Convergence Properties of Solitary Tract Neurons Responsive to Cardiac Receptor Stimulation in the Anesthetized Cat

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Silva-Carvalho, L., J.F.R. Paton, I. Rocha, G. E. Goldsmith and K. M. Spyer. Convergence properties of solitary tract neurons responsive to cardiac receptor stimulation in the anesthetized cat. J. Neurophysiol. 79: 2374 ± 2382, 1998. The convergence pattern of cardiac receptors, pulmonary C-fibers, carotid chemoreceptor, and baroreceptor afferents onto neurons within the nucleus of the solitary tract (NTS) was studied in the anesthetized (pentobarbitone sodium, 40 mg/kg,) paralyzed and artificially ventilated cat. Extra- and intracellular recordings were made from NTS neurons while stimulating both cardiac receptors by aortic root injections of veratridine (1–3 μg/kg) and pulmonary C-fibers by a right atrial injection of phenylbiguanide (10–20 μg/kg). The ipsilateral carotid body was stimulated by using arterial injection of CO2-saturated bicarbonate solution, whereas inflation of the ipsilateral carotid sinus was used to activate baroreceptors. The ipsilateral cardiac vagal branch, cervical vagus, and carotid sinus nerves were stimulated electrically (1 Hz, 0.2–1 ms, 1–35 V). In 78 NTS neurons recorded either extracellularly (n = 47) or intracellularly (n = 31), electrical stimulation of the cardiac branch of the vagus nerve evoked synaptic potentials (spikes and/or excitatory postsynaptic potentials) with an onset latency between 4 and 220 ms. Some neurons displayed both short and long latency inputs (15.5 ± 1.8 and 160.0 ± 8.5 ms; n = 14). Of these 78 neurons, 24 responded to veratridine stimulation of cardiac receptors (i.e., cardiorespiratory neurons) by exhibiting an augmenting–decteasing discharge of 37 ± 4 s in duration with a peak frequency of 30 ± 5 Hz. Convergence from other cardiorespiratory receptors was noted involving either carotid chemoreceptors (n = 7) or pulmonary C-fibers (n = 4) or from both carotid chemoreceptors and pulmonary C-fibers (n = 6). In contrast, only one cardioresceptive NTS neuron was activated by distension of the carotid sinus. Recording sites recovered were confined to the medial NTS at the level of the area postrema and extended caudally into the commissural subnucleus. Our results indicate a convergence of carotid chemoreceptor and pulmonary C-fiber afferent inputs to cardioresceptive NTS neurons. With the paucity of baroreceptor inputs to these neurons it is suggested that sensory integration within the NTS may reflect regulatory versus defensive or protective reflex control.

INTRODUCTION

Cardiac vagal receptors may play roles in cardiovascular homeostasis in both physiological and pathophysiological conditions (Armour 1994; Hainsworth 1991; Smith and Thames 1994). From peripheral afferent recordings of cardiac vagal receptors it appears that the receptors are sensitive to both chemical and/or mechanical stimulation. Cardiac vagal receptors with unmyelinated axons have been shown to be stimulated chemically by either foreign substances such as veratridine, capsaicin, nicotine, or phenylbiguanide (PBG; a serotergic subtype 3 receptor agonist) or by employing naturally occurring compounds such as adenosine, bradykinin, and prostaglandins applied topically to the myocardium or injected into either the coronary arteries or pericardial sac (Coleridge et al. 1964; Drinkhill 1993; Öberg and Thoren 1972; Sleight et al. 1969; Sleight and Widdicombe 1965). Unmyelinated cardiac afferents relaying in the vagus nerve are also excited by veratridine (see Öberg and Thoren 1972; Paintal 1955). Both adenosine and bradykinin are released during myocardial ischemia (e.g., Kaufman et al. 1980; Ustina and Schultz 1994), whereas mechanical distension of the left ventricle releases prostaglandins (Block et al. 1979). Recently it was estimated that there was a predominance of chemically sensitive cardiac vagal endings (70%) with a small multimodal contribution (i.e., mechano- and chemically sensitive; 10%) (Armour 1994).

Whether the heterogeneity in these cardiac vagal afferents has physiological significance is not clear, but the finding that stimulation of chemically sensitive cardiac vagal afferents evoked a potent reflex bradycardia that was not produced consistently during mechanical stimulation of the left heart (cf. McGregor et al. 1986 with Tutt et al. 1988) may indicate separate reflex pathways that may or may not be common with other cardiorespiratory reflexes. Because the projection target of cardiac vagal afferents overlap with the termination zones of other cardiorespiratory afferents in the nucleus of the solitary tract (NTS) (e.g., Kalia and Mesulam 1980; Kubin and Davies 1995; Loewy 1990; Mifflin 1996; Spyer 1994), we have investigated the origin of convergence to NTS neurons driven by chemical stimulation of cardiac receptors in the in vivo anesthetized cat. Our data suggest that mechanoreceptors and chemosensitive cardiorespiratory receptors converge onto different NTS neurons, consistent with previous studies in the mouse (Paton 1998).

A preliminary report of part of this study was communicated to the British Physiological Society (Silva-Carvalho et al. 1997).

METHODS

Surgical procedures and monitoring of cardiorespiratory variables

Cats (2.2–4.4 kg) of either sex were anesthetized with pentobarbitone sodium (60 mg/kg ip) and supplemented (10 mg bolus iv) as

Acknowledgments: This investigation was supported by grants from the British Medical Research Council (A28129) and the American Heart Association (Grants-in-Aid 9319028 and 9103487).
necessary by testing corneal and limb withdrawal reflexes. The trachea was intubated below the larynx. The bladder was cannulated and drained. Femoral blood vessels (artery and vein) were cannulated for monitoring of arterial pressure and for intravenous drug administration. The right atrium was cannulated via the right external jugular vein for measurement of right atrial pressure and injection of PBG (see Pulmonary C-fiber). The electrocardiogram (ECG) was measured with the use of bipolar percutaneous electrodes placed in a fore- and hindlimb. The ECG was amplified and filtered and heart rate derived with the use of an instantaneous rate meter. The right phrenic nerve was recorded with a bipolar silver wire hook electrode; signals were amplified and filtered. Rectal temperature was monitored and maintained at 37 ± 0.5°C. Animals were placed into a stereotactic head holder and spinal clamps (thoracic and lumbal) were used for support. During central recording cats were paralyzed by using either gallamine (Flaxedil 4 mg/kg iv) or vecuronium bromide (250 μg/kg iv) every 40 min and ventilated by changing minute volume and/or infusing HCO3 solution (0.5 M). A bilateral pneumothoracotomy was performed and an end-expiratory pressure of 1–2 cm water maintained. During neuromuscular blockade, anesthetic levels were assessed from recordings of arterial pressure, heart rate, and central respiratory activity.

**Electrical stimulation of afferent nerves**

The right carotid sinus nerve was stimulated electrically (0.2–0.5 ms, 1 Hz, 1–20 V). Additionally, cardiac vagal branches were identified anatomically and isolated following ligation of the azygous vein as approached from the right side (see McAllen and Spyer 1976). Fine insulated silver wires bared at their ends were wrapped around single or multiple cardiac vagal branches and insulated with dental impression compound (Reprosyl). As a physiological test these vagal branches were stimulated electrically (0.2 ms, 20 Hz, 0.5–3 V), which produced an immediate and pronounced bradycardia. In some control experiments (n = 3) pulmonary projecting vagal branches were also isolated and placed on stimulating electrodes. During central NTS recording, cardiac vagal branches were stimulated by using five times the intensity used to evoke cardiovascular responses (i.e., 2.5–15 V) at 1 Hz. The right cervical vagus nerve was also stimulated (0.1–0.5 ms; 1–0.5 Hz; 2–15 V). Stimuli were delivered with the use of a pulse generator (Digitimer) and isolated stimulators (Digitimer DS2A). All nerves were insulated in semisolid paraffin.

**Stimulation of cardiorespiratory reflexes**

**BARORECEPTOR AND PERIPHERAL CHEMORECEPTOR REFLEXES.** A balloon tipped cannula (Swann Ganz, Edwards size 4F) was placed within the right carotid sinus by retrograde cannulation of the external carotid artery. The balloon was inflated with the use of saline (0.1–0.2 ml). Stimulation of carotid body chemoreceptors was achieved by injection of CO2-saturated HCO3 solution (0.5 M, 50–200 μl) via the central lumen of the balloon-tipped catheter.

**CARDIAC RECEPTORS.** A cannula was advanced down the left common carotid artery so that its tip lay either at the root of the aortic arch or into the left ventricle. During its positioning arterial pressure was monitored. Placement of this cannula was determined by 1) the “knocking” felt through the cannula caused by the mitral valve, 2) further advancement that resulted in the cannula either entering the left ventricle or a coronary artery as seen from the pressure recording, and 3) injection of veratridine (1–3 μg/kg) to stimulate cardiac/coronary receptors and produce potent bradycardia and a depressor response. Misplacement of this cannula was clearly demonstrated by a complete absence of cardiovascular responses after an injection of veratridine. Five- to ten-minute inter-

vals were allowed between subsequent veratridine injections to prevent tachyphylaxis of cardiac receptors.

**PULMONARY C-FIBERS.** PBG was injected into the right atrium to activate pulmonary C-fibers (PCF) in the cat (10–20 μg/kg). A positive neuronal response was accepted only if the firing of the NTS neuron under study occurred within <5 s from the start of the injection and therefore within the estimated pulmonary circulation time (Daly 1991). Right atrial pressure was measured in many experiments via a second cannula. Because pressure changes in the right atrium can also activate NTS cells (Hines et al. 1994), a control injection of saline was given in most experiments to raise pressure to levels produced during PBG injection. This was necessary to delineate between right atrial stretch and PCF-driven NTS cells.

**Recording peripheral vagal afferent activity**

The time to effect of both veratridine and PBG injections on cardiac and pulmonary vagal receptors was assessed by recordings of the activity of afferent fibers taken from either the cervical vagus or the pulmonary and cardiac intrathoracic branches of the vagus. Single- and multunit discharges were recorded with either bipolar silver wire or suction electrodes. In the case of those recorded from the cervical vagus, conduction velocities were calculated from both the latency of an evoked action potential after electrical stimulation (0.2–0.5 ms; 1–5 V, 1 Hz) of either the cardiac or pulmonary branch and also the measured conduction distance between the recording and stimulating electrodes (see RESULTS).

**Recording central neuronal activity**

Intracellular current clamp recordings were made of NTS neurons with sharp microelectrodes (50–80 MΩ) filled with 3 M KCl by using an Axoclamp 2A amplifier. The pia was removed and recordings made with the use of a stabilizing foot placed on the surface of the dorsal medulla. NTS neurons were also recorded extracellularly with the use of either glass microelectrodes filled with 3 M NaCl (0.9–11 MΩ). 1 M Na acetate with Pontamine sky blue (2%) to mark a number of recording sites ionophoretically (−1 to −5 μA; 5–10 min), or tungsten steel microelectrodes (5–5.5 MΩ; World Precision Instruments). Signals were amplified (Neurolog 104), filtered (8 Hz-3 KHz; Neurolog 125), and displayed on an oscilloscope and/or computer monitor. Recording electrodes were
Peripheral vagal afferent activity

Both veratridine (into the left ventricle) and PBG (into the right atrium) excited vagal fibers in the cervical vagus; however, individual or few-fiber preparations responded to either but not to both at latencies \( \sim 1 \) s (Figs. 1 and 2). In total 49 single or few-fiber preparations were challenged with these stimuli. Four pulmonary branch fibers excited on pulmonary branch stimulation responded to PBG injections into the right atrium but not to veratridine given to the aortic root or left ventricle (Fig. 1). Three fibers or groups of fibers in the cervical vagus excited on cardiac branch stimulation responded to veratridine alone, but three further fibers in the cervical vagus responded to veratridine and also PBG but only after a considerably longer delay (i.e., \( >5 \) s; Fig. 2). This response we take as a direct effect of PBG or cardiac stimulation were quantified off-line with the use of Spike2 CED software. Rate histogram plots of firing frequency were constructed with the use of 1-s bins. Poststimulus time histograms were plotted for synaptically evoked spikes following cardiac vagal branch stimulation. All data are expressed as mean \( \pm \) SE; a Student’s \( t \)-test was used to test statistical significance by using paired data.

RESULTS

Cardiorespiratory reflex responses to cardiac and pulmonary C-fiber stimulation

In every experiment the cardiopulmonary responses that were evoked on the application of veratridine to either the aortic root or left ventricle and PBG into the right atrium were assessed. Both stimuli elicited an abrupt bradycardia (55–130 bpm) and systemic hypotension (35–65 mmHg; see Figs. 1, 2, 5, and 6). PBG produced a marked suppression of ventilation consistently (i.e., apnea with or without tachypnea), whereas there was a modest slowing and reduction in phrenic nerve amplitude with veratridine. The onset of the cardiovascular responses was between 2–3 s. These responses were analogous to those described before in the anesthetized cat (Daly 1991) and dog (Crisp et al. 1989).

Histological procedures

A proportion of recording sites were marked either by breaking off the tip of the intracellular recording electrode or by ionophoretic deposition of 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) and stained with neutral red and recording sites were documented with the use of a microscope fitted with a camera lucida.

Analysis

All recorded variables were digitized (Instrutech VR100B) and stored on VCR tape for off-line analysis. Peak neuronal firing frequency and response durations of single units responding to cardiac stimulation were quantified off-line with the use of Spike2 CED software. Rate histogram plots of firing frequency were constructed with the use of 1-s bins. Poststimulus time histograms were plotted for synaptically evoked spikes following cardiac vagal branch stimulation. All data are expressed as mean \( \pm \) SE; a Student’s \( t \)-test was used to test statistical significance by using paired data.
The majority of all neurons received a synaptic input following cardiac branch stimulation \( (n = 73) \), whereas the remainder \( (n = 5) \) were antidromically activated at latencies ranging from 7–180 ms. The latter group of neurons did not respond to injections of veratridine into the aortic root or left ventricle or to PBG and are not considered further.

**Evoked synaptic responses following cardiac vagal branch stimulation**

Stimulation of the cardiac branch of the vagus nerve evoked an excitatory response consisting of spikes (recorded extracellularly) or excitatory postsynaptic potentials (EPSPs)/spikes as seen intracellularly over a latency range of 4–220 ms \( (Figs. 3, 4, 6, \text{ and } 7; n = 59) \). (In one NTS cell recorded intracellularly an inhibitory postsynaptic potential was observed at a latency of 12 ms.) Many cells displayed multiple action potentials per stimulus (maximally 12). In 14 cells cardiac branch stimulation evoked both short- and long-latency synaptic responses (action potentials or EPSPs) of 15.5 ± 1.8 and 160.0 ± 8.5 ms, respectively (e.g., Fig. 6).

Because it is unlikely that the cardiac branch of the vagus nerve originates solely from receptors located in the heart (see Bennett et al. 1985) it was necessary to characterize physiologically NTS neurons that were synaptically driven by cardiac vagal branch stimulation by recording their responses to an injection of veratridine as a means of chemically activating cardiac receptors.

**Characterizing cardioreceptive NTS neurons**

From the 73 neurons responding to electrical stimulation of the cardiac vagal branch, 24 were also excited by veratridine injected into the aortic root or left ventricle \( (n = 18 \text{ extracellular}; n = 6 \text{ intracellular}) \). There was a latency of between 1–2 s from the start of the injection to the response \( (Figs. 4–7) \). Longer latency responses were not included in this analysis for the reasons described earlier. Thus neurons responding to both cardiac vagal branch stimulation and veratridine were termed “cardioreceptive.” The use of this
FIG. 6. An example of a cardioreceptive NTS neuron that was both synaptically driven by electrical stimulation of the ipsilateral cardiac branch, as revealed in a peristimulus time histogram (10-ms binwidth, 60 sweeps) and excited by aortic root injection of veratridine (2 μg/kg; bottom left panel). Neuron activity is displayed in a rate histogram (1-s binwidth). Neurons in this group exhibited convergent excitatory inputs from carotid chemoreceptors and pulmonary C-fibers stimulated by CO2-saturated bicarbonate solution and PBG (20 μg/kg), respectively. These neurons were not influenced by inflation of the ipsilateral carotid sinus. Reflex falls in heart rate are depicted for each of 4 stimuli.

Firing response of cardioreceptive NTS neurons following veratridine injection

The pattern of firing elicited by 1–3 μg/kg veratridine injections recorded extracellularly or intracellularly consisted of at least two components (i.e., rapidly augmenting and slow decrementing) with often an intermediate plateau phase in both extra- and intracellularly recorded neurons (Figs. 4–6). These responses lasted between 20–50 s in duration (mean 36.9 ± 4.5 s) with a peak frequency of 30.2 ± 4.9 Hz (range 17–60 Hz; Figs. 4–6). From intracellular recordings veratridine evoked a membrane depolarization of 9–11 mV (n = 6; Fig. 7), which led to action potential discharge in four neurons (Fig. 7). The firing response commenced either 0.5–2 s before or was coincident with the reflex cardiovascular changes described in the previous paragraphs (Figs. 5 and 6).

Electrically evoked synaptic responses in cardioreceptive NTS neurons

In cardioreceptive NTS neurons the latency of the synaptic input (spikes or onset of an EPSP) following stimulation of the cardiac branch of the vagus nerve was 7–182 ms (Figs. 3, 4, 6, and 7) with a maximum of 12 spikes being evoked per stimulus. In six neurons two synaptic responses were
observed, one early (30.9 ± 5 ms) and the other late (151.3 ± 7.0 ms; Fig. 6). From the six intracellular recordings, cardiac vagal branch stimulation evoked either EPSPs of 5.5 ± 0.4 mV in amplitude and 12–26 ms in duration (n = 4) or action potentials (n = 2; Fig. 7) over the stimulus intensity range used (see METHODS).

All cardioceptive neurons either discharged spikes or produced EPSPs (6.9 ± 0.5 mV; n = 6) to electrical stimulation of the cervical vagus nerve at a mean latency of 13.3 ± 1.5 ms (n = 16) and 42.4 ± 7.1 ms (n = 6; Fig. 7). On the basis of the differences between these latencies and those evoked from the cardiac vagal branch (see previous paragraphs), conduction velocities of 7.9 ± 1.1 m s⁻¹ and 1.3 ± 0.2 m s⁻¹ were calculated for the early and late synaptic responses, respectively.

In 16 of the 24 cardioceptive NTS neurons (12 extracellular; 4 intracellular) there were a convergent synaptic input following stimulation of the carotid sinus nerve (mean latency 10.6 ± 1.9 ms, range 5–21 ms; Figs. 4 and 7). In neurons recorded intracellularly, carotid sinus nerve either evoked spike discharge (n = 2; Figs. 4 and 7) or EPSPs (4.5–9 mV; n = 2).

Characterization of convergent inputs to cardioceptive NTS neurons

In addition to testing for a convergent input from pulmonary vagal C-fibers, the carotid sinus nerve inputs were characterized further by stimulating the carotid body chemoreceptors with CO₂-saturated bicarbonate solution and distending the carotid sinus to stimulate baroreceptors afferent endings. From extracellular recordings, 18 of the 24 cardioceptive NTS neurons were shown to receive convergent inputs from other cardiorespiratory receptors (Figs. 5–8). These included carotid chemoreceptors (excitatory n = 7; Figs. 5–8), pulmonary C-fibers (excitatory n = 4, Figs. 6 and 8; inhibitory n = 1; Fig. 5) or jointly from both carotid chemoreceptors and pulmonary C-fibers (both excitatory n = 6; Figs. 6 and 8). In contrast, it was rare to find convergence from the ipsilateral carotid sinus baroreceptors (Figs. 5–8). Of the 20 cardioceptive NTS neurons tested only one responded to inflation of the ipsilateral carotid sinus.

The technical difficulty of maintaining stable intracellular recordings during the reflex cardiovascular responses limited the number of observations with a full characterization of the response pattern to physiological stimuli. However, consistent with the extracellular data two intracellularly recorded cardioceptive neurons were shown to receive excitatory inputs following carotid body but not carotid sinus stimulation (Fig. 7). In both cases cardiac and chemoreceptor stimulation depolarized the membrane potential leading to action potential discharge (Fig. 7).

Concomitant with the neuronal responses of NTS neurons evoked by carotid chemo- and baroreceptor stimulation, there were reflex changes in the cardiorespiratory variables recorded that are consistent with those reported previously in the cat (e.g., Daly 1991). For example, stimulation of carotid chemoreceptors augmented the rate and amplitude of phrenic nerve discharge and produced a pressor response (17–22 mmHg; Fig. 5) and bradycardia (13–25 bpm; Fig. 6). Baroreceptor stimulation produced no obvious change in phrenic nerve activity but decreased both heart rate (5–18 bpm) and arterial pressure (8–15 mmHg; Figs. 5 and 6).
Cardiorespiratory inputs to noncardioreceptive NTS neurons

Of the 73 NTS neurons excited by stimulation of the cardiac vagal branch, 49 failed to respond to veratridine injection; 19 of these 49 neurons were both tested and excited by electrical stimulation of the cervical vagus nerve with many also responding to carotid sinus nerve stimulation (excitatory, \( n = 14 \); inhibitory, \( n = 1 \)).

Of these 19 neurons (16 extracellular; 3 intracellular), 4 received convergent inputs from carotid chemoreceptors, 2 cells were driven by both chemoreceptors and pulmonary C-fibers, and 2 by pulmonary chemoreceptors alone. Four of the 19 neurons responded to distension of the ipsilateral carotid sinus but failed to respond to either carotid chemoreceptor or pulmonary C-fiber stimulation. The latency of the vagus nerve–evoked synaptic responses in cells activated by pulmonary C-fiber stimulation was 54 ± 6.6 ms (\( n = 4 \)), whereas cells activated by carotid body or carotid sinus stimulation received an input from the carotid sinus nerve at 7.2 ± 1.1 ms (\( n = 10 \)).

Recording sites of cardiac-receptive NTS neurons

The recording sites of six neurons driven synaptically following cardiac branch stimulation and excited by chemical stimulation of cardiac receptors were marked and found to be within the NTS (Fig. 9). The positions of other neurons, determined by extrapolation relative to the marked recording sites, were restricted to regions dorsomedial, medial, and ventromedial to the solitary tract at rostro-caudal levels coinciding with the area postrema (particularly its caudal most half) and extending into the commissural subnucleus (Fig. 9).

Discussion

Our results give the first description of the response characteristics of NTS neurons following chemical stimulation of cardiac (ventricular) vagal receptors in the anesthetized cat. The major finding of this study was that the majority of cardiac vagal receptor-driven NTS neurons received convergent input from other cardiorespiratory receptors originating from carotid chemoreceptors and pulmonary vagal C-fibers and to a far lesser extent from baroreceptors. These data support the observations of Paton (1998a,b) in the mouse who showed that NTS neurons appear to receive either chemo- or mechanosensory information.

Technical considerations

With regard to the cardiac receptors stimulated with veratridine it is likely that these will include receptors close to the coronary arteries as well as within the ventricular wall because we injected veratridine into either the root of the aorta or the left ventricle. Stimulation of cardiac receptors with aortic root injections of veratridine depressed phrenic nerve activity and elicited a reflex bradycardia and depressor response that is similar to that described in other reports that used comparable methods for stimulating these receptors (Daly 1986; Hainsworth 1991; Paton 1998a,b). We believe that this stimulus is effective in activating receptors that relay with unmyelinated axons, as we were able to record such activity in cervical vagal fibers where we also measured the conduction delay after stimulation of their axons in the cardiac vagal branches, and it is known that myelinated vagal afferents are also affected (Oberg and Thorén 1972; Paton 1955). Notably such stimulation failed to activate fibers in pulmonary vagal branches. Interestingly, the excitatory responses observed in NTS neurons on veratridine application appeared to be mediated over pathways involving unmyelinated vagal afferents although there was often a short latency excitatory response to cardiac branch or cervical vagal stimulation. We measured the conduction velocity of the afferents involved with some accuracy as we had electrodes around both cardiac branches and the cervical vagus and could thus measure the conduction distance between them as well as the latencies of the responses. In many cases we had good evidence of both unmyelinated (i.e., latency >45 ms) and myelinated inputs. This is in direct contradiction to the observations of Bennett et al. (1985) who claimed discrete actions of myelinated and unmyelinated cardiac vagus nerve afferent inputs. In those cases where we fail to report an unmyelinated component to the response (10 cardiorespiratory neurons with latencies of <45 ms following cardiac vagus stimulation), these neurons were not subjected to exhaustive tests of all the cardiac vagal branches with increasing intensities of stimulation. Thus we cannot exclude the possibility that they also received an unmyelinated input that might contribute to the evoked effects of veratridine. The heterogeneity of afferents in the cardiac vagal branch may contribute to our observations as only cells driven by cardiac vagal branch stimulation (24 of 73) responded to aortic root or ventricular injections of veratridine. Thus the cardiac vagal branch may contain other afferents of noncardiac origin (i.e., oesophageal and pulmonary) as was discussed by Bennett et al. (1985) and Donoghue et al. (1981). However, we believe that convergence of myelinated and unmyelinated inputs is the norm rather than the exception and it is possible that this convergence involves chemosensitive inputs (see Peripheral vagal afferent activity).

We believe that right atrial injections of PBG affect primarily receptors within the pulmonary bed that relay to the medulla with unmyelinated axons; the receptors correspond to J receptors as defined by Paton (1955). Fibers in pulmonary vagal branches were activated by such injections and the activity of unmyelinated fibers in the cervical vagus were recorded that also responded abruptly (latency <1 s) to PBG. These injections failed to excite cardiac vagal branches at short latency with effects observed only after >6 s when as a consequence of circulation through the pulmonary bed they could directly affect cardiac receptors (Daly 1991). Because NTS responses following right atrial injection of PBG were evoked well within 5 s, we believe that the responses can be attributed to activation of pulmonary receptors. The cardiorespiratory responses to both veratridine and PBG were abolished by vagal section implying that they activate only receptor endings of vagal afferents.

Cardiorespiratory afferent convergence to cardiorespiratory NTS neurons

The observations that NTS neurons received convergent inputs from cardiac receptors stimulated by veratridine, pul-
monary chemosensitive receptors, and carotid chemoreceptors may add to our understanding of the organization and integration of reflex inputs. Recently distinctive and complementary patterns of cardiorespiratory afferent convergence based on peripheral receptor modality were demonstrated (Dawid-Milner et al. 1995; Paton 1998a,b). This data was based on the finding that mechanoreceptors and chemosensitive receptors affected different NTS neurons in an apparently ordered manner. This is both further supported and extended by the present data. In the present studies in the anesthetized cat, stimulation of both baroreceptors and distension of the right atrium failed to excite cardiorespiratory NTS neurons. However, these cardiorespiratory NTS neurons were synaptically driven by inputs from carotid chemoreceptors and/or pulmonary C-fibers. This evidence further substantiates the idea that cardiovascular mechanoreceptors do not excite those NTS neurons that are involved in the integration of chemosensitive cardiorespiratory receptor inputs. Baroreceptor activation did on occasion inhibit NTS neurons that were excited by chemosensory inputs (see also Silva-Carvalho et al. 1995). It is, however, notable that Mifflin (1996) describes a less distinct pattern of convergence between laryngeal mechanoreceptor inputs and carotid sinus nerve inputs. He reports convergence of laryngeal mechanoreceptive and carotid chemoreceptor inputs and a separate convergence of laryngeal mechanoreceptor and carotid baroreceptor input on the other. He argues that the convergence pattern is output related (i.e., related to the effects on heart rate of the various inputs as an example). He did not, however, distinguish between mechanical and chemical activation of the larynx as in the study of Dawid-Milner et al. (1995) and a marked mechanical stimulus might elicit secondary chemical effects within the larynx that could affect his interpretation. Clearly more studies are required to elucidate the discrepancies between these observations.

Our finding of separate central reflex pathways for chemosensory and mechanoreceptor inputs from a range of receptors within the cardiorespiratory system also is contentious; there is evidence, at least with cardiac receptor inputs, to suggest that they comprise a spectrum ranging from pure chemical to pure mechanical sensitivity (Kaufman et al. 1980; Oberg and Thoren 1972; Sleight et al. 1969). Armour (1994) suggests a ratio of 70:30% chemical to mechanoreceptor. Although we did not attempt to delineate the nature of the cardiac receptor input in the present study, stimulation of cardiac mechanoreceptors in the mouse proved ineffective in exciting NTS neurons responding to both intraventricular injection of veratridine and pulmonary C-fiber stimulation (companion paper Paton 1998b). We do have preliminary data indicating that unmyelinated vagal afferents excited by balloon inflation of the left ventricle are not affected by intraventricular injections of either veratridine or adenosine 5’-triphosphate (Silva-Carvalho, Rocha, Paton, and Spyer, unpublished observations) It follows, therefore, that cardiac receptors may provide the central nervous system with information containing different sensory modalities as recently described by Paton (1998a) in the mouse. Consistent with this possibility is the finding that selective activation of chemically versus mechanically sensitive cardiac receptors produced different reflex effects on the cardiovascular system (cf. McGregor et al. 1986 with Tutt et al. 1988). At present we have not investigated the interaction of cardiac mechanoreceptor inputs and chemical stimuli in the cat beyond the controls in the present study that involved control saline injections, but this is an important issue that requires detailed attention in further investigations. This interaction may parallel the situation with laryngeal receptors (see Widicombe 1986) and affect the interpretation of earlier published accounts (see Dawid-Milner et al. 1995; Mifflin 1996). However more compelling support comes from the observations outlined in studies in the mouse (Paton 1998a,b).

**Functional consequences of patterns of convergence**

The tendency for cardiac chemically sensitive inputs to converge onto neurons receiving either or both arterial chemosensory input and pulmonary C-fiber input is comparable with that seen in the mouse (Paton 1998a,b) and supports the notion of information channeling in the NTS. This channeling may indicate dedicated projection targets of NTS neurons to specific outputs such as cardiac vagal motoneurons, premotor sympathetic, or the respiratory network (Mifflin 1992, 1996; Paton 1998a,b; Spyer 1994) implying that the NTS is coded for output rather than input. There may, however, be additional consequences of the patterns of convergence revealed in the present investigation. It is perhaps surprising that afferent inputs that have distinctly different effects on certain physiological variables converge so early in the central processing network. This is exemplified with regard to the cardiac and pulmonary effects on respiration that are to slow partially respiratory rate by exciting postinspiratory activity (Paton 1997). Arterial chemoreceptors act to enhance respiratory rate and depth but also excite postinspiratory neurons. The former two reflex inputs could be considered to be protective in function and are related to primitive reflex functions in our fish ancestors. In this context it is notable that with respiration, controlled peripheral chemoreceptor stimulation provokes a bradycardia as with the other two reflex inputs (Daly 1991). This allows us to speculate that the arterial chemoreceptor reflex may also function as a protective reflex as well as acting as a primary homeostatic mechanism as suggested previously (Coleridge et al. 1991). This dual role may well underlie the facilitatory interaction between the arterial chemoreceptor reflex and the hypothalamic defense response (Marshall 1977; Silva-Carvalho et al. 1995) that is mediated at least in part in the NTS. Indeed many of the neurons in the present study that were shown to have convergent excitatory inputs from different chemosensory afferents were also excited upon stimulation within the hypothalamic defense area (Silva-Carvalho, Paton, Rocha, and Spyer, unpublished observations).

This study was supported by a Wellcome Trust Program grant to K. M. Spyer. J.F.R. Paton was supported by British Heart Foundation Grant BS/93003 and Royal Society Grant 14349.

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

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