VAGAL PREGANGLIONIC INNERVATION OF THE CAT HEART* **

—An attempt to identify the medullary 'heart area' by the retrograde axoplasmic transport of horseradish peroxidase—

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The medullary 'heart area', origins of the vagal preganglionic fibers innervating the heart, were identified in 19 cats with the use of horseradish peroxidase (HRP) as a retrograde axonal tracer.

When HRP was injected in the A-V node area, S-A node area, right atrial wall and right ventricular wall, the labeled neurons were principally found in the lateral half of the dorsal motor nucleus (DMN) and in the medial solitary nucleus (MSN), were almost the same in number bilaterally, and were chiefly from the calamus scriptorius to the upper cervical cord. They showed no anatomical proof for asymmetric distribution. When HRP was injected in the left ventricular wall or the apex, few labeled neurons were identified in the DMN. Labeled neurons were not found in the nucleus ambiguus.

This study suggests the labeled neurons in the DMN and MSN are origins of the dually innervating vagal preganglionic fibers to the heart.

THE cardioinhibitory parasympathetic nerves are believed to originate from the dorsal motor nucleus of the vagus located in the medulla oblongata.^{14,27,51} This concept is based on the observation of retrograde neuronal degeneration in the nucleus following cardiac denervations. However, there is strong physiological evidence suggesting that the dorsal motor nucleus is not a major source of visceromotor fibers to the heart. In fact its electrical stimulation does not produce

bradycardia^{1,7,10,28} and bilateral destruction of the nucleus does not abolish vagal cardioinhibitory reflexes⁷ nor affect the responses obtained by vagus nerve stimulation.³⁹ Some authors believe vagal preganglionic fibers to the heart originate in the nucleus ambiguus,^{40,68} whereas others believe excitation of the nucleus ambiguus neurons probably involves interneurons in the nucleus of the tractus solitarius.^{55,69} Therefore, the presence of a topographically organized 'heart area' in the medulla oblongata is still a subject of controversy since the results of retrograde neuronal degeneration studies are not conclusive and are contradictory to electrophysiological studies.

Since Ellison and Clark²¹ indicated that the horseradish peroxidase (HRP), an exogenous marker protein, is a useful retrograde tracer for various autonomic nervous pathways, HRP was

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injected in the sinoatrial (S-A) and atrioventricular (A-V) node areas, the lower part of the atrial wall, right and left ventricular walls and the apex of the adult cat heart to demonstrate the origins of the vagal preganglionic fibers to the cardiac ganglia. Labeled neurons containing retrogradely transported HRP in their perikarya were visualized by diaminobenzidine (DAB) reaction and were identified in the medulla oblongata. This paper reports the experimental results to indicate the 'heart area' in the medulla oblongata, location of which, however, does not conform fully with previous observations made by physiological and morphological methods.

MATERIALS AND METHODS

Nineteen adult cats weighing 2 to 5 kg were used (Table I). After atropin sulfate (0.2 mg, I.M.) was administered, they were placed into a plastic box filled with ether. When the anesthesia

was induced, an intravenous infusion was started and a endotracheal tube was connected to a intermittent positive pressure respirator.⁷⁰ For maintenance of anesthesia 3 mg/kg of sodium thiopental was administered intravenously. A right fifth intercostal thoracotomy was made. The first group (5 cats) received heparin intravenously (1 mg/kg) and positive pressure hyper-ventilation, after opening the pericardial sac. The superior and inferior vena cavae were then temporarily (4 to 9 min.) clamped by arterial forceps. After the right atriotomy (Fig. 1), the atrial septum, the ostium of the coronary sinus, the tricuspid valve and the ventricular septum were identified. A freshly prepared 100 mg of HRP (type II; Sigma Chemical Co.) was diluted to a 30–50% concentration in normal saline and was injected in the A-V node area using a Hamilton microsyringe with a 27 gauge needle. The atriotomy and thoracotomy were closed in

TABLE I MATERIALS WHICH WERE INJECTED HRP IN VARIOUS PARTS OF THE HEART

Case No.	Sex	Body weight (kg)	Anesthesia	Injected HRP (mg)	Interruption of the venous return (min.)	Injected heparin (mg)
SA- 1	M	4.5	PB	40		
SA- 4	F	2.5	TP	40		
SA- 5	F	3	TP	40		
SA- 7	F	3	TP	40		
SA- 8	F	2.5	TP	40		
SA- 9	F	3	TP	40		
SA-11	M	3	TP	40		
AV-13	M	2.2	TP	100	6	2
AV-15	F	2.8	TP	100	5	3
AV-16	M	1.7	TP	50	5	2
AV-17	M	5	TP	100	5	5
AV-18	F	3	TP	100	5	3
RA-21	M	3	TP	100		
RA-22	F	4	TP	100		
RA-23	M	2	TP	100		
RV-24	M	4	TP	100		
LV-25	M	2.2	TP	100		
AP-26	M	5	TP	100		
AP-27	F	2.8	TP	100		

SA-: cat which was injected HRP in the S-A node, AV-: cat which was injected HRP in the A-V node, RA-: cat which was injected HRP in the subepicardium of the right atrium, RV-: cat which was injected HRP in the subepicardium of the right ventricle, LV-: cat which was injected HRP in the subepicardium of the left ventricle, AP-: cat which was injected HRP in the apex of the heart, PB: sodium pentobarbital, TP: sodium thiopental.

Figs. 1a and b. a: A schematic illustration showing the procedure for opening the right atrium. After a right thoracotomy was made, four sets of stay sutures were placed on the atrial wall. The superior and inferior vena cavae (SVC and IVC) were temporarily clamped by two pairs of arterial forceps and then a right atriotomy was made for about 1 cm. (cited from Todo et al., *Acta Anat. Nippon.* 52: 197, 1977). b: A photograph showing an atriotomy. HRP was injected in the atrioventricular node area using a Hamilton microsyringe under direct vision.

Fig. 2. The heart which had been injected HRP in the A-V node area was incubated overnight and the area of injection was identified (arrowhead). SVC, superior vena cava; RA, right atrium; CS, orifice of coronary sinus; IVC, inferior vena cava; ANT. CUSP TV., anterior cusp of tricuspid valve; MEMB. SEPT., atrioventricular part of membranous septum; MED. CUSP TV., medial cusp of tricuspid valve; RV, right ventricle.

this order. The second group (7 cats) were injected HRP (40–100 mg) in the S-A node area. The third group were injected in the right atrial wall (3 cases), the right ventricular wall (1 case), the left ventricular wall (1 case) and the apex (2 cases). Then, the thoracotomy was closed, lungs were inflated until sufficient spontaneous respiration was restored, and the endotracheal and chest

tubes were removed. As a control, 5 cats received intravenous injection of 40–100 mg of HRP. The cats were sacrificed two days later by intracardiac perfusion of 2% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer (pH 7.3). The brain stem and the upper cervical spinal cord were removed and stored overnight in wash buffer with 20% sucrose. Frozen sections of

Figs. 3a and b. The locations of labeled neurons in the medulla oblongata and the upper cervical spinal cord after injections of HRP in the A-V node area (a: 5 cases) and in the S-A node area (b: 7 cases). The abbreviations in this and subsequent figures are as follows: AP, area postrema; D, dorsal motor nucleus; G, nucleus gracilis; H, hypoglossal nucleus; TS, tractus solitarius. The darkened part of the illustrated heart is the HRP injected site.

60 μ m thick, were made transversely through the medulla oblongata and upper cervical cord and were processed in 3, 3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide dissolved in phosphate buffer (pH 7.3, 37°C, 40 min.).

The heart was also incubated overnight in the same DAB solution and the area of HRP injection was identified (Fig. 2). The sections were mounted on gelatin-coated slides and lightly counterstained with cresyl violet acetate. Observation was made in bright- and dark-field illumination and a projection drawing apparatus was utilized for delineating nuclear configurations on tracing paper and for plotting HRP labeled neurons.

RESULTS

Group 1. In 5 cats which received 100 mg of HRP injection in the A-V node area, HRP labeled neurons were distributed bilaterally in the dorsal motor nucleus of the vagus (Fig. 3a). They appeared stippled due to fine brownish granules

in their perikarya (Fig. 5b), and, in dark-field illumination these neurons were brightly demonstrated by HRP reaction products (Fig. 5d). This conformed with the criteria of the retrogradely labeled neurons by HRP⁴⁵. The intensity of the reaction products was generally not marked. Therefore, for their visual confirmation, 400–1000 magnification was required. The majority of the labeled neurons were found evenly in the bilateral caudal portion of the dorsal motor nucleus below the calamus scriptorius. They were principally distributed in the lateral half of the dorsal motor nucleus (Fig. 5a), although some were found in the medial portion as well (Fig. 5c). In addition, HRP labeled neurons were found in the bilateral medial nucleus of the tractus solitarius, caudal to the level of the calamus scriptorius (Fig. 3a). The caudal medial solitary nucleus is composed of loosely packed neurons of small diameter containing weakly stained cytoplasm and oval nuclei in standard Nissl preparations. They are easily distinguished

from the large and more strongly stained neurons in the dorsal motor nucleus. In the medial solitary nucleus, the labeled neurons tended to gather not close to the central canal but mainly in the area near the tractus solitarius (Fig. 6a). Labeled neurons were also identified in the caudalmost medial solitary nucleus where the dorsal motor nucleus was no longer present and the central gray of the spinal cord was gradually replacing the nucleus (Fig. 6b). The retrogradely labeled neurons in the medial solitary nucleus were generally weakly labeled but were more in number than those in the dorsal motor nucleus. No labeled neurons were found in the nucleus ambiguus.

Group 2. In 7 cats which received 40–100 mg of HRP injection in the S-A node area, labeled neurons were distributed bilaterally in the dorsal motor nucleus (Fig. 3b). While the neurons were present chiefly below the calamus scriptorius, some were found infrequently above this level. HRP labeled neurons were also found in the medial nucleus of the tractus solitarius, below the calamus scriptorius (Fig. 3b), in an area medial to the tractus solitarius and immediately dorsal to the dorsal motor nucleus. Some were found in the immediate vicinity of the tractus

solitarius as well. The neurons in the medial solitary nucleus were generally more weakly labeled but more in number than those in the dorsal motor nucleus (Figs. 6c and d). They were also found in the transitional zone where the dorsal motor nucleus was no longer present and the medial solitary nucleus gradually merged into the central gray of the upper cervical spinal cord. Labeled neurons were not observed in the nucleus ambiguus.

Group 3. In 3 cats which received 100 mg of HRP injection in the anterior right atrial wall and in one cat in the anterior right ventricular wall, labeled neurons were observed, similar to groups 1 and 2, in the bilateral dorsal motor and medial solitary nuclei below the calamus scriptorius (Fig. 4). The labeled neurons were distributed essentially in accord with the cases of S-A and A-V node injections. However, in the cases which received 100 mg of HRP injection in the left ventricular wall (1 case) and in the apex of the heart (2 cases), only two HRP labeled cells were observed in the dorsal motor nucleus of one of the cats (apex case). But labeled neurons were observed neither in the dorsal motor nucleus nor in the medial solitary nucleus in the other two cats.

Figs. 4a and b. The locations of labeled neurons in the medulla oblongata and the upper cervical spinal cord after injections of HRP in the subepicardium of the right atrial wall (a: 3 cases) and the right ventricular wall (b: 1 case).

Figs. 5a–d. HRP labeled neurons in the dorsal motor nucleus (AV-15). These labeled neurons were principally distributed in the lateral half of the dorsal motor nucleus (a: arrowhead), although some were found in the medial portion as well (c: arrowhead). b: A higher magnification view of the HRP labeled neuron in 5a. d: A higher magnification view of the neuron in 5c. (a, c, d: dark-field illumination, b: bright-field illumination). a: $\times 55$, b: $\times 670$, c: $\times 117$, d: $\times 670$.

Group 4. In 5 cats which received 40–100 mg of HRP as a single intravenous injection, labeled neurons were found neither in the dorsal motor nucleus nor in the medial solitary nucleus.

DISCUSSION

On the method employed

The HRP method was originally described by Kristensson and Olsson^{41,42}. They had observed HRP labeled neurons in the hypoglossal nucleus after the injection of HRP into the rat tongue muscles. This was presumably due to retrograde intraaxonal transport of tracer protein molecules. Since then many studies with the use of the HRP method have appeared^{2,21,35,53,77} although the precise mechanism of retrograde axoplasmic transport of HRP is not clear. Ellison and Clark²¹ demonstrated that HRP was taken up by the peripheral autonomic nerve endings and that the marker protein could be identified in the neuronal somata which give rise to preganglionic fibers. Since then, the HRP method has been applied to

studies of autonomic nervous pathways^{12,63,71,76}. However, this is still a new technique and the interpretation of HRP labeled neurons deserves some comments. First, there is a possibility of blood-born HRP in the neurons. Broadwell and Brightman⁸ reported that highly concentrated HRP in the blood stream may be taken up by the nerve terminals and result in the retrograde labeling of certain groups of neurons. Some^{37,75} have even stated that several brain areas consistently contain cells with DAB staining granules, even in control animals without HRP injection. In my experimental studies, none of the cats receiving HRP intravenously showed labeled neurons in the nucleus. Therefore, the labeling was due to retrograde transport of HRP from the vagus nerve. Second, the survival period after HRP injection should be considered. As it has been known that the retrograde transport of HRP is in excess of 120 mm per day^{41,44} and the distance between the heart and the medulla oblongata in the adult cat is approximately 150–

Figs. 6a–d. a: HRP labeled neurons in the medial nucleus of the tractus solitarius (arrowheads). The labeled neurons tended to gather in the area near the tractus solitarius (AV-17). b: A labeled neuron was also observed in the caudal end of the medial solitary nucleus where the dorsal motor nucleus was no longer identifiable and the central gray of the spinal cord was gradually replacing the nucleus (AV-17). d: HRP labeled neurons in the medial solitary nucleus (arrowhead) were generally more weakly labeled than those in the dorsal motor nucleus as pointed to by the double arrowhead (SA-8), but in Fig. 6c they were more numerous than those in the dorsal motor nucleus (SA-9). (a, b, c: dark-field illumination, d: bright-field illumination). a: $\times 280$, b: $\times 280$, c: $\times 280$, d: $\times 300$.

200 mm, the cats were sacrificed on the 2nd or 3rd day after injection of HRP. A third problem is the locations of the injection and diffusion of HRP. The cardiac ganglia are scattered around the origins of the major vessels, in the atrial subepicardium, S-A node area, atrial septum and A-V node area.^{25,27} Therefore, all of these areas presumably receive vagal preganglionic fibers. The reason why the author had selected only the S-A and A-V node areas and the atrial and ventricular subepicardium for the study was because only these areas were amenable to HRP injection without diffusion to the adjacent structures. This means that this study did not disclose to the full extent the vagal preganglionic innervations of the cat heart.

Origins of the vagal preganglionic fibers to the heart

It has been understood that the dorsal motor nucleus gives rise to efferent vagal preganglionic fibers in many species including the cat.^{14,27,54}

In anatomical studies neurons representing vagal cardioinhibitory function are thought to be partly localized in the dorsal motor nucleus^{11,47,51} or, on the contrary, in the region of the nucleus ambiguus.^{40,68} On the basis of physiological studies in dogs, vagal cardioinhibitory fibers arise from both the dorsal motor nucleus and the region of the nucleus ambiguus.^{28,49} In cats, they are found in the region of the nucleus ambiguus,^{1,10,28,39} although there have been no retrograde degeneration studies to support these physiological observations. Efferent fibers from the nucleus ambiguus to the vagus nerve have been clearly described in the cat,⁷⁴ but only a few authors have stated on anatomical grounds, that the nucleus ambiguus contains cardioinhibitory nerves.^{40,68} The author has observed the HRP labeled neurons in the medial nucleus of the tractus solitarius as well as in the dorsal motor nucleus of the vagus but not in the nucleus ambiguus. This may indicate that vagal preganglionic

fibers to the heart partly originate from the dorsal motor nucleus as reported by some other authors previously,^{14,27,51} but not from the nucleus ambiguus. A recent study with the HRP method suggests the nucleus ambiguus innervates the striated muscles in the larynx, pharynx and upper esophageal wall,²¹ whereas the dorsal motor nucleus may innervate the stomach^{61,76} and the lower alimentary canal²⁷ via vagal preganglionic fibers. The distribution of dorsal motor nucleus neurons to the stomach was reported to be principally in the medial portion of the nucleus.⁷⁶ However, the author has found labeled neurons in the lateral caudal dorsal motor nucleus. This indicates the 'heart area' may exist in the lateral portion of the dorsal motor nucleus. The presence of labeled neurons in the medial nucleus of the tractus solitarius was unexpected although a similar finding was observed in the HRP study of the cat stomach.⁷⁶ Therefore, it can be said that the vagal preganglionic fibers to the cardiac ganglia originate from both dorsal motor and medial solitary nuclei.

On the physiological considerations

The parasympathetic preganglionic nerve endings may be found in the subepicardial tissue of the atria and auricular appendages, around the roots of the great vessels, along the interatrial and atrioventricular sulci, in the interatrial septum and in the vicinity of the S-A and A-V nodes. At first, the cardiac ganglion cells were thought to be localized in the atrial walls,¹⁸ particularly in the proximity of the S-A and A-V nodes,³⁶ but more recently, they have also been found in smaller numbers in the ventricular subepicardium.^{3,50,58,65,66}

Physiologists have generally accepted the fact that the right vagus acts mainly in the S-A node and the left vagus on the A-V node.^{24,26,60} The right vagus has a profound negative chronotropic effect and little negative dromotropic effect on conduction through the A-V node, whereas the left vagus exerts its effects dominantly on the A-V node.¹³ Hamlin and Smith³¹ have observed in dogs that stimulation of the left or right vagus equally slowed heart rate, although a section of the left vagus did. They, therefore, concluded that, although bilateral vagi have equal physiologic distribution to the S-A node, activity over the right vagus normally contributes more than the left. On the other hand, Bachmann⁴ indicated that the relation between both vagi and the various ganglion cell groups in S-A junction are widely different in species of animals. The

author has observed even distribution of the labeled cells on both sides in the dorsal motor and the caudal medial solitary nuclei. Thus, there is no anatomical evidence suggesting that the right vagus acts more on the S-A node and the left on the A-V node in the cat. There is no significant difference between the S-A and A-V node areas in distribution of the neurons in the dorsal motor and medial solitary nuclei.

Although the cardioinhibitory character of the peripheral vagal fibers has been well demonstrated,^{19,20,67,73} the stimulation of the dorsal motor nucleus itself has failed to express vagal effects^{7,39,69} with the exception of Cohen and Schenalls' work.¹⁵ Bilateral destruction of the dorsal motor nucleus has also failed to alter the visceromotor function of the vagus nerves.³⁸

The rostral half of the solitary nucleus is thought to be gustatory and the caudal half is visceral sensory in function.^{16,34,72} However, the solitary nucleus may not be a simple sensory nucleus. The silver degeneration study of the caudal solitary nucleus by Morest⁵⁵ did not show any ascending projections to the thalamus. This nucleus is, therefore, different from many other secondary sensory nuclei which do maintain thalamic connections. The solitary nucleus is also known to contain descending components to the spinal cord, the solitariospinal tract.^{9,43} A portion of the medial solitary nucleus adjacent to the area postrema called area subpostrema^{29,30} appears to contain vagal efferent neurons, since specific and pseudocholinesterase activity diminish rapidly following the axotomy of the vagus nerve.⁵⁶ Thus the caudal solitary nucleus may contain heterogenous groups of neurons, some of which may lead to vagal preganglionic fiber.

The author indicated that the cat heart is dually innervated by the dorsal motor and caudal medial solitary nuclei (Fig. 7). The dorsal motor nucleus may not have cardioinhibitory function but the preganglionic neurons may have some other visceral functions such as those of the smooth muscles of the coronary arteries.^{5,23} The medial solitary nucleus may supply visceromotor preganglionic fibers to the cardiac ganglion cells which regulate cardiomotor functions through the cardiac conduction system. Electrical stimulation of nucleus solitarius has been shown to elicit vagal bradycardia. This has been thought to be due to stimulation of primary afferent fibers at baroreceptors and chemoreceptors which are known to terminate in the nucleus of the tractus solitarius.^{6,17,52,60,65} However, by

Fig.7. A schematic drawing of the nervous pathways of the present study. The results show that the cat heart is dually innervated by the dorsal motor and caudal medial solitary nuclei bilaterally. N.X., nervus vagus; D.M.N., dorsal motor nucleus; N.T.S., nucleus tractus solitarius.

the antidromic collision technique, Harding and Leek³² demonstrated that the visceromotor preganglionic neurons certainly exist in the area dorsal and lateral to the dorsal motor nucleus, which corresponds to the medial solitary nucleus. *On functional implications*

Regulations of heart rate by direct stimulations of nerve elements or by sympathectomy have been tried experimentally and clinically. Value of cervico-thoracic sympathectomy for paroxysmal ventricular tachycardia was supported by reduced incidence of ventricular arrhythmias and death following sympathectomy in experimental myocardial infarction.^{33,62} Bilateral cervico-thoracic sympathectomy has also been reported as successful therapy for ventricular tachycardia

in man.^{22,48,78} Lazzara et al.⁴⁶ reported the method for the selective alteration of parasympathetic control over the S-A or the A-V node by differential electric stimulations in anesthetized dogs. Therefore, it appears possible to alter the cardiac functions in normal and diseased status by controlling the vagal and sympathetic outflow to the heart. This will, however, require precise knowledge on the central connectivity of the autonomic nervous system for the heart.

The dual 'heart area' in the medulla oblongata demonstrated by the HRP study here does not conform fully to the previous anatomical and physiological observations and this may give a clue to further investigation on its cardiac effects. This might include the effects not only upon cardiac conduction and contractility but also the behavior of the smooth muscles of the coronary vessels.^{5,23}

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