# Effect of pulmonary C-fibre afferent stimulation on cardiac vagal neurones in the nucleus ambiguus in anaesthetized cats

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(Received 16 February 2000; accepted after revision 7 April 2000)

1. It has been demonstrated previously that the vagal bradycardia evoked by activation of pulmonary C-fibres is not respiratory modulated. Experiments were carried out in α-chloralose anaesthetized cats to determine if these cardiac vagal preganglionic neurones (CVPNs) in the nucleus ambiguus (NA), which have respiratory modulated activity, can be activated when pulmonary C-fibre afferents are stimulated by right atrial injections of phenylbiguanide (PBG).

2. Eleven CVPNs with B-fibre axons in the right cardiac vagal branches were identified and found to be localized within or ventrolateral to the nucleus ambiguus. Ionophoretic application of a high current of DL-homocysteic acid (DLH) induced a vagally mediated bradycardia and hypotension in six of eight sites from which CVPNs were recorded.

3. The activity of B-fibre CVPNs, whether spontaneous ($n = 4$) or induced by ionophoresis of DLH ($n = 7$) was respiratory modulated, firing perferentially during post-inspiration and stage 2 expiration. This activity also correlated with the rising phase of the arterial blood pressure wave consistent with these CVPNs receiving an arterial baroreceptor input.

4. Right atrial injections of PBG excited nine of eleven CVPNs tested. In eight of these activated neurones the onset latency of the excitation was within the pulmonary circulation time, consistent with being activated only by pulmonary C-fibre afferents. In two neurones the PBG-evoked excitation still occurred when central inspiratory drive was inhibited, as indicated by the disappearance of phrenic nerve activity.

5. In conclusion, B-fibre respiratory modulated CVPNs can be activated following stimulation of pulmonary C-fibre afferents.

Most vagal cardiac reflexes are modulated by central respiratory drive such that cardiac slowing is greater during expiration than during inspiration. This is believed to be due to neural coupling between the brainstem respiratory system and the cardiovascular control system (see Taylor *et al.* 1999). This respiratory modulation has been considered to be due to inhibition of cardiac vagal preganglionic neurones in the nucleus ambiguus by central respiratory neurones (Gilbey *et al.* 1984) and disfacilitation of baroreceptor and chemoreceptor inputs by lung stretch afferents (Potter, 1981). These cardiac vagal preganglionic neurones have B-fibre axons which run in the cardiac branches of the vagus and receive an input from arterial baroreceptors (McAllen & Spyer, 1976, 1978*a*,*b*; Gilbey *et al.* 1984). However, the bradycardia evoked by injection of phenylbiguanide (PBG) into the right atrium, to activate pulmonary C-fibre afferents (Coleridge & Coleridge, 1984), is not influenced by either central respiratory drive or lung inflation (Daly & Kirkman, 1988, 1989; Daly, 1991; Daly *et al.* 1992). This led Daly (1991) to postulate that the pulmonary C-fibre-evoked bradycardia was mediated by a different group of cardiac vagal preganglionic neurones, possibly those with non-myelinated axons and located in the dorsal vagal nucleus (Donoghue *et al.* 1981; Jordan *et al.* 1986; Ford *et al.* 1990). Those neurones have been shown to be cardio-inhibitory in function (Jones *et al.* 1995) and to be activated synaptically by electrical stimulation of non-myelinated pulmonary vagal afferents (Bennett *et al.* 1985), but probably not by lung inflation (Jones *et al.* 1998). More recently, Jones *et al.* (1998) demonstrated in both cats and rats that these C-fibre cardiac vagal preganglionic neurones in the dorsal vagal nucleus lack respiratory modulation and are indeed excited by right atrial injection of PBG. However, such stimuli evoked only a short burst of excitation, which

was of a much shorter duration than that of the evoked bradycardia. Further, selective electrical stimulation of non-myelinated vagal efferents evoked a bradycardia in the cat of 11 beats $min^{-1}$ (Jones *et al.* 1995) which is much smaller than that evoked by stimulating pulmonary C-fibre afferents, for instance activation of these afferents with PBG (6–15 $\mu g\ kg^{-1}$) injected in right atrium evoked a bradycardia of around 55 beats $min^{-1}$ (Daly & Kirkman, 1988). Thus, it is difficult to explain how non-respiratory modulated C-fibre cardiac vagal preganglionic neurones in the dorsal vagal nucleus could be solely responsible for the vagal bradycardia evoked by pulmonary C-fibre afferent activation. Therefore the present study has been designed to determine whether respiratory modulated B-fibre cardiac vagal preganglionic neurones in the nucleus ambiguus are also activated by stimulation of pulmonary C-fibre nerve endings. A preliminary report of some of these observations has been published (Wang & Ramage, 1999).

## METHODS

The experiments were carried out under the Animals (Scientific Procedures) Act, 1986 and at the end of the experiment the animals were killed by an overdose of anaesthetic and exsanguination.

### General preparation

Experiments were carried out on seven adult cats (2·5–4·5 kg) of either sex, anaesthetized with a mixture of α-chloralose (70 mg $kg^{-1}$) and pentobarbitone sodium (6 mg $kg^{-1}$) injected I.V. Before and after neuromuscular blockade (see below), the level of anaesthesia was assessed by the absence of a withdrawal reflex and/or the cardiovascular response to paw-pinch and the stability of resting cardiovascular and respiratory variables and pupil size; if and when required, additional anaesthetic (α-chloralose, 10–15 mg $kg^{-1}$, I.V.) was administered.

Rectal temperature was monitored and maintained between 38–39 °C with a Harvard homeothermic blanket. When surgical anaesthesia was established, the brachial veins and arteries on both sides and one femoral vein were cannulated for administration or withdrawal of drugs/fluids and for recording blood pressure using a pressure transducer (Gould) connected to a Grass Model 7D Polygraph (Grass Medical Instruments, Quincy, MA, USA). The bladder was cannulated to prevent undue filling during the period of the experiment, avoiding reflex effects associated with bladder distension. A cervical tracheotomy was performed and the trachea cannulated just below the larynx. Tracheal pressure was monitored by a pressure transducer (Gould) connected to a side arm of the tracheal cannula. A silicone cannula, pre-filled with PBG (400 $\mu g\ ml^{-1}$), was advanced into the right atrium via the right external jugular vein. An ECG was recorded by leads attached to each of the forepaws of the animal from which heart rate was derived. The animals were placed in a stereotaxic frame and ventilated artificially (Harvard Ventilator model 551) with $O_2$-enriched air, maintaining a small positive end-expiratory pressure (1–2 $cmH_2O$). As soon as the ventilation had started, the animals were neuromuscularly blocked using vecuronium bromide (200 $\mu g\ kg^{-1}$, I.V.) and supplemented with an I.V. infusion of 480 $\mu g\ kg^{-1}\ h^{-1}$. This infusion (6 ml $kg^{-1}\ h^{-1}$) comprised 500 ml plasma substitute Gelofusine, 500 ml $H_2O$, 8·4 g $NaHCO_3$, 2 g glucose and 80 mg vecuronium bromide and was given to maintain blood volume, counteract the development of non-respiratory acidosis and maintain neuromuscular blockade. Arterial blood gas variables were measured using a Corning Blood Gas Analyser (Model 238). The blood gases and pH were regularly monitored and maintained at 100–180 mmHg $P_{O_2}$, 35–45 mmHg $P_{CO_2}$, and pH 7·3–7·4 by I.V. injection of sodium bicarbonate (1 M) and/or adjusting the volume and frequency of ventilation. In all experiments, animals were pretreated with the $\beta_1$-adrenoceptor antagonist atenolol (1 mg $kg^{-1}$, I.V.) to block sympathetic drive to the heart. Thus changes in heart rate could be presumed to be due to changes in activity in cardiac vagal efferents.

The right phrenic nerve was dissected from a dorsolateral approach, cut peripherally and desheathed. The cut central end of the nerve was placed on bipolar silver wire recording electrodes. Clamps applied to the vertebral spines at C7 and L2 or L3 were used to elevate and stabilize the animal. To expose the brainstem the nuchal muscles were removed, the occipital bone opened and the dura overlying the brainstem and cerebellum cut and reflected laterally. In some experiments the cerebellum was displaced rostrally with a small retractor to allow access to the region of the nucleus ambiguus.

### Preparation of cardiac and pulmonary vagal branches

A thoracotomy was performed between the fourth and sixth ribs to gain access to the right cranial, caudal cardiac and pulmonary branches, as previously described (McAllen & Spyer, 1976). The intact cardiac and pulmonary branches and the vagus nerve between the cranio- and caudal cardiac branches were placed on fine silver wire (0·125 mm in diameter) bipolar electrodes with a 2 mm gap. The wires were insulated from one another with wax and sealed round the nerves with President light body dental polyvinyl-siloxane (Coltene UK Ltd, West Sussex, UK). These silver wires had been soldered onto insulated copper wires, which were secured to the thorax. The electrodes were connected to an isolated stimulator (DS2A, Digitimer Ltd, Welwyn Garden City) triggered by Digitimer D4030 Programmer. The vagal branches were left intact and typically, stimulation of the main cardiac branch (1 ms pulses at 100 $\mu$A, 50 Hz) evoked 'cardiac arrest' without change in tracheal pressure, whilst stimulation of the pulmonary branches evoked changes in tracheal pressure but not heart rate.

### Single unit recording and identification of cardiac vagal preganglionic neurones

Extracellular recordings were made from neurones in the region of the nucleus ambiguus using 'piggy-back' electrodes which were assembled from a single glass recording electrode and a multi-barrelled glass electrode (Wang *et al.* 1998). The recording barrel contained 4 M sodium chloride. One of the barrels contained Pontamine Sky Blue dye (2% dissolved in 0·5 M sodium acetate) for automatic current balancing and marking the recording sites and the other barrel was filled with the glutamate receptor agonist DL-homocysteic acid (DLH, 100 mM, pH 8·5). Cardiac vagal preganglionic neurones were identified by their antidromic activation following electrical stimulation of the thoracic cardiac branches of the vagus (100–500 $\mu$A, 1 ms pulses, 0·2–1·0 Hz) as previously described (McAllen & Spyer, 1976, 1978*a*). The criteria used to determine antidromic activation were the constant latency of the evoked response and its collision with appropriately timed ongoing activity (Fig. 1*A*). The possibility of current spread from the cardiac branch to the whole vagus nerve was checked periodically during the experiment by the absence of antidromic activation to stimuli applied to the vagus nerve below the pulmonary nerve branching point. Pulmonary C-fibre afferents were stimulated by injection of a bolus of phenylbiguanide (PBG; 14–32 $\mu g\ kg^{-1}$ in 100–200 $\mu$l) into the right atrium. The minimum interval between two PBG injections was 5 min and the volume for a single injection

was restricted to less than 200 $\mu$l to avoid stimulation of receptors in the atrial wall by volume expansion.

### Data capture and analysis

Neuronal activity, phrenic nerve activity and ECG were amplified (× 2000, × 20 000 and × 5000, respectively) and filtered (0·5–5 kHz; Neurolog, AC preamplifier NL104 and filter NL125; Neurolog System, Digitimer Ltd, Welwyn Garden City, UK). Phrenic nerve activity was then integrated using an EMG integrator (NL 703, Neurolog System). Arterial blood pressure (BP), heart rate (HR), tracheal pressure (TP), ECG, raw and integrated phrenic nerve activity and neuronal activity were displayed on a computer using a 1401 interface (1401 Plus, Cambridge Electronic Design (CED), Cambridge, UK) and Spike2 software (CED) and stored on videotape using a digital data recorder (VR100B, Instrutech Corp., Great Neck, NY, USA). Offline analysis of the recorded data (phrenic-, tracheal pressure-, and ECG-triggered correlations) was made using Spike2 software. Baseline values for mean arterial pressure (MAP) and HR were taken as the mean over 40 s before the administration of PBG. The maximal overall changes evoked by PBG were compared with baseline. The mean baseline neuronal firing rate was measured over 40 s. In addition, the mean number of spikes per burst and mean burst duration of the four respiratory cycles before PBG injection were taken as the control. The number of spikes and duration of the first burst after the PBG injection were compared with the control and if the changes in either burst number and/or duration were greater than 20%, this was considered to be excitation. This excitation was then re-analysed to determine if it occurred within 5 s; neuronal responses to PBG occurring within this latency can be taken as resulting from pulmonary C-fibre stimulation (5 s window, see Daly & Kirkman, 1988; Jones *et al.* 1998). Beyond this duration changes in activity could be attributed to activation by PBG of other afferents that are downstream of the pulmonary circulation (Daly & Kirkman, 1988). However, since the B-fibre cardiac vagal preganglionic neurones were firing in the post-inspiratory and stage 2 expiratory (PI–E2) phases of the respiratory cycle (Gilbey *et al.* 1984), it was difficult to analyse the mean change in firing rate after PBG injection, as in most cases the burst of firing after PBG injection overlapped the 5 s window. Therefore the 1st second of the PBG-evoked response, which fell within the 5 s window, was analysed and compared with the mean of the 1st second of the previous four bursts. All data are presented as means ± S.E.M. except where indicated, and all

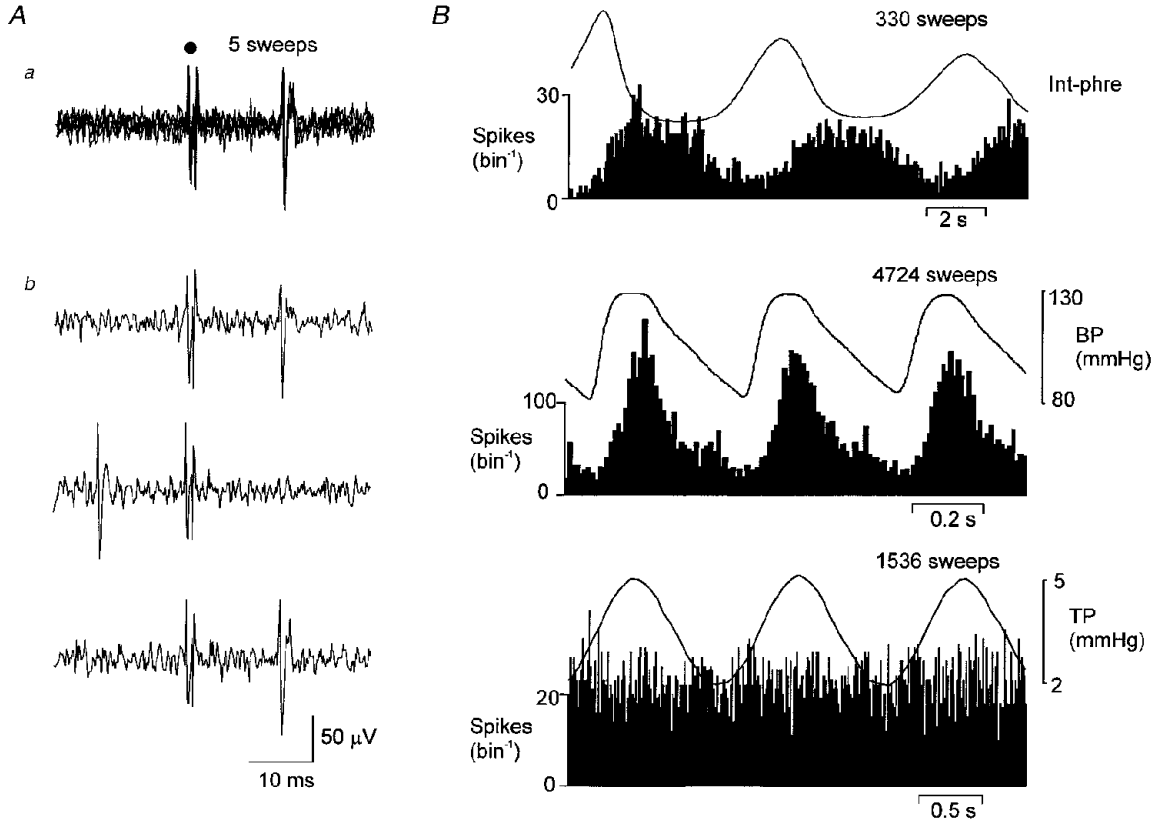


**Figure 1. Identification of a B-fibre cardiac vagal preganglionic neurone in the nucleus ambiguus**

*A*, traces showing a cardiac vagal preganglionic neurone antidromically activated (latency, 15 ms) by stimulating the right cardiac branch (200 $\mu$A, 1 ms, 0·5 Hz). *Aa*, five consecutive sweeps superimposed to show the constant latency of the evoked spike; *Ab*, three consecutive sweeps showing that the evoked spike (see top and bottom trace) was cancelled by the spontaneous spike (see middle trace). The ● indicates the stimulus artefacts. *B*, histograms of the activity (with DLH at 20 nA) of the same CVPN as in *A* triggered by integrated phrenic nerve activity (Int-phre; 50 ms bin width; top panel), the *R*-wave of the ECG (10 ms bin width; middle panel) and by tracheal pressure (10 ms bin width; lower panel). Above the histograms is an average of integrated phrenic activity, ECG triggered arterial blood pressure (BP) and the tracheal pressure (TP) wave, respectively. The number of sweeps on top of each panel refers to both the average and to the histogram.

comparisons of the means were made using Student's paired $t$ test. Differences between means were taken as significant when $P < 0{\cdot}05$.

### Localization of recording sites

Recording sites were marked by ionophoretic ejection of Pontamine Sky Blue. Following the experiments, brainstems were removed and fixed in 10% formal saline, and serial frozen sections (80 $\mu$m) were cut and stained with Neutral Red. The marked recording sites were visualized and displayed on standard sections of brainstem taken from the stereotaxic atlas of the cat (Berman, 1968, Fig. 2).

### Drugs

Drugs were obtained from the following sources: $\alpha$-chloralose, DL-homocysteic acid and atenolol from Sigma Aldrich Chemical Co., Poole, Dorset, UK; pentobarbitone sodium from Rhône Mérieux Ltd, Harlow, Essex, UK; Pontamine Sky Blue dye from BDH, Poole, Dorset, UK; Gelofusine from Braun Medical Ltd, Aylesbury, Bucks, UK; phenylbiguanide from Research Biochemicals, Semat Technical Ltd, St Albans, Herts, UK, and vecuronium bromide from Organon Technika Ltd, Cambridge, UK.

## RESULTS

A total of 11 antidromically identified vagal preganglionic neurones with axons in the cardiac branches of the vagus nerve were recorded in this study. They had calculated axon conduction velocities within the B-fibre range (5·9–18·0 m s$^{-1}$, a mean of 11·4 ± 0·9 m s$^{-1}$). The recording sites of six of these were localized by pontamine injection and another two were recorded in very close proximity to a previously marked site. These sites were located within or ventrolateral to the nucleus ambiguus (Fig. 2). Based on the depth and rostro-caudal position of the recording electrode the other three B-fibre cardiac projecting neurones were considered also to be in this same region. Baseline values (means ± S.D.) were; mean arterial blood pressure 101 ± 5 mmHg; heart rate 160 ± 23 beats min$^{-1}$; tracheal pressure, inflation and deflation, 5·4 ± 2·5 and 2·1 ± 0·7 mmHg, respectively; $P_{O_2}$ 141 ± 27 mmHg; $P_{CO_2}$ 38 ± 10 mmHg and pH 7·32 ± 0·05.

### B-fibre cardiac vagal preganglionic neurones

Ten of the B-fibre cardiac vagal preganglionic neurones had little or no ongoing spontaneous activity, whereas one neurone had an average firing rate of 6·3 spikes s$^{-1}$. The profile of the activity was analysed in detail in neurones either with ($n = 7$) or without ($n = 4$) DLH (10–120 nA) ionophoretically applied to induce or increase firing rate. This combined group had a mean firing rate of 2·0 ± 0·7 spikes s$^{-1}$. The activity in all 11 neurones showed a strong pulse-related rhythm (Fig. 1*B*). In addition, a component of this activity was correlated with central respiratory drive, being maximal during post-inspiration and stage 2 expiration (E2; Fig. 1*B*). Even during high discharge rates (up to 20 spikes s$^{-1}$) evoked by DLH application at high currents (60–120 nA) the activity in neurones remained respiratory modulated (Fig. 3*C*). This respiratory modulation of the activity was abolished in two neurones tested when inhibition of central inspiratory activity caused ongoing activity to become continuous (Fig. 4*B*). The ongoing activity of the majority of these neurones (8 out of 11) showed no obvious relationship to tracheal pressure (Fig. 1*B*), but in the other three neurones there was a correlation between neuronal activity and tracheal pressure, the maximal discharge occurring during the phase of lung deflation. In all the experiments phrenic nerve activity was locked to lung inflation.

At six out of the eight sites from which B-fibre cardiac vagal preganglionic neurones were recorded, ionophoretic application of DLH at a high current (30–160 nA) induced a

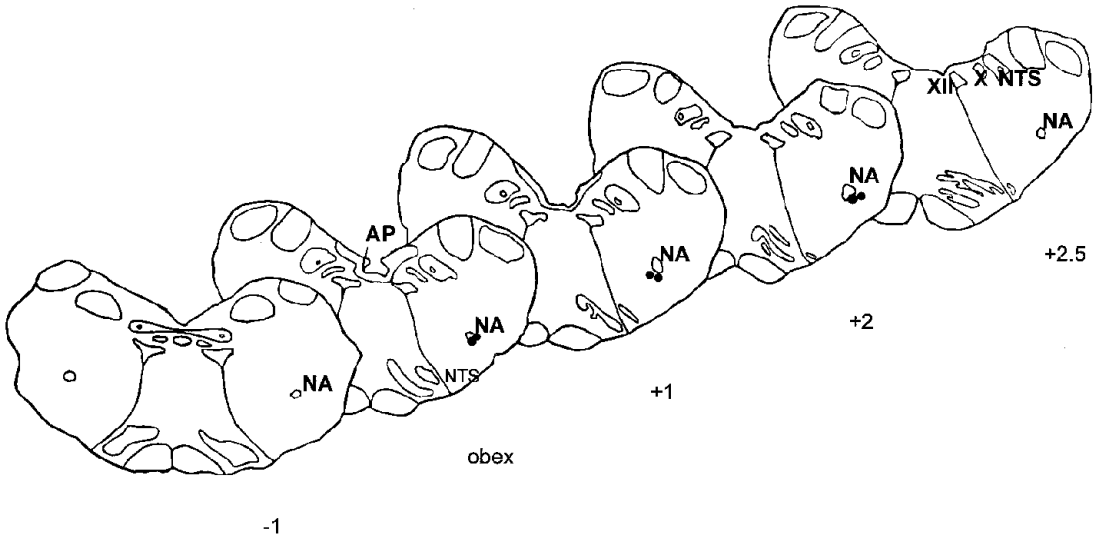


**Figure 2. Pontamine Sky Blue marked locations of recordings from the medulla**

The position of six B-fibre (●) cardiac vagal preganglionic neurones from which recordings were made are shown on five standard sections of the medulla taken from −1 to +2·5 mm caudal to rostral at the level of the obex. Abbreviations: NA, nucleus ambiguus; NTS, nucleus tractus solitarius; X, dorsal vagal nucleus; XII, hypoglossal nucleus; AP, area postrema.

significant bradycardia and hypotension. During ionophoresis of DLH, the heart rate decreased from $153 \pm 6$ to $139 \pm 6$ beats min$^{-1}$ ($P < 0{\cdot}01$, $n = 6$) and the mean arterial blood pressure fell from $106 \pm 4$ to $90 \pm 3$ mmHg ($P < 0{\cdot}05$, $n = 6$). At three sites neuronal activity was monitored during and following these DLH applications. In all these cases the increase in firing rate was associated with a bradycardia and hypotension (Fig. 3).

### Effect of right atrial injection of PBG on B-fibre cardiac vagal preganglionic neurones

**Overall effect of PBG.** PBG injections excited nine of the eleven B-fibre cardiac vagal preganglionic neurones. In these nine responding neurones the combined mean ongoing activity was $1{\cdot}9 \pm 0{\cdot}7$ spikes s$^{-1}$ which comprised means of $2{\cdot}0 \pm 1{\cdot}4$ spikes s$^{-1}$ ($n = 4$) for spontaneously firing neurones and $1{\cdot}9 \pm 0{\cdot}8$ spikes s$^{-1}$ ($n = 5$) for those activated by DLH.

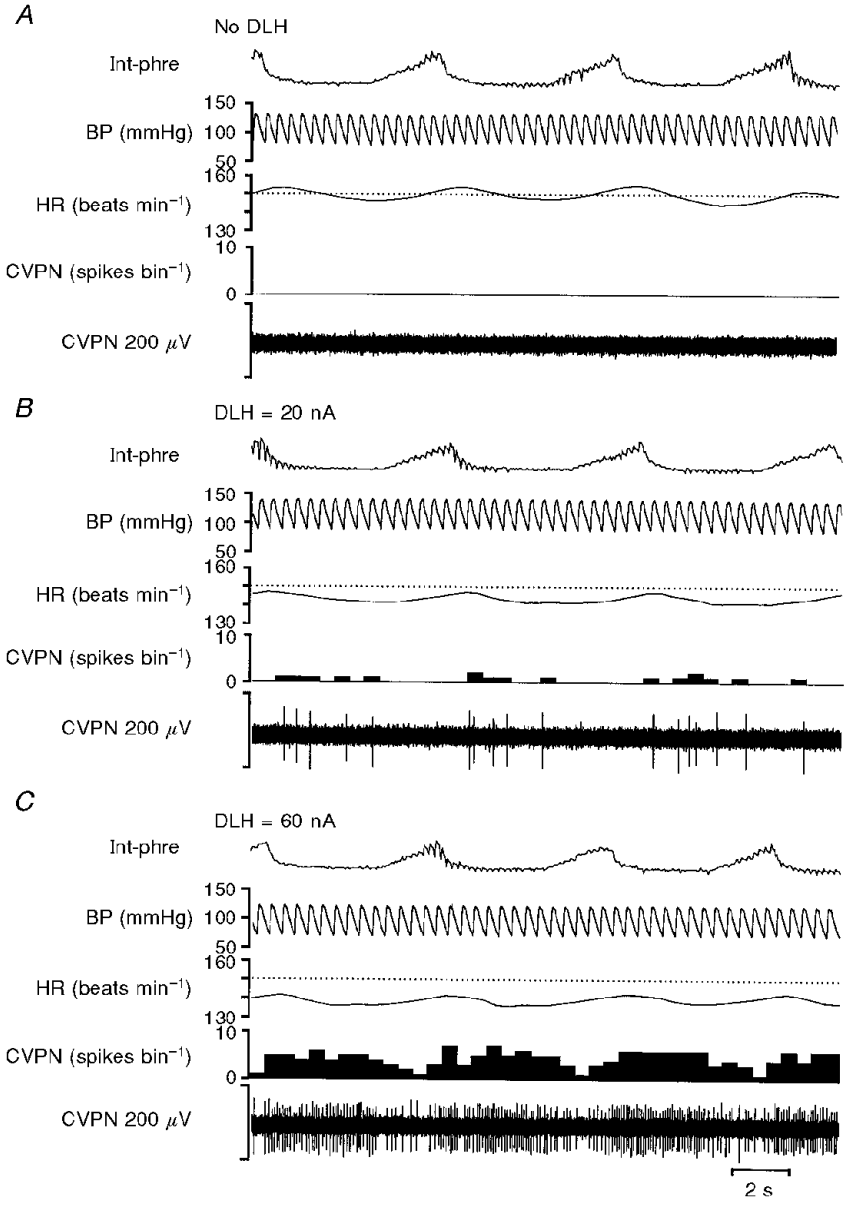


**Figure 3. Traces showing the effects of ionophoretic application of DLH onto a CVPN with a B-fibre axon**

Anaesthetized cat pretreated with atenolol (1 mg kg$^{-1}$, I.V.). Records from top downwards: Int-phre, integrated phrenic nerve activity; BP, arterial blood pressure (mmHg); HR, heart rate (beats min$^{-1}$) and rate histogram (0·5 s bin) and recording of the activity of a B-fibre CVPN. *A*, ongoing activity, no DLH; *B* and *C*, activity in the presence of 20 nA DLH and 60 nA DLH, respectively. Note the heart rate fell as CVPN discharge increased (the dotted lines on the heart rate traces represent the mean heart rate in control without DLH, 150 beats min$^{-1}$) and even at a high level of excitation the activity was still respiratory modulated (*C*).

Right atrial injection of PBG (14–32 $\mu$g kg$^{-1}$ in 100–200 $\mu$l) increased both the number of spikes per burst from 11 ± 4 to 27 ± 6 ($P < 0{\cdot}01$, $n = 9$) and the burst duration from 1·9 ± 0·5 to 3·6 ± 0·8 ms ($P < 0{\cdot}01$, $n = 9$), respectively (Fig. 4*A*). In association with the increased neuronal activity, PBG also evoked a vagally mediated bradycardia of 69 ± 6 beats min$^{-1}$ (heart rate fell from 159 ± 7 to 90 ± 6 beats min$^{-1}$, $P < 0{\cdot}001$, $n = 9$) and a reduction in arterial blood pressure of 22 ± 1 mmHg (mean arterial blood pressure decreased from 96 ± 5 to 74 ± 4 mmHg, $P < 0{\cdot}001$, $n = 9$). The latency for the evoked excitation in the cardiac vagal preganglionic neurones was 3·4 ± 0·3 s (range 1·8–4·5 s) which was significantly ($P < 0{\cdot}01$, $n = 9$) shorter than that of the latency for the evoked bradycardia (3·7 ± 0·3 s; range 2·5–4·5 s). Of the nine neurones, six were excited before the appearance of the bradycardia.

**Effect of PBG within pulmonary circulation time.** In order to establish that pulmonary C-fibre afferent-evoked responses in the cardiac vagal preganglionic neurones occurred within the pulmonary circulation time, the number of spikes in the 1st second of the evoked excitation within the 5 s window were analysed (see Methods). In eight out of nine neurones, PBG increased the number of spikes

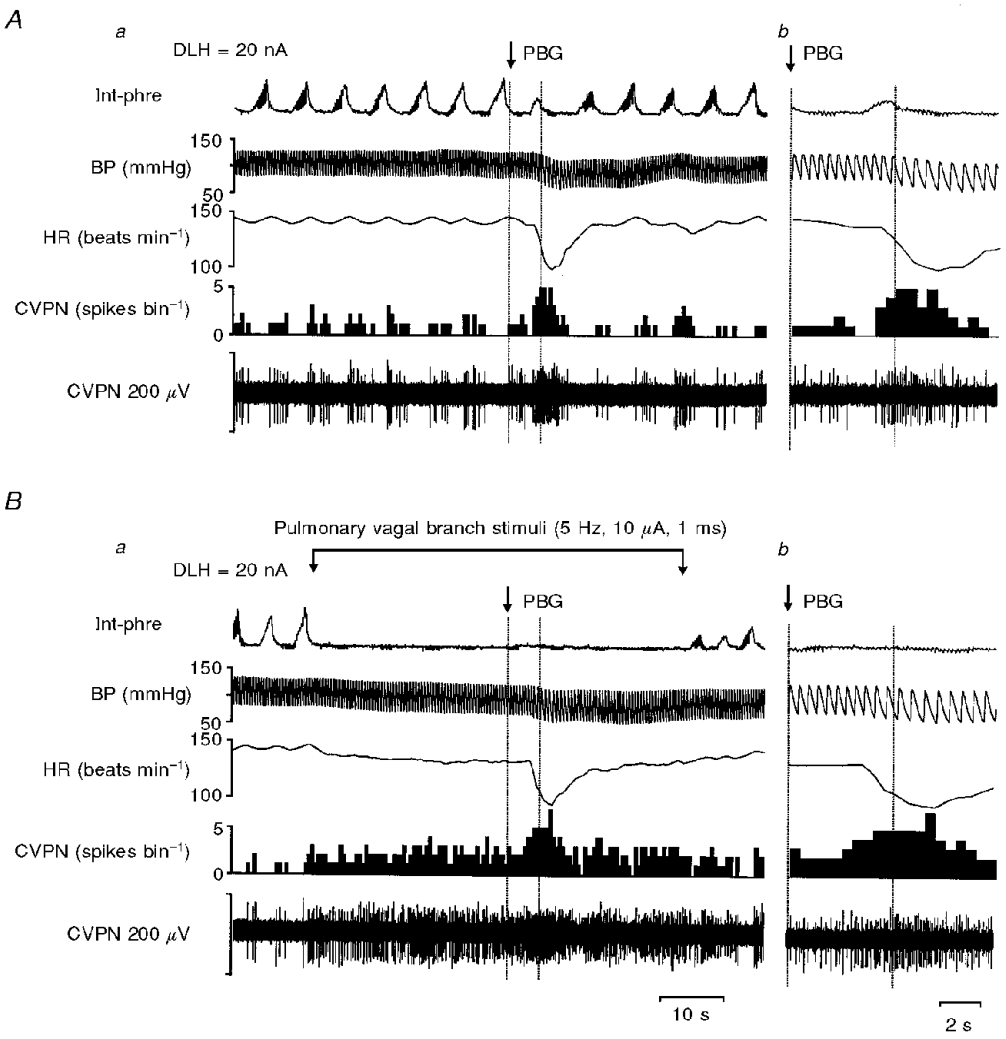


**Figure 4. Traces comparing the effect of right atrial injections (at the point marked by arrow) of PBG (20 $\mu$g kg$^{-1}$) on a CVPN with a B-fibre axon in the presence (*A*) and absence (*B*) of central respiratory drive**

Anaesthetized cat pretreated with atenolol (1 mg kg$^{-1}$, I.V.). Records from top downwards: Int-phre, integrated phrenic nerve activity; BP, arterial blood pressure (mmHg); HR, heart rate (beats min$^{-1}$) and CVPN rate histogram (0·5 s bin) and on-going activity. The 5 s window following the PBG injection (see Methods) is shown by the two vertical dotted lines. *Ab* and *Bb* are expanded traces after the PBG injections shown in *Aa* and *Ba*. Note in *B*: (1) low intensity electrical stimulation of the pulmonary vagal branch inhibits central respiratory drive, as indicated by the lack of phrenic nerve activity, and (2) the increase in neuronal activity during this stimulation is due to the inhibition of central respiratory drive.

within the 1st second of the burst from $4 \pm 1$ to $10 \pm 1$ ($P < 0{\cdot}01$, $n = 8$; Fig. 4*A*). The firing frequency in the 1st second in the one remaining neurone was not affected by PBG, although the burst discharge was increased and prolonged (data not illustrated). In all nine neurones the phrenic nerve activity was attenuated (Fig. 4*A*).

In one neurone, low intensity electrical stimulation of a pulmonary vagal branch (10 $\mu$A), was used to inhibit phrenic nerve activity. The firing pattern of this neurone changed from respiratory related bursts to a continuous firing pattern (Fig. 4*B*). Under these conditions PBG still caused excitation (Fig. 4*B*). Similarly, in a second neurone, PBG still evoked excitation of the cardiac vagal preganglionic neurone when phrenic nerve activity was inhibited by hyperventilation (data not shown).

### Effect of electrical stimulation of cardiac and pulmonary vagal afferents on B-fibre cardiac vagal preganglionic neurones

Electrical stimulation of either thoracic cardiac or pulmonary vagal branches evoked an excitatory synaptic input in six out of the eight cardiac vagal preganglionic neurones activated by phenylbiguanide (Fig. 5*A* and *B*). Among these six neurones, three received inputs from both cardiac and pulmonary branches, one received an input from only the pulmonary branch and the other two received an input only from the cardiac vagal branch. These orthodromic vagal afferent synaptic inputs had longer onset latencies than the antidromic responses, i.e. onset latencies were between 125 and 230 ms for orthodromic excitation *versus* between 11 and 21 ms for the antidromic responses. There was only one exception, where activation of pulmonary vagal afferents evoked both a short (18 ms) and a long (180 ms) latency excitatory input (Fig. 5*A*).

## DISCUSSION

### Characteristics of cardiac vagal preganglionic neurones

In the present experiments recordings have been made from neurones located in or ventrolateral to the nucleus ambiguus that were antidromically activated following stimulation of one of the vagal cardiac branches. Their activity, either spontaneous or DLH evoked was primarily in the post-inspiratory and/or stage 2 expiratory phases of the respiratory cycle and was positively correlated to the arterial blood pressure wave. Thus these neurones have the same characteristics as those that have been recorded in this area previously (McAllen & Spyer, 1976, 1978*a*,*b*; Gilbey *et al.* 1984) and can be classified as cardiac vagal preganglionic neurones. However, the precise cardiac function of these neurones has not been determined directly. Although ionophoretic application of high currents of DLH at six of these recording sites caused a bradycardia, an atrial inotropic, dromotropic or coronary vasomotor function cannot be ruled out. As these animals were pretreated with the $\beta$-adrenoceptor antagonist atenolol this bradycardia can be attributed to an increase in vagal tone. Interestingly, in two neurones the changes in activity caused by DLH paralleled the accompanying changes in heart rate confirming a similar observation made by McAllen & Spyer (1978*a*).

### Responses to stimulation of pulmonary C-fibre afferents

Nine of these eleven B-fibre cardiac vagal preganglionic neurones were activated by PBG injected into the right atrium indicating that they receive a synaptic input from cardiopulmonary C-fibres. It should be emphasized that right atrial injections of PBG, as well as activating

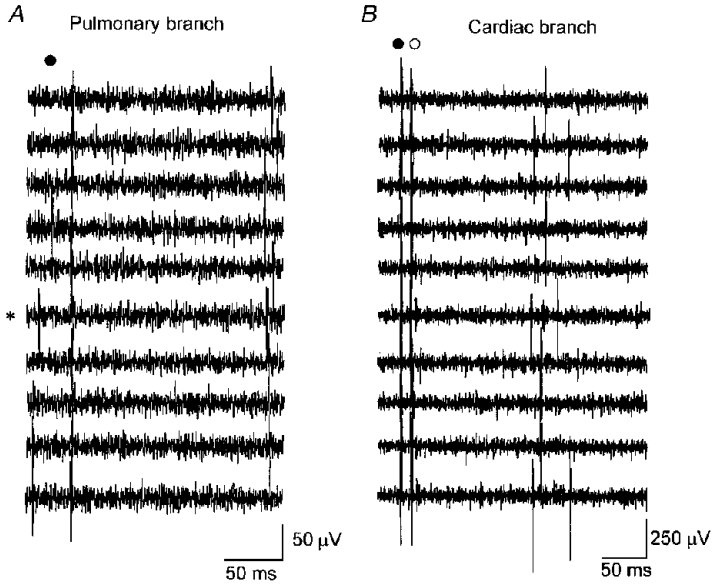


**Figure 5. Synaptic input from vagal branches to two different CVPNs with B-fibre axons**

Two sets of traces containing ten consecutive sweeps each showing the effect of stimulation (●) of the pulmonary branch (200 $\mu$A, 1 Hz, 1 ms) (*A*), which evoked both a short and a long latency excitatory input (Note: in the 6th trace (*) the spontaneous spike did not cancel the short latency input from the pulmonary nerve), and the cardiac branch (150 $\mu$A, 0·5 Hz, 1 ms) (*B*), which evoked a short latency antidromic spike (○) and a long latency synaptic input.

pulmonary C-fibres, may also activate afferents in the systemic circulation (see Coleridge & Coleridge, 1979). In the cat it has been demonstrated (Daly & Kirkman, 1988) that the onset latency, depending on the level of the cardiac output, for right atrial injections of PBG to evoke a bradycardia due to pulmonary C-fibre afferent stimulation alone ranges between 2 and 5 s. Thus, using a 5 s window for analysis in order to exclude any systemic effects of the PBG (see Methods), right atrial injection of this agent evoked an increase in activity in eight of these neurones, which could be considered to be due to pulmonary C-fibre activation alone. In addition, activity in six of these eight neurones was also elicited by orthodromic electrical stimulation of the cardiac and pulmonary vagal branches of the vagus, which would also indicate that they received an input from C-fibre afferents running in these branches. The failure to see excitation evoked by electrical stimulation in the other two neurones may be due to sampling, that is the afferents innervating these neurones may be running in cardiac and pulmonary branches other than those being stimulated.

As right atrial injections of PBG inhibited inspiration, the excitation of cardiac vagal preganglionic neurones by PBG could, at least in part, be an indirect disinhibition resulting from inhibition of central respiratory drive. However, in the present experiments activation of pulmonary C-fibre afferents still excited cardiac vagal preganglionic neurones during periods of central apnoea, as indicated by the disappearance of phrenic nerve activity, suggesting that the excitation is independent of the inhibition of central respiratory drive. In view of the finding that background activity of these neurones is respiratory modulated, i.e. the activity is reduced during the phase of inspiration, it might be anticipated that the PBG evoked activity in these neurones would also be respiratory modulated. However, in the present experiments this could not be established due to the variability in the size and occurrence of phrenic nerve activity during PBG-evoked excitation of the neurone within the 5 s window, and the short duration of the evoked excitation.

### Pulmonary C-fibre activation and cardio-respiratory integration

The present results demonstrate that stimulation of pulmonary C-fibre afferents activates respiratory modulated cardiac vagal preganglionic neurones with B-fibre axons and that, at least part of this excitation, was not due to inhibition of central respiratory drive by these afferents. Thus this study, taken together with that of Jones *et al.* (1998) indicates that stimulation of pulmonary C-fibre endings with PBG activates simultaneously two groups of cardiac vagal preganglionic neurones: those with B-fibre axons located within or in the vicinity of the nucleus ambiguus and those with C-fibre axons located in the dorsal vagal nucleus. The question arises: how is it that the reflex vagal bradycardia resulting from stimulation of pulmonary C-fibres is not respiratory modulated (Daly & Kirkman, 1988, 1989; Daly 1991)? Since activity in cardiac vagal preganglionic neurones with B-fibre axons is known to be respiratory modulated (see Introduction; present experiments), it would be expected that pulmonary C-fibre evoked increases in activity would also be respiratory modulated, as are those evoked by baroreceptor and chemoreceptor inputs. However, since this was not tested in the present experiments, the excitation of the cardiac vagal preganglionic neurones may or may not be respiratory modulated. It is possible that respiratory modulation might be prevented if, in addition to exciting cardiac vagal preganglionic neurones, the pulmonary C-fibre afferents also prevented their respiratory modulation by inhibiting synaptic transmission of the inspiratory input to these neurones. However, it is unlikely that the high firing rate evoked in cardiac vagal preganglionic neurones by pulmonary C-fibre stimulation are overriding the respiratory modulation, since neurones activated with DLH to a similar level of activity as that caused by pulmonary C-fibre stimulation still showed respiratory modulation (compare Fig. 3*C* with Fig. 4*Ab*). A final possibility is that an interaction between activity in non-respiratory modulated cardiac vagal preganglionic neurones with C-fibre axons and that in respiratory modulated cardiac vagal preganglionic neurones with B-fibre axons occurs at the level of the cardiac ganglia and/or postganglionic nerve endings in the sino-atrial node (Jones, 1993; Jones *et al.* 1998). Clearly, this study demonstrates that respiratory modulated cardiac vagal preganglionic neurones with B-fibre projecting axons are excited by the activation of pulmonary C-fibre afferents. However, the precise mechanism by which the evoked bradycardia escapes respiratory modulation remains to be determined.

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**Acknowledgements**

The work is supported by the Wellcome Trust (Grant 050894/Z) and a Health Research Board (Ireland)/British Council travel grant. R.D.J is a British Heart Foundation PhD Student. We are also grateful for the technical assistance of Mr S. Wilkinson.

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