Cardiac Sympathetic Afferent Cell Bodies Are Located in the Peripheral Nervous System of the Cat

Zeljko J. Bosnjak and John P. Kampine

Studies of the stellate ganglion and middle cervical ganglion indicate that sympathetic efferent nerve activity can be modified by peripheral excitatory inputs and that these neural connections may function as pathways for a peripheral reflex at the level of the thoracic sympathetic ganglia. This excitatory synaptic input could have a soma in either the central or the peripheral nervous system. A study was designed to determine whether chronic decentralization (3 weeks) of the stellate ganglion in cats would 1) abolish sympathetic cardiac afferent nerve activity recorded at the stellate cardiac nerve and 2) abolish local thoracic reflexes that are generated by stimulation of peripheral nerves. The ansae subclaviae, T3 and T4 rami, and stellate ganglion were also examined by electron microscopy for the extent of Wallerian degeneration. Afferent cardiac activation of the axon collaterals arising from cell bodies located in the dorsal root ganglia was abolished due to degeneration. However, sympathetic afferent nerve activity from the left ventricular receptors was still present and was recorded from the stellate cardiac nerve in all cats. Cardiac receptors were sensitive to mechanical distortion, increases in the left ventricular pressure, and epicardial application of veratrine hydrochloride. These data imply that 1) cardiovascular afferent input to the stellate ganglion persists following chronic decentralization and 2) the sensory neurons are located in the peripheral sympathetic nervous system. Thus, we find that regulation of the heart occurs in part via thoracic ganglia, independently of the central nervous system. (Circulation Research 1989;64:554-562)

There is substantial evidence that excitatory cardiovascular reflexes can take place in animals with an intact innervation and that significant activation of cardiovascular sympathetic afferent fibers can occur in the conscious animal even in the absence of pain. Change in efferent cardiac sympathetic discharge can result in increased neural activity in several cardiac sympathetic nerves, with a simultaneous decrease in neural activity in others. The asynchronous activation of cardiac neural input may be of importance in the generation of early ventricular arrhythmias. This excitatory reflex may involve synapses located in the spinal cord, but ganglionic mechanisms appear to be involved as well. For instance, coronary artery occlusion may produce arrhythmias that are greatly reduced by bilateral stellactomy.

However, subsequent studies have suggested that such simple cardiac denervation is far more complex than previously realized. Unilateral interruption of efferent and afferent sympathetic activity may be more arrhythmogenic than leaving the nerves intact, possibly because of the differential anatomical distribution of the neural fibers. Earlier research has shown that stimulation of sympathetic afferents, known to occur with coronary occlusion, may activate a cardio-cardiac reflex. The spinal cord appears to play a part in this reflex along with the thoracic ganglia.

There is electrophysiological evidence that excitation of afferent cardiac sympathetic nerve fibers initiates reflex increases in sympathetic efferent discharge. This evidence suggests that the sympathetic ganglia closest to the heart contain the majority of efferent neurons whose fibers are present in the cardiac nerves. Thus, afferent neurons that control the heart and lungs may be activated by afferent neural elements in the thoracic autonomic nerves via synaptic mechanisms in the thoracic autonomic ganglia. The soma for this afferent pathway could be located either in the central nervous system (dorsal root ganglia) or in the periphery (i.e.,
The chronic decentralization of the stellate and middle cervical ganglia would interrupt the afferent axons from their cell bodies in the dorsal root ganglia. These afferent fibers would degenerate and, no longer being present, could not be activated by afferent nerve stimulation.

On the other hand, if there were afferent cell bodies present in the thoracic autonomic ganglia whose axons terminate on the efferent sympathetic neurons in these structures, chronic decentralization of the thoracic autonomic nerves and ganglia might modify, but not abolish, their transsynaptic activity. Other research has shown that preliminary destruction of ganglionic connections with the central nervous system is fatal only to a small proportion of intraganglionic synaptic contacts, while the rest of them persist. If the neurons are located in the SGs that project their axons in the thoracic rami (T1–T4), we would be able to evoke an antidromically stimulated action potential.

Experiments In Vitro

Following a 3-week recovery, six of the cats were anesthetized with 3% halothane. The chest was opened, and the left SG was quickly dissected and cut, together with connecting nerves, from the surrounding tissue. Each preparation was immediately placed in a preheated organ bath. The bath was maintained at 37±0.5°C, as measured near the preparation with a thermistor probe, and superfused with Krebs solution of the following ionic composition (mM): Na+ 137, K+ 3.0, Ca2+ 2.5, Mg2+ 1.2, Cl− 134, HCO3− 15.5, HPO42− 1.2, and glucose 11.5. The solution was bubbled continuously with a 97% O2-3% CO2 gas mixture (pH 7.4±0.05).

In order to minimize motion trauma to the SG and its associated nerves, surrounding connective tissue was pinned to a transparent silastic rubber floor (Sylgard, Dow Corning, Midland, Michigan) using fine (25 μm) tungsten wire pins. With the aid of a dissecting microscope, the connective tissue located on the top of the SG was removed. The associated nerves (T1 ramus, ventral ansa, dorsal ansa, and stellate cardiac nerve) were desheathed and placed on the stimulating electrodes.

The nerves to be stimulated were placed on bipolar tungsten electrodes connected to a stimulus isolation unit (WP Instruments, New Haven, Connecticut). The voltage used for nerve stimulation was dependent on the size of the nerve trunk and the amount of adherent connective tissue. To rule out the possibility that the stimulating electrodes might be indirectly affecting the neurons, we applied a supramaximal stimulus to the electrodes when they were not touching any nerve trunks. The ongoing electrical activity of the ganglion was unaffected.

We measured transmembrane potentials by means of short, tapered, ultrafine-tip glass microelectrodes. The microelectrodes were filled with 3 M KCl (50–80 MΩ resistance) and were placed on an electrode
holder attached to a hydraulic microdrive and a micromanipulator. We detected electrical activity by means of an electrometer (model M707, WP Instruments) and a Tektronix Model R5113 storage oscilloscope (Beaverton, Oregon).

Electrical activity was recorded simultaneously on an FM tape recorder (series 100, Tandberg, Oslo, Norway) and displayed on a digital oscilloscope ( Nicolet Instrument Corp, Madison, Wisconsin) whose output was plotted on an X-Y recorder.

Experiments In Situ

For in situ experiments we anesthetized eight of the cats with sodium pentobarbital (20-25 mg/kg) 3 weeks following a SG decentralization. We recorded peripheral blood pressure with a pressure transducer (Statham model P23D, Statham Medical Instruments, Hato Rey, Puerto Rico) connected to a catheter that was advanced through the femoral artery. Left ventricular pressure (LVP) was monitored by placing a polyethylene catheter into the left ventricle through the apex of the heart. To allow for intravenous infusion, we introduced a polyethylene cannula through a femoral vein. Following tracheal intubation, respiration was controlled via a Bird respirator. The upper six or seven ribs from the left side were removed from the costovertebral joint to the costochondral junction.

We cut the stellate cardiac nerve close to the SG, desheathed it, and placed it in a small recording chamber containing warm mineral oil. For recording purposes, the whole nerve was mounted on bipolar metal electrodes that were connected to a high-input impedance preamplifier and amplifier equipped with high- and low-pass filters (100-2,000 Hz). The moving time average was obtained by processing full wave rectified nerve activity through a fourth-order Bessel linear phase averaging filter (analog averaging window, 100 msec) and displayed on the Gould recorder (Brush 260, Cleveland, Ohio) for visual monitoring. Further quantification was obtained by processing the rectified nerve activity through a voltage-to-frequency converter whose output was counted in 1-second intervals using an eight-bit digital counter/timer with D/A output. The output was scaled into microvolts using the total system gain and counting interval. We increased peripheral sympathetic afferent input to the SG by either occluding the descending aorta, probing the epicardial surfaces of the heart, or injecting veratrine hydrochloride (50-100 µg) intravenicularly.

Nerve traffic and LVP were also examined by analyzing the data on an Ortec averaging computer using the LVP as a trigger. Nerve activity during 16-32 consecutive heart beats was accumulated at 5-msec increments by the computer and printed out with the LVP changes for the same heart beats using an X-Y plotter. Once receptor location was found on the heart, conduction velocities were also determined by placing a bipolar stimulating electrode on that area of the epicardium and electrically stimulating the afferent fibers. We recorded the evoked action potentials at the cut end of the stellate cardiac nerve and determined the overall distance between the stimulating and recording electrodes by using a wet thread.

Each experiment served as its own control for treatment effects. All data were expressed as mean±SD and evaluated by appropriate one- or two-way analysis of variance. Means were compared by least significant difference tests.

Results

Experiments In Vitro

Postganglionic electrical stimulation of the stellate cardiac nerve evoked graded excitatory post-synaptic potentials or action potentials in cells of the SG in proportion to the stimulus strength (Figure 1). At the lowest effective stimulus strength, small synaptic potentials were evoked; increasing the stimulus strength via dorsal ansa or ventral ansa increased their amplitude and also recruited postganglionic fibers of slower conduction velocity. Once a synaptic potential reached a critical depolarization threshold level of 12±5 mV, an action potential was initiated. The resting membrane potential obtained from 185 neurons impaled was 58±7 mV.

These recordings from the chronically decentralized SG are abolished in the presence of hexamethonium and similar to those obtained in the acute ganglion preparation. Therefore, even after the central input has been degenerated, postganglionic neurons of the SG still receive synaptic input from more than one postganglionic fiber, and these fibers have different conduction velocities and thresholds.

The only apparent difference between the acute and chronic ganglia preparations is the number of neurons that receive such peripheral synaptic input.
Electrical stimulation of the peripheral nerves following chronic decentralization of the SG evoked graded synaptic responses in 28±5% of the neurons studied; this compares with 90±8% of the neurons during an acute decentralization of the SG. Therefore, chronic decentralization decreases but does not abolish peripheral synaptic input.

On the other hand, stimulation of the preganglionic nerves (T3, Figure 1) also produced graded synaptic responses in 20% of the neurons tested in chronically decentralized preparation as well as antidromic discharges in some neurons. Since these evoked synaptic responses are not due to a preganglionic efferent input, which degenerated during the 3-week period, they must have originated from the projections of the cell bodies located in the peripheral nervous system.

This is a surprising finding since no synaptic input was expected while stimulating the stubs of the T3 or T4 rami. An antidromic activation of the soma is expected in some of the neurons that constitute a sympathetic efferent innervation of the vessels in the spinal cord. Such antidromic stimulation is shown in the upper part of Figure 2 (A–C) during the increase in T3 stimulation. An antidromic nature of these action potentials was verified with a collision technique as reported previously. Briefly, this method can detect the presence of an axonal action potential collision traveling in the opposite direction between the intracellularly evoked action potential and the peripherally/centrally evoked action potential when they are separated by an appropriate time interval. If an action potential is a result of an antidromic stimulation, it follows that after simultaneous intracellular and peripheral/central nerve stimulation, only one action potential will be evoked (intracellularly evoked). That is not the case if the peripheral/central stimulation produces a synaptic-induced action potential. Under these conditions, the first action potential will be intracellularly evoked (no conduction delay), followed by a synthetically induced action potential. The only limitation in the latter example is the refractoriness of the neuron after the initial action potential.

Increasing the stimulus strength also recruited some fibers and induced synaptic depolarization following an antidromic action potential hyperpolarization (four traces are superimposed in Figure 2C). The left part of Figure 2A–E shows graded synaptic responses of the cell during increasing central stimulation. It is apparent that the removal of the efferent preganglionic sympathetic axons did not eliminate graded synaptic responses in 18±9% of the neurons tested. Since these synaptic potentials are present after the chronic decentralization, they probably also exist in the acute SG preparation. Therefore, not all of the synaptic activity recorded in the neurons of the SG is from the central origin.

**Experiments In Situ**

We removed the chronically decentralized SG in eight cats for the extracellular recordings as described previously. All of the sympathetic afferent recordings were done using the stellate cardiac nerve. Figure 3 compares changes in sympathetic afferent nerve activity, time-averaged nerve activity, LVP, and systemic blood pressure during two probings of the left ventricular epicardium. Such left ventricular probings produced large and variable increases in the afferent nerve activity, depending on the severity of the left ventricular deformation, the level of peripheral blood pressure, the size of the receptor area, and the number of fibers from which the recording was being made. The approximate location of the receptor area was confirmed by gentle probing of the epicardial surface of the left ventricle with a soft insulated probe. The receptive fields of these receptors varied up to 1 cm². Most of
the receptors were located in the area of the left ventricle supplied by the left circumflex coronary artery. Since the afferent axons of the soma located in the dorsal root ganglia had presumably degenerated, the observed afferent input to the SG must have its cell body in the peripheral nervous system.

Figure 4 depicts the changes in the sympathetic afferent activity using a multifiber preparation with a receptor in the left circumflex region before and during occlusion of the descending aorta (indicated by the arrows). Occlusions were performed during the lower (110/50 mm Hg) and higher (150/80 mm Hg) peripheral pressures, producing respective peak LVP increases from 90 to 150±10 mm Hg and from 120 to 176±21 mm Hg. As seen in Figure 4B, higher LVP during the aortic occlusion also produced higher afferent nerve activity. The time-averaged increases in the sympathetic afferent nerve activity was 60±28% and 105±40% during the lower and higher LVP increases, respectively. Increased firing could be due to either a greater stress in the left ventricular wall or the altered mechanics during the diastole. After the aortic occlusions, left ventricular and peripheral blood pressures are briefly lower, while afferent activity remains depressed.

In addition to the above-mentioned stimuli, these cardiac receptors were sensitive to chemical stimulation with veratrine hydrochloride, as seen in Figure 5. Two doses of veratrine were injected directly into the left ventricle (at the arrows). As seen, there was a dose-dependent increase in the afferent nerve activity that averaged 126±21% and 266±73% after 50 and 100 μg of veratrine, respectively. The smaller dose of veratrine (50 μg) had no effect on the systemic blood pressure, which averaged 110/50, while the higher dose caused a slight depression of the LVP (5±2%) and peripheral blood pressure (11±5%), along with a small decrease in heart rate (8±3%).

This marked increase in the afferent activity following veratrine (100 μg) was further examined using...
FIGURE 5. Injection of veratrine hydrochloride into left ventricle (at the arrows) caused a dose-dependent increase in sympathetic afferent nerve activity (SANA). LVP, left ventricular pressure; BP, blood pressure.

LVP-triggered histograms (Figure 6), where we compared the occurrence of action potentials during 16 consecutive heart beats. As shown, the action potential frequency was closely related to the diastolic portion of the LVP curve (dark neurohistogram). Since it took an average of 125 msec for the action potential to reach the recording site close to the removed SG, the shape of the neurohistogram was drawn (dotted) to the left in order to compensate for the conduction delay. Therefore, the greatest activity of these receptors would occur during a sudden decrease in the wall tension of the left ventricle.

The same pattern of the afferent nerve activity as recorded from the stellate cardiac nerve was observed in all eight preparations following a veratrine excitation. Conduction velocities of this pathway were measured at the end of each experiment by placing the bipolar stimulating electrodes over the receptive field of the left ventricular receptor. Figure 7 represents the evoked action potential histogram from which conduction velocity was calculated, along with the measured distance from the stimulating to the recording electrodes. The conduction velocity of this afferent pathway ranged from 0.5 to 0.9 m/sec and averaged 0.67 m/sec.

The ansae subclaviae, stellate cardiac nerve, T3, and T4 rami, and SG were examined under an electron microscope to determine the extent of Wallerian degeneration and the presence of synapses in the chronically decentralized ganglion. As expected, synaptic contacts were observed in the ganglion (Figure 8A). In addition, an electron micrograph of the T3 rami is shown in Figure 8B. This micrograph represents the cross-section of the T3 stub left on the SG (approximately 5 mm long) after 3 weeks of decentralization. The total number of myelinated and unmyelinated fibers was compared with the number of fibers counted in six micrographs (at the same magnification) obtained from the acute (n=3) and chronically decentralized (n=3) SG. Although some axons were degenerated (both myelinated and unmyelinated), 78% of these fibers survived chronic decentralization.

Some portion of these axons constitute a normal efferent sympathetic innervation of the spinal cord.
The rest of the fibers are most likely afferent projections of the neurons located either in the thoracic ganglia or closer to the target organ itself. These afferent projections must branch on the neurons of the SG on their way to the central nervous system, and therefore, the stimulation of the T3 or T4 rami evokes graded synaptic responses.

Discussion

The main finding of this study is that cardiovascular afferent input to the SG persists following chronic decentralization and that sensory neurons responsible for this input are located in the peripheral sympathetic nervous system.

Although neural control of the heart has been associated mainly with autonomic integrative mechanisms present within spinal and supraspinal segments of the central nervous system, recent evidence suggests that integrative regulatory mechanisms exist in prevertebral and paravertebral thoracic ganglia. Bosnjak et al5 have examined the change in the heart rate during the stimulation of the peripheral nerves (dorsal ansa) toward the SG using a decentralized preparation in vivo. We observed a positive chronotropic effect on the heart rate during the stimulation. The cholinergic nature of the synaptic transmission was confirmed with the use of the acetylcholinesterase inhibitor, physostigmine, and the ganglionic blocker, hexamethonium. Furthermore, in vitro studies3-7-34 were conducted on the middle cervical ganglion and the SG by recording intracellular action potentials from its neurons. The excitatory postsynaptic potentials (EPSPs) produced by simultaneous stimulation of the central and peripheral neural input were shown to summate and discharge the cells.

Intracellular recordings were also made in situ4 from neurons of the cat SG attached via the postganglionic nerves to the rest of the animal. When peripheral sympathetic afferent input to the SG was increased by occluding the descending aorta, hyperventilation, or increase in the peripheral blood pressure, some of the neurons exhibited an increase in EPSPs and/or action potentials. Most of the synaptic input recorded from the ganglion cells in situ had a close relationship with the cardiac cycle and/or respiration.

Other studies7 have examined the general morphology of cat SG cells using a horseradish peroxidase injection, in relation to the synaptic input that each neuron receives. It was shown that neurons receiving synaptic input from both preganglionic and postganglionic nerves have a complex dendritic morphology. Some of these neurons are located close to the postganglionic nerves having an emerging axon. As shown by Armour and Hopkins,11 these sympathetic efferent cell bodies, which are located near the cranial pole of the left SG, innervate the left heart and lungs. Other neurons with complex dendritic morphology found closer to the preganglionic nerves, in the caudal pole of the SG, had no identifiable axons leaving the ganglion.7 This population of neurons could not be excited antidromically by electrical stimulation, and we suggested...
that they are interneurons. It has been postulated that these interneurons play an active part in modifying cardiac glucose use within specific areas of the heart, as measured with 2-[^14]C]deoxyglucose use, during a local cardiac reflex.

All of these studies have suggested that neurons in the thoracic autonomic ganglia are organized in a complex fashion and that they play an important part in the integrative properties of these ganglia. The existence of a true peripheral reflex arc is well established for the prevertebral sympathetic ganglia such as the solar, abdominal, and inferior mesenteric plexuses and inferior mesenteric ganglia. In addition, middle and superior cervical ganglia have been shown to be a part of the local reflex. Morphological and electrophysiological studies have confirmed the persistence of these peripheral reflexes after the chronic decentralization of the above prevertebral sympathetic ganglia.

Unlike studies on the prevertebral sympathetic ganglia, early work did not document a peripheral reflex in the paravertebral (lateral chain) ganglia. Although preganglionic axon reflexes have been reported to be mediated by the SG, they would irreversibly disappear after the degeneration of preganglionic fibers. More recently, evidence has shown that the SG of the cat and dog participates in the local reflexes and probably plays a more dominant role in the thorax than the prevertebral ganglion (middle cervical ganglion).

The results of the present study using chronic decentralization of the SG are compatible with our previous finding that this ganglion receives synaptic information from peripheral nerves in the form of a true reflex arc. The afferent neurons responsible for this local reflex are C-fiber afferents with a conduction velocity of 0.5–0.9 m/sec. As shown, they remain functional and intact in the peripheral nerves and preganglionic stumps, while efferent fibers and afferent fibers of the central origin degenerate. The function and afferent nature of the remaining C-fibers of peripheral origin was confirmed by exciting their receptors in the left ventricular wall chemically and mechanically. The present study did not establish the location of the afferent neurons of this peripheral reflex. It has been shown that these cells within the abdominal ganglia are located in either the prevertebral ganglia or the visceral organ close to the receptor site, with a long axon projecting to the prevertebral ganglion.

Histochemically and pharmacologically, it has been established that these peripheral C-afferents are cholinergic in nature in both prevertebral and paravertebral ganglia. It appears that up to 15% of the neurons in the SG of the cat are rich in acetylcholinesterase, suggesting a cholinergic nature. The results from this and our previous studies suggest that these peripheral afferent nerves not only synapse with postganglionic efferent cells in the SG, but also send their information to the central nervous system.

Therefore, the present data show that the neural connection of the SG with the central nervous system is not unidirectional but bidirectional. On the one hand, stimulation of peripheral afferent neurons stimulates the efferent (postganglionic) cells and, therefore, closes the local arc of the thoracic reflex; on the other hand, some sensory projections proceed to the spinal cord. The spinal projections also appear to be excitatory in nature as far as the sympathetic efferent flow is concerned, resulting in increases in arterial blood pressure, myocardial contractility, and heart rate. Stimulation of afferent cardiac sympathetic nerve fibers might also reflexively modify the sympathetic control of the mechanical properties of the thoracic aorta, the sensitivity of aortic mechanoreceptors, and the characteristics of those reflexes initiated by them. Conversely, stimulation of the ventricular wall receptors may be involved in the reflex fall in blood pressure and bradycardia that may accompany acute myocardial infarction.

This study provides the most convincing evidence thus far for the presence of peripheral synaptic input to the paravertebral ganglion. Since synaptic activity has persisted after chronic SG decentralization, we believe that at least two types of neurons exist in the peripheral thoracic ganglia, one afferent and the other efferent. The afferent cardiac nerve activity recorded at the stellate cardiac nerve after chronic decentralization cannot be due to vagal or preganglionic projections. To date there is no evidence to show that preganglionic fibers could reach the stellate cardiac nerve without passing through the sympathetic chain and the SG. The "looping" of the vagal fibers (axonic reflex) was not found to exist in the cat or dog SG since the reflex could be augmented after acetylcholinesterase inhibitor and abolished after the administration of ganglionic blockers.

At this time we cannot fully assess the functional importance of the peripheral reflex at the SG, and further studies are necessary to establish its overall importance. It is likely, however, that possible physiologic actions of this peripheral reflex could include local early responses to changing systemic or pulmonary venous return as well as regulation of blood volume distribution within the cardiopulmonary region. In addition, this local feedback would lead to augmentation of low and high levels of sympathetic efferent nerve activity along with alteration of sensitivity of thoracic mechanoreceptors. On the other hand, pathophysiologic activation of this reflex could initiate cardiac arrhythmias as well as an increase in cardiac contractility and heart rate in the presence of cardiac ischemia, leading to further propagation of myocardial damage.

References

34. Bosnjak ZJ, Kampine JP: Cardiac sympathetic afferent cell bodies located in the peripheral autonomic nervous system (abstract). *Physiologist* 1986;29(4):109
36. Sjoqvist F: The correlation between the occurrence and localization of acetylcholinesterase rich cell bodies in the stellate ganglion and the outflow of cholinergic secretory fibers to the forepaw of the cat. *Acta Physiol Scand* 1963;57:339-351

Key Words: intracellular recordings, local reflex, peripheral nervous system, afferent nerves, veratrine hydrochloride, stellate ganglion
Cardiac sympathetic afferent cell bodies are located in the peripheral nervous system of the cat.

Z J Bosnjak and J P Kampine

*Circ Res.* 1989;64:554-562
doi: 10.1161/01.RES.64.3.554

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/64/3/554