# Horseradish Peroxidase Localization of Sympathetic Postganglionic and Parasympathetic Preganglionic Neurons Innervating the Monkey Heart

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## Abstract

**The localization of the sympathetic postganglionic and parasympathetic preganglionic neurons innervating the monkey heart were investigated through retrograde axonal transport with horseradish peroxidase (HRP). HRP (4 mg or 30 mg) was injected into the subepicardial and myocardial layers in four different cardiac regions. The animals were euthanized 84-96 hours later and fixed by paraformaldehyde perfusion *via* the left ventricle. The brain stem and the paravertebral sympathetic ganglia from the superior cervical, middle cervical, and stellate ganglia down to the T9 ganglia were removed and processed for HRP identification. Following injection of HRP into the apex of the heart, the sinoatrial nodal region, or the right ventricle, HRP-labeled sympathetic neurons were found exclusively in the right superior cervical ganglion (64.8%) or in the left superior cervical ganglion (35%). Fewer labeled cells were found in the right stellate ganglia. After HRP injection into the left ventricle, labeled sympathetic cells were found chiefly in the left superior cervical ganglion (51%) or in the right superior cervical ganglion (38.6%); a few labeled cells were seen in the stellate ganglion bilaterally and in the left middle cervical ganglion. Also, in response to administration of HRP into the anterior part of the apex, anterior middle part of the right ventricle, posterior upper part of the left ventricle, or sinoatrial nodal region, HRP-labeled parasympathetic neurons were found in the nucleus ambiguus on both the right (74.8%) and left (25.2%) sides. No HRP-labeled cells were found in the dorsal motor nucleus of the vagus on either side.**

**Key Words: sympathetic postganglionic neurons, parasympathetic preganglionic neurons, horseradish peroxidase (HRP), monkey heart**

## Introduction

We described in a previous paper (11) how the application of HRP in cats to the apex of the heart, the ventral wall of the right ventricle, the dorsal wall of the left ventricle, and the sinoatrial nodal region

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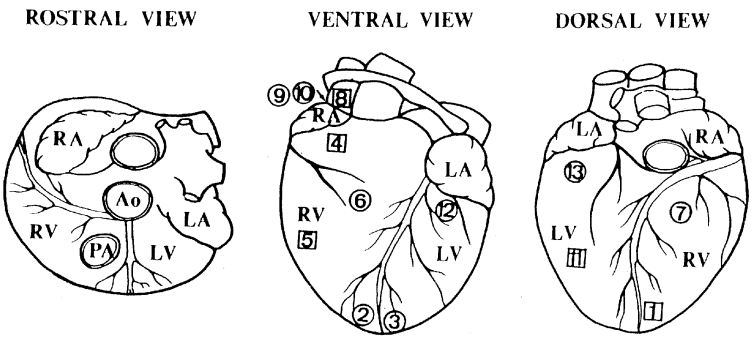


Fig. 1. Quadrangle and circle represent HRP injection sites at the apex (AP), the right ventricle (RV), the left ventricle (LV), and the sinoatrial nodal region (SA). RA: right atrium. LA: left atrium. Ao: aorta. Pa: pulmonary artery. □: 4 mg HRP. ○: 30 mg HRP.

revealed a localization of HRP-labeled sympathetic postganglionic neurons predominantly (more than 88.5%) in the stellate ganglion on both sides. Only a small number of labeled cells (fewer than 11.5%) were observed in the superior and middle cervical ganglia and in the fourth, fifth, sixth, eighth, and ninth thoracic ganglia. By contrast, in dogs the sympathetic postganglionic neurons innervating the heart have been found more in the middle cervical ganglion bilaterally (3, 5, 10). The difference may be a result of species variation. In cats, the stellate ganglion contains the inferior cervical and the first, second, and third thoracic ganglia; likewise, in dogs the stellate ganglion comprises the inferior cervical and the first and second thoracic ganglia. In rhesus monkeys, the stellate ganglion includes the inferior cervical and the first thoracic ganglia only (7, 8, 9). Since in some respects cardiac innervation in the monkey resembles that of humans (9), it is worth investigating the autonomic neurons innervating the heart in the Taiwan monkey (*Macaca cyclopis*) using HRP retrograde localization. We hope that the results may provide fundamental insights for the surgical treatment of cardiovascular diseases in humans (12).

## Materials and Methods

*Experimental Procedures*

Sixteen adult male and female Taiwan monkeys weighing 4.0-8.0 kg were used. They were divided into five groups. Under aseptic conditions, all were anesthetized with sodium pentobarbital (35 mg/kg, i.p.), tracheostomized, and artificially ventilated. Additional anesthetic was administered as needed. A right or left thoracotomy at the fourth or fifth intercostal space was made and the pericardium was opened. A total of 4 mg or 30 mg of horseradish peroxidase (HRP; Sigma, Type VI) in saline was injected subepicardially (3, 10) with a 10 µl Hamilton syringe at the apex of the heart (Group 1), the right ventricular wall (Group 2), the left ventricular wall (Group 3), the sinoatrial nodal region (Group 4), or the pericardial sac (Group 5). Injection sites and doses are shown in Figure 1. After the pericardium was closed, a chest intubation with underwater seated drainage was made, and the wound sutured. The animal was given 500 mg of ampicillin intramuscularly and was allowed to survive 84-96 hours.

*Histochemical Procedures*

The monkey was reanesthetized with sodium pentobarbital (40-50 mg/kg, i.p.) and perfused through the left ventricle or the ascending aorta, first with 2,000 ml of 0.9% saline, then with 3,000 ml of fixative (1.25% glutaraldehyde + 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) at room temperature, and subsequently with 2,500 ml of 10% sucrose in a 0.1 M phosphate buffer (pH 7.4) at 4°C. The paravertebral sympathetic ganglia from the superior cervical, middle cervical, and stellate ganglia down to the T9 ganglion were identified and removed under a dissecting microscope. The brain stem from the apex to the upper part of the striae medullares were also removed, as well as the dorsal root ganglia from C5 to T5. All removed materials were stored in a fresh 10% sucrose-phosphate buffer at 0-4°C for 24 hours, then sectioned to 40 µm thick with a cryostat freezing microtome at -20°C. Free-floating sections were incubated with tetramethyl benzidine (Sigma, ST, Louis, MO, USA) following the procedure described by Mesulam (7). The sections were counterstained with a 1% neutral red solution. Labeled cells, identified carefully in each section by the presence of a fine, dark blue HRP reaction product, were scanned at a magnification of 150x and counted at 600x under a light microscope.

## Results

Group 1: Following injection of HRP into the

**Table 1. Summary of application sites and number of retrogradely labeled neurons in sympathetic ganglia and nucleus ambiguus in the hearts of four groups of monkeys.**

| Group | No. of monkey | Injection location | SCG | | MCG | | SG | | Nu ambiguus | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | R | L | R | L | R | L | R | L |
| 1 | 1 | Apex | 340 | 280 | 0 | 0 | 0 | 0 | 0 | 0 |
| AP | 2* | Apex | 312 | 108 | 0 | 0 | 0 | 0 | 38 | 26 |
| | 3* | Apex | 74 | 29 | 0 | 0 | 7 | 0 | 0 | 0 |
| 2 | 4 | Anterior upper | 254 | 126 | 0 | 0 | 0 | 0 | 0 | 0 |
| RV | 5 | Lateral | 102 | 84 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6* | Anterior middle | 312 | 202 | 0 | 0 | 0 | 0 | 40 | 12 |
| | 7* | Posterior middle | 256 | 98 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 8 | SA node | 408 | 208 | 0 | 0 | 0 | 0 | 0 | 0 |
| SA | 9* | SA node | 284 | 142 | 0 | 0 | 0 | 0 | 56 | 14 |
| | 10* | SA node | 176 | 82 | 0 | 0 | 0 | 0 | 20 | 0 |
| 4 | 11 | Posterior middle | 140 | 208 | 0 | 0 | 0 | 0 | 0 | 0 |
| LV | 12* | Anterior upper | 117 | 129 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 13* | Posterior upper | 0 | 3 | 0 | 18 | 23 | 27 | 1 | 0 |

*Thirty mg HRP injection into the subepicardial and intramuscular regions SCG: superior cervical ganglion. MCG: middle cervical ganglion. SG: stellate ganglion. Nu ambiguus: nucleus ambiguus. R: right. L: left. AP: apex. RV: right ventricle. SA: sinoatrial node. LV: left ventricle.

apex of the heart (Fig. 1) in three monkeys, labeled sympathetic postganglionic neurons were found to be localized in the superior cervical ganglion on both sides (Table 1). The right superior cervical ganglion appeared more heavily labeled (63.1% of all labeled cells) than the left (36.3% of all labeled cells). Considerably fewer cells were labeled in the right stellate ganglion in one of the three monkeys than in the other two. On the other hand, labeled parasympathetic neurons were observed in one of the two monkeys given 30mg of HRP, all of them localized in the nucleus ambiguus on both sides. The right nucleus ambiguus (59.4%) appeared more heavily labeled than the left (40.6%). No HRP-positive neurons were found on either side in the middle cervical ganglion, in the T1-T9 ganglia, or in the dorsal motor nucleus of the vagus.

Group 2: Following the injection of HRP into various parts of the right ventricular wall in four monkeys (Fig. 1), we found that a majority (64.4%) of HRP-labeled sympathetic cells were located in the right superior cervical ganglion, and fewer in the left superior cervical ganglion (35.6%) (Table 1). On the other hand, labeled parasympathetic neurons were observed in one of the two monkeys given 30 mg of HRP, all of them in the nucleus ambiguus on both sides; they were localized predominantly (76.9%) in the right nucleus ambiguus and to a lesser extent (23.1%) in the left. There were no HRP-labeled neurons in the dorsal motor nucleus of the vagus or below the middle cervical ganglion on either side.

Group 3: After HRP was injected in three monkeys into the sinoatrial nodal region at the junction between the superior vena cava and the right atrium (7) (Fig. 1), HRP-labeled sympathetic neurons were found to be most numerous (66.8%) in the right superior cervical ganglion (Fig. 2) and fewer in the left superior cervical ganglion (33.2%) (Fig. 3) (Table 1). On the other hand, HRP-labeled parasympathetic neurons were observed in both of the monkeys given 30 mg of HRP-most of them (84.4%) in the right nucleus ambiguus and the remainder (15.6%) in the left nucleus ambiguus. However, no labeled cells were found in the dorsal motor nucleus of the vagus or below the middle cervical ganglion on either side.

Group 4: Following the HRP injections into various parts of the left ventricle in three monkeys (Fig. 1), HRP-labeled sympathetic neurons were found to be most numerous (51.1%) in the left superior cervical ganglion; fewer were discovered in the right superior cervical ganglion (38.7%) (Table 1). In one of the three monkeys, some cells were labeled in the left middle cervical ganglion and in the bilateral stellate ganglia. No labeled neurons were found in the ganglia below T2 or in the dorsal motor nucleus of the vagus on either side. One labeled neuron was found in the right nucleus ambiguus in one of the two monkeys given 30 mg of HRP.

Group 5: No HRP-labeled cells were found in the sympathetic ganglia, the nucleus ambiguus, or in the dorsal motor nucleus of the vagus on either side.

In the ganglia, HRP-labeled neurons were oval

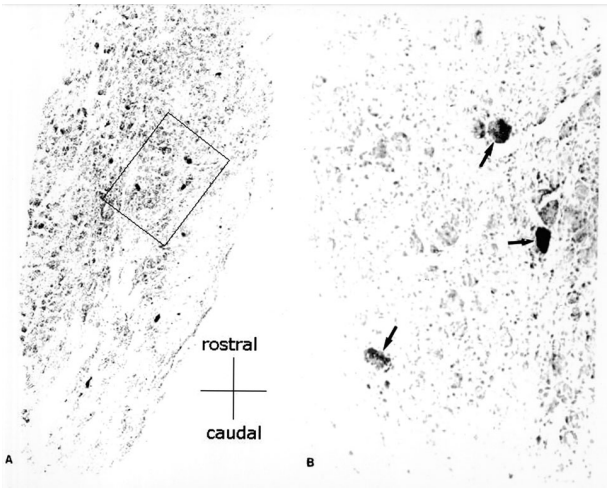


Fig. 2. Photomicrography of the caudal part of the right superior cervical ganglion (A) illustrating retrogradely labeled neurons in one monkey after application of HRP to the sinoatrial nodal region. A magnification of HRP labeled neuron (arrow) in the insertion (B). (A photograph X100, B photograph X400)

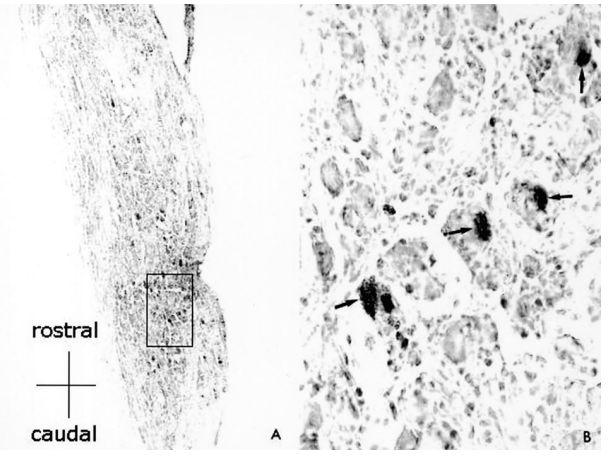


Fig. 3. Photomicrography of the caudal part of the left superior cervical ganglion (B) illustrating retrogradely labeled neurons in one monkey after application of HRP to the sinoatrial nodal region. A magnification of HRP labeled neuron (arrow) in the insertion (B). (A photograph X100, B photograph X400)

to round, ranging from 21-60 μm in cell diameter, with an average value of 38 μm.

## Discussion

Armour and Hopkin (1) report that following HRP injection into a left medial cardiac nerve of the macaque, labeled sympathetic postganglionic neurons were distributed relatively evenly among the superior and middle cervical ganglia and the stellate ganglion. The present study provides more specific information about the localization of sympathetic postganglionic neurons innervating the sinoatrial nodal region, the right ventricular wall, the apex of the heart, and the left ventricular wall. Our results show that the sympathetic postganglionic neurons innervating the regions cited are located predominantly (89.8-100%) in the superior cervical ganglion (Table 1). The right superior cervical ganglion appeared to be more densely labeled (63.1-66.8%) than the left (33.2-36.3%) in Groups 1, 2, and 3, whereas in Group 4 the left superior cervical ganglion was more densely labeled (51.1%) than the right (38.6%). In Group 4, some labeled cells (7.5%) were located in the stellate ganglion on both sides, and a few labeled cells (2.7%) in the left middle cervical ganglion. Very few labeled neurons (0.6%) innervated the right stellate ganglion in Group 1. Surprisingly, no labeling was observed in any of the thoracic paravertebral ganglia caudal to the stellate ganglion. These results are similar to those of our earlier investigation into the monkey's cardiac coronary artery (2) and also similar to the results reported by Randall (9).

The present study also demonstrates that the injection of HRP into four different cardiac regions (namely, the anterior part of the apex, the anterior middle part of the right ventricle, the posterior upper part of the left ventricle, and the sinoatrial nodal region) results in retrograde labeling in the nucleus ambiguus of the medulla. Labeled neurons were observed in 5 of the 13 monkeys, predominantly in the right nucleus ambiguus and to a lesser extent in the left nucleus ambiguus. These findings are consistent with findings in studies of dog hearts (1) and cat hearts (6). In the dog and cat, the majority of the cells of origin of vagal efferent preganglionic fibers innervating the heart are located in the nucleus ambiguus. Our findings are inconsistent with the findings in the study of Hopkins and Armour (4), who reported that after HRP injection into cardiopulmonary nerves, retrogradely labeled cells were concentrated ipsilaterally in the most lateral parts of the dorsal motor nucleus of the vagus nerve (DMV) and in the ventrolateral nucleus ambiguus (NA). Fewer labeled neurons were identified close to or in the principal (dorsal) division of the NA and in the intermediate zone between the DMV and NA. Their results indicated that monkey cardiopulmonary nerves have multiple origins; their somata are located primarily in the ventrolateral NA and to a lesser extent in the lateral DMV. The different results between the present study and Hopkins and Armour's study might be due to the difference in HRP injection location (i.e. Hopkins and Armour injected HRP into cardiopulmonary nerves and we injected HRP into the apex of the heart, the right and left ventricular wall and the sinoatrial nodal region). However, in human beings,

the efferent cardiac parasympathetic fibers are derived from the dorsal nucleus of the vagus and from cells near the nucleus ambiguus, and run in the cardiac branches of the vagus to synapse about cells in the cardiac plexuses and in the walls of the atria (13).

In our laboratory, Shih (11) has reported that in cats, sympathetic postganglionic innervation of the apex of the heart, the ventral wall of the right ventricle, the sinoatrial nodal region, and the dorsal wall of the left ventricle arises chiefly from bilateral stellate ganglia (88.5-93.6%). Only a few HRP-labeled neurons were seen in the middle cervical ganglion on either side. In contrast, Hopkins and Armour (5) report that following injections of HRP into the dog heart, the majority of labeled sympathetic postganglionic neurons were observed in the middle cervical ganglion bilaterally, although labeled neurons were also found in the stellate ganglion bilaterally, primarily in the cranial poles. The superior cervical ganglion had labeled cells in only eight of the cases studied with less than ten labeled neurons being found in seven of 18 cases. In contrast, our results reveal that the superior cervical ganglion is the key ganglion innervating the monkey heart, with the right superior cervical ganglion predominant in Groups 1, 2, and 3, and the left superior cervical ganglion predominant in Group 4.

In conclusion, our findings indicate that the superior cervical ganglion is more important to innervate the heart in primates than it appears to be in dogs or cats. The nucleus ambiguus is equally important in primates, dogs, and cats.

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