbations that may arise from input from peripheral sensory receptors (Ogilvie, Gottschalk, Anders, Richter & Pack, 1992; Balis,

* To whom correspondence should be addressed.

Morris, Koleski & Lindsey, 1994). This triphasic model suggests a more complex behaviour of respiratory neuronal activity than a parallel inhibition of inspiratory and expiratory firing activity. Accordingly, the purpose of this study was to determine the effect of cardiopulmonary C fibre receptor stimulation on the firing activity of expiratory neurones with decrementing activity (E_{dec}) that began to discharge in the early expiratory (post-inspiratory) phase, of expiratory neurones with augmenting activity (E_{aug}) that began to discharge in the late expiratory phase, and of inspiratory neurones. We further determined whether some of these neurones sent axons to the spinal cord, contralateral or ipsilateral ventrolateral medulla or cervical vagus nerve by applying antidromic activation tests.

METHODS

General animal preparation

Experimental protocols followed in this work were reviewed and approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Experiments were performed in forty-eight male Sprague–Dawley rats (382–512 g). Each rat was anaesthetized with an intraperitoneal injection of 1·7 g kg^{-1} of urethane, which was adequate to eliminate flinch or withdrawal of the hindpaw in response to a hard pinch. The animals were placed on a servo-controlled water blanket; body temperature was monitored via a rectal temperature probe and maintained within 37 ± 1 °C. Catheters were introduced into the femoral vein for administering fluids and into the femoral artery for measuring arterial blood pressure and withdrawing blood samples for arterial blood gases. Before neuromuscular blockade the adequacy of anaesthesia was assessed every half-hour by pinching the hindlimb paw and monitoring for a hindlimb flinch or withdrawal, and/or a sudden fluctuation in arterial blood pressure or heart rate. When any hindlimb flinch or withdrawal or any obvious change in arterial blood pressure (≥5 mmHg) or heart rate (≥10%) was observed in response to paw pinch, a supplemental dose of pentobarbitone (10–20 mg kg^{-1} I.V.) was given. A few minutes after pentobarbitone, paw pinch was reapplied to assure adequacy of anaesthesia.

The trachea was cannulated below the larynx, and a catheter connected to a side port of the endotracheal tube to monitor intratracheal pressure. A catheter of premeasured length was advanced through the right external jugular vein into the right atrium for injections into the pulmonary circulation. The right atrial catheter was prefilled with phenylbiguanide (50 μg ml^{-1}); the first injection was made before commencement of recording to assure that the tip was filled and that there was a rapid-onset, low-amplitude phrenic nerve discharge and/or apnoea. The cervical vagus nerve ipsilateral to the recording site was isolated. The fourth cervical (C4) branch of the phrenic nerve was isolated in the neck and cut distally.

Each rat was placed in a stereotaxic head frame. The endotracheal tube was then attached to the ventilator, and the rat mechanically ventilated with oxygen-enriched air at 45–55 breaths min^{-1}. The expiratory line of the ventilator was placed under 2 cmH_2O to prevent atelectasis. The ventilator rate was adjusted and/or sodium bicarbonate infused in order to maintain blood gases and pH within normal limits, which were defined as arterial blood P_{O_2} above 100 mmHg; P_{CO_2} between 35 and 45 mmHg; and pH between 7·35 and 7·45.

The vagus nerve was placed on a bipolar silver hook electrode and covered with a mixture of warm petroleum jelly and mineral oil and connected to a stimulus isolation unit driven by a Grass S48 stimulator. The central end of the phrenic nerve was placed on a bipolar silver hook electrode and covered with a mixture of warm petroleum jelly and mineral oil. Phrenic nerve activity was full-wave rectified and smoothed with a leaky integrator (Paynter filter with a 50 ms time constant). A vertebral clamp was placed on the T2 spinal process and an occipital craniotomy performed. The caudal portion of the fourth ventricle was exposed by removing the dura and arachnoid membranes and then covered with warm mineral oil. In experiments where antidromic activation was attempted, a cervical laminectomy was performed. The spinous processes and dorsal laminae of C1–C4 were removed and the dura cut to expose the spinal cord. The resulting incision was then filled with mineral oil and the spinal cord covered with warm petroleum jelly.

After completion of the surgical preparation, at the commencement of recording, the animals were paralysed with gallamine (10 mg kg^{-1} I.V.). During neuromuscular blockade, the adequacy of anaesthesia was assessed every half-hour by pinching the hindlimb paw and monitoring for a sudden fluctuation in arterial blood pressure or heart rate. When any obvious change in arterial blood pressure (≥5 mmHg) or heart rate (≥10%) was observed in response to paw pinch, a supplemental dose of pentobarbitone (10–20 mg kg^{-1} I.V.) was given. A few minutes after pentobarbitone, the paw pinch test was reapplied to assure adequacy of anaesthesia. In addition, sudden spontaneous increases in arterial blood pressure (≥5 mmHg), any irregular heart rate rhythm, or increases in heart rate (≥10%) were also set as criteria for a supplemental dose of pentobarbitone (10–20 mg kg^{-1} I.V.). About every hour, the effect of the neuromuscular blockade began to wane, as evidenced by an occasional, small but perceptible spontaneous chest wall movement. At that time, before the next dose of gallamine was given, the animals were administered the paw pinch test and monitored for hindlimb flinch or withdrawal in addition to changes in arterial blood pressure (≥5 mmHg) or heart rate (≥10%). When any one of such responses to paw pinch was observed, a supplemental dose of pentobarbitone (10–20 mg kg^{-1} I.V.) was given. At the end of the experiment the rats were killed with an overdose of pentobarbitone.

Extracellular unit recording and antidromic activation

Extracellular recordings of single unit activity were made through a glass electrode filled with 2% Chicago Sky Blue dye (Sigma) in 0·5 M sodium acetate. For recording in the ventral respiratory group (VRG) the reference point on the dorsal surface of the brainstem was the calamus scriptorius. The searching co-ordinates were from 1·0 mm caudal to 3·0 mm rostral to the calamus, from 1·4 to 2·5 mm lateral to mid-line, and from 1·8 to 4·8 mm ventral to the dorsal surface.

Recognizing that classifying respiratory neurones by discharge pattern alone is somewhat arbitrary based on the variability and state dependence of the neuronal activity, various groups have used different criteria and terminology (Richter, 1982; Lindsey, Segers & Shannon, 1987; Ezure, 1990). We used the more general nomenclature of Ezure that reflects phase and discharge patterns in relation to phrenic nerve activity for the respiratory neurones (Ezure, 1990). Respiratory neurones were classified as having decrementing (dec), augmenting (aug) or constant or plateau-like patterns (con).

Recordings were made from expiratory and inspiratory neurones with decrementing (dec), augmenting (aug) and constant (con) discharge patterns. Decrementing inspiratory neurones (I_{dec}) exhibited maximal discharge frequencies at the onset of the inspiratory phase, which then declined; these neurones are termed early-inspiratory in the Richter model (Richter, 1982; Richter *et al.* 1986; Richter, Ballanyi & Schwarzacher, 1992), inspiratory to expiratory/inspiratory (inspiratory driver) cells by Lindsey *et al.* (1987), and pre-inspiratory cells by Onimaru, Arata & Homma (1989) and Smith, Ellenberger, Ballanyi, Richter & Feldman (1991). Augmenting inspiratory neurones (I_{aug}) exhibited increases in discharge frequency during inspiration; these correspond to the I_{aug} cells of Lindsey *et al.* (1987) and are included in the ramp inspiratory (I_r) group in the models by Richter and colleagues (Richter, 1982; Richter *et al.* 1986, 1992) and Ogilvie *et al.* (1992). Other inspiratory neurones exhibited a constant discharge frequency throughout inspiration (I_{con}); these are included in the I_{aug} population by Lindsey *et al.* and the I_r group by Richter and Ogilvie and colleagues. E_{dec} neurones discharged with maximal frequencies either at the end of inspiration or during the post-inspiratory phrenic nerve discharge; these neurones, also termed E_{dec} by Lindsey *et al.* (1987) and Balis *et al.* (1994), correspond to the post-inspiratory neurones of Richter *et al.*, as simulated by Ogilvie *et al.* E_{aug} neurones began to discharge at various times during expiration, with augmenting frequencies that stopped at around the beginning of the phrenic nerve discharge; this classification corresponds to the general category of expiratory cells by Richter *et al.* and Ogilvie *et al.* (Richter, 1982; Richter *et al.* 1986, 1992; Ogilvie *et al.* 1992), and to the E_{aug} late of Lindsey *et al.* (1987) and Balis *et al.* (1994). The E_{con} neurones exhibited a constant discharge pattern throughout expiration and correspond to the E_{aug} symmetric of Lindsey *et al.* and Balis *et al.* and to the general expiratory group by Richter *et al.* and Ogilvie *et al.*

To determine whether neurones sent axons to or through the spinal cord, the contralateral VRG, the ipsilateral VRG caudal to the recording site, or to the vagus nerve, we used antidromic activation tests. An array of four monopolar electrodes (Rhodes Medical Instruments, Woodland Hills, CA, USA), two on each side of the spinal cord, was inserted at C2–C3. The electrodes were inserted at the origin of the dorsal rootlets and advanced ventrally through the spinal cord while stimulating at 1 Hz, until the smallest current evoked the largest field potential in the ventrolateral medulla. Two monopolar electrodes were placed in the contralateral VRG in the rostrocaudal plane with the rostral electrode positioned at the same co-ordinate as the recording electrode and the second electrode positioned ~500 μm caudally. One monopolar electrode was placed in the ipsilateral VRG ~500 μm caudal to the recording electrode. The cervical vagus nerve ipsilateral to the recording site was placed on a bipolar stimulating electrode. Individual electrodes within the spinal cord array and the contralateral VRG array could not be independently stimulated.

The criteria for antidromic activation included: (1) short, invariant latency (latency varied by $< 0{\cdot}2$ ms) of the evoked response, (2) a stable threshold, (3) faithful response to high stimulation rates, and (4) a positive collision test. If the cell met the first two criteria, it was tested with high-frequency stimulation: two stimuli (delivered at ~1·2 × threshold current) separated by 5 ms were applied to determine whether the cell could follow stimulation rates of at least 200 Hz; this was done to discriminate antidromic from orthodromic activation of the cell. The interval between the paired stimuli was then usually decreased to roughly estimate the highest stimulation rate that the cell could follow (refractory period). The collision test was then applied: the stimulator was triggered by a spontaneous orthodromic action potential to determine whether stimulation evoked an antidromic action potential. The interval between the spontaneous orthodromic action potential and the triggered stimulus was decreased until there was a collision of the antidromic with the orthodromic action potential. The presence of an initial segment depolarization of the action potential was used to verify that the recording was made from a cell body (Fuller & Schlag, 1976; Lipski, 1981). Even if a cell followed stimulation rates of up to 1000 Hz, it was not considered antidromically activated unless collision could be demonstrated.

Protocol

Once a respiratory cell was isolated, we determined whether its discharge pattern was augmenting, decrementing or constant and then attempted to antidromically activate the cell by stimulating successively through the spinal cord electrode array, the contralateral VRG array, the ipsilateral VRG electrode and the vagus nerve electrode. The cell was then tested with one or two doses of phenylbiguanide injected in the right atrium to stimulate cardiopulmonary C fibre receptors. Phenylbiguanide (2·5–10 μg kg^{-1}) was administered no more often than once every 5 min. We have shown previously that tachyphylaxis does not occur under these conditions (Wilson, Zhang & Bonham, 1996). In order to limit the findings to neurones related to breathing, we excluded those in which changes in impulse activity occurred after the onset of phrenic nerve changes or those that were antidromically activated from the vagus cervical nerve.

Cardiopulmonary C fibre receptor stimulation produces apnoea and/or rapid shallow breathing, but the latter is more frequently observed in spontaneously breathing rats than in paralysed, ventilated rats. Therefore in order to determine the change in firing activity of respiratory neurones during cardiopulmonary C fibre-evoked rapid shallow breathing, we also recorded unit activity in spontaneously breathing rats that received low doses of phenylbiguanide (2·5 mg kg^{-1}). Higher doses in the spontaneously breathing animals tended to evoke an apnoea with the same regularity as in the paralysed, ventilated rats.

Histology

Recording sites were marked by passing current (10 μA for 7 s every 14 s for 15 min, electrode negative) through the recording electrode to deposit 2% Chicago Sky Blue dye. At the end of an experiment, the brainstem was removed and fixed in 4% paraformaldehyde and 20% sucrose. The brainstems were cut in 40 μm coronal sections and counterstained. Recording sites were reconstructed from dye spots with the aid of a drawing tube.

Data analyses

Unit activity and phrenic nerve activity were fed via high-impedance source followers to second stage amplifiers, filtered (0·3–3 kHz) and fed in parallel to an oscilloscope, chart recorder, audio-monitor, and a PCM digital tape-recorder with a sampling rate of 11 kHz per channel (Neuro-Corder, DR886; Neuro Data Instruments, New York) for off-line analyses. Arterial blood pressure was also stored on tape for analysis.

Unit activity was analysed in terms of burst duration, impulses within a burst, mean impulse frequency and interburst interval using EGAA/Computerscope software from RC Electronics, Inc. (Goleta, CA, USA). Phrenic nerve activity was analysed in terms of burst duration, interburst interval and amplitude of the full wave-rectified moving average of the raw nerve activity. Stimulation of cardiopulmonary C fibre receptors with phenylbiguanide injected even in low doses (2·5 μg kg^{-1}) in paralysed, ventilated animals typically evoked either an apnoea or extremely low-amplitude

phrenic nerve bursts, which often were barely perceptible and not distinguishable from a phrenic nerve apnoea. For the purposes of analysis, apnoea was defined as the period when the amplitude of the phrenic nerve burst was $< 20\%$ of the baseline, such that small phrenic nerve bursts were considered part of the apnoea (Fig. 6). The phrenic nerve apnoea was followed by a period of irregular activity before baseline activity was restored. The duration of the unit and phrenic nerve response was taken as the time from the onset of the response to the time when two successive parameters (burst duration and interburst interval for the unit and phrenic nerve, number of impulses per burst of the unit and amplitude of the integrated phrenic nerve burst) were within 10% of baseline activity.

To determine the effect of cardiopulmonary C fibre activation on unit activity, we examined the burst duration, number of impulses per burst, and interburst interval for ten unitary bursts during the control period, during the period that corresponded to the phrenic nerve apnoea, and during a recovery period. Comparisons were made using ANOVA with repeated measures, followed by Fisher's LSD *post hoc* test for significant differences. Significant levels were set as $P < 0{\cdot}05$. All data are reported as means ± S.E.M. unless otherwise indicated.

To verify that the number of spikes of inspiratory neurones corresponded to the magnitude of the phrenic nerve activity during cardiopulmonary C fibre-evoked rapid shallow breathing, we correlated the number of the impulses per burst to the magnitude of the area of integrated phrenic nerve bursts by using linear regression. Measurements were made averaged over five control phrenic nerve bursts and for three separate bursts during the period of rapid shallow breathing. To obtain the area of integrated phrenic nerve bursts, we used a digitizing tablet (Kurta Corporation, Phoenix, AZ, USA) and computer software (SigmaScan, Jandel Scientific, San Rafael, CA, USA).

RESULTS

Respiratory neurones recorded in the VRG

Extracellular recordings were obtained in 114 respiratory neurones in the VRG in which right atrial injections of phenylbiguanide (2·5–10 μg kg^{-1}) evoked rapid-onset changes in impulse activity that coincided with the abolition of phrenic nerve activity (the neural equivalent of apnoea) or rapid low-amplitude phrenic nerve bursts (the neural equivalent of rapid shallow breathing). At the commencement of recordings, arterial blood pH was 7·44 ± 0·06, P_{CO_2} was 39·4 ± 7·8 mmHg, and P_{O_2} was 340 ± 80 mmHg (means ± S.D.).

Recordings were made in the ventrolateral medulla extending from 1 mm caudal to 3 mm rostral to the obex and from 1·5 to 2·5 mm lateral to the mid-line, and from 1·9 to 4·5 mm ventral to the dorsal surface of the medulla (Fig. 1). This region corresponds to previous co-ordinates for

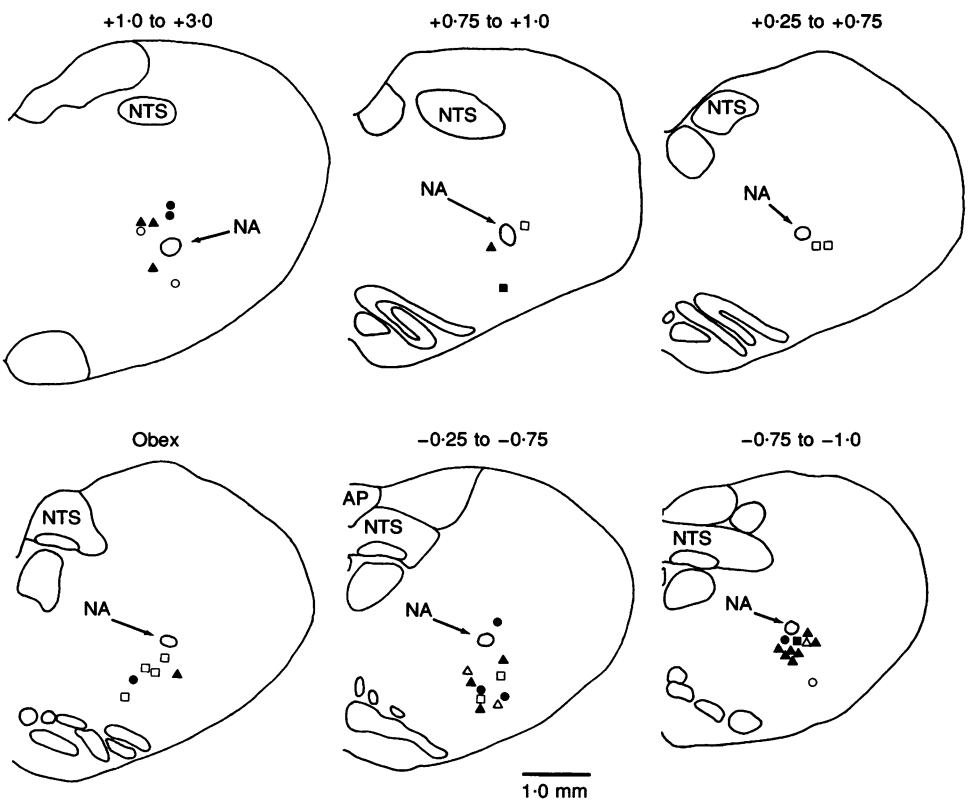

Figure 1. Schematic coronal sections illustrating histologically verified recording sites for neurones in the ventrolateral medulla ($n = 39$) that responded to cardiopulmonary C fibre receptor stimulation

Sections are composites labelled rostrocaudally with respect to the obex. □, E_{dec}; △, E_{aug}; ○, E_{con}; ■, I_{dec}; ▲, I_{aug}; ●, I_{con}. AP, area postrema; NTS, nucleus tractus solitarii; NA, nucleus ambiguus.

the VRG and Bötzinger complex in rats (Ezure, Manabe & Yamada, 1988). Seven of eight E_{dec} neurones that were histologically verified with dye spots were located rostral to the obex, in the region corresponding to rostral VRG (Ezure *et al.* 1988) (Fig. 1). Only three E_{aug} neurones were verified histologically with dye spots; all were located caudal to the obex in the caudal VRG (Ezure *et al.* 1988). Three E_{con} neurones were verified at the rostral and caudal extents of the VRG. Inspiratory neurones, regardless of discharge pattern were encountered throughout the VRG.

Firing behaviour of E_{dec} neurones during cardiopulmonary C fibre receptor stimulation

Single unit activity was recorded from nine E_{dec} neurones in paralysed, ventilated rats. Cardiopulmonary C fibre receptor stimulation resulted in a tonic excitation of the E_{dec} neurones that usually continued throughout the phrenic nerve apnoea, as shown in the example in Fig. 2. The grouped data are shown in Fig. 3. Concurrently with the phrenic apnoea (which lasted $7{\cdot}6 \pm 4{\cdot}1$ s; mean ± S.D.), cardiopulmonary C fibre stimulation increased the burst duration ($P = 0{\cdot}006$, ANOVA) and number of impulses per burst ($P = 0{\cdot}001$, ANOVA) of the E_{dec} neurones. During the prolonged burst durations, the mean impulse frequency was also increased ($P = 0{\cdot}019$, ANOVA). Six of the nine E_{dec} neurones discharged continuously throughout the apnoea, while the remaining three displayed prolonged bursts separated by brief interburst intervals.

Two E_{dec} neurones were antidromically activated from the contralateral VRG with antidromic latencies of 0·5 and 3·1 ms; two others were antidromically activated from the spinal cord with antidromic latencies of 1·0 and 1·35 ms. None were antidromically activated from the ipsilateral VRG.

Eight E_{dec} neurones were recorded in spontaneously breathing animals in which cardiopulmonary C fibre activation with low doses of phenylbiguanide ($2{\cdot}5\ \mu g\ kg^{-1}$) produced rapid low-amplitude phrenic nerve bursts rather than an apnoea. The E_{dec} neurones discharged tonically throughout the low-amplitude phrenic nerve bursts, as they did during apnoea. Interruption of the prolonged bursts of the E_{dec} neurones was only seen when the area of the phrenic nerve activity was 56% of the control area ($31{\cdot}8 \pm 19\ mm^2$ compared with the control value $57{\cdot}5 \pm 26{\cdot}3\ mm^2$; $n = 10$; $P = 0{\cdot}02$; Student's paired t test).

The prolonged burst durations of the E_{dec} neurones did not show a uniform recovery following C fibre stimulation. In five E_{dec} neurones, the burst duration (23 ± 7 s) and phrenic nerve interburst interval (26 ± 3 s) recovered simultaneously ($P = 0{\cdot}15$). In the remaining four neurones, the burst duration recovered significantly more slowly (216 ± 71 s) than the phrenic nerve interburst interval (28 ± 8 s; $P = 0{\cdot}036$; paired t test) or the fast-recovering E_{dec} neurones ($P = 0{\cdot}009$, unpaired t test).

Firing behaviour of E_{aug} neurones during cardiopulmonary C fibre receptor stimulation

Single unit activity was recorded from twenty E_{aug} neurones. In contrast to the tonic discharge of E_{dec} neurones during cardiopulmonary C fibre activation, E_{aug} neuronal activity was decreased. An example is shown in Fig. 4. The

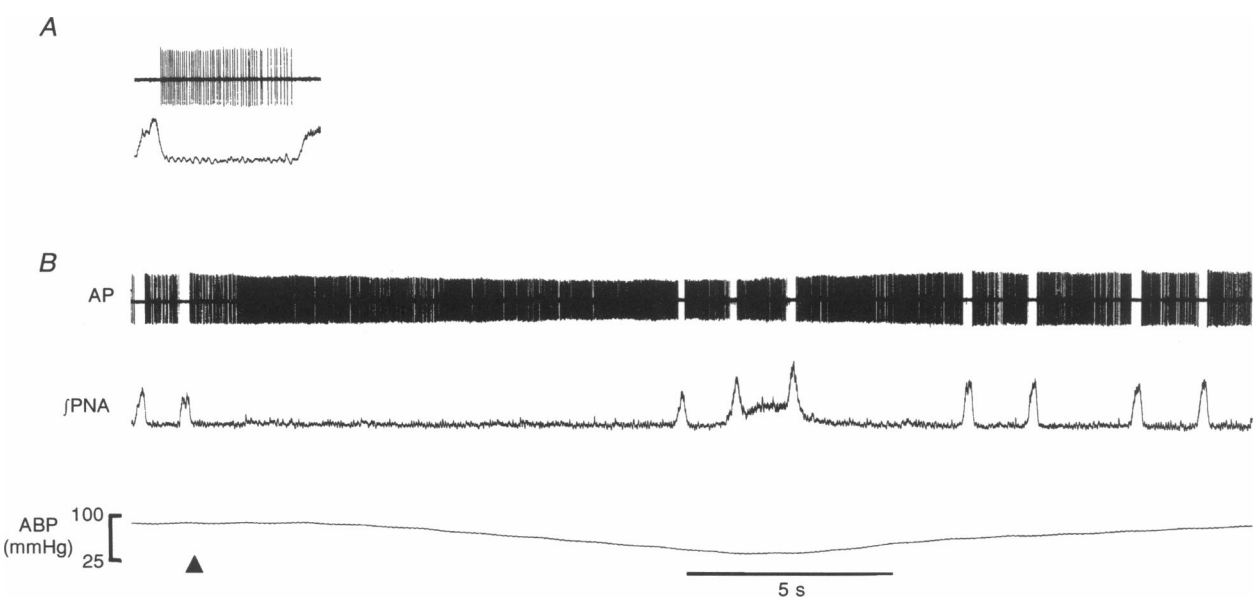

Figure 2. Response of E_{dec} neurone during cardiopulmonary C fibre receptor stimulation

A shows discharge pattern of cell. *B*, right atrial injection of phenylbiguanide (▲; $5\ \mu g\ kg^{-1}$) evoked a tonic discharge of the cell concurrently with the phrenic nerve apnoea. Decrease in mean arterial blood pressure (ABP) had a slower onset than the change in phrenic nerve activity. AP, action potentials; ∫PNA, integrated phrenic nerve activity.

E_{aug} neurone projected to the spinal cord (Fig. 4*B*). Right atrial phenylbiguanide injection abolished both the unit and phrenic nerve activity, but phrenic activity recovered sooner than unit activity. The grouped data are summarized in Fig. 5. Concurrently with evoking a phrenic nerve apnoea (which lasted 8·1 ± 4·2 s; mean ± S.D.), cardiopulmonary C fibre stimulation decreased the burst duration ($P = 0·0001$, ANOVA) and number of impulses per burst ($P = 0·0001$, ANOVA) and significantly increased the interburst interval of the E_{aug} neurones ($P = 0·013$, ANOVA).

The decreases in E_{aug} unit activity during cardiopulmonary C fibre receptor stimulation lasted significantly longer than the changes in phrenic nerve activity (Fig. 4); for the grouped data the phrenic nerve interburst interval returned to baseline within 27·0 ± 5·5 s while the interburst interval of the E_{aug} neurones returned to baseline within 179 ± 25 s ($P < 0·0001$; paired t test). The decreases in the burst duration of the E_{aug} neurones also recovered significantly more slowly (174 ± 28 s; $P = 0·0001$; paired t test) than did the phrenic nerve interburst interval.

Thirteen of the twenty E_{aug} neurones were antidromically activated from the spinal cord with antidromic latencies that averaged 1·2 ± 0·3 and ranged from 0·75 to 1·6 ms; five were antidromically activated from the contralateral VRG with antidromic latencies that ranged from 0·9 to 3·7 ms; and two of thirteen neurones were activated from both sites. None were antidromically activated from the ipsilateral VRG. The activity of E_{aug} neurones that sent axons to the spinal cord and of those that sent axons to the contralateral VRG was uniformly decreased during cardiopulmonary C fibre receptor stimulation.

Firing behaviour of E_{con} neurones during cardiopulmonary C fibre receptor stimulation

Single unit activity was recorded from nine E_{con} neurones. In these nine trials, cardiopulmonary C fibre stimulation evoked an apnoea that lasted 12·3 ± 9·3 s (mean ± S.D.). There was no consistent response of the E_{con} neurones; four responded similarly to the E_{dec} neurones, discharging almost continuously throughout the apnoea, with increased burst durations and number of impulses per burst and decreased interburst intervals. Three neurones discharged similarly to E_{aug} neurones, with decreased burst durations and number of impulses per burst and increased interburst intervals. Two did not respond. Four E_{con} neurones, two of which behaved like E_{dec} neurones and two like E_{aug} neurones, were antidromically activated from the spinal cord with antidromic latencies that ranged from 0·6 to 1·5 ms. None were antidromically activated from the ipsilateral VRG.

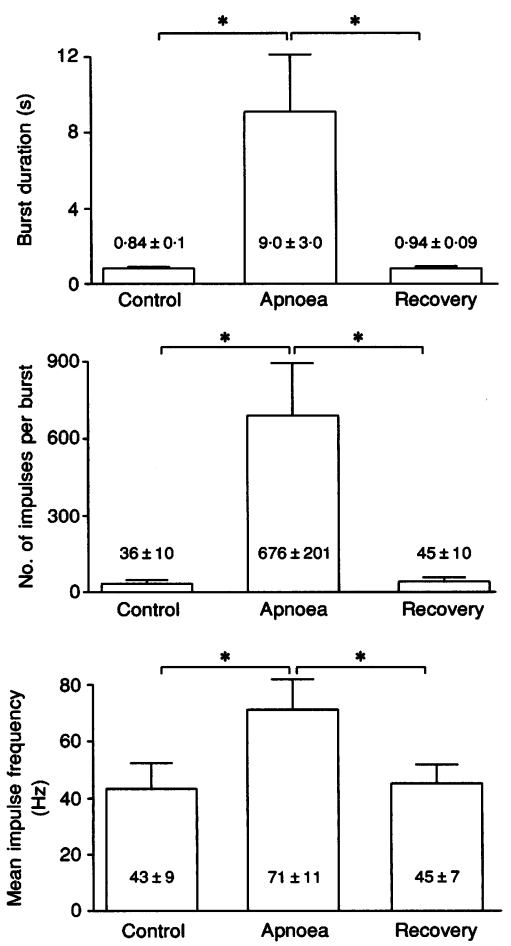

Figure 3. Summary of responses of E_{dec} neurones ($n = 9$) during cardiopulmonary C fibre receptor stimulation

Right atrial injections of phenylbiguanide (5 $\mu g\ kg^{-1}$) increased the burst duration (* $P = 0·006$, ANOVA; $P < 0·05$, Fisher's LSD *post hoc* test), increased the number of impulses per burst (* $P = 0·001$, ANOVA; $P < 0·05$, Fisher's LSD *post hoc* test) and increased the mean impulse frequency (* $P = 0·019$, ANOVA; $P < 0·05$, Fisher's LSD *post hoc* test) coincident with a phrenic nerve apnoea.

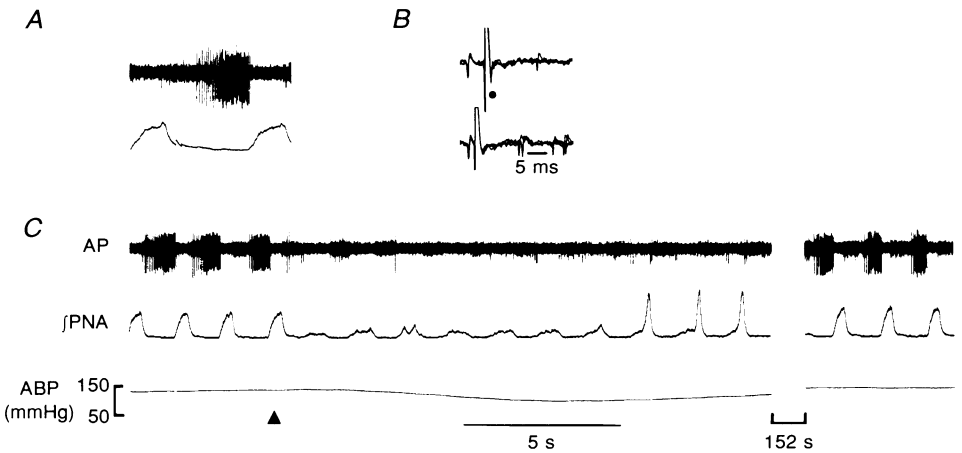

Figure 4. Response of E_{aug} neurone during cardiopulmonary C fibre receptor stimulation

A shows augmenting pattern of unit discharge. *B*, antidromically evoked action potential (top trace) following stimulation of the contralateral VRG when the stimulus was presented at 4·8 ms after the spontaneous orthodromic action potential. Collision (bottom trace) when the stimulus interval was reduced to 1·4 ms. Four superimposed sweeps in each trace. Dot indicates antidromically evoked action potential. Stimulating current was 500 μA, 0·1 ms pulses. *C*, right atrial injection of phenylbiguanide (▲; 5 μg kg^{-1}) abolished the unit activity (action potentials, AP) concurrently with the phrenic nerve activity (∫PNA), but the unit remained silent after phrenic nerve activity recommenced.

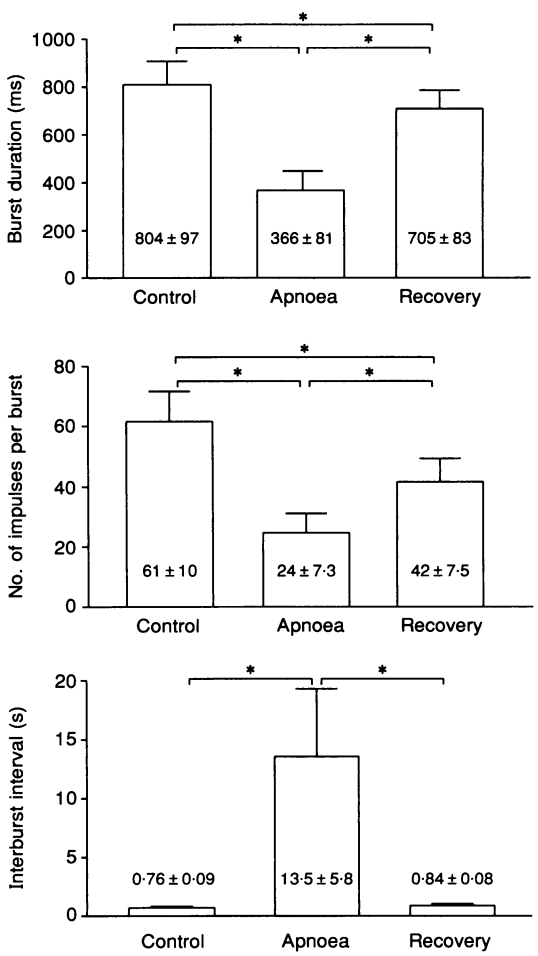

Figure 5. Summary of responses of E_{aug} neurones ($n = 20$) during cardiopulmonary C fibre receptor stimulation

Right atrial injections of phenylbiguanide (5 μg kg^{-1}) decreased the burst duration (* $P = 0{\cdot}0001$, ANOVA; $P < 0{\cdot}05$, Fisher's LSD *post hoc* test), decreased the number of impulses per burst (* $P = 0{\cdot}0001$, ANOVA; $P < 0{\cdot}05$, Fisher's LSD *post hoc* test) and increased the interburst interval (* $P = 0{\cdot}013$ ANOVA; $P < 0{\cdot}05$, Fisher's LSD *post hoc* test) coincident with evoking a phrenic nerve apnoea.

Firing behaviour of inspiratory neurones during cardiopulmonary C fibre receptor stimulation

Single unit activity was recorded from fifty-five inspiratory neurones (34 I_{aug}, 2 I_{dec}, 19 I_{con}). The inspiratory neurones exhibited two types of responses during the cardiopulmonary C fibre-evoked apnoea. The majority (45 of 55) showed decreases in activity corresponding to the phrenic nerve apnoea, regardless of the discharge pattern (26 I_{aug}, 2 I_{dec}, 17 I_{con}). Eleven (6 I_{aug}, 1 I_{dec}, 4 I_{con}) of these forty-five inspiratory neurones projected to or through the contralateral VRG with antidromic latencies averaging 2·3 ± 1·3 ms and ranging from 0·26 to 4·0 ms. Ten (5 I_{aug}, 1 I_{dec}, 4 I_{con}) projected to the spinal cord with antidromic latencies averaging 1·9 ± 1·5 ms and ranging from 0·5 to 5·1 ms. None projected to the ipsilateral VRG. Examples of neurones sending axons to the contralateral VRG or the spinal cord are shown in Fig. 6. In the upper panel (*A*), the I_{aug} cell (*Aa*) was antidromically activated from the

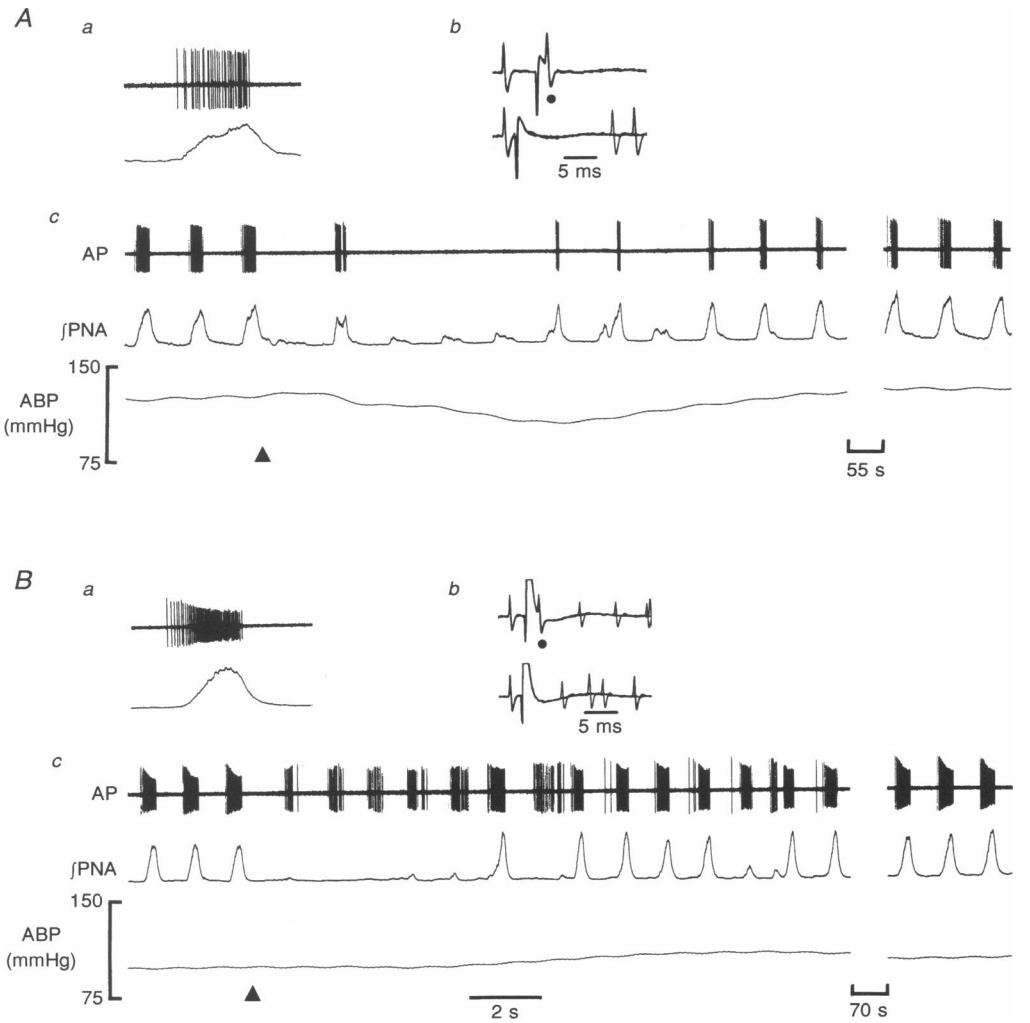

Figure 6. Responses of inspiratory neurones ($n = 2$) during cardiopulmonary C fibre receptor stimulation

Aa shows augmenting discharge pattern. *Ab*, antidromically evoked action potential (top trace) following stimulation of the contralateral VRG when the stimulus was presented at 5 ms after the spontaneous orthodromic action potential. Collision (bottom trace) when the stimulus interval was reduced to 1·9 ms. Four superimposed sweeps in each trace. Stimulating current was 100 μA, 0·15 ms pulses. Dot indicates antidromically evoked action potential. *Ac*, right atrial phenylbiguanide injection (▲; 5 μg kg^{-1}) abolished unit activity (action potentials, AP) and evoked low-amplitude integrated phrenic nerve bursts (∫PNA). Decrease in mean arterial blood pressure (ABP) had a slower onset. *Ba* shows augmenting discharge pattern. *Bb*, antidromic response (top trace) following stimulation of the spinal cord when the stimulus was presented at 2·2 ms after the spontaneous orthodromic action potential. Collision (bottom trace) when the stimulus interval was reduced to 1·8 ms. Four superimposed sweeps in each trace. Dot indicates antidromic action potential. Stimulating current was 1000 μA, 0·2 ms pulses. *Bc*, right atrial phenylbiguanide injection (▲; 5 μg kg^{-1}) decreased unit activity (AP) and abolished ∫PNA. ABP did not change during the apnoea, and then increased slightly.

contralateral VRG (*Ab*). Its activity was abolished by stimulation of the cardiopulmonary C fibre receptors coincidentally with the apnoea (*Ac*). In the lower panel (*B*), the activity of an I_{aug} cell (*Ba*) that was antidromically activated from the spinal cord (*Bb*) was diminished but not abolished coincidentally with the phrenic nerve apnoea (*Bc*). The grouped data for these forty-five inspiratory neurones that were inhibited during C fibre stimulation are summarized in Fig. 7. During the phrenic nerve apnoea (which lasted 8·8 ± 6·7 s; mean ± S.D.), cardiopulmonary C fibre stimulation decreased the burst duration ($P = 0·0001$, ANOVA) and the number of impulses per burst ($P = 0·0001$, ANOVA) and increased the interburst interval ($P = 0·0001$, ANOVA). The prolonged interburst interval of the inspiratory neurones (9·5 ± 2·3 s) was similar to the prolonged increased in the phrenic nerve interburst interval (the phrenic nerve apnoea).

Inspiratory unit activity required about twice as long to return to baseline as did the phrenic nerve activity. The inspiratory cell burst durations recovered in 69 ± 10 s compared with 28 ± 4 s for phrenic nerve burst durations ($P = 0·006$, paired t test); the inspiratory cell interburst interval recovered in 60 ± 11 s compared with 27 ± 3 s for the phrenic interburst interval ($P = 0·026$); and the number of impulses per burst recovered in 79 ± 11 s compared with 39 ± 8 s for the amplitude of the integrated phrenic nerve bursts ($P = 0·007$). The recovery times for the burst durations, number of impulses per burst and interburst interval did not differ for the bulbospinal neurones *versus* neurones that sent axons to the contralateral VRG ($P > 0·05$, unpaired t test). However, the recovery time for the inspiratory neurones was significantly shorter than that for the E_{aug} neurones: the burst duration recovery times were 69 ± 10 s for the inspiratory neurones *vs.* 174 ± 38 s for the E_{aug} neurones ($P = 0·0001$; unpaired t test) and the interburst interval recovery times were 60 ± 11 and 179 ± 25, respectively ($P = 0·0001$, unpaired t test). These differences in recovery times occurred despite similar recovery times for phrenic nerve interburst intervals, burst durations and amplitudes.

The remaining ten (8 I_{aug}, 2 I_{con}) of these fifty-five inspiratory neurones fired almost tonically but with a decreased mean impulse frequency throughout the cardiopulmonary C fibre receptor-evoked phrenic apnoea. The neurones showed an increase in burst duration from 0·38 ± 0·06 to 3·2 ± 1·3 s ($P = 0·02$, ANOVA), with no change in number of impulses per burst ($P = 0·158$, ANOVA) and a decrease in the mean impulse frequency from 170 ± 33 to 69 ± 12 Hz ($P = 0·001$, ANOVA). The interburst interval also decreased from 833 ± 81 ms to 377 ± 92 ms ($P = 0·0001$, ANOVA). All parameters recovered from the effects of cardiopulmonary C fibre stimulation. One I_{con} neurone projected to the spinal cord.

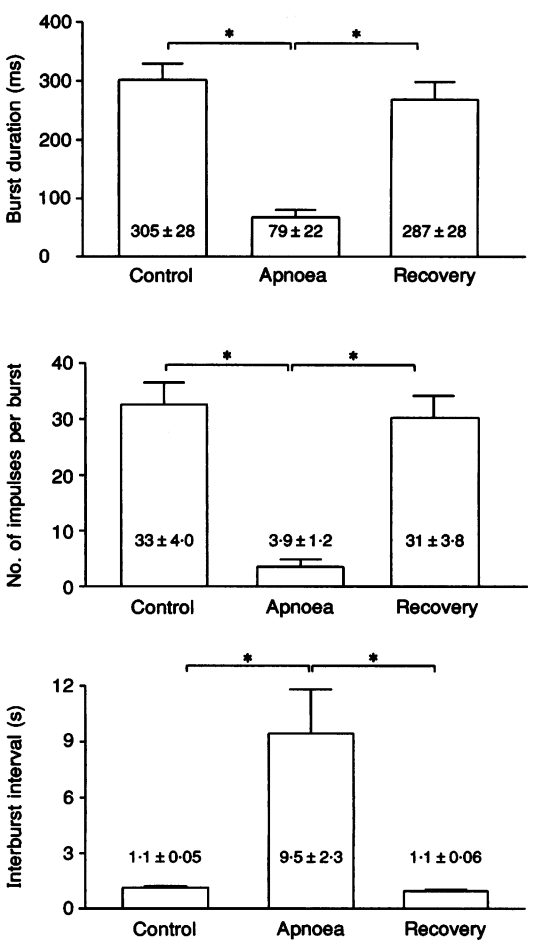

Figure 7. Summary of responses of inspiratory neurones ($n = 45$) during cardiopulmonary C fibre receptor stimulation

Right atrial injections of phenylbiguanide (5 $\mu g\ kg^{-1}$) decreased the burst duration (* $P = 0·0001$, ANOVA; $P < 0·05$, Fisher's LSD *post hoc* test), decreased the number of impulses per burst (* $P = 0·0001$, ANOVA; $P < 0·05$, Fisher's LSD *post hoc* test), and increased the interburst interval (* $P < 0·0001$ ANOVA; $P < 0·05$, Fisher's LSD *post hoc* test) coincident with the phrenic nerve apnoea.

Thirteen inspiratory neurones were recorded in spontaneously breathing animals, in which cardiopulmonary C fibre receptor stimulation with low doses of phenylbiguanide (2·5 μg kg^{-1}) evoked rapid low-amplitude phrenic nerve bursts rather than an apnoea. An example is shown in Fig. 8. In the upper panel, 2·5 μg kg^{-1} phenylbiguanide produced an initial increase in impulses in a single burst that corresponded to a single phrenic nerve burst with an increased amplitude followed by a decreased number of impulses per burst which corresponded to rapid low-amplitude phrenic nerve bursts. Injection of 5 μg kg^{-1} phenylbiguanide abolished unit and phrenic nerve activity. Following the cessation of activity, the unit discharged a few action potentials corresponding to very rapid and shallow phrenic nerve bursts. For all thirteen inspiratory neurones, the decrease in the number of impulses per burst was correlated with the decrease in the area of the integrated phrenic nerve bursts during the rapid low-amplitude phrenic nerve bursts ($r = 0{\cdot}557$; $P = 0{\cdot}0001$).

DISCUSSION

This study examined the changes in firing activity of VRG neurones during the activation of cardiopulmonary C fibre receptors. E_{dec} neurones exhibited tonic increases in activity that accompanied the cardiopulmonary C fibre-evoked apnoea or rapid shallow breathing. By contrast, the E_{aug} neurones and the majority of the inspiratory neurones displayed decreases in firing activity, characterized by decreases in burst durations and number of impulses per burst. How these changes in respiratory neuronal activity can be interpreted with respect to current models for the central respiratory network and with perturbations in respiratory neuronal activity in response to stimulation of other peripheral sensory receptors is discussed below.

Responses of E_{dec} neurones

The tonic increase in activity of E_{dec} neurones during cardiopulmonary C fibre receptor-evoked apnoea fits with current models for the respiratory network in which E_{dec} neurones (identified as post-inspiratory neurones in the Richter model and equated with E_{dec} neurones by Ogilvie *et al.* (1992)) have a major role in controlling expiratory time (Richter, 1982; Richter *et al.* 1986; Segers, Shannon, Saporta & Lindsey, 1987; Lindsey *et al.* 1987; Ogilvie *et al.* 1992; Balis *et al.* 1994). In the current models by Richter and colleagues (Richter, 1982; Richter *et al.* 1986; Ogilvie *et al.* 1992) and by Lindsey and colleagues (Segers *et al.* 1987; Lindsey *et al.* 1987; Balis *et al.* 1994), apnoea can result from a tonic increase in the activity of E_{dec} neurones that is coupled with the synaptic inhibition of inspiratory and late expiratory elements of the network.

The current findings are also consistent with experimental results regarding the responses of E_{dec} neurones to activation of other peripheral sensory afferents that cause apnoea; stimulation of superior laryngeal afferent fibres (Remmers, Richter, Ballantyne, Bainton & Klein, 1985; Lawson, Richter, Czyzyk-Krzeska, Bischoff & Rudesill,

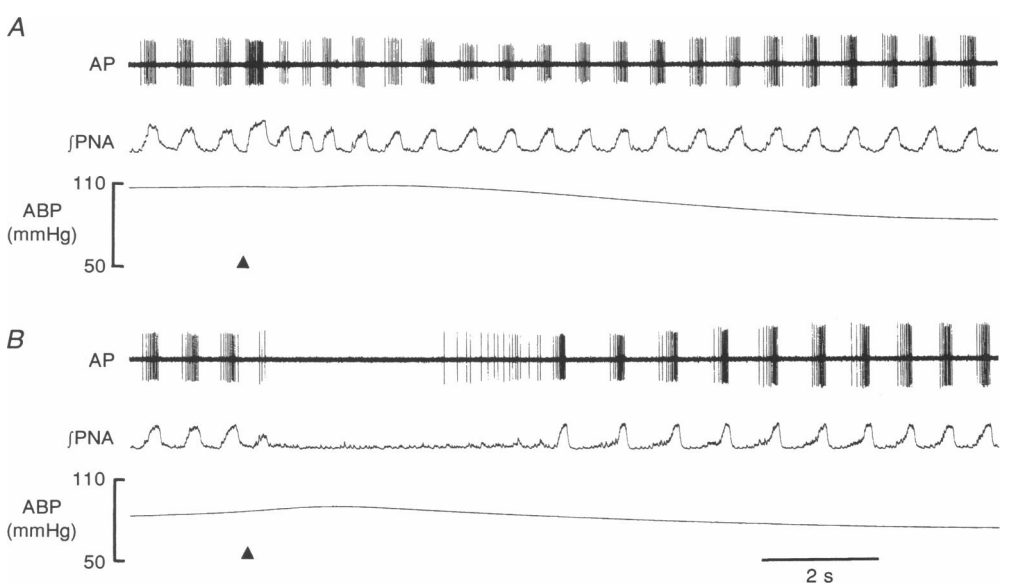

Figure 8. Response of inspiratory neurone during cardiopulmonary C fibre-evoked rapid shallow breathing

A, phenylbiguanide (▲; 2·5 μg kg^{-1}) produced a decrease in the action potentials (AP) that corresponded to rapid low-amplitude integrated phrenic nerve bursts (∫PNA). Mean arterial blood pressure (ABP) increased slightly and then fell. *B*, phenylbiguanide (▲; 5 μg kg^{-1}) abolished unit activity (AP) and ∫PNA. After cessation of activity, the unit discharged a few APs at the end of the phrenic apnoea when very small phrenic nerve bursts were discernible.

1991; Czyzyk-Krzeska & Lawson, 1991) or slowly adapting pulmonary stretch receptors (Manabe & Ezure, 1988) has been shown to depolarize or increase the spiking activity of E_{dec} neurones (termed post-inspiratory neurones in (Remmers *et al.* 1985; Czyzyk-Krzeska & Lawson, 1991) coincidentally with increasing the phrenic nerve interburst intervals or producing apnoea. Together, the experimental data and simulations suggest that E_{dec} neurones may be pivotal in mediating responses of the respiratory network to activation of peripheral sensory receptors to produce apnoea. This pivotal role for E_{dec} neurones is further strengthened by the widespread inhibitory inputs of these neurones to other respiratory neurones: inspiratory neurones in the contralateral VRG (Lindsey *et al.* 1987; Ezure & Manabe, 1988; Fedorko, Duffin & England, 1989; Jiang & Lipski, 1990; Balis *et al.* 1994), expiratory bulbospinal neurones in the caudal VRG (Jodkowski & Berger, 1988; Ezure & Manabe, 1988; Jiang & Lipski, 1990), and vagal motoneurones with expiratory activity in the VRG (Ezure & Manabe, 1988). In the current study, two E_{dec} neurones could be antidromically activated from the contralateral VRG, and thus may represent propriobulbar E_{dec} neurones in the network described by Lindsey *et al.* (1987) or the post-inspiratory neurones described by Richter (1982).

On the other hand, these neurones may have been pharyngeal motoneurones, sending their axons through the contralateral VRG to the pharyngeal branch of the vagus nerve. We did not test the neurones for antidromic activation from the pharyngeal nerve, and some pharyngeal motoneurones have been shown to exhibit E_{dec} patterns of activity (Grélot, Barillot & Bianchi, 1989). In that regard, Haxhiu, Lunteren, Deal & Cherniack (1988) observed a continuous discharge of both pharyngeal constrictor and thoracic expiratory muscles during cardiopulmonary C fibre receptor stimulation that occurred concurrently with phrenic nerve apnoea. Their study was performed in non-paralysed animals, and as the authors pointed out, bronchoconstriction evoked by cardiopulmonary C fibre receptor stimulation may have activated other vagal pulmonary receptors which could have contributed to some of the effects on the expiratory muscles. This experimental condition may limit the comparisons that can be made with respect to the current findings. Nonetheless, the current observation that two E_{dec} neurones could be antidromically activated from the spinal cord, as has been previously documented in cats (Fedorko & Merrill, 1984), suggests that some of the E_{dec} neurones that displayed tonic discharges in response to cardiopulmonary C fibre receptor stimulation may have excited thoracic expiratory motoneurones that, in turn, relayed a tonic excitatory input to the thoracic expiratory muscles studied by Haxhiu *et al.* (1988). Only four of the nine E_{dec} neurones could be antidromically activated from either the contralateral VRG or the spinal cord. However, the number of neurones with identifiable projections may be underestimated by the technique of antidromic activation due to mismatches of membrane impedance at branching points of axons or phasic changes in excitability of the cells, or because the stimulating electrode was not close enough to the axon (Lipski, 1981).

The E_{dec} neurones also discharged with prolonged bursts during the neural equivalent of rapid shallow breathing, as long as the area of the phrenic nerve bursts was less than ~56% of the control area. This finding is also consistent with the models of Richter and colleagues (Richter, 1982; Ogilvie *et al.* 1992) and of Lindsey and colleagues (Lindsey *et al.* 1987; Balis *et al.* 1994) in which either tachypnoea or apnoea could be modelled by variable increases in post-inspiratory (E_{dec}) neuronal activity coupled with variable reductions in expiratory (E_{con} and E_{aug}) neuronal activity.

The activity of five of the nine E_{dec} neurones recovered concurrently with phrenic nerve activity (~25 s). However, four E_{dec} neurones recovered in ~216 s, far more slowly than did phrenic nerve activity. The significance of the two different recovery times is not known. Interestingly, the E_{aug} neurones recovered with a similarly slow time course (~175 s), suggesting that some E_{dec} neurones and the majority of E_{aug} neurones recover more slowly than inspiratory drive following cardiopulmonary C fibre receptor stimulation. Considered in the context of connectivity studies demonstrating synaptic input from E_{dec} neurones to E_{aug} neurones, the E_{dec} neurones that recovered more slowly may have sent projections to the E_{aug} neurones (Jodkowski & Berger, 1988; Ezure & Manabe, 1988; Jiang & Lipski, 1990); alternatively, both groups of neurones may have received a shared input.

Responses of E_{aug} neurones

In contrast to the tonic discharge of the E_{dec} neurones, the E_{aug} neurones exhibited decreases in activity that were qualitatively similar to the decreases in inspiratory neuronal activity during cardiopulmonary C fibre receptor stimulation. Like the responses of the E_{dec} neurones, these decreases in E_{aug} activity are also consistent with current models of the respiratory network in which expiratory neurones that fire in late expiration (including E_{aug} neurones) are under inhibitory control by post-inspiratory or E_{dec} neurones (Richter, 1982; Lindsey *et al.* 1987; Ogilvie *et al.* 1992; Balis *et al.* 1994).

The decreases in firing activity of the E_{aug} neurones are also consistent with previous studies in which stimulation of pulmonary stretch receptors was shown to decrease the firing rates of E_{aug} neurones (Manabe & Ezure, 1988) and stimulation of superior laryngeal afferent fibres decreased the firing activity of bulbospinal E_{aug} neurones coincidentally with evoking a phrenic nerve apnoea and a tonic discharge of thoracic expiratory muscle activity (Jodkowski & Berger, 1988; Czyzyk-Krzeska & Lawson, 1991). Thus, the experimental data and simulations suggest that the unit activity of E_{aug} neurones may be decreased during

perturbations that produce apnoea, such as those resulting from stimulation of slowly adapting lung stretch receptors, superior laryngeal afferent fibres and cardiopulmonary C fibre receptors.

The E_{aug} neurones were recorded for the most part in the intermediate and caudal VRG, and the majority (13 of 20) projected to the spinal cord. Many expiratory neurones in this part of the VRG project to the spinal cord in both cats and rats, and in some cases have been shown to make excitatory connections with thoracic (internal intercostal) and abdominal expiratory motoneurones (Kirkwood & Sears, 1973; Miller, Ezure & Suzuki, 1985; Onai, Saji & Miura, 1987; Jodkowski & Berger, 1988; Yamada, Ezure & Manabe, 1988). The decreased activity of the bulbospinal E_{aug} neurones may explain the relaxation of abdominal expiratory muscle activity produced by cardiopulmonary C fibre stimulation (Haxhiu *et al.* 1988). Five E_{aug} neurones sent axons to the contralateral VRG, findings that agree with those of Zheng, Barillot & Bianchi (1992*a*) in the rat; and two sent axons to both the contralateral VRG and spinal cord. While intramedullary arborizations are not evidence that respiratory neurones are part of the central respiratory network, propriobulbar E_{aug} neurones with mutual inhibitory connections with I_{dec} neurones have been proposed in some models of the central respiratory network (Balis *et al.* 1994).

E_{aug} neuronal activity was also decreased when cardiopulmonary C fibre stimulation evoked the neural equivalent of rapid shallow breathing. This response is also consistent with the Richter and Lindsey models which predict that either an apnoea or tachypnoea can result from variable increases in the activity of E_{dec} (post-inspiratory) neurones coupled with variable decreases in late expiratory (E_{aug} or E_{con}) activity (Richter, 1982; Lindsey *et al.* 1987; Ogilvie *et al.* 1992; Balis *et al.* 1994). Previous experimental observations also suggest that, during either rapid shallow breathing or tachypnoea, the activity of neurones that discharge during late expiration, including E_{aug} neurones, is decreased. In that regard, Richter & Spyer (1990) observed that when the vagus nerve was stimulated at intensities sufficient to activate vagal cardiopulmonary C fibres and evoke the neural equivalent of rapid shallow breathing, the membrane potentials of bulbospinal expiratory (E_{aug}) neurones showed smaller phasic membrane depolarizations that were characterized by an absence of late expiratory depolarization imposed from elements of the respiratory network, but a persistence of inspiratory and post-inspiratory hyperpolarizations. Parkes, Lara-Muñoz, Izzo & Spyer (1994) similarly found that during spontaneous increases in respiratory rate, late expiratory neurones failed to discharge or to depolarize fully.

Like the responses of some of the E_{dec} neurones, the activity of all the E_{aug} neurones remained below baseline level long after (~175 s) phrenic nerve activity recovered to baseline values (~25 s). Interestingly, Haxhiu *et al.* (1988) reported that the depression of abdominal expiratory activity often outlasted that of diaphragmatic activity. Taken together, the findings suggest that there may be temporal differences in the influence of cardiopulmonary C fibre receptor stimulation on the outputs to inspiratory muscles and abdominal expiratory muscles.

Responses of E_{con} neurones

The variability of the responses of the E_{con} neurones during cardiopulmonary C fibre receptor stimulation was in distinct contrast to the invariable responses of the E_{dec} and E_{aug} neurones. Subtle variations in peripheral sensory input, e.g. changes in end-expiratory load, can change expiratory firing patterns from decrementing to constant (Ezure, 1990). Thus, multiple sensory inputs may have blurred the firing patterns of these neurones, such that those that responded with tonic firing may belong functionally to the group of E_{dec} neurones, while those that responded with decreased burst durations and number of impulses per burst may belong to the group of E_{aug} neurones.

Responses of inspiratory neurones

The firing activity of inspiratory neurones having either intramedullary axons or axons descending to the spinal cord, was, for the most part and as expected, decreased during cardiopulmonary C fibre receptor stimulation. The magnitude of the decrease in inspiratory neuronal activity paralleled the degree of inhibition of phrenic nerve activity; the units had decreases in number of impulses per burst and burst durations during rapid shallow breathing that progressively diminished or were abolished during the cessation of phrenic nerve activity (Fig. 8). The time for recovery for the inspiratory neurones was significantly shorter than that for the E_{aug} neurones and for the slowly recovering group of E_{dec} neurones, despite similar recovery times for phrenic nerve interburst intervals.

A minority of inspiratory neurones (most of which were I_{aug}) behaved differently from phrenic nerve activity, displaying a tonic discharge with a decreased impulse frequency throughout the phrenic nerve apnoea. There were no detectable differences in the baseline firing activity of these I_{aug} neurones compared with the I_{aug} neurones that were silenced by cardiopulmonary C fibre stimulation. The function of these neurones is not known. They were not antidromically activated by the vagus nerve, but could have been pharyngeal motoneurones that may have fired continuously during cardiopulmonary C fibre receptor stimulation (Haxhiu *et al.* 1988). Alternatively, the neurones may represent an element of the central respiratory network that was relatively insensitive to perturbations from sensory receptor stimulation or that in the face of wide-scale inhibition of inspiratory drive discharged tonically to restart inspiratory drive.

The antidromic latencies reported in this study are consistent with previous finding in rats (Saether, Hilaire & Monteau, 1987; Zheng, Barillot & Bianchi, 1991; Zheng *et al.* 1992*a*; Zheng, Barillot & Bianchi, 1992*b*; Lipski, Zhang, Kruszewska & Kanjhan, 1994; Kanjhan, Lipski,

Kruszewska & Rong, 1995). Kanjhan *et al.* (1995) reported that expiratory neurones in the Bötzinger complex were antidromically activated from the contralateral medulla with latencies that averaged ~1·3 ms, and Zheng *et al.* (1991) reported antidromic latencies ranging from 0·98 to 3·4 ms. For E_{aug} and E_{con} cells, Zheng *et al.* (1992*a*, *b*) reported latencies of 0·6 and 0·92 ms for antidromic activation from the spinal cord, and Saether *et al.* (1987) reported latencies of ~1·1 ms. For VRG inspiratory cells antidromically activated from the spinal cord, Zheng *et al.* (1992*a*, *b*) reported latencies of 0·6 ms with averages of 1·1 ms and 1·3 ms when stimulating in the contralateral and ipsilateral spinal cord, respectively; Lipski *et al.* (1994) reported antidromic latencies of 1–2·8 ms; and Saether *et al.* (1987) reported similar latencies averaging ~1·1 ms. It should be acknowledged, however, that with short antidromic latencies $\leqslant$ 1 ms, large collision errors may be encountered, possibly resulting in false antidromic tests (Lipski, 1981).

In conclusion, the present study documents the responses of VRG expiratory and inspiratory neurones having either decrementing, augmenting or constant discharge patterns to activation of the cardiopulmonary C fibre receptors. We have previously described a site in the caudomedial region of the nucleus tractus solitarii (NTS) that contained lower-order neurones in the cardiopulmonary C fibre afferent pathway in the rat (Bonham & Joad, 1991; Wilson *et al.* 1996). The current findings provide no data on connectivity among the respiratory neurones recorded. However, placed within the context of current models of the central respiratory network and studies of the connectivity of neurones in the respiratory network (Richter, 1982; Segers *et al.* 1987; Lindsey *et al.* 1987; Ogilvie *et al.* 1992; Balis *et al.* 1994), the findings are consistent with the working hypothesis that cardiopulmonary C fibre stimulation activates second-order neurones in the NTS and also results in a tonic discharge of E_{dec} neurones, perhaps via a paucisynaptic pathway from the NTS. A portion of these E_{dec} neurones, via intramedullary connections, may provide inhibitory inputs to inspiratory neurones as well as to expiratory or cranial motoneurones (some of the E_{aug} neurones) resulting in the synchronous relaxation of inspiratory muscles, abdominal expiratory muscles and upper airway dilating muscles, respectively, during activation of the cardiopulmonary C fibre receptors. The E_{dec} neurones with bulbospinal projections may relay a tonic excitation to thoracic expiratory muscles during cardiopulmonary C fibre receptor stimulation.

Balis, U. J., Morris, K. F., Koleski, J. & Lindsey, B. G. (1994). Simulations of a ventrolateral medullary neural network for respiratory rhythmogenesis inferred from spike train cross-correlation. *Biological Cybernetics* **70**, 311–327.

Bonham, A. C. & Joad, J. P. (1991). Neurones in commissural nucleus tractus solitarii required for full expression of the pulmonary C-fibre reflex in rat. *Journal of Physiology* **441**, 95–112.

Coleridge, H. M. & Coleridge, J. C. G. (1986). Reflexes evoked from the tracheobronchial tree and lungs. In *Handbook of Physiology*, section 3, *Respiratory System*, vol. II, part 1, ed. Cherniack, N. & Widdicombe, J. G., pp. 395–429. American Physiological Society, Bethesda.

Coleridge, H. M. & Coleridge, J. C. G. (1994). Pulmonary reflexes: neural mechanisms of pulmonary defense. *Annual Review of Physiology* **56**, 69–91.

Czyzyk-Krzeska, M. F. & Lawson, E. E. (1991). Synaptic events in ventral respiratory neurones during apnoea induced by laryngeal nerve stimulation in neonatal pig. *Journal of Physiology* **436**, 131–137.

Ezure, K. (1990). Synaptic connections between medullary respiratory neurons and considerations on the genesis of respiratory rhythm. *Progress in Neurobiology* **35**, 429–450.

Ezure, K. & Manabe, M. (1988). Decrementing expiratory neurons of the Bötzinger complex. II. Direct inhibitory synaptic linkage with ventral respiratory group neurons. *Experimental Brain Research* **72**, 159–166.

Ezure, K., Manabe, M. & Yamada, H. (1988). Distribution of medullary respiratory neurons in the rat. *Brain Research* **455**, 262–270.

Fedorko, L., Duffin, J. & England, S. (1989). Inhibition of inspiratory neurons of the nucleus retroambigualis by expiratory neurons of the Bötzinger complex in the cat. *Experimental Neurology* **106**, 74–77.

Fedorko, L. & Merrill, E. G. (1984). Axonal projections from rostral expiratory neurons of Bötzinger complex to medulla and spinal cord. *Journal of Physiology* **350**, 487–496.

Fuller, J. H. & Schlag, J. D. (1976). Determination of antidromic excitation by the collision test: problems of interpretation. *Brain Research* **112**, 283–298.

Grélot, L., Barillot, J. C. & Bianchi, A. L. (1989). Pharyngeal motoneurons: respiratory-related activity and responses to laryngeal afferents in the decerebrate cat. *Experimental Brain Research* **78**, 336–344.

Haxhiu, M. A., Lunteren, E. V., Deal, E. C. & Cherniack, N. S. (1988). Effect of stimulation of pulmonary C-fiber receptors on canine respiratory muscles. *Journal of Applied Physiology* **65**, 1087–1092.

Jiang, C. & Lipski, J. (1990). Extensive monosynaptic inhibition of ventral respiratory group neurons by augmenting neurons in the Bötzinger complex in the rat. *Experimental Brain Research* **81**, 639–648.

Jodkowski, J. S. & Berger, A. J. (1988). Influences from laryngeal afferents on expiratory bulbospinal neurons and motoneurons. *Journal of Applied Physiology* **64**, 1337–1345.

Kanjhan, R., Lipski, J., Kruszewska, B. & Rong, W. (1995). A comparative study of pre-sympathetic and Bötzinger neurons in the rostral ventrolateral medulla (RVLM) of the rat. *Brain Research* **699**, 19–32.

Kirkwood, P. A. & Sears, T. A. (1973). Monosynaptic excitation of thoracic expiratory motoneurones from lateral respiratory neurones in the medulla of the cat. *Journal of Physiology* **234**, 87–89*P*.

Koepchen, H. P., Kalia, M., Sommer, D. & Klussendorf, D. (1977). Action of type J afferents on the discharge pattern of medullary respiratory neurons. In *Krogh Centenary Symposium on Respiratory Adaptations, Capillary Exchange and Reflex Mechanisms*, ed. Paintal, A. S. & Gill-Kumar, P., pp. 407–425. Vallabhbai Patel Chest Institute, Delhi.

Lawson, E. E., Richter, D. W., Czyzyk-Krzeska, M. F., Bischoff, A. & Rudesill, R. C. (1991). Respiratory neuronal activity during apnea and other breathing patterns induced by laryngeal stimulation. *Journal of Applied Physiology* **70**, 2742–2749.

Lindsey, B. G., Segers, L. S. & Shannon, R. (1987). Functional associations among simultaneously monitored lateral medullary respiratory neurons in the cat. II. Evidence for inhibitory actions of expiratory neurons. *Journal of Neurophysiology* **57**, 1101–1117.

Lipski, J. (1981). Antidromic activation of neurones as an analytic tool in the study of the central nervous system. *Journal of Neuroscience Methods* **4**, 1–32.

Lipski, J., Zhang, X., Kruszewska, B. & Kanjhan, R. (1994). Morphological study of long axonal projections of ventral medullary inspiratory neurons in the rat. *Brain Research* **640**, 171–184.

Manabe, M. & Ezure, K. (1988). Decrementing expiratory neurons in the Bötzinger complex. I. Response to lung inflation and axonal projection. *Experimental Brain Research* **72**, 150–158.

Miller, A. D., Ezure, K. & Suzuki, I. (1985). Control of abdominal muscles by brain stem respiratory neurons in the cat. *Journal of Neurophysiology* **54**, 155–167.

Ogilvie, M. D., Gottschalk, A., Anders, K., Richter, D. W. & Pack, A. I. (1992). A network model of respiratory rhythmogenesis. *American Journal of Physiology* **263**, 962–975.

Onai, T., Saji, M. & Miura, M. (1987). Projections of supraspinal structures to the phrenic motor nucleus in rats studied by a horseradish peroxidase microinjection method. *Journal of the Autonomic Nervous System* **21**, 233–239.

Onimaru, H., Arata, A. & Homma, I. (1989). Firing properties of respiratory rhythm generating neurons in the absence of synaptic transmission in rat medulla *in vitro*. *Experimental Brain Research* **76**, 530–536.

Parkes, M. J., Lara-Muñoz, J. P., Izzo, P. N. & Spyer, K. M. (1994). Responses of ventral respiratory neurones in the rat to vagus stimulation and the functional division of expiration. *Journal of Physiology* **476**, 131–139.

Remmers, J. E., Richter, D. W., Ballantyne, D., Bainton, C. R. & Klein, J. P. (1985). Activation of bulbar post-inspiratory neurones by upper airway stimulation. In *Neurogenesis of Central Respiratory Rhythm*, ed. Bianchi, A. L. & Denavit-Saubié, M., pp. 290–291. MTP Press, Lancaster, UK; Boston, MA, USA.

Richter, D. W. (1982). Generation and maintenance of the respiratory rhythm. *Journal of Experimental Biology* **100**, 93–107.

Richter, D. W., Ballantyne, D. & Remmers, J. E. (1986). How is the respiratory rhythm generated? A Model. *News In Physiological Sciences* **1**, 109–112.

Richter, D. W., Ballanyi, K. & Schwarzacher, S. (1992). Mechanisms of respiratory rhythm generation. *Current Opinion in Neurobiology* **2**, 788–793.

Richter, D. W. & Spyer, K. M. (1990). Cardiorespiratory Control. In *Central Regulation of Autonomic Functions*, ed. Loewy, A. D. & Spyer, K. M., pp. 189–207. Oxford University Press, New York.

Saether, K., Hilaire, G. & Monteau, R. (1987). Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Research* **419**, 87–96.

Segers, L. S., Shannon, R., Saporta, S. & Lindsey, B. G. (1987). Functional associations among simultaneously monitored lateral medullary respiratory neurons in the cat. I. Evidence for excitatory and inhibitory actions of inspiratory neurons. *Journal of Neurophysiology* **57**, 1078–1100.

Smith, J. C., Ellenberger, H., Ballanyi, K., Richter, D. W. & Feldman, J. L. (1991). Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* **254**, 726–729.

Thames, M. D. & Schmid, P. G. (1979). Cardiopulmonary receptors with vagal afferents tonically inhibit ADH release in the dog. *American Journal of Physiology* **237**, H299–304.

Thorén, P. (1979). Role of cardiac vagal C fibers in cardiovascular control. *Reviews of Physiology Biochemistry and Pharmacology* **86**, 1–94.

Vardhan, A., Kachroo, A. & Sapru, H. N. (1993). Excitatory amino acid receptors in the nucleus tractus solitarius mediate the response to the stimulation of cardio-pulmonary vagal afferent C fiber endings. *Brain Research* **618**, 23–31.

Verberne, A. J. M. & Guyenet, P. G. (1992). Medullary pathway of the Bezold-Järisch reflex in the rat. *American Journal of Physiology* **263**, R1195–1202.

Wilson, C. G., Zhang, Z. & Bonham, A. C. (1996). Non-NMDA receptors transmit cardiopulmonary C-fibre input in nucleus tractus solitarii (NTS) in rats. *Journal of Physiology* **496**, 773–785.

Yamada, H., Ezure, K. & Manabe, M. (1988). Efferent projections of inspiratory neurons of the ventral respiratory group. A dual labeling study in the rat. *Brain Research* **455**, 283–294.

Zheng, Y., Barillot, J. C. & Bianchi, A. L. (1991). Are the post-inspiratory neurons in the decerebrate rat cranial motoneurons or interneurons? *Brain Research* **551**, 256–266.

Zheng, Y., Barillot, J. C. & Bianchi, A. L. (1992*a*). Intracellular electrophysiological and morphological study of the medullary inspiratory neurons of the decerebrate rat. *Brain Research* **576**, 235–244.

Zheng, Y., Barillot, J. C. & Bianchi, A. L. (1992*b*). Medullary expiratory neurons in the decerebrate rat: an intracellular study. *Brain Research* **576**, 245–253.

Acknowledgements

This work was supported by NIH HL 48584 and HL52165. The authors gratefully acknowledge Judy Stewart and Stephanie Perino for technical support, Dr Jesse Joad for reviewing the manuscript, and Elizabeth Walker and Sally DiVecchia for clerical support.

Author's email address

A.C. Bonham: acbonham@ucdavis.edu

Received 13 January 1996; accepted 7 July 1997.