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THE CHARACTERISTICS OF CARDIAC VAGAL PREGANGLIONIC MOTONEURONES IN THE DOGFISH

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SUMMARY

Preganglionic vagal motoneurones supplying the heart of the dogfish have been located in the medulla by antidromic stimulation of the central cut end of the branchial cardiac branch of the vagus. They supplied axons with conduction velocities between 4·75 and 16·3 m s^{-1}, which is similar to mammalian B fibres. Motoneurones were found in two locations: the rostromedial ($N = 5$) and lateral ($N = 12$) divisions of the vagal motor column. Their measured depths and rostrocaudal distributions with respect to obex corresponded with the location of branchial cardiac motoneurones determined by horseradish peroxidase (HRP) histochemistry. All the neurones located in the rostromedial division of the vagal motor column were spontaneously rhythmically active. Their activity contributed to the rhythmic, respiratory-related bursts in peripheral recordings of efferent activity from the branchial cardiac vagus. They could be induced to fire in a prolonged burst by mechanical stimulation of the gill arches. The neurones located lateral to the rostromedial division of the vagal motor column could be divided into three categories: (1) spontaneously, continuously active cells which could be induced to fire more frequently by mechanoreceptor stimulation, (2) silent cells which could be induced to fire by mechanoreceptor stimulation, (3) silent cells which did not respond to mechanoreceptor stimulation. It is concluded, from the response of the medial and two categories of lateral cells to mechanoreceptor stimulation (which results in a transient bradycardia), that branchial cardiac motoneurones from both these central locations exert a chronotropic influence on the heart.

INTRODUCTION

In all vertebrates the brain stem harbours the centres and termination of all cranial nerves, except for cranial nerve I. The motor column of the Xth vagus nerve in the dogfish consists of four divisions: rostromedial, lateral, dorsomedial and ventromedial (Barrett, Roberts & Taylor, 1984). In cartilaginous fish the brain stem also contains a fairly well developed reticular formation and a number of relay centres and their associated ascending and descending connections (Smeets, Niewenhuys & Roberts, 1983).

Key words: Cardiac vagal motoneurones, fish.

Elasmobranchs possess two distinct pairs of cardiac vagal rami; the visceral cardiac (Marshall & Hurst, 1905) and the branchial cardiac branches (Norris & Hughes, 1920; Lutz, 1930; Young, 1933). Branchial cardiac preganglionic vagal motoneurones are located in the rostromedial and lateral divisions of the vagal motor column, whereas the cell bodies of visceral cardiac neurones are confined to a medial location (Barrett, Roberts & Taylor, 1983*a*). Peripheral recordings from the branchial cardiac branch of the vagus consist of two easily distinguishable types of efferent activity: units that fire regularly in a respiratory-related manner and units that fire sporadically and whose activity increases during an induced bradycardia (Taylor & Butler, 1982). The present study was undertaken in an attempt to locate electrophysiologically and to describe the properties of branchial cardiac preganglionic vagal motoneurones. The impetus for this investigation was the possibility that the dual origin of these cardiac motoneurones results in the two types of activity found in the preceding paper to be conducted by the branchial cardiac branch of the vagus (Barrett & Taylor, 1985*b*). A preliminary account of this work has been published (Barrett & Taylor, 1984*b*).

METHODS

The experiments were performed on 20 dogfish, *Scyliorhinus canicula* L., of either sex and body length between 58 and 74 cm. The fish were maintained at the experimental temperature (15 ± 1 °C) for a minimum of 2 weeks, and not fed for 1 week, prior to the experiments. They were anaesthetized in sea water containing 0·17 g l^{-1} MS 222 (Sandoz). After approximately 20 min the fish were transferred to an operating tray, packed in ice and left for a further 5 min (Williamson & Roberts, 1981). The skin between the eyes was removed and a flap cut from the chondrocranium to expose the forebrain. A complete transection was made through the diencephalon immediately rostral to the optic tectum and the forebrain was removed after all the main blood vessels had been cauterized. The cartilage above the cerebellum was carefully removed to allow a pair of retractors to be inserted into the dorsal walls of the chrondrocranium, lateral to the cerebellum. Gentle opening of the retractors allowed sufficient force to be applied to the cartilage above the hindbrain to facilitate its removal. Care was taken not to damage any blood vessels in the choroid plexus.

The branchial cardiac branch of the vagus nerve was exposed on the left side of the fish by a lateral incision, starting from a point approximately 2 cm behind the spiracle and extending posteriorly just below and parallel with the lateral line to a point dorsal to the 5th gill slit (Taylor & Butler, 1982). This incision opened into the dorsal wall of the anterior cardinal sinus on the floor of which runs the glossopharyngeal nerve and the major branches of the vagus nerve. The branchial cardiac branch of the vagus was identified as it entered the ductus Cuveri, cleared of connective tissue, cut distally and replaced on the floor of the sinus. The incision was then tightly sutured to prevent the admission of sea water whilst the fish was transferred to the experimental tank, which was filled with oxygenated, filtered and recirculated sea water. The fish was prepared for the experiment by clamping it into a stereotaxic frame (Narishige Instruments) with a plate inserted in the mouth and clamped dorsally, a lateral clamp holding the body of the fish caudal to the pectoral fins and dorsal to the midline to

avoid constriction of the posterior cardinal sinuses, and a clamp around the posterior part of the tail.

As the decerebrate fish recovered from the anaesthetic and commenced to ventilate its gills a small volume (< 1 ml) of curare solution [(+)-tubocurarine chloride, Wellcome] was injected intravenously into the suborbital sinus of each fish at a concentration of 7 mg kg^{-1}. In the curarized fish spontaneous respiratory and locomotor movements ceased almost immediately. The gills were force ventilated with oxygenated sea water *via* a tube inserted into the mouth below the clamp. In the majority of cases heparin (Evan's Medicals) was administered at a level of 150 i.u. kg^{-1}.

The incision into the anterior cardinal sinus was then reopened and held in position by a pair of lacrymal duct retractors (McCarthy's Surgical). By the careful positioning of the retractors the incision was orientated so that blood continued to flow across the floor of the cardinal sinus and drain into the ductus Cuveri, thus maintaining venous return to the heart.

The branchial cardiac branch of the vagus was placed over a pair of platinum bipolar stimulating and recording electrodes and was stimulated with 0·02-ms pulses (0·5–15 V) delivered regularly (0·5 Hz), singly or in trains, using physiological stimulators (Farnell and Neurolog systems, Digitimer). Activity in the branchial

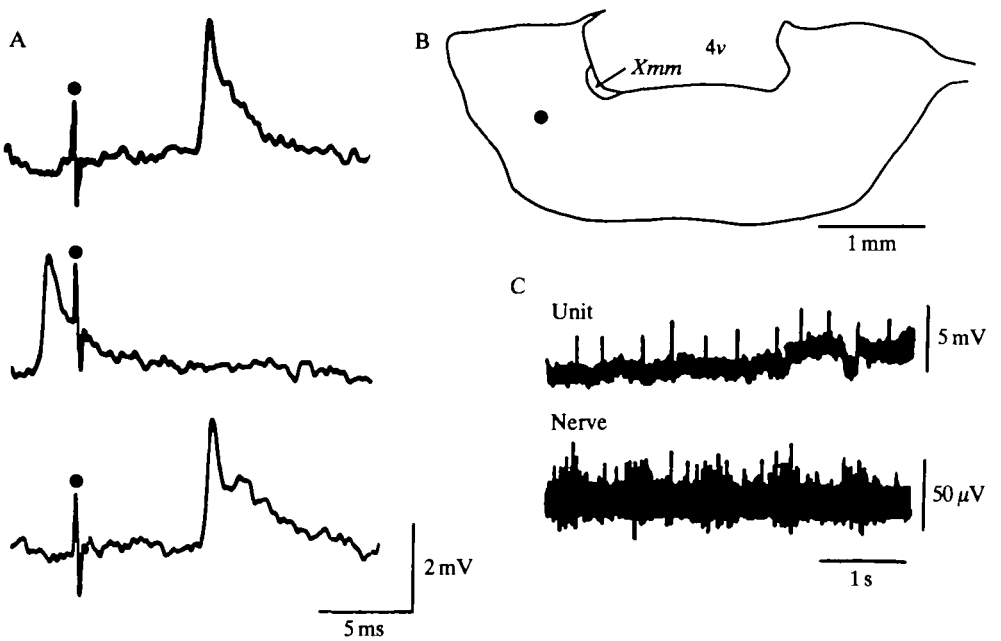

Fig. 1. The location and properties of an antidromically identified, spontaneously active branchial cardiac motoneurone. (A) Three successive sweeps of activity induced by antidromic stimulation of the branchial cardiac nerve at 0·5 Hz (stimulus artifact marked by dot). During the second sweep a spontaneous spike occurring within the period of stimulation cancelled the normally occurring antidromic response shown in the first and third sweeps. (Absolute refractory period of this unit was approximately 4 ms.) (B) Transverse section through the hindbrain 240 μm rostral to obex with the position of the identified unit marked by a dot. (*Xmm*, medial vagal motor nucleus; 4*v*, 4th ventricle). (C) Spontaneous activity recorded from this unit (upper trace) together with activity in the branchial cardiac nerve (lower trace). The unit can be seen to fire regularly both within and between the rhythmic bursts of activity in the cardiac nerve.

cardiac branch was relayed *via* a pre-amplifier (Isleworth, type A101) with suitable filters and an additional amplifier (Neurolog systems, Digitimer) to a dual beam storage oscilloscope (Tetronix R5031) and recorded on magnetic tape (4-channel FM tape recorder, SE Laboratories).

Micropipettes filled with 4 mol l^{-1} NaCl, having resistances of 3–25 MΩ, were driven into the hindbrain with a calibrated micromanipulator (Narishige Instruments) and the approximate surface locations were marked on a diagram of the dorsal surface of the brain during each experiment. Antidromically evoked responses were identified by (i) the 'all or nothing' nature of the responses; (ii) the constant latency at a specific frequency of stimulation; (iii) their ability to follow high frequencies of stimulation (up to 200 Hz) and (iv) cancellation of the evoked response by collision with a spontaneous spike occurring at the appropriate time (i.e. around the period of stimulation but outside the absolute refractory period of the unit, Fig. 1A). Neurones identified as antidromically activated were observed during a period of spontaneous activity (if present) along with peripheral activity in the branchial cardiac branch (Fig. 1C) and during mechanoreceptor stimulation such as a prod to the gill septa, and recorded on magnetic tape.

The recording position was identified either by leaving the microelectrode tip *in situ* or with pontamine sky blue dye spots electrolytically deposited from the electrode (Hellon, 1971). The electrode position was then determined from 120-μm frozen sections of the brain cut after fixation in 10% formal saline.

In one experiment on a decerebrate fish, the conduction velocities of the axons in

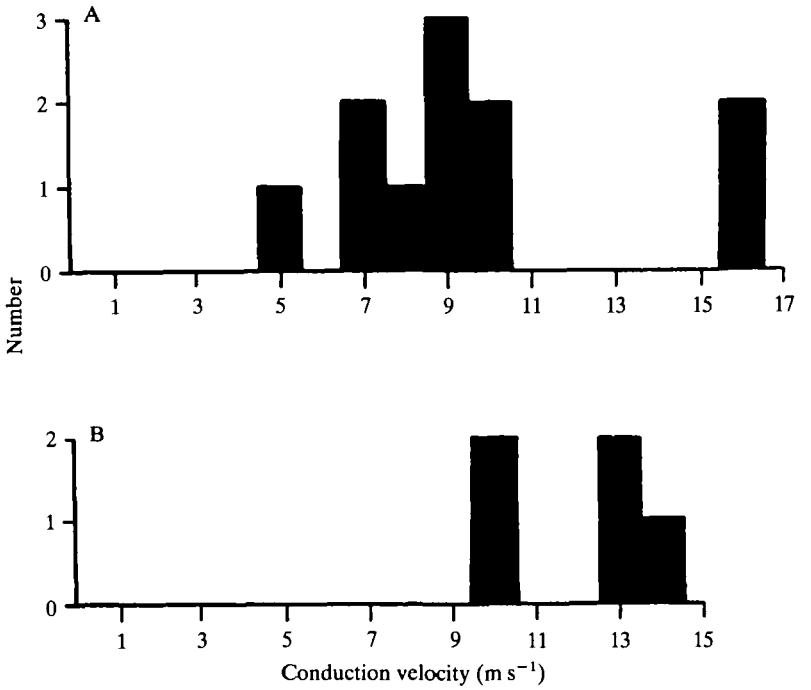

Fig. 2. Frequency histograms of the axonal conduction velocities calculated for 12 cardiac vagal efferent neurones in the lateral division of the vagal motor column (A) and five cardiac vagal efferent neurones in the rostromedial division of the vagal motor column (B).

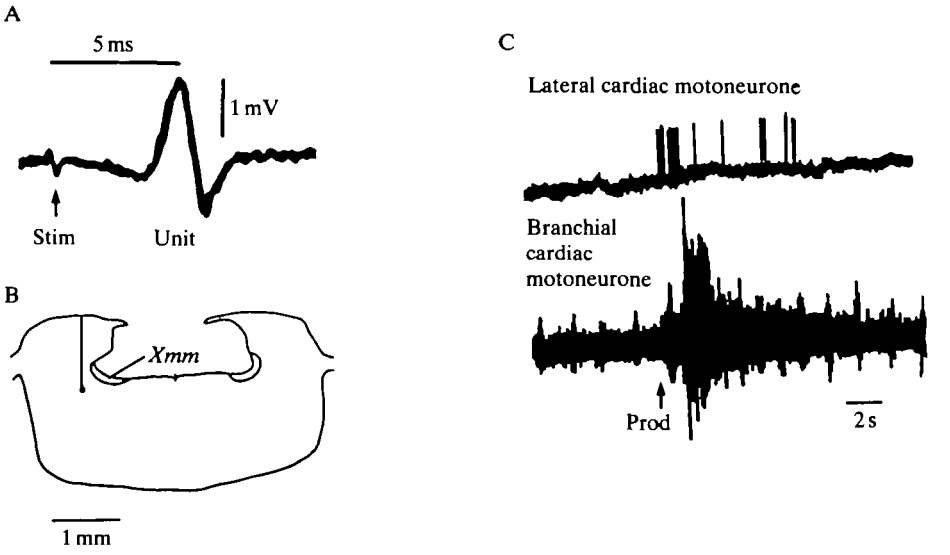

Fig. 3. The location and properties of an antidromically identified silent branchial cardiac motoneurone which could be induced to fire by mechanoreceptor stimulation. (A) An antidromically activated potential from stimulation of the central cut end of the branchial cardiac vagus. Stim, stimulus artefact. (B) A 60-μm transverse section through the hindbrain, just rostral to obex. The recording position from the unit is marked (●) along with the track of the electrode. *Xmm*, medial vagal motor nucleus. (C) The upper trace shows activity from the laterally located unit. Normally there was no spontaneous activity but a prod to the gill septa (Prod) evoked a transient non-rhythmical bout of action potentials from the unit which coincided with the increase in activity in the peripheral recording from the branchial cardiac branch of the vagus (lower trace).

the branchial cardiac branch of the vagus were determined by stimulating the branchial cardiac branch (1–24 V) at frequencies between 0·5 and 500 Hz and recording from the vagal trunk 3·1 cm proximal to the stimulating site.

RESULTS

The location of vagal efferent neurones

In the present study two areas of the medulla were searched for unitary responses during electrical stimulation of the central cut end of the branchial cardiac branch of the vagus. This was based on the dual origin of branchial motoneurones determined by using the retrograde axonal transport of horseradish peroxidase (Barrett & Taylor, 1985*b*). These encompassed an area approximating to the rostromedial and lateral divisions of the vagal motor column. The position of the middle vagal rootlets was used as a guide to the placement of the microelectrode since we know that these contain cardiac efferent fibres (Barrett & Taylor, 1985*b*).

Lateral cardiac vagal motoneurones

Twelve neurones located lateral to the rostromedial division of the vagal motor column were shown to project to the ipsilateral branchial cardiac branch of the vagus by their antidromic responses to electrical stimulation at 0·5 Hz with 0·02-ms pulses.

The threshold voltages for activating these cells fell within the range 0·5–11 V. The latency of these responses, measured from the stimulus artifact, was between 3 and 10 ms. On the basis of conduction distances measured in each fish (mean 5·05 ± 0·2 cm), the calculated conduction velocities were in the range 4·75 to 16·3 m s^{-1} (Fig. 2A).

Three categories of antidromically activated vagal efferent neurones were identified. There were five units which were sporadically active (Fig. 1C) and which increased their firing rate in response to the mechanoreceptive stimulus of a prod to the gill septa. There were two units which were not spontaneously active, but were induced to fire when the gill septa were prodded (Fig. 3). Finally, there were five units that were not spontaneously active and could not be induced to fire by a prod to the gill septa.

The rostrocaudal distribution of these lateral cardiac efferent neurones, determined from serial sections of the hindbrain, which included the pontamine blue dye spot or the *in situ* electrode tip position, was between 0·6 and 0·06 mm rostral to obex with the exception of one unit which was located 1·08 mm rostral to obex. Their mean depth, taken from micrometer readings, was 1·66 ± 0·15 mm (range 0·75–2·0 mm).

Medial cardiac vagal motoneurones

On electrical stimulation of the central cut end of the branchial cardiac branch of the vagus, five vagal efferent neurones were identified within the ipsilateral rostromedial division of the vagal motor column. The threshold voltages for the antidromic activation of these cells fell within the range 4–6 V. The latency of these responses, measured from the stimulus artifact, was in each case either 4 or 5 ms. Calculated conduction velocities based on the conduction distance (mean 5 ± 0·24 cm) were in the range 9·5–12·75 m s^{-1} (Fig. 2B).

All antidromically identified cardiac vagal motoneurones in the rostromedial

Fig. 4. Branchial cardiac motoneurones were found, by their antidromic activation, which exhibited regular tonic activity (middle trace) and contributed two or three action potentials to the regular bursts of efferent activity recorded from the central cut end of the branchial cardiac branch of the vagus (lower trace).

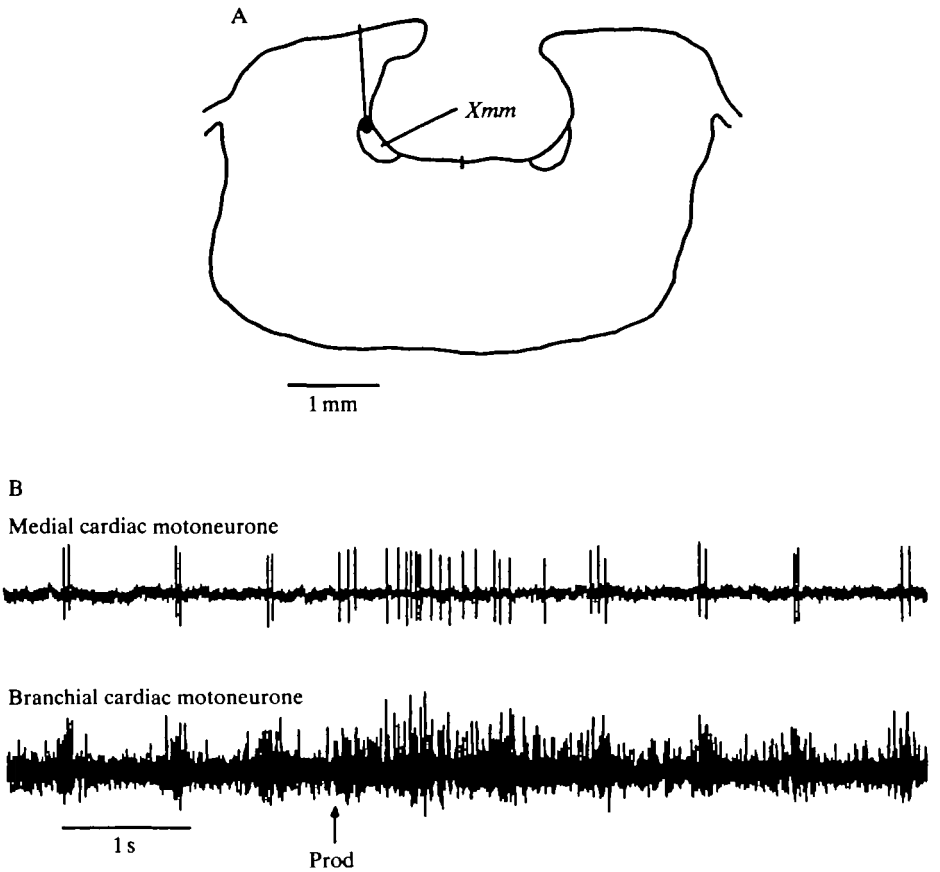

Fig. 5. The location and properties of an antidromically identified rhythmically bursting branchial cardiac motoneurone. On the transverse section through the hindbrain, just rostral to obex, (A) the recording position from the unit is marked (●) along with the track of the electrode. (B) The upper trace shows activity from the medially located unit. Normally the unit fired action potentials in a regular manner coincident with the burst of efferent activity in the branchial cardiac branch of the vagus (lower trace). The administration of the mechanoreceptive stimulus, a prod to the gill septa (Prod), evoked a brief increase in the discharge rate of the unit which was synchronous with the transient increase of activity in the nerve.

division of the vagal motor column were spontaneously active and fired in rhythmic bursts, synchronous with the bursts of action potentials recorded simultaneously from the branchial cardiac branch of the vagus (Fig. 4). Fig. 5 is an example of spontaneous activity from a medial cardiac motoneurone which contributed two spikes to each burst of activity in the nerve and which responded to the mechanoreceptive stimulus of a prod to a gill septum by an increase in firing, which was also reflected in the peripheral efferent activity in the nerve.

The rostrocaudal location was determined for two units both located approximately 120 μm rostral to obex. The search, with subsequent location, for the medial cardiac neurones on three occasions contained a relatively silent period, as the electrode penetrated and traversed the 4th ventricle, before making contact with a medially

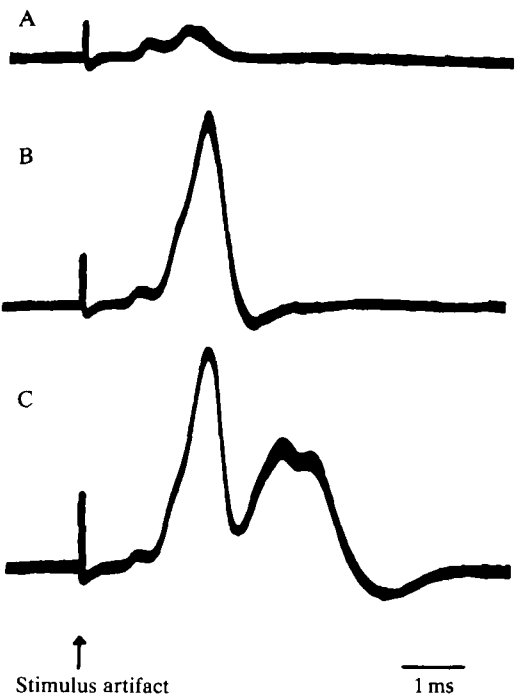

Fig. 6. Responses obtained, recording 3·1 cm proximal to the stimulation site, to stimulating the central cut end of the branchial cardiac branch of the vagus. (A) 6 V; (B) 12 V; (C) supramaximal stimulation, 23 V. The traces are from polaroid photographs of the oscilloscope screen.

located cell. The mean depth, taken from micrometer readings, at which the medial cardiac motoneurones were located was $1{\cdot}3 \pm 0{\cdot}12$ mm (range 1·0–1·75 mm).

Conduction velocities of axons comprising the branchial cardiac branch of the vagus

Stimulation of the branchial cardiac branch of the vagus nerve at gradually increasing voltages (1–24 V), demonstrated that there were four populations of axons with different threshold voltages comprising the branchial cardiac branch (Fig. 6). The first response was activated by a threshold voltage of 5·5 V. Maximal stimulation (23 V) resulted in four responses with latencies of 0·9, 2·0, 3·5 and 4·2 ms. The conduction velocities of these axons, calculated from the conduction distance of 3·1 cm, were 34·4, 15·5, 8·9 and 7·4 m s^{-1} respectively.

DISCUSSION

A point that needs to be answered is whether the units antidromically activated by stimulation of the branchial cardiac branch do indeed represent the activity of cell bodies, or merely that of axons of cells situated elsewhere. A number of identified units showed an inflection on the rising phase of the spike potential (Fig. 3A), indicating that the recordings were from close to cell bodies rather than axons (Eccles, 1964). The mean depth at which the medial and lateral units were recorded, 1·3 and

1·66 mm respectively, correlates well with the location of the cell bodies in the brain determined with HRP histochemistry in the preceding paper (Barrett & Taylor, 1985*b*). In the present series of experiments all electrode penetrations that reached the lateral location of branchial cardiac cells would have passed through the motor nerve tract of the vagus (Barrett, 1984), yet none of the antidromically activated units were found there. While it is not possible to answer unequivocally the question of whether the recordings were from cell bodies or axons, the evidence put forward above provides support for the contention that the recordings were from somata.

The cell bodies contributing axons to the branchial cardiac branch of the vagus nerve have been shown to originate in two locations in the medulla of the dogfish: the rostromedial and lateral divisions of the vagal motor column (Barrett *et al.* 1983*a*; Barrett & Taylor, 1985*b*). The rostrocaudal distribution of the antidromically identified branchial cardiac motoneurones complements the location of these neurones determined by HRP histochemistry (Barrett & Taylor, 1985*b*). Peripheral recordings from the branchial cardiac branch contain two easily distinguishable types of efferent activity (Taylor & Butler, 1982) both of which continue in the paralysed fish (Barrett & Taylor, 1985*a*), implying that they can be centrally generated. The peripheral efferent activity consists of rhythmic bursts of action potentials, synchronous with ventilatory movements or bursts of activity in nerves supplying the expiratory muscles, and sporadically active units which accelerate during hypoxia. The hypothesis that the two locations of branchial cardiac cell bodies were responsible for different peripheral activity in the nerve (Barrett & Taylor, 1985*b*), the medial cells being responsible for the rhythmic bursts of action potentials and the lateral cells for the sporadic units, is supported by the data presented in this paper. The respiratory-related rhythm of the tonic activity of the medial cardiac cells must be of central origin, since in all cases the recordings were from paralysed fish. A central origin for the phasic respiratory modulation of the cardiomotor neurone activity has been shown in the cat (Garcia, Jordan & Spyer, 1978) and postulated in the rabbit (Jordan, Khalid, Schneiderman & Spyer, 1982). The identification of sporadically active lateral units, which accelerate during an induced bradycardia, also confirms the hypothesis that different activities characterize each of the central locations of branchial cardiac motoneurones. The presence of two other types of lateral units, the silent cells which can be induced to fire in response to a mechanoreceptive stimulus and the silent cells which cannot be induced to fire in response to this stimulus, results perhaps not surprisingly, in a more complex situation than had previously been proposed.

Two of the types of lateral cardiac motoneurones and the medial cardiac cells, respond to a mechanoreceptive stimulus. Study of the peripheral efferent activity in the branchial cardiac branch does not allow discrimination between the units contributing to the response to a prod of the gill septa (i.e. the bursting and the sporadically firing units). This study has revealed that both types of units fire in response to this stimulus. This has interesting implications for the local circuitry of these neurones. It is possible that there is an excitatory input to the medial branchial cardiac motoneurones from the adjacent branchial vagal motoneurones which fire during expiration (Barrett & Taylor, 1985*a*). If this is the case, then the tonic respiratory-related activity of the medial branchial cardiac motoneurones and their response to the gill septa prod are to be expected. The prod of the gill septa would be

relayed along the afferent branchial neurones to respiratory rhythm generator cells, which in turn would affect the branchial motoneurones. This pathway is, however, unlikely to impinge on the lateral branchial cardiac motoneurones as they are not active in a respiratory-related manner. It is possible, therefore, that a pathway exists from either direct afferent terminations of neurones innervating gill mechanoreceptors or *via* interneurones. A similar pathway could also exist to the medial branchial cardiac motoneurones. In the dogfish, branchial vagal and glossopharyngeal motoneurones are confined to a medial location (Barrett *et al.* 1984), whereas in mammals respiratory vagal motoneurones are located in the nucleus ambiguus, the dorsal motor vagal nucleus and the region between these nuclei (e.g. Bennett, Ford, Kidd & McWilliam, 1982).

The increase in activity in the branchial cardiac branch of the vagus radiating from both the medial and lateral motoneurones in response to a prod of the gill septa is accompanied by a transient bradycardia, implying that both of the central locations of the branchial cardiac motoneurones are responsible for the chronotropic responses of the heart. The cardiac vagi of the dogfish are thought solely to exert a chronotropic effect on the heart. The only increases in stroke flow that occur in normal circumstances are thought to be a manifestation of Starling's Law of the heart (Short, Butler & Taylor, 1977). This is different to the situation found in mammals where the cardiac vagus has both a negative chronotropic and inotropic effect (e.g. Middleton, Middleton & Grundfest, 1950).

Another important difference between the cardiac vagi of dogfish and mammals is that those in the dogfish contain only myelinated axons (Short *et al.* 1977), whereas those in mammals contain both myelinated and unmyelinated axons (Agostini, Chinnock, Daly & Murray, 1957). Furthermore, it is proposed that in mammals, the vagal neurones with unmyelinated axons are those located in the dorsal vagal motor nucleus, which may be responsible for inotropic control of the heart (Spyer, 1982) and that the myelinated axons arise from cells located in the nucleus ambiguus (Geis & Wurster, 1980). The dual origin of dogfish cardiac vagal motoneurones cannot be attributed to different gross peripheral functions, nor is it reflected in the nature of their fibres.

The conduction velocities of the axons supplied by the lateral, and more particularly, the medial branchial cardiac neurones fall within the range described for mammalian B fibres (Iriuchijima & Kumada, 1963; Jewett, 1964; Katona, Poitras, Barnett & Terry, 1970; Kunze, 1972; McAllen & Spyer, 1976, 1978*a*; Jordan *et al.* 1982) and definitely above the range of cardiac unmyelinated fibres reported by Bennett, Ford, Kidd & McWilliam (1984). It is, however, important to realise that conduction temperature will be much lower in the poikilothermic dogfish compared to mammals, but this is unlikely to alter severely the broad statement made above.

Four populations of axons, with different conduction velocities, were identified as contributing to the branchial cardiac vagus. Although the central location of branchial cardiac motoneurones resulted in similar conduction velocities to three of the populations identified, no units were found, either medially or laterally, whose conduction velocity corresponded with the population that had the fastest conduction velocity.

Thirty percent of the cardiac motoneurones antidromically activated had no natural, or induced, spontaneous activity. All of these silent cells were located in the lateral division of the vagal motor column. The role of these cells in cardiac responses

can only be speculated upon. Ideally this study would have included systematic testing of the response of these neurones to a chemoreceptive stimulus such as hypoxia, which is monitored in the dogfish by oxygen receptors distributed widely in the orobranchial cavity (e.g. Butler, Taylor & Short, 1977) and possibly in the venous system (Barrett, Roberts & Taylor, 1983*b*; Barrett & Taylor, 1984*a*). The spontaneously active medial and lateral branchial cardiac motoneurones may also be affected by chemoreceptive input.

As no other work has been reported on the location and properties of cardiac motoneurones in lower vertebrates, this has necessitated frequent comparison with the abundant data available from mammalian studies. One of the prime stimuli for the excitation of mammalian cardiac motoneurones is the baroreceptor reflex. Bilateral section of cranial nerves IX and X in the dogfish (physiologically similar to a reduction in blood pressure) had no effect on heart rate, implying that baroreceptor reflexes are not important in determining heart rate (Butler *et al.* 1977). Experiments in which blood volume was either reduced or increased (Barrett & Taylor, 1984*a*) caused a fall or rise in blood pressure with no compensatory chronotropic response, also implying that baroreceptors do not play an important role in determining heart rate in the dogfish.

In mammals cardiac vagal motoneurone activity can be affected by respiratory influences, arterial baroreceptors or the hypothalamus (see for example, McAllen & Spyer, 1978*b*; Coote, Hilton & Perez-Gonzalez, 1979; McCloskey & Potter, 1981; Potter, 1981; Spyer, 1981, 1982). In the dogfish, cardiac vagal motoneurone activity is affected by respiratory influences, mechanoreceptor stimulation (which may act indirectly *via* respiratory influence) and probably by chemoreceptor stimulation. An influence from higher centres in the dogfish brain remains to be investigated. The application of more detailed electrophysiological, pharmacological and neuroanatomical techniques to the study of the cardiac vagal motoneurones in the dogfish will enable further elucidation of the local circuitry controlling the output of cardiac vagal motoneurones and its subsequent effect on the heart, in higher and lower vertebrates.

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