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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., cardioceptive neurons) by exhibiting an augmenting–decrementing 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 cardioceptive 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 cardioceptive 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 serotonergic 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 Thorén 1972; Sleight et al. 1969; Sleight and Widdicombe 1965). Myelinated cardiac afferents relaying in the vagus nerve are also excited by veratridine (see Öberg and Thorén 1972; Paintal 1955). Both adenosine and bradykinin are released during myocardial ischemia (e.g., Kaufman et al. 1980; Ustinova and Schultz 1994), whereas mechanical distension of the left ventricle releases prostaglandins (Block et al. 1974). 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

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 $37 \pm 0.5^\circ\text{C}$. Animals were placed into a stereotaxic head holder and spinal clamps (thoracic and lumbar) were used for support. During central recording cats were paralyzed by using either gallamine (Flaxedil 4 mg/kg iv) or vecuronium bromide (250 $\mu\text{g}/\text{kg}$ iv) every 40 min and ventilated by changing minute volume and/or infusing HCO_3^- 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 (Reprosil). 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 CO_2 -saturated HCO_3^- 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 $\mu\text{g}/\text{kg}$) to stimulate cardiac 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 $\mu\text{g}/\text{kg}$). 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 multiunit 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 $\text{M}\Omega$) 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 $\text{M}\Omega$), 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 $\text{M}\Omega$; 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

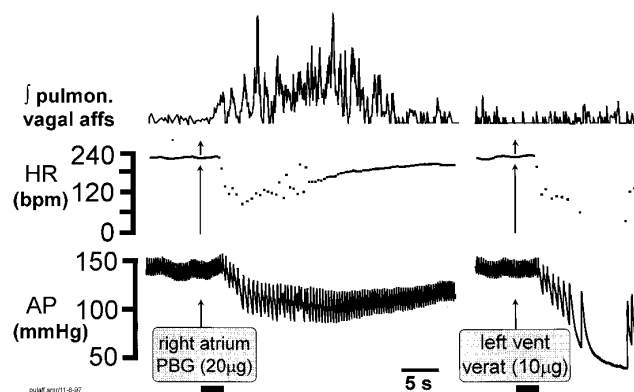


FIG. 1. Right atrial injection of phenylbiguanide (PBG) induced afferent activity in a multifiber preparation from a pulmonary vagal branch recorded intrathoracically. This evoked afferent barrage occurred just before the reflex bradycardia and depressor response. Note the onset time of this evoked activity was <2 s. Importantly, intraventricular injection of veratridine (verat) to stimulate cardiac receptors did not increase pulmonary vagal activity but did produce potent bradycardia and hypotension. The conduction velocity of these units was estimated to be 0.8–1.0 m/s.

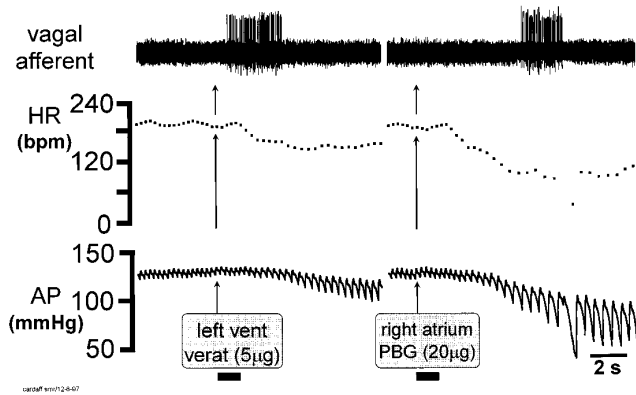


FIG. 2. A suction electrode recording from an unmyelinated vagal afferent recorded from the cervical vagus that responded to aortic root injection of veratridine (verat) at a latency of ~ 1 s. All cardiorespiratory neurons (i.e., cells responding to veratridine) contained in this study fired 0.5–2 s from the time of the injection. Note that the reflex cardiovascular responses occurred just after the afferent volley. This single fiber also responded to right atrial injection of PBG but the time to effect was 5–6 s after the injection. It is likely that this delayed afferent discharge reflects an action of PBG on a cardiac vagal receptor because the delay closely reflects the circulation time through the pulmonary circulation (Daly 1991). The PBG evoked reflex arterial pressure and heart changes were almost fully manifested at the time of the afferent discharge. The conduction velocity of this fiber was 1.1 m/s. On the basis of recordings of this kind NTS neurons were only considered as receiving a synaptic input from pulmonary C-fibers if they responded within 5 s of an injection of PBG.

placed into the NTS under visual guidance by using a binocular microscope and driven with a stepping motor using 1- to 2- μm steps. Surface landmarks of the dorsal medulla (e.g., midline and area postrema) were used for orientation. Microelectrodes were positioned at rostro-caudal sites were corresponding to area postrema and 1–2 mm caudal to it and up to 2 mm lateral to midline.

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 $-5 \mu\text{A}$; 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 μm) 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.

RESULTS

Cardiorespiratory reflex responses to cardiac and pulmonary C-fiber stimulation

In every experiment the cardiorespiratory 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).

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 ~ 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 receptors because this latency is accounted for by the circulation time through the lungs (see Daly 1991). Control saline injections via each route failed to evoke changes in vagal afferent activity and cardiorespiratory variables. Notably all cardiorespiratory responses to both PBG and veratridine were abolished by bilateral section of the cervical vagi ($n = 3$).

The fibers in the cervical vagus that were excited by the

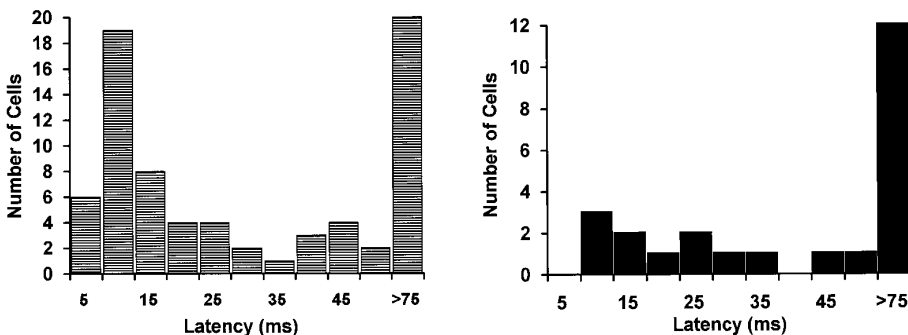


FIG. 3. The latencies of the synaptic response (spikes, extracellular; EPSPs, intracellular) evoked following electrical stimulation of the cardiac branches of the vagus nerve are compared for the total population of NTS neurons recorded ($n = 73$) and those that also responded to intraventricular or aortic root injection of veratridine ($n = 24$). The finding that only $\frac{1}{3}$ of cardiac vagal branch-driven NTS also responded to veratridine stimulation of cardiac receptors reflects the heterogeneity of afferents contained within this nerve.

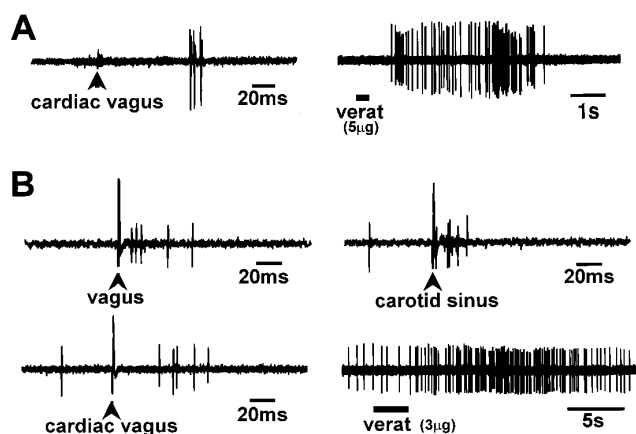


FIG. 4. Cardioreceptive NTS neurons were characterized by their evoked excitatory synaptic responses following both electrical stimulation of the ipsilateral cardiac vagal branch and chemical stimulation of cardiac receptors by aortic root injection of veratridine (verat; $2 \mu\text{g}/\text{kg}$). Two neurons are depicted. *A*: cardiac vagal branch was stimulated with 8 V (0.5 ms; 3 consecutive superimposed sweeps). This neuron also received a synaptic input following cervical vagus nerve stimulation (not shown). *B*: cardiac vagal branch was stimulated with 11 V (0.5 ms; 4 consecutive superimposed sweeps). The NTS neuron in *B* also received convergent synaptic inputs following electrical stimulation of the carotid sinus (0.2 ms; 15 V) and cervical vagus nerves (7 V, 0.5 ms).

application of these two chemoexcitants had conduction velocities in the range to be expected of C-fibers (i.e., 0.6–1.6 m/s, $n = 10$). We did not make exhaustive recordings from peripheral fibers and so do not have data regarding the involvement of myelinated afferents in any of the responses to cardiac afferent activation, although there is well-established evidence in the literature for their involvement (see Oborg and Thoren 1972; Paintal 1955) (also see *Technical considerations*).

Central electrophysiological studies

The present observations are based on 78 neurons recorded either extracellularly ($n = 47$) or intracellularly ($n = 31$). From intracellular data the mean resting membrane potential was below -45 mV [i.e., -47 ± 1.7 (SE)

mV]. 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, 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 cardioresponsive 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 ($n = 18$ extracellular; $n = 6$ 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

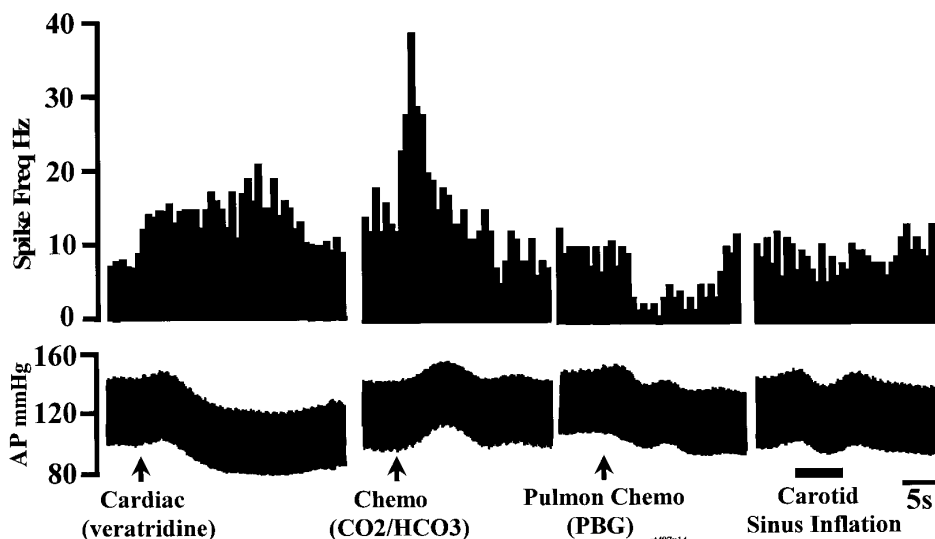


FIG. 5. Convergence of carotid chemoreceptor inputs to a cardioresponsive NTS neuron. Firing frequency is depicted as a rate histogram and the reflex responses in arterial pressure are shown during stimulation of cardiac receptors (left, intraventricular veratridine $2 \mu\text{g}/\text{kg}$), carotid chemoreceptors (close arterial injection of CO_2 -saturated bicarbonate solution into the carotid body), pulmonary C-fiber stimulation with PBG; $15 \mu\text{g}/\text{kg}$), and baroreceptor stimulation by inflation of the ipsilateral carotid sinus. In this group neurons failed to respond to PBG and baroreceptor stimulation. In all cases the stimuli produced reflex falls in arterial pressure. The evoked synaptic input from the cardiac vagal branch occurred at a latency of 90 ms. Rate histogram binwidth was 1 s.

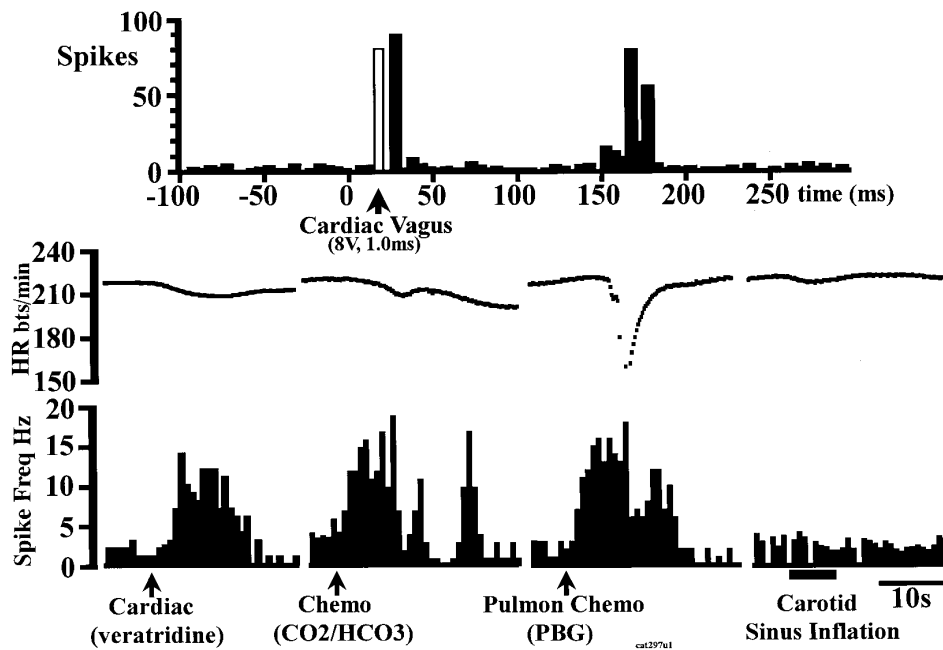


FIG. 6. An example of a cardioceptive NTS neuron that was both synaptically driven by electrical stimulation of the ipsilateral cardiac branch, as revealed in a peristimulus time histogram (10-ms binwidth, 60 sweeps) and excited by aortic root injection of veratridine ($2 \mu\text{g}/\text{kg}$; bottom 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 CO_2 -saturated bicarbonate solution and PBG ($20 \mu\text{g}/\text{kg}$), 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.

term is for convenience and is not meant to imply that the only input impinging on these cells was from cardiac receptors.

Firing response of cardioceptive NTS neurons following veratridine injection

The pattern of firing elicited by $1\text{--}3 \mu\text{g}/\text{kg}$ 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 intracel-

lular 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 cardioceptive NTS neurons

In cardioceptive 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

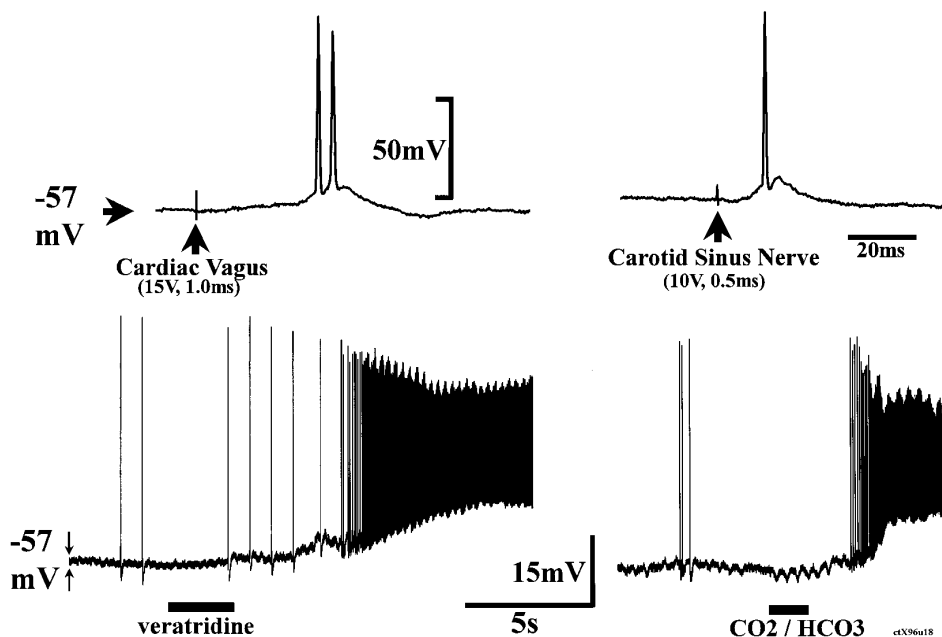


FIG. 7. An intracellularly recorded NTS neuron that was synaptically driven following electrical stimulation of the cardiac vagus (top left) and carotid sinus nerves. This neuron was depolarized and discharged following chemical stimulation of cardiac receptors (aortic root injection of veratridine $2 \mu\text{g}/\text{kg}$) and carotid chemoreceptors with CO_2 -saturated bicarbonate solution. Carotid sinus inflation did not evoke a synaptic response (not shown).

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.

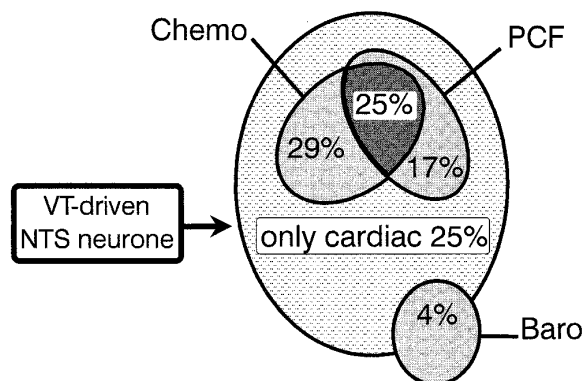


FIG. 8. A Venn diagram summarizing the convergence of synaptic inputs to 24 characterized cardioceptive NTS neurons from either carotid chemoreceptors (Chemo) and/or pulmonary C-fibers (PCF) and baroreceptors (Baro). The greatest convergence was from carotid chemoreceptors and jointly from both chemoreceptors and PCF. Converging inputs from baroreceptors were rarely observed. VT, veratridine (1 – 3 μ g/kg), which was injected into the left ventricle or aortic root.

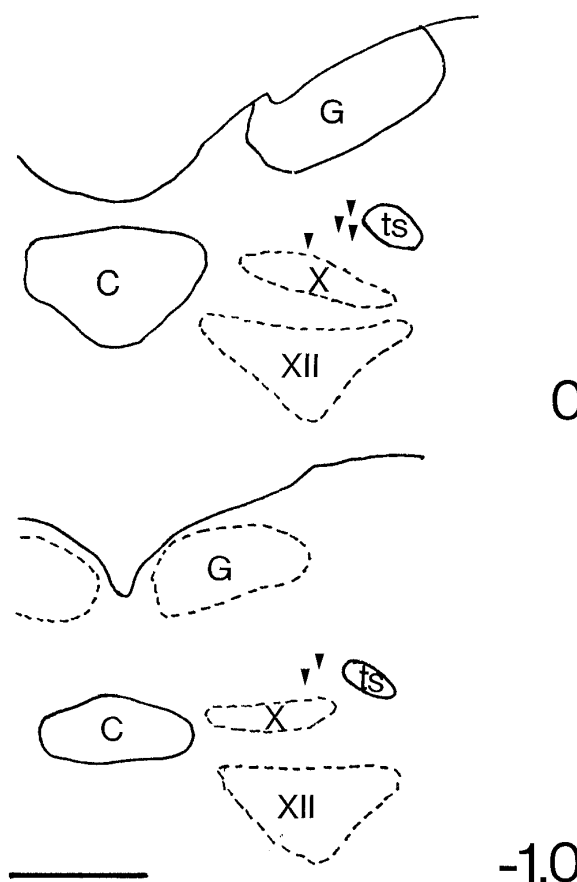


FIG. 9. Representative transverse sections of the dorsomedial medulla of the cat depicting the recording sites of six characterized cardioceptive NTS neurons. Recording sites are from both extracellular (pontamine sky blue dye) and intracellular recordings: in the latter the glass microelectrode was cut and left in place. Abbreviations: C, central canal; G, gracile nucleus; ts, solitary tract; X, dorsal vagal nucleus; XII, hypoglossal motor nucleus. Numbers refer to distance from the obex in mm. Scale bar = 1 mm.

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 rostral-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 (Öberg and Thorén 1972; Paintal 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 cardioresceptive 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 Paintal (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 cardioresceptive 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 cardioceptive NTS neurons. However, these cardioceptive 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).

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REFERENCES

AL-TIMMAN, J.K.A., DRINKHILL, M. J., AND HAINSWORTH, R. Reflex responses to stimulation of mechanoreceptors in the left ventricle and coro-

- nary arteries in anaesthetized dogs. *J. Physiol. (Lond.)* 472: 769–783, 1993.
- ARMOUR, J. A. Peripheral autonomic neuronal interactions in cardiac regulation. In: *Neurocardiology*, edited by J. A. Armour and J. L. Ardell. New York: Oxford Univ. Press, 1994, p. 219–244.
- BENNETT, J. A., GOODCHILD, C. S., KIDD, C., AND MCWILLIAM, P. N. Neurons in the brainstem of the cat excited by vagal afferent fibers from the heart and lungs. *J. Physiol. (Lond.)* 369: 1–15, 1985.
- BLOCK, A. J., POOLE, S., AND VANE, J. R. Modification of basal release of prostaglandins from rabbit isolated hearts. *Prostaglandins* 7: 473–486, 1974.
- COLERIDGE, H. M., COLERIDGE, J.C.G., AND JORDAN, D. Integration of ventilation and cardiovascular control systems. In: *The Lung: Scientific Foundations*, edited by R. G. Crystal and J. B. West. New York: Raven, 1991, p. 1405–1418.
- COLERIDGE, H. M., COLERIDGE, J.C.G., AND KIDD, C. Cardiac receptors in the dog, with particular reference to two types of afferent endings in the ventricular wall. *J. Physiol. (Lond.)* 174: 323–329, 1964.
- CRISP, A. J., TUTT, S. M., MCGREGOR, K. H., AND HAINSWORTH, R. The effects of changes in left ventricular pressure on respiratory activity in anaesthetized dogs. *Q. J. Exp. Physiol.* 74: 291–300, 1989.
- DALY, M. DE BURGH. Interactions between respiration and circulation. In: *Handbook of Physiology. The Respiratory System. Control of Breathing*, edited by N. S. Cherniack and J. G. Widdicombe. Washington, DC: Am. Physiol. Soc., 1986, sect. 3, vol. II, p. 529–594.
- DALY, M. DE BURGH. Some cardioinhibitory responses in the cat and their modulation by central inspiratory neuronal activity. *J. Physiol. (Lond.)* 439: 559–577, 1991.
- DAWID-MILNER, S., SILVA-CARVALHO, L., GOLDSMITH, G. E., AND SPYER, K. M. Hypothalamic modulation of laryngeal reflexes in the anaesthetized cat: role of the nucleus tractus solitarius. *J. Physiol. (Lond.)* 487: 739–749, 1995.
- DONOGHUE, S., FOX, R. E., KIDD, C., AND KOLEY, B. N. The distribution in the cat brain stem of neurons activated by vagal non-myelinated fibers from the heart and lungs. *Q. J. Exp. Physiol.* 66: 391–404, 1981.
- DRINKHILL, M. J., MOORE, J., AND HAINSWORTH, R. Afferent discharges from coronary arterial and ventricular receptors in anaesthetized dogs. *J. Physiol. (Lond.)* 472: 785–799, 1993.
- HAINSWORTH, R. Reflexes from the heart. *Physiol. Rev.* 71: 617–658, 1991.
- HINES, T., TONEY, G. M., AND MIFFLIN, S. W. Responses of neurons in the nucleus tractus solitarius to stimulation of heart and lung receptors in the rat. *Circ. Res.* 74: 1188–1196, 1994.
- KALIA, M. AND MESULAM, M. Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac and gastrointestinal branches. *J. Comp. Neurol.* 193: 467–508, 1980.
- KAUFMAN, M. P., BAKER, D. G., COLERIDGE, H. M., AND COLERIDGE J.C.G. Stimulation by bradykinin of afferent vagal C-fibers with chemosensitive endings in the heart and aorta of the dog. *Circ. Res.* 46: 476–484, 1980.
- KUBIN, L. AND DAVIES R. O. Central pathways of pulmonary and airway vagal afferents. In: *Regulation of Breathing*, edited by J. A. Dempsey and A. I. Pack. New York: Dekker, 1995, p. 219–284.
- LOEWY, A. D. Central autonomic pathways. In: *Central Regulation of Autonomic Functions*, edited by A. D. Loewy and K. M. Spyer. New York: Oxford Univ. Press, 1990, p. 88–103.
- MARSHALL, J. M. Contribution to overall cardiovascular control made by the chemoreceptor-induced alerting defense response. In: *Neurobiology of the Cardiorespiratory System*, edited by E. W. Taylor. Manchester UK: Manchester Univ. Press, 1987, p. 221–249.
- MCALLEN, R. M. AND SPYER, K. M. The location of cardiac vagal preganglionic motoneurons in the medulla of the cat. *J. Physiol. (Lond.)* 258: 187–204, 1976.
- MCGREGOR, K. H., HAINSWORTH R., AND FORD, R. Hindlimb vascular responses in anaesthetized dogs to aortic root injections of veratridine. *Q. J. Exp. Physiol.* 71: 577–587, 1986.
- MIFFLIN, S. W. Arterial chemoreceptor input to nucleus tractus solitarius. *Am. J. Physiol.* 263: R368–R375, 1992.
- MIFFLIN, S. W. Convergent carotid sinus nerve and superior laryngeal nerve afferent inputs to neurons in the NTS. *Am. J. Physiol.* 271: R870–R880, 1996.
- ÖBERG, B. AND THORÉN, P. Studies on left ventricular receptors, signaling in non-medullated vagal afferents. *Acta Physiol. Scand.* 85: 145–163, 1972.
- PAINTAL, A. S. Impulses in vagal afferent fibers from specific pulmonary deflation receptors. The response of these receptors to phenyl diguanide, potato starch, 5-hydroxytryptamine and nicotine and their role in respiratory and cardiovascular reflexes. *Q. J. Physiol.* 40: 89–111, 1955.
- PATON, J.F.R. Rhythmic bursting of pre- and post-inspiratory neurons during central apnoea in mature mice. *J. Physiol. (Lond.)* 502.3: 623–639, 1997.
- PATON, J.F.R. Convergence properties of solitary tract neurons synaptically driven by cardiac vagal receptors in the mouse. *J. Physiol. (Lond.)* 508.1: 237–252, 1998a.
- PATON, J.F.R. Pattern of cardiorespiratory afferents convergence to solitary tract neurons driven by pulmonary vagal C-fiber stimulation in the mouse. *J. Neurophysiol.* 79: 2365–2373, 1998b.
- SILVA-CARVALHO, L., DAWID-MILNER, M. S., GOLDSMITH, G. E., AND SPYER, K. M. Hypothalamic modulation of the arterial chemoreceptor reflex in the anaesthetized cat: role of the nucleus tractus solitarius. *J. Physiol. (Lond.)* 487.3: 751–760, 1995.
- SILVA-CARVALHO, L., PATON, J.F.R., ROCHA, I., GOLDSMITH, G. E., AND SPYER, K. M. Cardiac afferent inputs to the nucleus tractus solitarius (NTS) in the cat. *J. Physiol. (Lond.)* 504P: 198P, 1995.
- SLEIGHT, P., LALL, A., AND MUERS, M. F. Reflex cardiovascular effects of epicardial stimulation by acetylcholine in dogs. *Circ. Res.* 25: 705–711, 1969.
- SLEIGHT, P. AND WIDDICOMBE, J. G. Action potentials in fibers from receptors in the epicardium and myocardium of the dog's left ventricle. *J. Physiol. (Lond.)* 181: 235–258, 1965.
- SMITH, M. L. AND THAMES, M. D. Cardiac receptors: discharge characteristics and reflex effects. In: *Neurocardiology*, edited by J. A. Armour and J. L. Ardell. New York: Oxford Univ. Press, 1994, p. 19–52.
- SPYER, K. M. Central nervous mechanisms contributing to cardiovascular control. *J. Physiol. (Lond.)* 474: 1–19, 1994.
- TUTT, S. M., MCGREGOR, K. H., AND HAINSWORTH, R. Reflex vascular responses to changes in left ventricular pressure in anaesthetized dogs. *Q. J. Exp. Physiol.* 73: 425–437, 1988.
- USTINOVA, E. E. AND SCHULTZ, H. D. Activation of cardiac vagal afferents in ischemia and reperfusion. *Circ. Res.* 74: 904–911, 1994.
- WIDDICOMBE, J. G. Reflexes from the upper respiratory tract. In: *Handbook of Physiology. The Respiratory System. Control of Breathing*, edited by N. S. Cherniack and J. G. Widdicombe. Washington, DC: Am. Physiol. Soc., sect. 3, vol II, 1986, p. 363–429.