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# Pattern of Cardiorespiratory Afferent Convergence to Solitary Tract Neurons Driven by Pulmonary Vagal C-Fiber Stimulation in the Mouse

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**Paton, Julian F. R.** Pattern of cardiorespiratory afferent convergence to solitary tract neurons driven by pulmonary vagal C-fiber stimulation in the mouse. *J. Neurophysiol.* 79: 2365–2373, 1998. The central integration of signals from pulmonary vagal C-fibers (or type-J receptors) with those arising from cardiac, peripheral chemoreceptor, and baroreceptor afferents to neurons within the nucleus of the solitary tract (NTS) was studied in an arterially perfused working heart–brain stem preparation of adult mouse. Pulmonary vagal C-fibers were excited by right atrial injection of phenylbiguanide (PBG) while cardiac receptors were stimulated by left ventricular injection of veratridine ( $1\text{--}3\ \mu\text{g}/\text{kg}$ ) or mechanically by distension of the left ventricle ( $20\text{--}50\ \mu\text{l}$  perfusate) using an indwelling cannula. Carotid body chemoreceptors were activated by aortic injection of Na cyanide, whereas baroreceptors were stimulated by increasing arterial perfusion pressure. Stimulation of pulmonary C-fibers and cardiac, chemo-, and baroreceptors all produced a reflex bradycardia ( $23\text{--}133\ \text{bpm}$ ). Central respiratory activity, as recorded from the phrenic nerve, was depressed by stimulating pulmonary C-fibers and cardiac and baroreceptors but enhanced in amplitude and frequency during chemoreceptor stimulation. Twenty-seven NTS neurons were excited and three were inhibited after pulmonary C-fiber stimulation displaying decrementing discharges with a peak firing frequency of up to  $42\ \text{Hz}$  ( $15 \pm 2.2\ \text{Hz}$ , mean  $\pm$  SE) that lasted for  $8.8 \pm 0.9\ \text{s}$ . These responses occurred  $<1\ \text{s}$  from the end of the PBG injection that was within the pulmonary circulation time. None of these cells responded to increases in right atrial pressure. All cells excited by PBG were also driven synaptically after electrical stimulation of the ipsilateral cervical vagus nerve at a latency of  $32.9 \pm 3.2\ \text{ms}$  (range  $20\text{--}62\ \text{ms}$ ). None of these neurons had ongoing activity related to central respiratory activity. Convergence from cardiorespiratory afferents to 21 neurons driven by pulmonary C-fibers was tested. Twenty-five percent of cells were selectively excited by chemical stimulation of cardiac receptors alone, 19% were driven by peripheral chemoreceptors, and 38% responded to both cardiac and chemoreceptor activation. In contrast, only 13% of the cells activated by PBG injection responded to stimulation of baroreceptors and only 6% to cardiac mechanoreceptor stimulation. None of these neurons were activated by increasing right atrial pressure. The data indicate a high proportion of afferent convergence from pulmonary C-fibers, cardiac receptors, and peripheral chemoreceptors in the NTS. However, these neurons appear not to integrate inputs from cardiovascular mechanoreceptors. The significance of the data is discussed in relation to pathological disease states such as pulmonary congestion and cardiac failure.

## INTRODUCTION

Recently the notion of common afferent modality convergence within the nucleus of the solitary tract (NTS) was

proposed on the basis of cardiac receptor inputs in the mouse (Paton 1998). This was portrayed by the finding that NTS neurons responding to chemical stimulation of the left ventricle were also excited by peripheral chemoreceptors but not baroreceptors, whereas mechanosensitive vagal receptors within the left heart converged on neurons driven by baroreceptors but not chemoreceptors (Paton 1998). Because mechanoventricular- and baroreceptors are responsive to changes in pressure, whereas chemically sensitive cardiac receptors and peripheral chemoreceptors are stimulated during ischemia or hypoxia, an organization based of sensory modality was postulated. This functional segregation of inputs was also found between mechano- and nonmechanosensitive laryngeal receptor inputs that converged onto NTS neurons excited by baro- and chemoreceptor stimulation, respectively (Dawid-Milner et al. 1995). The present study extends these previous observations and considers the pattern of cardiorespiratory afferents convergence onto NTS neurons activated by pulmonary vagal C-fibers (PCF; i.e., J receptors) (Paintal 1955, 1969).

The pulmonary chemoreflex can be evoked by stimulation of PCF. Physiological stimuli of PCF include hyperinflation of the lungs and pulmonary edema (Coleridge and Coleridge 1984, 1994; Paintal 1955, 1969) but foreign chemicals such as capsaicin (dog: Coleridge et al. 1965) and a serotonin ( $5\text{-HT}_3$ ) agonist (phenylbiguanide) were used experimentally (cat: Paintal 1955; rat: Butcher and Paton 1998; Wilson et al. 1996; mouse: Paton 1997a; Paton and Butcher 1998). Stimulation of PCF evokes a characteristic and potent pattern of reflex cardiorespiratory response including a pronounced bradycardia, hypotension, and apnea in the dog (Coleridge et al. 1965), cat (Daly 1991; Paintal 1955), rabbit (Jones and Jordan 1993), rat (Butcher and Paton 1998; Wilson et al. 1996) and mouse (Eglen et al. 1994; Paton 1997a,b; Paton and Butcher 1998). In addition, bronchoconstriction, mucus secretion, and an inhibition of somatic motor tone are also produced (e.g., Coleridge and Coleridge 1984, 1994; Ginzler and Eldred 1977). Despite the extensive literature on afferent activation, there is limited information (see Wilson et al. 1996) describing the response of NTS neurons receiving synaptic inputs from PCF.

There is both neurophysiological (for review see Kubin and Davies 1995) and neuroanatomic evidence (Kalia and Mesulam 1980) that pulmonary vagal afferents terminate within regions of the NTS coinciding with area postrema and extend into the commissural subnucleus. Functionally,

blockade of synaptic transmission within these NTS regions abolished the pulmonary chemoreflex evoked by right atrial injection of phenylbiguanide in the rat (Bonham and Joad 1991). However, these NTS regions are also important for mediating reflexes originating from cardiac receptors (Kalia and Mesulam 1980; Paton 1998) and peripheral chemoreceptors (Chitravanshi and Sapru 1995; Mifflin 1992), which raises the question concerning the degree of convergence of these cardiorespiratory afferents with pulmonary C-fibers. Although NTS neurons were shown to receive convergent synaptic inputs after pulmonary C-fiber activation and electrical stimulation of the cardiac branch of the vagus nerve in the rat (Jones et al. 1995), the origin of the cardiac vagal receptor was not characterized.

The present data reveal that NTS neurons integrating information from PCF receive a predominance of converging inputs from chemically sensitive cardiac receptors and peripheral chemoreceptors.

A preliminary report of this study was communicated to the British Physiological Society (Paton 1997b).

## METHODS

For a full description of the working heart–brain stem preparation (WHBP) see Paton (1996). Here only a brief description of the preparation is given.

### *Surgical procedures and monitoring of cardiorespiratory variables*

Mice (strain MF1; 3–6 wk) were anesthetized deeply with either ether or halothane. Once the animal failed to respond to a noxious pinch of a paw or the tail, it was bisected subdiaphragmatically and its upper body placed in ice-chilled artificial cerebrospinal fluid (aCSF) gassed with 95% O<sub>2</sub>–5% CO<sub>2</sub> (carbogen). Mice were decerebrated at the precollicular level using aspiration through a parietal craniotomy. The preparation was skinned before transferring to a recording chamber. The descending aorta was cannulated (0.8–1.0 mm OD) and perfused at constant flow (18–22 ml/min) with carbogen gassed solution (see text below; Fig. 1) with the use of a roller pump. The perfusate was warmed to 31°C, filtered (40- $\mu$ m pore size; Millipore), and passed through two bubble traps to remove gas bubbles and dampen pulsations originating from both the pump and heart. Perfusion pressure was monitored close to the tip of the perfusion cannula. In all experiments two cannulas (0.63 mm OD) were placed into the right atrium via the inferior vena cava to record pressure and/or to inject drugs. Left ventricular pressure was recorded transmurally via a stainless steel cannula (25-gauge hypodermic needle) placed through the apex of the heart. This cannula was fitted with a side arm through which perfusate was injected to raise left ventricular pressure or cardiac receptor stimulants were injected. Perfusion pressure was set between 85–95 mmHg by adjusting flow rate, which gave a right atrial pressure of between 5–8 mmHg and a left ventricular pressure of 80–90 mmHg. These pressure recordings ensured that perfusion of the pulmonary circulation was within a physiological range and gave an index of cardiac performance, respectively. Pressure signals were transduced (Gould Statham), amplified, and displayed (Gould TA11). The electrocardiographic (ECG) and phrenic nerve activity were recorded via glass suction electrodes; in some experiments the recurrent laryngeal nerve was recorded also. The phrenic motor pattern was used to gauge the adequacy of oxygenation of the brain stem and the viability of the preparation. Both the ECG and phrenic nerve discharges were amplified and filtered (NL 104 and 125 Neurolog mod-

ules). Heart rate was derived from the R wave of the ECG by using an instantaneous rate meter or computer and Spike 2 software (CED).

### *Stimulation of cardiorespiratory reflexes*

**BARORECEPTOR REFLEX.** Baroreceptors were stimulated by transient increases in perfusion pressure (30–65 mmHg above control). Before its entrance into the descending aorta, the perfusion cannula bifurcated with a branch to the descending aorta and another to a bypass circuit. Flow in the bypass line was controlled by an adjustable resistor. By increasing resistance to flow on the bypass circuit the perfusion pressure was increased. Alternatively, increasing the flow rate of the perfusion pump also produced increases in perfusion pressure.

**PERIPHERAL CHEMORECEPTOR REFLEX.** Na cyanide (0.05%; 50–100  $\mu$ l) was injected into the descending aorta via a side arm port of the perfusion cannula (Fig. 1) to stimulate carotid body chemoreceptors because there are no aortic bodies in the mouse (Hollinshead 1941). Because arterial perfusion is retrograde in the descending aorta in the WHBP (see Fig. 1), Na cyanide delivered into the perfusate was carried in the arterial circulation to the carotid body chemoreceptors.

**PULMONARY C-FIBER REFLEX** ( $n = 23$ ). It should be emphasized that there is a venous return in the working heart–brain stem preparation via the superior vena cava and this produced a normal pulsatile right atrial pressure (mean: 4–8 mmHg), which perfused the pulmonary circulation and filled the left ventricle (Paton 1996). Right atrial injection of phenylbiguanide (10–40  $\mu$ g/kg dissolved in 10–25  $\mu$ l) was used to activate pulmonary C-fibers (Fig. 1). A positive neuronal response was only taken if the firing of an NTS neuron occurred within 1–1.5 s from the start of the injection and therefore within the pulmonary circulation time (Milnor 1982). Because the pressure changes in the right atrium can also activate NTS cells (Hines et al. 1994), a control injection of perfusate was used to raise pressure to levels produced during phenylbiguanide (PBG) administration. This was necessary to delineate between right atrial stretch and PCF-driven NTS cells. During recording of some NTS neurons ( $n = 4$ ) responsive to PCF stimulation, a similar dose of PBG was injected into the aorta to act as a control.

**CARDIAC RECEPTOR REFLEX** ( $n = 25$ ). Cardiac receptors were stimulated chemically by injection of veratridine (0.5–3  $\mu$ g/kg over a 1- to 2-s period) directly into the left ventricle via an indwelling cannula (Fig. 1). In addition, the left ventricle was distended to activate mechanoreceptors within the left heart by using bolus injections of perfusate (50–250  $\mu$ l). Stimulation of both left cardiac receptors (and pulmonary C-fibers) was only repeated after a 7- to 10-min interval to prevent tachyphylaxis (Daly 1986).

### *Recording central neuronal activity and electrical stimulation of vagal afferents*

NTS neurons were recorded extracellularly using glass microelectrodes filled with 3 M NaCl (0.9–8 M $\Omega$ ) or 1 M Na acetate with Pontamine sky blue (2%) to mark recording sites ionophoretically (–1 to –5  $\mu$ A; 5 min). Signals were amplified (Neurolog 104), filtered (8 Hz to 3 kHz; Neurolog 125), and displayed on an oscilloscope and/or computer monitor. Recording electrodes were placed into the NTS under visual guidance by using a binocular microscope and driven into the tissue with a stepping motor (1- to 2- $\mu$ m steps). Surface landmarks of the dorsal medulla (e.g., midline and area postrema) were used for orientation. Microelectrodes were driven into the dorsal medulla at an angle  $\sim$ 60° to rostro-caudal sites corresponding to area postrema and 1- to 1.5-

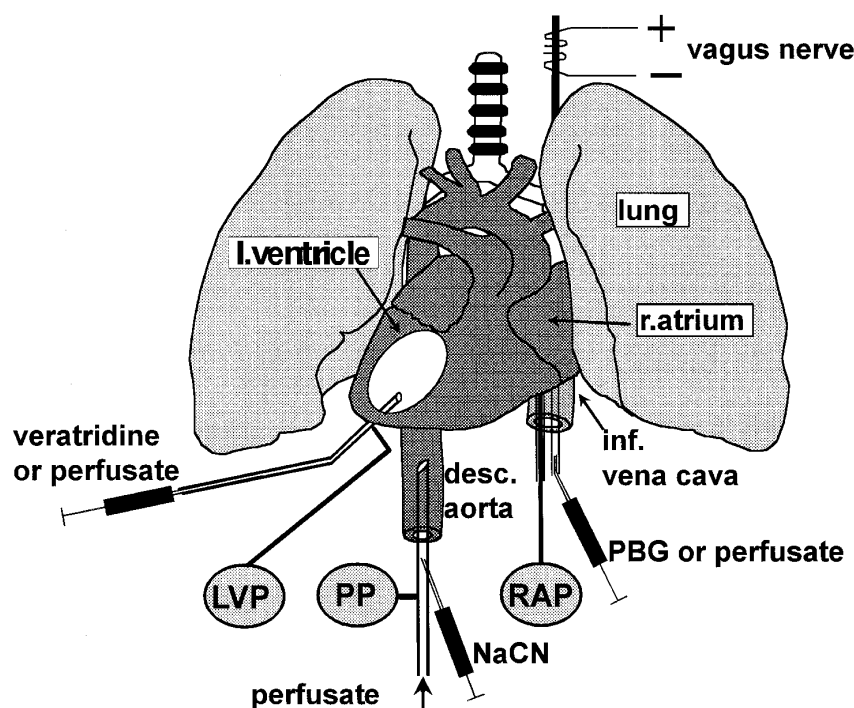


FIG. 1. Schematic to illustrate methods of stimulation of cardiorespiratory receptors in working heart-brain stem preparation (WHBP) of the mouse. Perfusate is pumped with a peristaltic pump via a heat exchanger (31°C), bubble traps, and filters into the descending aorta (see Paton 1996). There is a venous return via the superior vena cava and the pulmonary circulation is perfused by the working heart. Right atrial pressure and left ventricular pressures are within physiological range (Paton 1996). The perfusate is returned via a drain in the preparation chamber to the reservoir for regassing (95% O<sub>2</sub>-5% CO<sub>2</sub>) and recirculating. Baroreceptors were activated by elevating perfusion pressure (PP). Na cyanide (NaCN) was injected into the perfusion cannula to activate carotid body chemoreceptors, whereas pulmonary C-fibers were stimulated by injection of phenylbiguanide (PBG) into the right atrium. As a control against coactivation of right atrial stretch receptors, perfusate was injected into the right atrium to increase pressure to levels induced by PBG injections (see Fig. 3). Cardiac receptors were excited by intraventricular injection of veratridine and/or distension of the left ventricle by using perfusate. Left ventricular pressure (LVP) was monitored via a side arm of the transmyocardial cannula, whereas right atrial pressure (RAP) was measured via a 2nd cannula placed into the right atrium via inferior vena cava. Thoracic region of WHBP is shown only for clarity and is of a posterior view of the heart and lungs.

mm caudal to it. In all experiments the ipsilateral vagus nerve was isolated, insulated in low-melting point wax, and electrically stimulated (0.1–0.5 ms; 1–0.5 Hz; 2–15 V) by using a pulse generator (Neurodata 4000) and an isolated stimulator (Digitimer DS2A).

### Histological procedures

Recording sites were marked by either breaking off the tip of the microelectrode in the medulla or iontophoretically depositing Pontamine sky blue (–1 to –5  $\mu$ A; 5 min). The brain stem was removed and fixed in 2% paraformaldehyde overnight and then placed into 2% paraformaldehyde with 20% sucrose for >12 h. Tissue was sectioned transversely (50  $\mu$ m), stained with neutral red, and recording sites documented with a microscope fitted with a camera lucida.

### Analysis

All recorded variables were digitized (Instrutech VR100B; sampling rate 26 kHz) and stored on VCR tape (Panasonic) for off-line analysis. Neuronal firing frequency and response durations of single units were quantified either on- or off-line using Spike2 CED software. Poststimulus time histograms were constructed for synaptically evoked spikes after vagus nerve stimulation. Phrenic triggered NTS activity was compiled to assess any central respiratory modulation of ongoing activity over 8–10 respiratory cycles. All data are expressed as means  $\pm$  SE; a Student's *t*-test was used to test statistical significance using paired data.

### Solutions and drugs

The constituents of the aCSF were as follows (in mM): 10 dextrose, 125 NaCl, 24 NaHCO<sub>3</sub>, 5 KCl, 2.5 CaCl<sub>2</sub>, 1.25 MgSO<sub>4</sub>, and 1.25 KH<sub>2</sub>PO<sub>4</sub>. The perfusate consisted of the artificial cerebrospinal fluid + 2.0–2.2% dextran (average molecular weight 260K), an antibiotic cocktail containing penicillin (50 U/l), streptomycin (0.05 mg/l), neomycin (0.1 mg/l; Sigma) and, in some preparations, vecuronium bromide (0.04  $\mu$ g/ml; Organon Teknika)

to block neuromuscular transmission. At this dose vecuronium had a minimal effect on cardiac vagal motor transmission. Perfusate osmolarity was  $298 \pm 5$  mosM/kg H<sub>2</sub>O and on gassing with carbogen the pH was  $7.35 \pm 0.05$ . Na cyanide (1–10  $\mu$ g), phenylbiguanide (10–40  $\mu$ g/kg), and veratridine (1–3  $\mu$ g/kg) were warmed to preparation temperature before administration. Unless stated otherwise, all drugs were from Sigma.

## RESULTS

### Baseline cardiorespiratory variables

In 24 preparations basal heart rate was  $361 \pm 5$  bpm (at 31°C) and perfusion pressure was  $97 \pm 5$  mmHg. Right atrial pressure was pulsatile between 4–8 mmHg. Phrenic nerve activity comprised an incrementing inspiratory discharge of  $690 \pm 45$  ms in duration. The respiratory cycle length was  $3.1 \pm 0.2$  s ( $19.4 \pm 2.1$  cycles/min) with an inspiratory ( $T_i$ ) to total respiratory cycle time ( $T_{tot}$ ) ratio of 22%.

### Cardiopulmonary reflexes

**PULMONARY CHEMOREFLEX.** Right atrial injection of PBG elicited a bradycardia of  $130 \pm 7$  bpm from control and a significant slowing of central respiratory rate, i.e. an increase in the interphrenic discharge interval from  $3.1 \pm 0.2$  s to  $5.2 \pm 0.4$  s (Figs. 2, 4, and 6;  $P < 0.05$ ;  $n = 24$ ). These data are based on four respiratory cycles before injection of PBG and three trials per preparation. The increase in phrenic nerve cycle length was accompanied by an increase in the postinspiratory activity (amplitude and duration) recorded in the recurrent laryngeal nerve ( $n = 3$ ; Fig. 2). The onset of these responses was <1.5 s. Injection of an identical dose of PBG (1–3  $\mu$ g) into the arterial circulation (via the perfusion cannula) failed to affect heart rate or phrenic nerve activity in all preparations ( $n = 6$ ), ruling out the possible

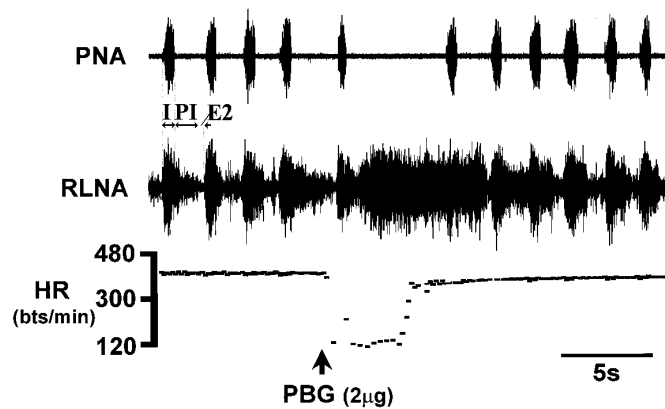


FIG. 2. Representative example of phrenic nerve activity (PNA), recurrent laryngeal nerve activity (RLNA), and bradycardiac response to stimulation of pulmonary C-fibers with phenylbiguanide in the WHBP of mouse. All 3 respiratory phases are clearly seen in the RLNA and consist of inspiration (I; also seen in the phrenic neurogram), postinspiration (PI), and stage 2 expiration (E2). Within 0.5 s of injection of PBG there was increase in cycle length of phrenic nerve activity. This was associated with an increase in duration and amplitude of postinspiratory activity as revealed in the RLNA. There was also a pronounced bradycardia that could be blocked with atropine (not shown). Notice enhanced sinus arrhythmia after recovery of heart rate indicative of an increased excitability of cardiac vagal motoneurons.

coactivation of receptors located within the coronary arteries/arterial circulation. However, doses  $>8 \mu\text{g}$  produced increases in phrenic nerve activity and variable changes in heart rate and were attributed to nonspecific action. In addition, increasing right atrial pressure to levels recorded during PBG injections failed to produce reflex changes in heart rate or phrenic nerve activity.

**CARDIAC RECEPTOR REFLEX.** In the WHBP chemical stimulation of cardiac receptors with an intraventricular injection of veratridine evoked a reduction in heart rate of  $133 \pm 5$  bpm ( $n = 24$ ) from resting levels of  $361 \pm 5$  bpm (Figs. 5 and 7). In 11 of 25 preparations this reflex bradycardia was modulated by central inspiratory activity (see Fig. 7). In 19 of 25 preparations the cycle length of phrenic nerve discharge increased by  $1.1 \pm 0.2$  s. As a control for the specificity of chemical stimulation of cardiac receptors, veratridine was injected into the descending aorta at doses comparable to those used transmurally. However, no measurable response was observed ( $n = 5$ ). In addition, distension of the left ventricle produced a mean reflex fall in heart rate of  $80 \pm 12$  bpm in 5 of 18 preparations (Fig. 5) and phrenic nerve cycle length increased by  $1.4 \pm 0.3$  s.

#### *Firing responses of NTS neurons synaptically driven by PCF and cardiac receptor stimulation*

This study was based on 30 NTS neurons that responded synaptically to both electrical stimulation of the ipsilateral vagus nerve and to right atrial injection of PBG (i.e., PCF-receptive cells) including both excitatory ( $n = 27$ ) and inhibitory ( $n = 3$ ) responses.

**VAGUS NERVE EVOKED SYNAPTIC RESPONSES.** All NTS neurons were activated synaptically after electrical stimulation of the ipsilateral vagus nerve at a mean latency of  $32.9 \pm$

$3.2$  (range 20–62 ms,  $n = 27$ ; Fig. 3). In all but one cell multiple action potentials were evoked to a single stimulus to the vagus nerve (mean  $3.6 \pm 0.4$ ; range 1–7 spikes; Fig. 3). Eleven cells had a relatively invariant latency of 2–7 ms, whereas another 12 showed  $<8$ -ms variance. In all cases tested there was no change in neuron activity after an injection of 1–3  $\mu\text{g}$  PBG into the aorta ( $n = 4$ ).

**PULMONARY C-FIBER STIMULATION.** Most PCF-receptive NTS neurons had ongoing sporadic discharge (2–9 Hz), but there was no obvious or consistent correlation of this firing to phrenic nerve discharge under control conditions. Neurons stimulated by right atrial injection of PBG exhibited either augmenting–decrementing or decrementing discharges with peak firing frequencies ranging from 8–42 Hz (mean  $15 \pm 2.2$  Hz). Responses remained above baseline firing for periods of  $8.8 \pm 0.9$  s (range 3–21 s; Figs. 4–7). The latency to onset after the start of a PBG injection was within 1–1.5 s. The firing response induced by PBG occurred just before the reflex bradycardia (Figs. 4, 5, and 7). There was a similarity between the duration of both the cell response and the reflex bradycardia in some of the PCF-receptive NTS neurons. In 14 of the 30 cells studied, the neuronal firing duration was  $8.23 \pm 1.8$  s, which coincided with and was not significantly different from the reflex bradycardia, which lasted  $8.43 \pm 1.4$  s (Figs. 4, 5, and 7).

As an injection of PBG increased right atrial pressure, it was essential to delineate whether the response of NTS neurons was due to the stretch of this cardiac chamber or to PCF stimulation. In all neurons studied, increasing right atrial pressure to levels either similar to or greater than those induced during PBG injections did not produce any obvious response in all 30 PCF-driven NTS neurons (Fig. 4).

**CARDIAC RECEPTOR STIMULATION.** Intraventricular injection of veratridine excited 13 NTS neurons occurring 1–1.5 s from the start of the injection; longer latency effects were attributed to activation of receptors of noncardiac origin. Veratridine injection evoked firing patterns consisting of either decrementing or augmenting–decrementing (Figs. 5 and 7). These responses had a peak discharge rate of  $14.7 \pm 1$  Hz (range 10–39 Hz), which lasted between 2–

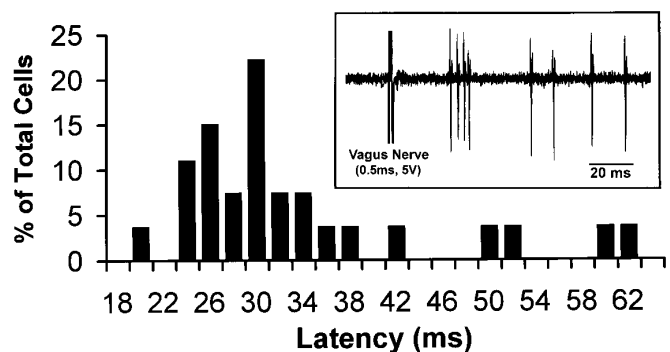


FIG. 3. Latency range of synaptically evoked excitatory inputs from the ipsilateral vagus nerve for all nucleus of the solitary tract (NTS) neurons responding to right atrial injection of phenylbiguanide ( $n = 27$ ). The mean latency of activation from the vagus nerve was  $32.9 \pm 3.2$  ms. *Insert:* vagus nerve-evoked synaptic response of NTS neuron also activated during pulmonary chemoreflex stimulation; 4 consecutive superimposed sweeps are shown. Note: it was typical that these neurons responded with multiple action potentials per stimulus.

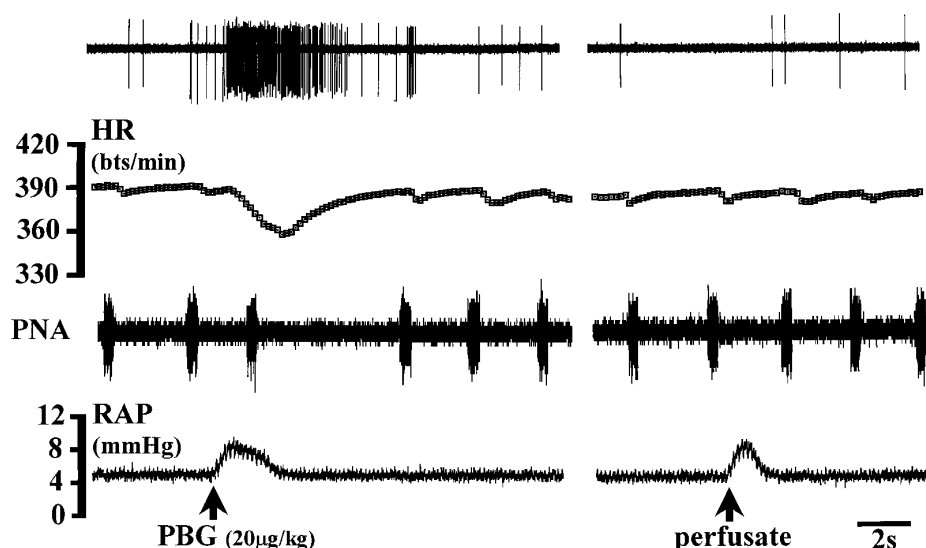


FIG. 4. Representative decrementing firing response of a pulmonary C-fiber-driven NTS neuron after a right atrial injection of PBG (*left side*). Latency of response after injection of PBG was within estimated pulmonary circulation time in the mouse (1.5 s) (Milnor 1982). Because RAP increased during injection of PBG, a control injection of perfusate was injected to raise RAP to comparable levels to ascertain the involvement of right atrial stretch receptor afferents in producing the cellular response. In all cells increasing RAP failed to activate PBG-driven NTS units. There is an ongoing sinus arrhythmia that is potentiated in magnitude after PBG administration. Note that the reflex fall in heart rate occurs just after the onset of firing and follows a similar time course to the duration of the NTS neuron's response. There is also a reflex increase in the cycle length of PNA.

40 s. As with the PCF inputs the neuronal firing response occurred before the onset of the reflex bradycardia (Figs. 5 and 7). In some cells ( $n = 4$ ) the duration of the reflex bradycardia appeared related to the time course of the NTS firing response (i.e., neuronal firing 19.1 s vs. reflex bradycardia lasting 20 s; Fig. 7). Most neurons displayed ongoing activity (26 of 30 neurons), which was typically irregular and single spiking (0.2–5 Hz), but showed no obvious correlation to the cardiac cycle. Electrical stimulation of the ipsilateral vagus nerve evoked synaptic discharge in all these neurons comprising between 1–10 spikes at a latency of  $33 \pm 2$  ms (range 20–75 ms). No measurable change in NTS unit activity occurred during nonselective injection of veratridine (1–2  $\mu$ g) into the descending aorta, indicating a relatively specific action on cardiac receptors ( $n = 3$ ).

#### Patterns of convergence to PCF-receptive NTS neurons from other cardiorespiratory receptors

With the exception of three PCF-driven NTS cells there was convergence after stimulation of other cardiorespiratory receptors.

#### Chemically sensitive cardiac receptors

Cardiac receptors were stimulated chemically by intra-left ventricular injection of veratridine. Of 21 PCF-receptive NTS units tested, 13 were excited (i.e., 62%), 2 inhibited (10%), and 6 (28%) showed no response after veratridine injection (Figs. 5–7).

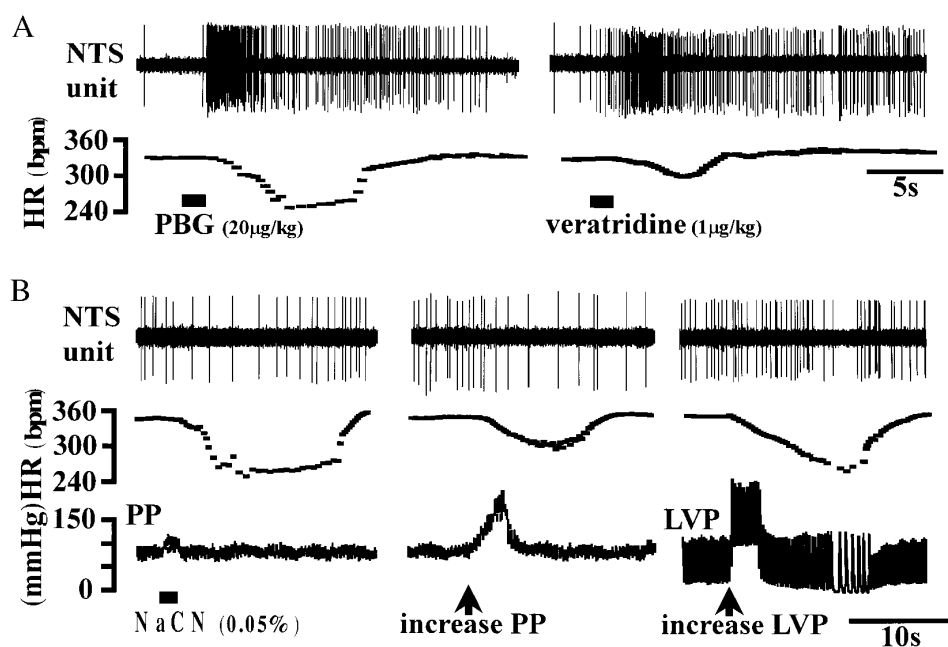


FIG. 5. *A*: example of convergence of input from pulmonary C-fibers and cardiac receptors (stimulated with veratridine) to an NTS neuron. Note decrementing patterns of discharge after both pulmonary C-fiber and cardiac receptor stimulation and the reflex bradycardia that occurred just after the neuronal response. *B*: absence of synaptic response evoked by stimulating cardiovascular mechanoreceptors (cardiac and baroreceptors *right and middle*). Increasing left ventricular pressure or perfusion pressure was without an immediate effect on this NTS neuron but both produced a reflex bradycardia. The reduced firing frequency after the increase in left ventricular pressure was delayed relative to the stimulus (and manifestation of the reflex bradycardia) and not a direct effect of stimulating cardiac mechanoreceptors. With an absence of immediate effect from stimulating cardiac mechanoreceptors, cardiac receptors stimulated with veratridine are unlikely to be mechanosensitive. This cell was not excited by stimulation of peripheral chemoreceptors using aortic injection of NaCN (30  $\mu$ l, 0.05% solution; *left*).

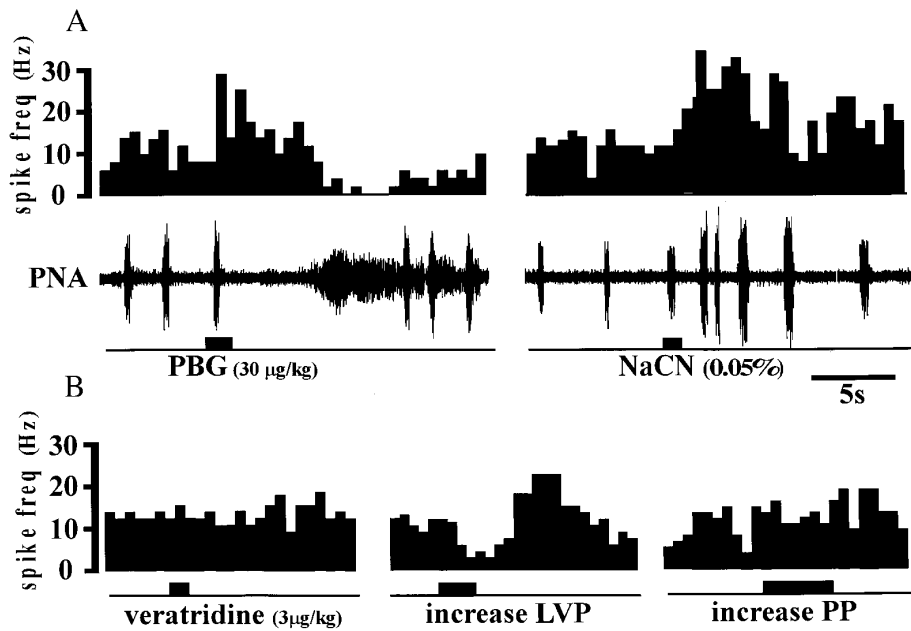


FIG. 6. *A*: convergence of afferent input from pulmonary C-fiber and peripheral chemoreceptors to an NTS neuron together with reflex changes in phrenic nerve discharge. NTS neuron firing illustrated as a rate histogram (0.5-s binwidth). Note increase in postinspiratory activity during the latter half of the apnea after PBG. *B*: no excitatory response of this NTS neuron during stimulation of both cardiac receptors, with veratridine (*left*) and by increasing left ventricular pressure (LVP), and after an increase in perfusion pressure (PP; from 85 to 175 mmHg) to stimulate baroreceptors. In this cell ongoing firing was reduced during increases in LVP to activate mechanoreceptors. Total volume of NaCN, 25  $\mu$ l.

#### Peripheral chemoreceptors

Twenty-one PCF-driven NTS neurons were tested for convergence from peripheral chemoreceptors stimulated by Na cyanide. These neurons were either excited ( $n = 12$  or 57%), inhibited ( $n = 3$  or 18%), or did not respond to peripheral chemoreceptor stimulation ( $n = 6$  or 25%; Figs. 5–7).

#### Baroreceptors

Convergence from baroreceptors was rarely found; 13 of 15 NTS neurons tested failed to respond during increases in perfusion pressure (Figs. 5–7). However, the remaining two PCF-activated NTS neurons were excited (i.e., Fig. 8).

#### Mechanically sensitive cardiac receptors

In contrast to the substantial convergence from chemically sensitive cardiac receptors, there was minimal convergence

after distension of the left ventricle to stimulate left heart mechanoreceptors. In 17 PCF-driven NTS neurons tested, 15 showed no excitatory response (Fig. 5), 1 was excited (Fig. 8), and 1 was inhibited as seen by the reduction in ongoing firing frequency.

#### Multiple convergence to PCF-receptive NTS neurons

It was of interest that 8 of the 21 PCF-driven NTS units tested received combined convergence from both chemically sensitive cardiac receptors and peripheral chemoreceptors (Figs. 7 and 8).

#### NTS neurons not activated by PCF stimulation

An additional 16 neurons failed to respond to stimulation of PCF but did respond to other cardiorespiratory afferents. These cells were characterized as receiving the following

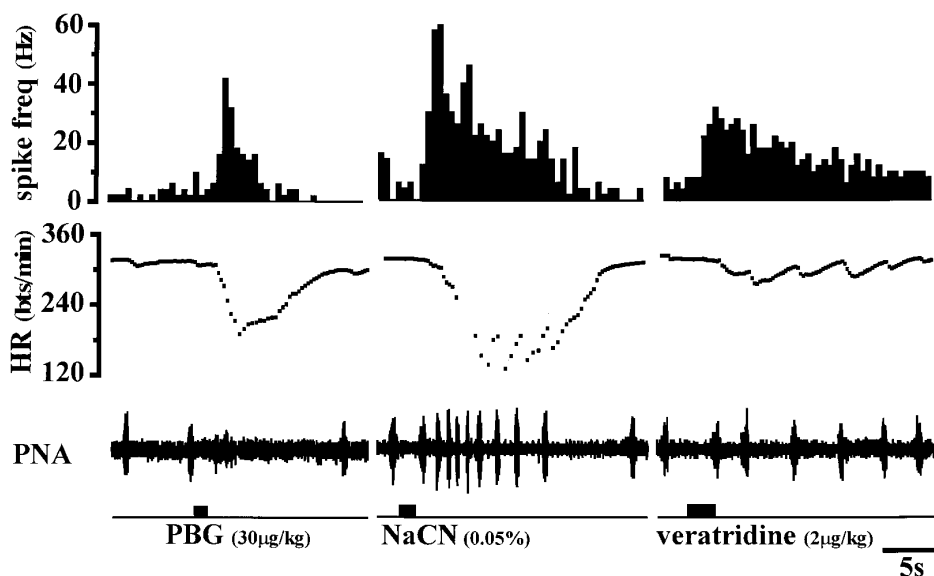


FIG. 7. Coconvergence from pulmonary C-fibers, peripheral chemoreceptors, and chemically sensitive cardiac receptors to NTS neuron. NTS neuron firing responses depicted as rate histogram (0.5-s binwidth). Firing frequency and duration correlated well with reflex changes in peak amplitude and duration of the evoked reflex bradycardia and phrenic nerve responses. This pattern of convergence was seen in 38% of cells tested. Note the augmenting–decrementing discharge patterns and ongoing sinus arrhythmia that are augmented during chemoreceptor and cardiac receptor stimulation. This cell failed to respond to right atrial distension, baroreceptor stimulation, or distension of the left ventricle (not shown).

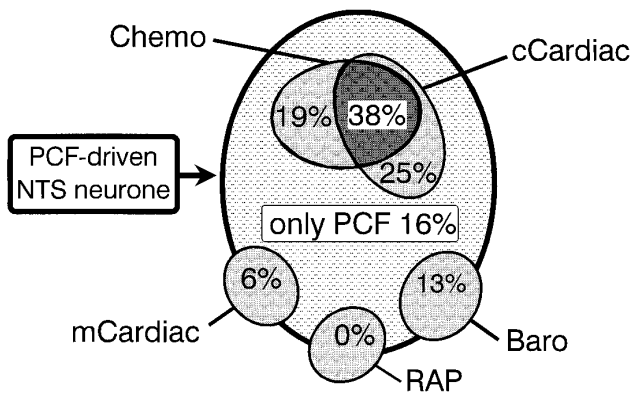


FIG. 8. Venn diagram summarizing excitatory synaptic convergence patterns from chemically sensitive cardiac receptors (cCardiac), peripheral chemoreceptors (Chemo), baroreceptors (Baro), mechanically sensitive cardiac receptors (mCardiac), and right atrial stretch receptors (RAP) to pulmonary C-fiber (PCF)-driven NTS neurons. The PCF-driven NTS neurons are depicted as the large oval structure; converging synaptic inputs are superimposed on it (lightly shaded small oval structures). The darkly shaded structure indicates coconvergence of synaptic inputs from both peripheral chemoreceptors and chemically sensitive cardiac vagal receptors (i.e., 38%). In the PCF-driven NTS neurons tested ( $n = 21$ ) the predominating finding was a convergence of either chemically sensitive cardiac receptors and/or peripheral chemoreceptor inputs. There was a notable paucity of excitatory convergence from cardiovascular mechanoreceptors to PCF-NTS neurons (i.e., mechanosensitive cardiac receptors,  $n = 1$  neuron of 17 tested; baroreceptors,  $n = 2$  neurons of 15 tested) and right atrial stretch receptors ( $n = 0$  neurons of 21 tested). Of the PCF-driven NTS neurons, 16% did not receive convergence from any of the receptors tested in this study.

inputs: cardiac chemically ( $n = 3$ ) or mechanically sensitive ( $n = 3$ ) left heart, baroreceptor ( $n = 6$ ), or peripheral chemoreceptors ( $n = 4$ ). These data indicate the relative specificity of PCF inputs to the NTS.

#### Recording sites of PCF-receptive NTS neurons

The recording sites of 15 NTS neurons driven by stimulation of PCF were recovered and found in regions of the commissural subdivision being located 0.5–1.0 mm caudal to the obex, 0.1–0.5 mm lateral to midline, and 0.1–0.5 mm below the dorsal surface (Fig. 9). These recording sites were intermingled with those reported recently for NTS neurons receiving inputs from left ventricular receptors (Paton 1998).

#### DISCUSSION

The major finding of the present study was that the majority of PCF-driven NTS neurons receive convergent inputs from chemically sensitive left cardiac receptors and peripheral chemoreceptors. There was a notable absence of convergence from mechanically sensitive cardiovascular receptors (i.e., left ventricular, baroreceptors, and right atrial stretch receptors). These data provide the first description of the response characteristics of NTS neurons after stimulation of PCF with PBG in the mouse.

#### PCF and cardiac receptor reflexes in WHBP of mice

The present evidence substantiates the presence of the pulmonary chemoreflex and cardiac receptor reflex in the mouse. Stimulation of PCF in the WHBP of mice evoked a

qualitatively identical response, including bradycardia and apnea, to the pattern reported in anesthetized mammals (mouse: Eglen et al. 1994; Paton and Butcher 1998; rat: Butcher and Paton 1998; Wilson et al. 1996; rabbit, cat, and dog: Coleridge and Coleridge 1994). On the basis of recent data, the evoked apnea is a “postinspiratory” apnea as seen by the augmentation and prolongation of this respiratory phase in recordings of recurrent laryngeal nerve (Paton 1997a). Although rapid shallow breathing was reported to follow the reflex apnea (Coleridge and Coleridge 1984, 1994; Paintal 1955, 1969), this was rarely seen in the working heart–brain stem preparation of mice (Paton 1997a; Paton and Butcher 1998), nor was it consistently evident in anesthetized mice (Butcher et al. 1998; Paton 1997a; Paton and Butcher 1998) or rats (Butcher and Paton 1998; Vardhan et al. 1993; Wilson et al. 1996). It is likely that the rapid shallow breathing component is dependent on depth of anesthesia, PBG dose, and species.

It is argued that the NTS responses after right atrial injection of PBG were evoked from receptors located within the pulmonary vascular bed. This is based on 1) a response latency within the pulmonary circulation time after a PBG injection into the right atrium and 2) the absence of a central neuronal response to aortic injections of PBG. Additionally, the characteristic triad of response (apnea, bradycardia, and depressor effect) further supports activation of PCF.

With regard to the cardiac receptors stimulated with veratridine, it is likely that these will include receptors close to the coronary arteries (Brown 1965) as well as receptors located within the myocardium (see Hainsworth 1991). It is accepted that raising pressure within the left ventricle will also increase the pressure in the left atrium. The finding that stimulation of cardiac receptors with veratridine depressed phrenic nerve activity and elicited a potent bradycardia in the WHBP is consistent with previous reports in other studies and species (Crisp et al. 1989; Daly 1986; Hainsworth 1991).

#### Characteristics of PCF-receptive NTS neurons

All PCF-driven neurons responded at relatively long latency (mean 33 ms) to electrical stimulation of the ipsilateral vagus nerve and in some cases the evoked responses were relatively invariant (i.e., 2–7 ms; 11 of 23 neurons). These findings suggest, but are not definitive of, an involvement of vagal C-fibers.

The firing pattern of NTS neurons after PCF activation included augmenting–decrementing and decrementing discharges with a mean peak frequency as high as 42 Hz lasting up to 9 s and are comparable with those described in the anesthetized rat (Wilson et al. 1996). These central firing responses are greater than those of single unit PCF vagal afferents recorded during comparable doses of PBG injected into the right atrium of other species (Coleridge and Coleridge 1984; Paintal 1955, 1969). Although this might suggest a major convergence of PCFs, the possibility is raised that the transfer function from PCF to NTS neurons is not linear but amplified. PCF inputs to NTS neurons may depend on the co-release of a neuromodulator that potentiates the synaptic response as recently reported for vagus nerve–evoked synaptic responses recorded from cardiac receptive NTS neurons (Paton 1997c).

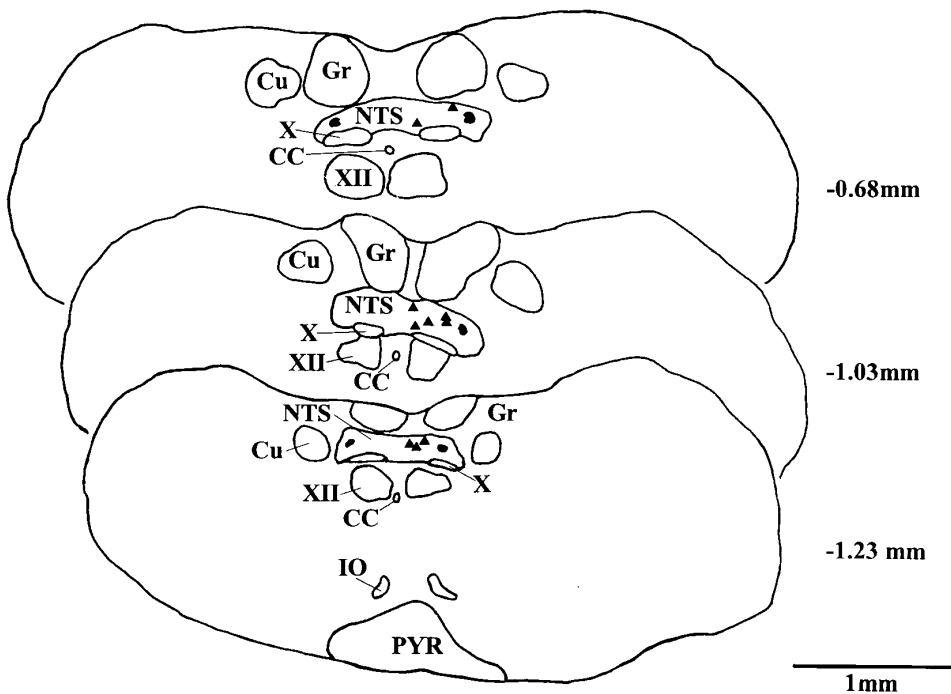


FIG. 9. Recording sites of 10 NTS neurons driven by stimulation of right atrial injection of phenylbiguanide were found in regions of the commissural subdivision of the NTS (▲). They were located 0.5–1.0 mm caudal to the obex, 0.1–0.5 mm lateral to midline, and 0.1–0.5 mm below the dorsal surface.

#### *Cardiorespiratory afferent convergence to PCF-receptive NTS neurons*

Figure 8 shows that the major source of converging afferent inputs to PCF-receptive NTS neurons was from chemically sensitive cardiac receptors and/or peripheral chemoreceptors and not from cardiovascular mechanoreceptors (baro- or cardiac receptors). Although PCF-receptive NTS neurons might receive subthreshold synaptic inputs from cardiovascular mechanoreceptors, the present extracellular data support the notion of a common afferent modality convergence in the NTS (Paton 1998) (see INTRODUCTION). This result is supported by intracellular recordings from NTS in both the cat (Silva-Carvalho et al. 1998) and mouse (unpublished observations) indicating an absence of subthreshold synaptic inputs after stimulation of cardiovascular mechanoreceptors in cardio-receptive- and PCF-driven neurons, respectively.

Peripheral chemoreceptors are known to sense changes in blood gas tension and blood flow and chemically sensitive cardiac receptors are responsive to myocardial ischemia. Unlike carotid and aortic body chemoreceptors, cardiac chemically sensitive receptors respond to chemicals such as bradykinin and prostaglandin (Armour 1994), which are released from the myocardium during ischemia (Ustinova and Schultz 1994). In contrast, the exact modality of PCF is not clear. It is known that PCFs are responsive to not only lung hyperinflation and pulmonary edema but also to increases in pulmonary arterial blood flow, carbon dioxide tension, lung irritants (ammonia, sulfur dioxide), lung inflammation, and emboli (Coleridge and Coleridge 1984). Thus PCFs appear to be multimodal. Furthermore, stimulation of PCF with PBG is an unphysiological stimulus that may be selective for only a specific population of PCF that may or may not have a distinct modality.

Under pathophysiological conditions the activity of both

PCF and chemically sensitive cardiac receptors might increase synergistically. A major stimulus to PCF is pulmonary edema that can be induced by pulmonary congestion. The latter is caused during left heart failure, a condition that can be produced by myocardial ischemia that in turn will stimulate cardiac receptors. In this condition a reflex decrease in both cardiac work and afterload may be a defensive response (Coleridge et al. 1991) to assist a failing heart. Indeed, increased vagal drive is beneficial to an ischemic heart and reduces arrhythmias and sudden cardiac death (Cerati and Schwartz 1991). Because a common component of the reflex cardiovascular response of PCF and chemically sensitive cardiac receptors includes a vagal bradycardia, the central neuronal pathways activated by both these receptors under conditions of heart failure might be similar and account for the convergence observed in the NTS. Moreover, this is upheld by the similarity of the time course of NTS neuron responses with the reflex heart rate changes (Figs. 4, 5, and 7). Convergence of both PCF and chemically sensitive cardiac afferents may be necessary to potentiate and maintain this reflex response. This is consistent with the idea that central reflex organization is based on regulatory versus defensive types (Coleridge and Coleridge 1994; Coleridge et al. 1991; Comroe 1954). The latter idea may explain the absence of any significant convergence from mechanically sensitive left ventricular, baro-, and right atrial stretch receptors to PCF-driven NTS neurons that are involved in short-term regulation.

The convergence between peripheral chemoreceptors and PCF may also represent common integration of defensive reflex inputs. It is known that the reflex respiratory response after chemoreceptor stimulation is blocked during a concomitant PCF-induced apnea (Paton 1997a); this might explain the inhibitory effects of chemoreceptor stimulation on the ongoing firing rate of a limited number of PCF-driven NTS cells recorded in this study. In the absence of central inspira-

tory drive and lung inflation, as is the case during a PCF reflex, stimulation of peripheral chemoreceptors evokes a bradycardia (Daly 1986). In conditions of pulmonary edema, an accompanying systemic hypoxia is likely and will therefore stimulate chemoreceptors. Thus during pulmonary edema PCF will reflexly reduce central inspiratory drive and block the chemoreceptor-induced respiratory excitation. Under these conditions chemoreceptor activity may act synergistically with PCF perhaps to increase cardiac vagal motor activity. Finally, although controversial (see Coleridge and Coleridge 1984), PCFs were reported to be sensitive to changes in pulmonary arterial PCO<sub>2</sub> (Delpierre et al. 1981) and, if true, then NTS convergence of PCF and peripheral chemoreceptors could also be explained on the basis of common modality (see Paton 1998).

I am most grateful to Prof. K. M. Spyer for comments on the manuscript.

This work was supported by British Heart Foundation Grant BS/93003 and Royal Society Grant 14349.

Received 1 October 1997; accepted in final form 26 January 1998.

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