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THE DISTRIBUTION IN THE CAT BRAIN STEM OF NEURONES ACTIVATED BY VAGAL NON-MYELINATED FIBRES FROM THE HEART AND LUNGS

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SUMMARY

In anaesthetized cats, right cardiac vagal branches were electrically stimulated and recordings of evoked 'slow wave' and single neurone activity were made in the brain stem. Short-latency 'slow wave' and multi-neuronal activity evoked by excitation of myelinated vagal afferent fibres were recorded in the medial and lateral subnuclei of the nucleus tractus solitarius, the area postrema, the dorsal vagal motor nucleus, the lateral reticular formation and the nucleus ambiguus. Long-latency responses evoked by vagal non-myelinated fibres were recorded in the medial subnucleus of the nucleus tractus solitarius, the area postrema, dorsal vagal motor nucleus, the parahypoglossal area and the lateral reticular formation dorsal to the nucleus ambiguus. A specific study was made of seventy-two single neurones activated by non-myelinated afferent fibres in the cardiac branch. Thirty-four were shown to be synaptically activated, twenty-one were activated nonsynaptically and seventeen could not be classified. One neurone was also activated by myelinated cardiac afferent fibres, and two by thoracic vagal (including pulmonary) afferent fibres. Neurones were not spontaneously active. Indirect evidence suggests that the majority of the recordings of nonsynaptically activated neurones were likely to be from cell bodies. Neurones were located from the level of the obex to 3·0 mm rostral to it in the medial subnucleus of the nucleus tractus solitarius (45), and in the lateral subnucleus (2), the area postrema and its border with the medial subnucleus of the nucleus tractus solitarius (13), the dorsal vagal motor nucleus (9), the parahypoglossal area (1) and the lateral reticular formation dorsal to nucleus ambiguus (2). Recordings were made from fifteen neurones activated by myelinated fibres in the cardiac vagal branches, and twelve were excited synaptically. The neurones were located in the medial (8) and lateral (3) subnuclei of the nucleus tractus solitarius, the dorsal vagal motor nucleus (1) and the lateral reticular formation (1). Four neurones were also excited by vagal afferent fibres in the thoracic vagal nerve immediately caudal to the caudal cardiac branch.

INTRODUCTION

The majority of afferent nerve fibres in cardiac and pulmonary vagal branches are non-myelinated (Agostoni, Chinnock, Daly & Murray, 1957). The location and physiological characteristics of receptors in the different chambers of the heart with such fibres are now well known (Coleridge, Coleridge & Kidd, 1964; Coleridge, Coleridge, Dangel, Kidd, Luck & Sleight, 1973; Oberg & Thorén, 1972; Thorén, 1976, 1977). There are, however, no accounts of the distribution of these afferent fibres in the brain stem nor of the neurones they influence.

Lam & Tyler (1952) recorded long latency responses in the nucleus of the tractus solitarius following electrical stimulation of the cervical vagal nerves in the rabbit, and Fussey, Kidd

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& Whitwam (1973) described multiple and single neuronal activity in the nucleus tractus solitarius and dorsal vagal motor nucleus of the dog in response to electrical stimulation of non-myelinated fibres of the cervical vagus. However, cervical vagal stimulation excites a mixed population of afferent fibres with a wide distribution of receptor sites in the thorax and abdomen. Since it is not easy to selectively activate, with physiological stimuli, cardiac receptors with non-myelinated afferent fibres we therefore attempted to restrict the origins of the afferent fibres activated by stimulating only cardiac vagal branches passing to the heart on the right side. Our aim was to define the distribution, within the brain stem, of neurones activated by electrical stimulation of such fibres; additionally, information on the location of neurones activated by myelinated fibres in this branch was also obtained. A preliminary account of these experiments has been communicated to the Physiological Society (Donoghue, Fox, Kidd & Koley, 1977).

METHODS

Experiments were performed on nineteen adult cats weighing 2·3–3·7 kg. Anaesthesia was induced with sodium thiopentone (40 mg.kg^{-1}, Intraval, May & Baker, injected I.P., and maintained with additional intermittant intravenous injections (5 mg.ml^{-1}), or with α-chloralose (10 mg.ml^{-1}). The level of anaesthesia was adjusted so that the withdrawal reflex was just abolished. Cannulae were placed in the femoral artery and vein and a tracheal cannula inserted. Positive pressure ventilation was applied with a pump (Starling 'Ideal') using oxygen enriched room air ($F_{I,O_2} = 0{\cdot}4$) and with the expiratory tube placed under 1–2 cm water. Arterial blood pressure, gastric temperature and end-tidal P_{CO_2} were routinely monitored and maintained within normal limits.

Portions of ribs 2–6 on the right side were removed, the upper lobe of the right lung was ligated and removed and the azygos vein was tied and divided. The caudal cardiac branch or branches of the right vagal nerve were dissected free of surrounding tissue. Only those branches in which electrical stimulation (rectangular pulses, 10·0 V, 1·0 ms) produced an immediate and intense bradycardia were used. The thoracic vagal nerve at the level of the lung root and its branches to the upper and middle lobe of the right lung were similarly isolated. The right cervical vagal nerve and, sometimes, the aortic nerve, were exposed and isolated by a lateral approach. The head was placed in a stereotaxic frame (La Précision Cinématographique, Ansieres, Paris) and the dorsal surface of the brain stem was exposed; in some preparations the caudal part of the cerebellum was removed by suction to allow access to the rostral parts of the medulla. The animal was then rotated 45° on its longitudinal axis.

Stimulating electrodes, consisting either of fine silver wire (20 μm diam.) insulated with Teflon (Medwire Corpn, N.Y.) to approximately 0·5 cm from the tips, or small silver wire hooks, were placed on the cervical vagal nerve, the thoracic vagal nerve and the cardiac and pulmonary branches. The nerve–electrode assembly was encapsulated with a silicone rubber (Silgel 604, Wacker Silicones; see Donoghue, Fox & Kidd, 1977*a*). Rectangular electrical stimuli (1·0 ms, 1–20 V) were delivered to the nerves from a stimulator (SPD8, Grass Instrument Co.) at a frequency of 0·25 Hz. Extracellular recordings were made of neuronal activity in the brain stem with platinum–iridium–glass micro-electrodes (Fussey, Kidd & Whitwam, 1970) attached to a micro-manipulator. A conventional f.e.t. follower system was attached close to the micro-electrode and the recorded signals were passed to a pre-amplifier (Tektronix 122) and displayed on an oscilloscope (Tektronix 565). The frequency response of the overall system was 0·8–10^4 Hz for 'slow wave' recording and 80–10^4 Hz for single neurone recording. A computer (DEC PDP12) was used for data analysis during the experiment. Photographic recordings were made from the oscilloscope face with a camera.

At the end of each experiment the brain was perfused via the thoracic aorta with 0·9% NaCl followed by 5% formalin in 0·9% NaCl. After 12–14 h the brain stem was transferred to formol-saline solution for another 1–2 weeks. It was then embedded in paraffin and sections (25 μm) were cut, stained with Cresyl violet and mounted. During each penetration by a micro-electrode careful recordings of the sequential appearance of characteristic spontaneous and physiologically evoked activity (e.g. from dorsal column nuclei) were made. This proved to be a useful method of identifying the location of the electrode tip. Final determination of the sites of recordings was achieved by comparison of the histological and physiological data. At the end of each experiment, the conduction distances along the nerve between the thoracic and cervical stimulation points and to the brainstem were measured.

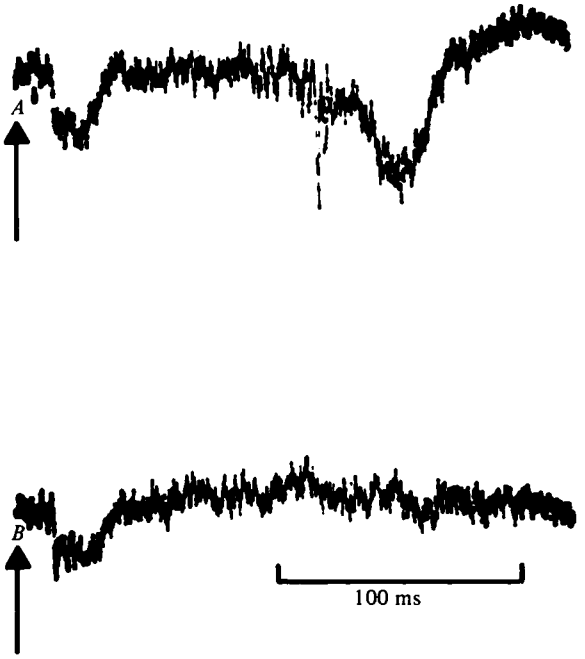

Fig. 1. 'Slow wave' and multi-neuronal activity evoked in the brain stem by stimulation of the caudal cardiac nerve. During the upper trace the nerve was stimulated at 10 V, 1·0 ms and the lower at 5 V, 1·0 ms. Two distinct components to the response can be observed. An 'early' response (latency 20 ms) and a 'late' response (latency 108 ms). *A*, 10 V, 1·0 ms; *B*, 5 V, 1·0 ms. A reduction in stimulation intensity resulted in disappearance of the late response. The stimulus artefacts are indicated by arrows.

RESULTS

'Slow wave' and multi-neuronal responses

In twenty-eight cats, 190 penetrations were made into the brain stem between the limits of 4·0 mm rostral and 4·0 mm caudal to the obex, from 0 to 5·0 mm lateral to the mid-line, and from the dorsal surface to a depth of 5·0 mm. Thus, much of the caudal brain stem was explored. In ninety penetrations, electrical stimulation of the caudal cardiac vagal branches evoked a negative 'slow wave' or a burst of electrical activity comprising a group of action potentials from many neurones. Characteristically, with suprathreshold stimulus intensities, the evoked response had two distinct components (Fig. 1). The short-latency early response began with a latency of 20 ms and lasted 10 ms. A second long-latency response occurred with an initial latency of 108 ms and lasted about 75 ms. A reduction in stimulus intensity caused the long latency response to disappear abruptly. In general, the late response was invariably clearly separated in time from the early response, and it disappeared when the stimulus intensity was reduced to a level at which the early response was unaffected. In these experiments, the conduction distance (caudal branch-medulla) was 182 mm (range 160–190 mm) and the estimated minimum conduction time for non-myelinated fibres ($> 2{\cdot}5\ m\,.\,s^{-1}$) would be less than 65 ms.

Thus, responses with such initial latencies of less than 65 ms could only be produced by myelinated nerve fibres in the cardiac branches. They were observed in seventy-five

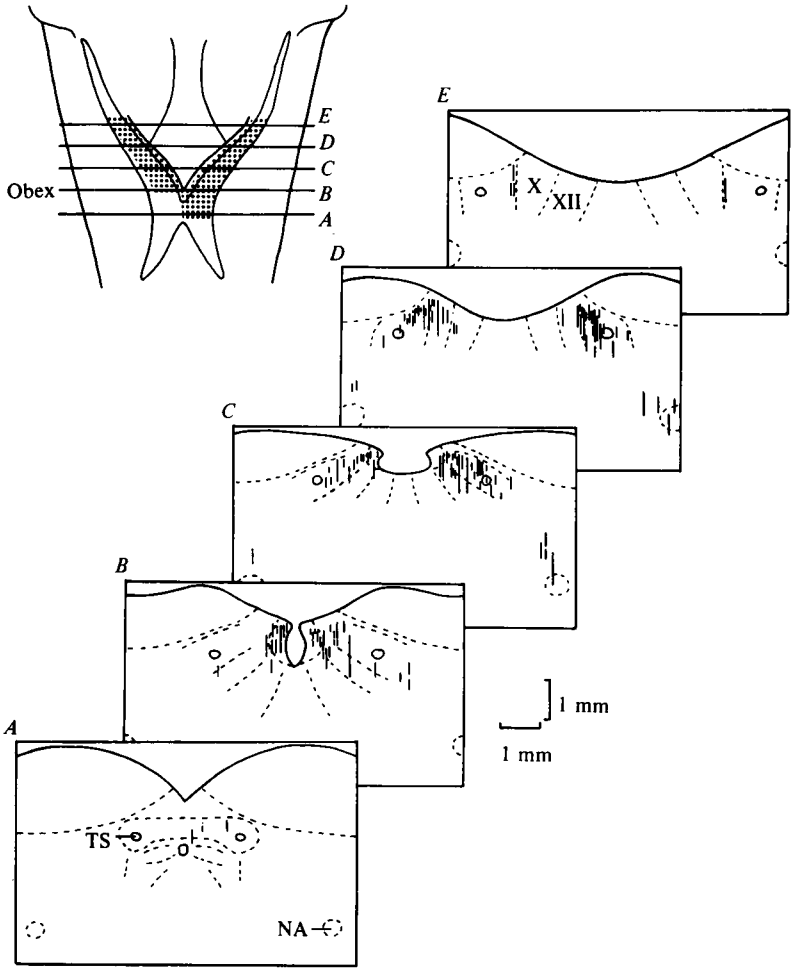

Fig. 2. Sites from which 'slow wave' and multi-neuronal activity was evoked by stimulation of the caudal cardiac vagal nerve. *A–E* represent sections of the brain stem, at 1 mm intervals, as shown on the dorsal view (insert). All recordings were made from the ipsilateral (right) side of the brain stem and the rostrocaudal extent is shown as the dotted area in the insert. On the left side are sites with long latency responses and on the right are shown short latency responses. The depth over which responses were recorded is indicated by the vertical lines. X, dorsal motor nucleus of vagus; XII, hypoglossal nucleus; TS, tractus solitarius; NA, nucleus tractus.

penetrations at recording sites between 1·0 mm caudal and 3·0 mm rostral to the obex and their location is shown in Fig. 2. Responses occurred most frequently in penetrations through the medial subnucleus of the nucleus tractus solitarius and the lateral edge of the area postrema (43 penetrations), although some were found in the lateral subnucleus of the nucleus tractus solitarius (9). Responses were also recorded in the region of the dorsal vagal motor nucleus vagus (15); in the reticular formation between the nucleus tractus solitarius and the nucleus ambiguus (5) and in the nucleus ambiguus itself (3).

The high threshold long-latency group of responses with initial latencies of 65–250 ms were likely to be due to activation of vagal non-myelinated fibres since their latencies are appropriate and they were only observed when stimulus intensity and duration were increased well above those required to evoke short-latency responses. In some cases late

responses were observed at sites where short latency responses did not simultaneously occur. These responses were recorded between the level of the obex and 3·0 mm rostral to the obex in sixty-one penetrations at the sites shown in Fig. 2. Most active sites lay within the medial subnucleus of the nucleus tractus solitarius (29 penetrations), the area postrema (12) and the dorsal vagal motor nucleus (15). In addition, responses were found in the parahypoglossal area (4) and the lateral reticular formation dorsal to nucleus ambiguus (3).

Despite an extensive search through the lateral and ventral areas of the brain stem, short and long-latency evoked responses following stimulation of the cardiac vagal nerves were not observed.

Although study of evoked 'slow' waves and multi-neuronal responses provides information about the regions of the brain stem to which cardiac vagal fibres project it is not possible to identify individual contributions to these responses of primary afferent fibres, efferent fibres or interneurones. Therefore, during the study major emphasis was placed on the characteristics of the activity recorded from individual neurones.

Responses from Single Neurones

Recordings were made from eighty-seven single neurones and under the conditions of the experiments only one was spontaneously active. The latency to the first spike in response to caudal cardiac branch stimulation varied from 7 to 250 ms. For fifteen neurones the latency ranged from 7 to 50 ms and with the conduction distances involved they could only have been activated by myelinated fibres (see later).

Neurones activated by non-myelinated fibres

For seventy-two neurones the range of latencies (67–250 ms; mean 144 ± 46 ms) was compatible with the hypothesis that they were activated by non-myelinated vagal fibres. The stimulus intensities were appropriate and well above those required to evoke short latency responses. In several intances, recordings were made of electrically evoked fibre activity in the cervical vagus and it was observed that the threshold for excitation of non-myelinated fibres was just below that of late responses in the brain stem. In order to confirm that the late responses were due to activation of non-myelinated afferent fibres and not to long polysynaptic central connexions the vagus was additionally stimulated in the neck; such two-point stimulation was performed for thirty-two neurones. The mean overall latency for these neurones was 164 ms (range 128–220 ms) and the mean branch medulla conduction distance was 184 mm (range 160–190 mm). The peripheral conduction velocity, calculated from the two-point stimulation experiments, of the fibres activating these neurones was 1·28 $m.s^{-1}$ (mean, range 0·7–2·0 $m.s^{-1}$). In each case the fibres activating these long-latency neurones were non-myelinated, in no case were myelinated fibres involved. The distribution of latencies for those neurones ($n = 43$) not tested with two-point stimulation was similar and we therefore suggest that they were also excited by non-myelinated fibres.

Classification of Neurones

Since there is no published data which would allow us to decide whether recordings were made from neurones activated synaptically or nonsynaptically by non-myelinated fibres we identified a number of criteria. At suprathreshold intensities, single shock stimulation of the cardiac vagal branches evoked 1–5 spikes. Those neurones in which two or more spikes were evoked by such stimulation were regarded as being excited synaptically. Those in which only a single spike could be evoked belong to one of three populations: they may be excited

Table 1. *Properties of neurones after final classification*

	Synaptic	Non-synaptic	Unclass.
No. of spikes	1–5	1	1
Latency variability at threshold (ms)	5–100	< 2	—
Latency shift, threshold to supramax (ms)	5–30	< 3	—
Intensity change for probability change from 0–100% (V)	< 0·2–> 10	< 0·2	—
Latency (ms) (mean ± S.E.)	168·5 ± 7·7	136·6 ± 6·2	122·5 ± 10·3
Range	67–250	72–164	70–210
Conduction velocity ($m.s^{-1}$) (mean ± S.E.)	1·19 ± 0·07	1·46 ± 0·08	1·61 ± 0·12
Range	0·7–2·5	0·9–2·4	0·9–2·5
No. of neurones	34	21	17

synaptically and respond with a single spike at up to 5 × threshold stimulus intensities, or they may be excited nonsynaptically, and be afferent fibres excited orthodromically or efferent neurones activated antidromically. To provide further evidence toward a separation of these categories we used criteria derived from those previously used in systems which involved myelinated fibres (e.g. Fussey *et al.* 1970).

The criteria tested were: the number of spikes evoked by a single supramaximal stimulus, variability of latency at threshold, latency change when the stimulus intensity was increased from threshold to supramaximal levels, and the range of stimulus intensities required to increase the probability of the response from 0 to 100%. The responses of the two groups are shown in Table 1. The primary considerations which governed whether neurones were defined as being synaptically activated was that they should respond with two or more spikes to a single, supramaximal stimulus. The population of such neurones had the following properties: variability of latency at threshold (100% probability) < 5 ms (range 5–50 ms), change of latency with increased stimulus intensity < 5 ms (range 5–30 ms) and an increase of stimulus intensity of 0·5 V (range 0·5–7·5 V) to achieve an increase in probability from 0 to 100%. Fig. 3 *A* shows an example of the evoked activity of a synaptically activated neurone and illustrates all of these properties. Fig. 3 *B* shows post-stimulus-time histograms of the neurone. Neurones that responded with one spike but fulfilled the above criteria were placed into the synaptically activated category, while neurones which did not exhibit these properties were placed in the nonsynaptic group (Table 1). An example of the activity of a nonsynaptically activated neurone and of post-stimulus-time histograms for such neurones is shown in Fig. 4.

Finally, thirty-four neurones were judged to be synaptically excited and twenty-one neurones were placed in the nonsynaptic group. For the remaining seventeen neurones, insufficient evidence was obtained and they therefore remained unclassified.

Convergence

The responses of all seventy-two neurones which responded to stimulation of non-myelinated fibres in the caudal cardiac branch were examined to identify additional excitation by myelinated fibres which would be revealed by responses at short latency in addition to those at long latency. Only one neurone showed such effects; it responded with a single spike at a latency of 47 ms to caudal cardiac branch stimulation which was followed by a burst of 5–6 spikes starting at about 110 ms.

Fifty-eight neurones responding to caudal cardiac nerve stimulation were tested to see

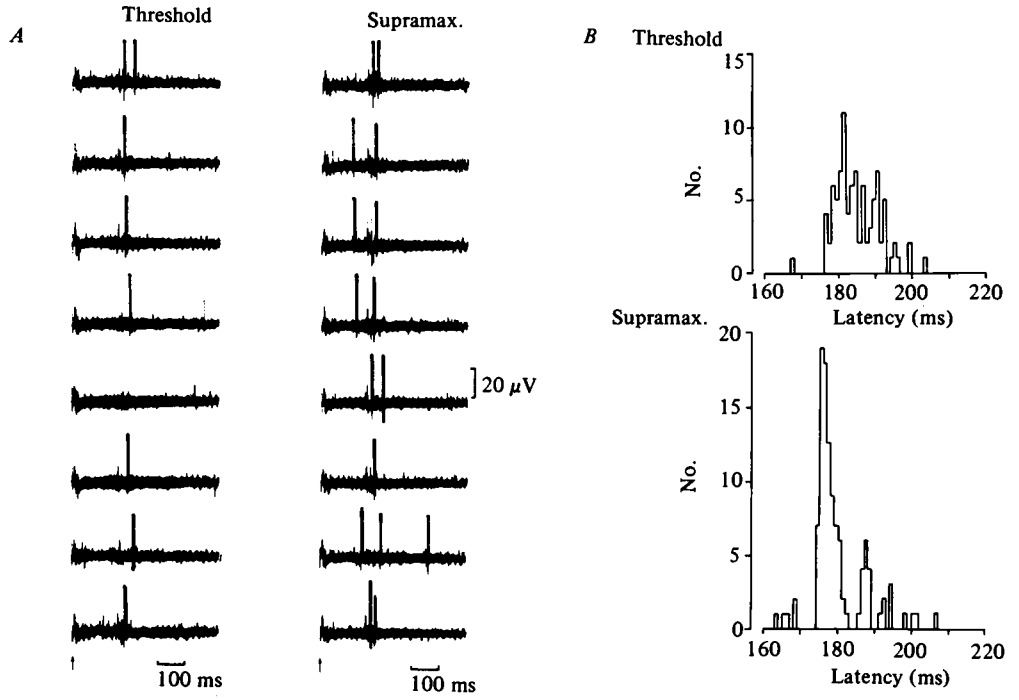

Fig. 3. Evoked activity of a neurone synaptically activated by non-myelinated fibres in the caudal vagal cardiac nerve. *A*, responses at threshold and supramaximal stimulus intensities. Note variability in latency and number of spikes. Stimulus artefact (†) is indicated. Action potentials have been retouched. *B*, post-stimulus time histograms at threshold (4·4 V, 1·0 msec) and supramaximal (12·0 V, 1·0 msec) intensities. Note the shortening of latency and reduction in variability which accompanies the increase in intensity.

whether early or late responses were induced by stimulation applied to the more caudal thoracic vagal nerve (including pulmonary afferent fibres); seven neurones were tested for potential activation by pulmonary vagal branches, and four by the aortic nerve. Only two neurones showed such convergence. One, activated synaptically from the cardiac branch with a latency of 145 ms, was also activated from the thoracic vagal nerve at the level of the lung root with a latency of 135 ms. The other, showing early convergence effects from the cardiac branch (see above) responded repeatedly at short latency following stimulation of the thoracic vagal nerve.

Location of neurones

The recording sites of all neurones activated by non-myelinated fibres in the caudal cardiac branches of the vagal nerve are shown in Fig. 5. They were all in the lower brainstem between the level of the obex and 3·0 mm rostral. Forty-five were located in the medial subnucleus of the nucleus tractus solitarius, two were in the lateral subnucleus of the nucleus tractus solitarius, thirteen were in the area postrema and on the border between the nucleus tractus solitarius and the area postrema. Nine neurones were in the dorsal vagal motor nucleus, one was in the parahypoglossal area and two in the lateral reticular formation, dorsal to the nucleus ambiguus. Many penetrations were made through nucleus ambiguus but no neurones excited by non-myelinated fibres were observed.

Neurones excited synaptically and nonsynaptically are indicated separately in Fig. 5.

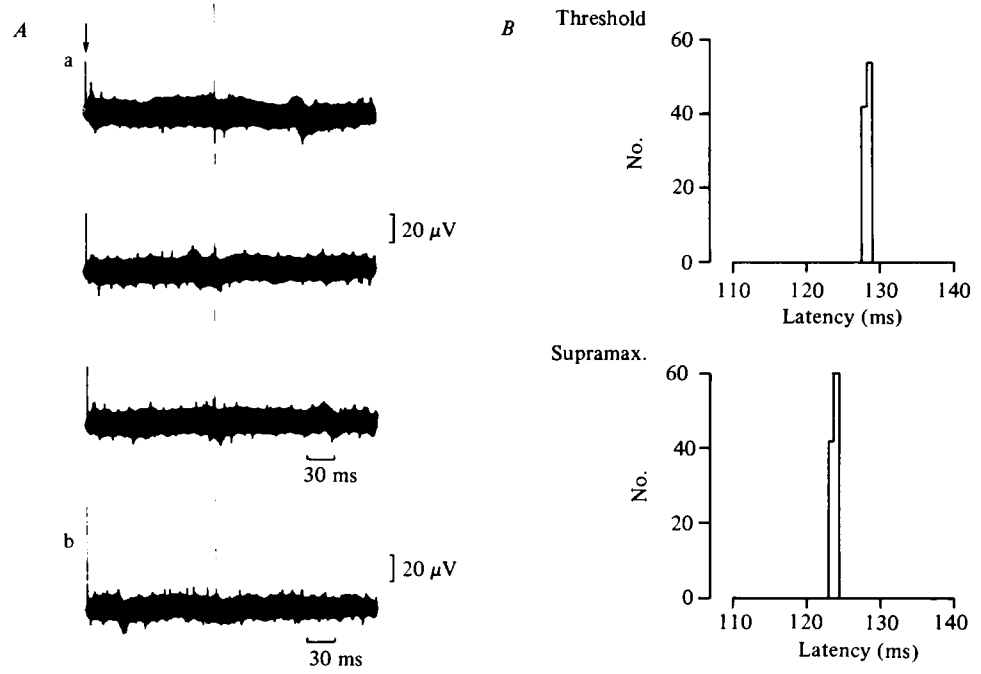

Fig. 4. Response of single neurone excited nonsynaptically by electrical stimulation of non-myelinated fibres in the caudal vagal cardiac nerve. *A*, a single action potential with constant latency with threshold (a, 6 V, 1·0 ms) and supramaximal; (b, 12 V, 1·0 ms) stimulus intensities. The stimulus artefact (↓) is indicated. *B*, post-stimulus-time histograms from another neurone responding to threshold (5 V, 1·0 ms) and supramaximal (12 V, 1·0 ms) stimulus intensities.

Neurones of both groups were recorded in the medial subnucleus of the nucleus tractus solitarius and dorsal vagal motor nucleus. Synaptically activated neurones were also recorded in the area postrema, whilst nonsynaptically activated neurones were not. Both neurones recorded in the lateral reticular formation were nonsynaptucally activated.

Neurones activated by myelinated fibres

Recordings were made from fifteen neurones at latencies compatible with excitation of myelinated fibres in the caudal cardiac branch. Using conventional criteria (Fussey *et al.* 1970), twelve were classified as being synaptically activated, whilst three remained unclassified. The mean initial latency of these neurones was 29·3 (± 14·0 ms; range 7–50 ms). For five neurones, two point stimulation of the vagus showed that the mean conduction velocity of the afferent fibres activating them was 8·9 ms (range 3·3–16·0 $m.s^{-1}$).

Eight neurones were tested to determine whether they were also activated by stimulation of the thoracic vagal nerve; three were activated by such stimulation at latencies of 17–40 ms, i.e. by myelinated fibres.

Location of neurones

The recording sites of thirteen neurones are shown in Fig. 6. Eleven were in the nucleus tractus solitarius, eight in the medial subnucleus, three in the lateral subnucleus. One neurone was located in the dorsal vagal motor nucleus and another was in the lateral reticular formation, lateral to the hypoglossal nucleus.

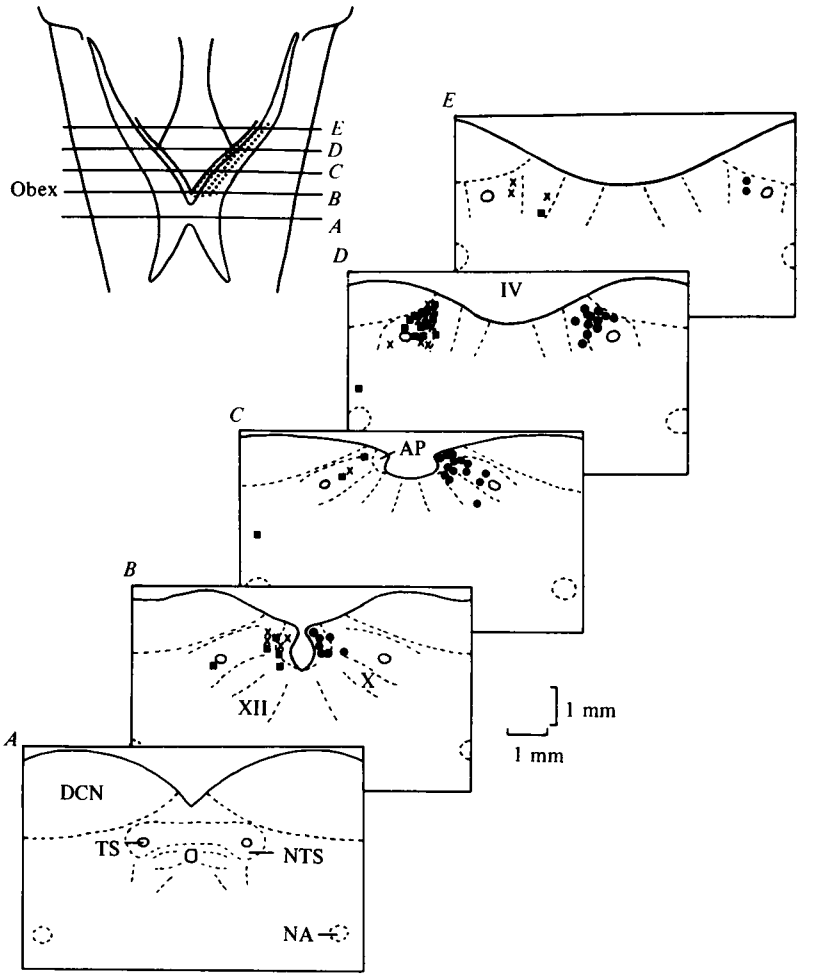

Fig. 5. Sites at which recordings were made from neurones activated by non-myelinated fibres of the caudal cardiac vagal nerve. *A–E* represent sections at 1 mm intervals shown on the dorsal view of the brain stem (insert). All recordings were made from the ipsilateral (right) side and overall extent is indicated on the plotted area. For clarity, synaptically activated neurones (●) are plotted on the right side, while the nonsynaptically activated (■) and unclassified (×) neurones are shown on the left side. TS, tractus solitarius; AP, area postrema; X, dorsal motor vagal nucleus; XII, hypoglossal nucleus; NA, nucleus ambiguus; DCN, dorsal column nuclei.

DISCUSSION

Neurones excited by afferent fibres in the caudal cardiac nerve have been demonstrated in the medial brain stem, particularly in the medial subnucleus of the nucleus tractus solitarius, the area postrema and the dorsal vagal motor nucleus. Although some neurones were excited by myelinated vagal afferent fibres, particular attention was paid to neurones which responded with a long latency, and several lines of evidence suggest that the afferent fibres activating these neurones were non-myelinated. The stimulus intensities were appropriate for excitation of such afferent fibres and the conduction velocities of vagal afferent fibres (determined by two-point stimulation) for a large population of neurones were less than $2{\cdot}5\ \mathrm{m}\ .\ \mathrm{s}^{-1}$. Furthermore, there was no evidence that these neurones were additionally excited

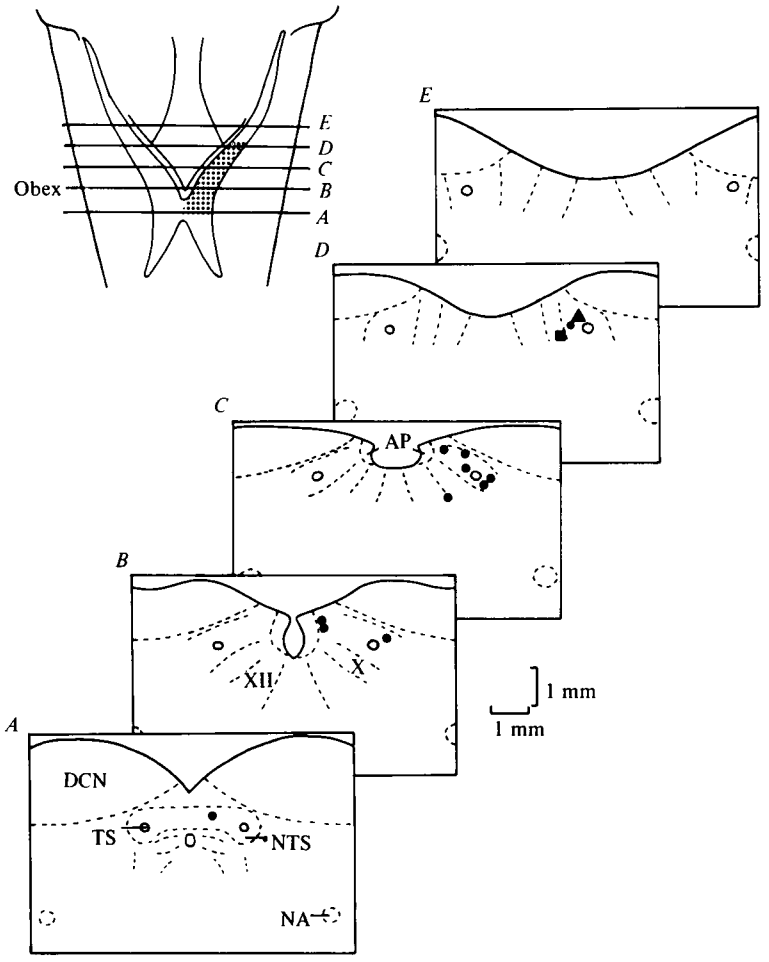

Fig. 6. Sites at which recordings were made from neurons activated by myelinated fibres of the caudal vagal cardiac nerve. Arrangement and abbreviations as Fig. 5.

by volleys in myelinated fibres within the caudal cardiac nerve. These characteristics were to be expected from neurones activated by the arrival of volleys in vagal non-myelinated afferent fibres rather than by myelinated fibres over a long polysynaptic pathway. Thus, for the first time evidence has been provided for the location of neurones in the lower brain stem which are influenced by non-myelinated afferent fibres in the cardiac vagal branches.

Neurones activated by myelinated fibres in cardiac vagal branches

These neurones, most of which were activated synaptically, were located mainly within the nucleus tractus solitarius, both medial and lateral to the tractus solitarius. A detailed study of convergent influences from other cardiac and pulmonary vagal branches onto these neurones was not made, other than to show that they were not excited by non-myelinated fibres of the same cardiac branch. The locations of some neurones activated by atrial receptors with myelinated afferent fibres have previously been described (Baertschi, Munzer, Ward, Johnson & Gann, 1975; Keith, Kidd, Linden & Snow, 1975; Ward, Baertschi &

Gann, 1977) and were in these regions; i.e., the medial subnucleus of nucleus tractus solitarius, the dorsal vagal motor nucleus, the parahypoglossal area and the reticular formation ventral to the nucleus tractus solitarius. Although the nature and origins of receptors attached to the vagal afferent fibres excited by the electrical stimuli to the cardiac branch remain to be established, it is known that fibres from the heart and lungs travel in this branch. Further experiments using physiological stimuli are clearly required.

Neurones activated by non-myelinated fibres in the cardiac branch

Criteria based on conventional electrophysiological techniques were used to determine whether recordings were from neuronal elements excited synaptically or nonsynaptically, since these had not been established for neurones activated by non-myelinated fibres. The nonsynaptic groups may be primary afferent fibres or may represent antidromically activated cell bodies with efferent fibres in the caudal cardiac nerve. Several indirect lines of evidence suggest that efferent neurones are likely to form a majority of this group. Most recordings from single cells could be maintained over depths of 100–130 μm whereas axonal recordings can be satisfactorily obtained only over depths of 10–20 μm (Fussey *et al.* 1970). The low ratio of synaptic to nonsynaptic recordings (34:21), compared to the high ratios usually observed using this type of metal electrode (e.g. 41:15 for dorsal column nuclei (Fussey *et al.* 1970), 45:4 for neurones activated by myelinated aortic nerve fibres and 29:6 for neurones activated by aortic nerve non-myelinated fibres (Donoghue, Kidd & McWilliam, 1978; Donoghue, Fox, Kidd & McWilliam, 1981), is also compatible with the proposition that the nonsynaptic recordings probably represent cell bodies of neurones with non-myelinated efferent axons in the caudal cardiac vagal branch. Histological evidence for the presence of cells in this region with axons in the caudal cardiac nerve exists. Recently, Bennett, Kidd, Latif & McWilliam (1979, 1981) have shown in the cat that horseradish peroxidase applied to the cut central end of these cardiac vagal branches labels cells in the dorsal vagal motor nucleus as well as in the region of the nucleus ambiguus. Similarly, Geis & Wurster (1980) have shown that horseradish peroxidase, injected subepicardially, labels cell bodies in both these areas. The existence of non-myelinated vagal efferent axons in cardiac and pulmonary branches has also been demonstrated histologically (Agostoni *et al.* 1957) and since we have demonstrated electrophysiologically that neurones in the dorsal vagal motor nucleus and adjacent areas have non-myelinated axons in the cardiac vagal branches, we suggest that it is likely that the nonsynaptic group represents the cell bodies of non-myelinated efferent axons passing towards the heart. Although there is electrophysiological evidence that cells of the nucleus ambiguus have myelinated axons in the cardiac vagal branches with patterns of discharge which suggest cardiomotor and bronchomotor functions (McAllen & Spyer, 1978), a functional role for the non-myelinated efferent fibres remains to be established.

Afferent fibres with non-myelinated vagal axons within these cardiac branches have been shown to originate in the atria and ventricles (Amman & Schaefer, 1943; Jarisch & Zottermann, 1948; Dickinson, 1950; Neil & Zottermann, 1950; Öberg & Thorén, 1972; Thorén, 1976, 1977), and these branches also contain afferent fibres from the lungs (Dickinson, 1950; Öberg & Thorén, 1973; Donoghue, Fox & Kidd, unpublished observations). The central projections of these afferent fibres have not yet been determined by electrophysiological techniques. However, Kalia & Mesulum (1980), using an anterograde horseradish peroxidase technique, have attempted to trace the projection to the brain stem of afferent fibres claimed to originate in the heart; labelling was found to be mainly confined

to the subnuclei of the nucleus tractus solitarius and the area postrema. A similar distribution was demonstrated for pulmonary afferent fibres.

In our study, synaptically excited neurones were located almost exclusively in the dorsomedial medulla at, and rostral to, the obex. Many were located in the medial subnucleus of the nucleus tractus solitarius, and were therefore located in the region of the medulla where myelinated and non-myelinated afferent fibres in the aortic nerve terminate (Crill & Reis, 1968; Donoghue, Fox & Kidd, 1977*b*; Jordan & Spyer, 1978; Donoghue *et al.* 1981; Kalia & Welles, 1980), and where carotid sinus nerve afferent fibres terminate (Lipski, McAllen & Spyer, 1975; Jordan & Spyer, 1977; Berger, 1979; Panneton & Loewy, 1980). Despite the convergence of afferent inputs to this nucleus, little evidence could be found in this study for convergence onto single neurones of afferent myelinated and non-myelinated fibres from different areas. Clearly, the use of extracellular recording technique limited the search for such convergence to suprathreshold excitatory effects and subliminal influences would not have been easily detected.

Neurones activated by non-myelinated cardiac afferent fibres were not found in the nucleus ambiguus. This is surprising since such afferent fibres are known to produce a profound vagal bradycardia when excited (Öberg & White, 1970; Öberg & Thorén, 1973), and it is known that cardiomotor efferent neurones lie in this area (McAllen & Spyer, 1976, 1978). However, the cells are sparse and in our study a specific search was not made for neurones with myelinated axons excited antidromically from the cardiac branch. We were unable to demonstrate that afferent fibres in the cardiac vagal branches excited neurones in the ventrolateral brain stem likely to form the bulbospinal projections to sympathetic efferent neurones (e.g. Coote & Macleod, 1974*a*, *b*; Henry & Calaresu, 1974). Nor have we tested whether neurones in the dorsomedial subnucleus of the nucleus tractus solitarius which are activated by afferent fibres in the caudal cardiac nerve project to the spinal cord (Armendt, Czachurski, Dembowsky & Seller, 1979).

The location of neurones activated by the caudal vagal cardiac nerve in the area postrema is interesting, and raises the question of a functional role. Recently it has been shown that myelinated and non-myelinated afferent fibres of the carotid sinus nerve and myelinated fibre aortic nerve afferents also project to this area (Jordan & Spyer, 1977; Berger, 1979; Donoghue *et al.* 1981; Wallach & Loewy, 1980; Kalia & Welles, 1980). Several functions have been suggested for the area postrema (see Koella & Sutin, 1967). It may act as a trigger zone for vomiting since gastric relaxation and vomiting reflexes can be induced by electrical stimulation of non-myelinated fibre in the cardiac branch, by intrapericardial infusion of nicotine or by coronary artery occlusion (Abrahamsson & Thorén, 1972, 1973). The projection of cardiac non-myelinated fibres to the area postrema would provide a pathway for these effects. It has also been postulated that the area postrema may mediate the central nervous hypertensive effects of angiotension II (Joy & Lowe, 1970; Ferrario, Gildenberg & McCubbin, 1972; Gildenberg, Ferrario & McCubbin, 1973). The projection of non-myelinated afferent fibres from cardiovascular receptor areas to the area postrema may indicate that angiotensin II exerts an action on neurones activated by these afferent fibres. Clearly, further observations would be needed to substantiate such a relationship between the synaptic connexions of non-myelinated vagal afferent fibres from the heart and lungs and the area postrema.

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