Differential control over postganglionic neurons in rat cardiac ganglia by NA and DmnX neurons: anatomical evidence

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1Departments of Pediatric, Kosair Children’s Hospital Research Institute and Departments of2Physiology and Biophysics and 4Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky 40202; and
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Cheng, Zixi (Jack), Hong Zhang, Shang Z. Guo, Robert Wurster, and David Gozal. Differential control over postganglionic neurons in rat cardiac ganglia by NA and DmnX neurons: anatomical evidence. Am J Physiol Regul Integr Comp Physiol 286: R625–R633, 2004. First published November 26, 2003; 10.1152/ajpregu.00143.2003.—In previous single-labeling experiments, we showed that neurons in the nucleus ambiguus (NA) and the dorsal motor nucleus of the vagus (DmnX) project to intrinsic cardiac ganglia. Neurons in these two motor nuclei differ significantly in the size of their projection fields, axon caliber, and endings in cardiac ganglia. These differences in NA and DmnX axon cardiac projections raise the question as to whether they target the same, distinct, or overlapping populations of cardiac principal neurons. To address this issue, we examined vagal terminals in cardiac ganglia and tracer injection sites in the brain stem using two different anterograde tracers [1,1'-dioleyl-3,3',3'-tetramethylindocarbocyanine methanesulfonate and 4-[4-(dihexadecylamino)-styryl]-N-methylpyridinium iodide] and confocal microscopy in male Sprague-Dawley rats. We found that 1) NA and DmnX neurons innervate the same cardiac ganglia, but these axons target separate subpopulations of principal neurons and 2) axons arising from neurons in the NA and DmnX in the contralateral sides of the brain stem enter the cardiac ganglionic plexus through separate bundles and preferentially innervate principal neurons near their entry regions, providing topographic mapping of vagal motor neurons in left and right brain stem vagal ganglia. Because the NA and DmnX project to distinct populations of cardiac principal neurons, we propose that they may play different roles in controlling cardiac function.

brain stem; parasympathetic; anterograde tracing; heart; baroreflex

VAGAL EFFERENT NERVE ACTIVATION induces negative chronotropic, dromotropic, and inotropic changes in the heart (13, 16). Our knowledge, however, of how the brain stem circuitry is organized to control the heart via the vagus is limited because of the complexity of brain-heart connections and limitations of previous anatomic techniques (8).

Vagal efferent preganglionic axons arise mainly from neurons in two brain stem nuclei [the nucleus ambiguus (NA) and the dorsal motor nucleus of the vagus (DmnX)], whereas a small number of vagal efferent axons arise from neurons in the intermediate zone between DmnX and NA. The functional roles of cardiac neurons in these nuclei are not well understood (6, 8, 11, 13, 16, 25). Furthermore, neurons in different cardiac ganglia control heart rate, arteriovenous (A-V) conduction, and myocardial contractility (17, 18, 20–22). In contrast to the conventional concept that cardiac ganglia are simple relay stations for central nervous system input, these structures are more likely operating as complex integration centers (19, 23). They consist of sensory neurons, motor neurons, and interneurons that employ complex chemical coding (12, 26) and are involved in local, as well as central, cardiac reflexes (1, 19, 23, 27). Thus a major question is how cardiac ganglia are innervated and controlled by neurons in the NA and DmnX in the brain stem.

Traditionally, it has been assumed that the NA is the major vagal motor neuron pool controlling the heart, whereas neurons in the DmnX play only a minor role in cardiac control (13, 16). Using anterograde tracing and confocal microscopic techniques, however, we recently demonstrated that neurons in both NA and DmnX project to each cardiac ganglion where their axons form extensive basket endings around ganglionic principal neurons (6, 8). Therefore, this anatomic evidence suggests that both nuclei could be important for cardiac function. Consistent with this morphological evidence, physiological data also support the functional significance of the two nuclei: 1) electrical stimulation of vagal efferent B fibers (presumably from NA) and vagal C fibers (presumably from DmnX) selectively evokes bradycardia, A-V block, and reduction of cardiac contractility (9, 14, 15, 29); 2) stimulation of the DmnX elicits bradycardia and reduces myocardial contractility (4, 10, 24); 3) activation of the NA induces negative chronotropic, dromotropic, and inotropic effects (2, 3, 17, 28); and 4) both DmnX and NA neurons are barosensitive (30). However, whether the two vagal motor nuclei perform different functional roles remains unclear.

To study the difference between the NA and DmnX, we first compared cardiac projections of the two nuclei (6, 8). DmnX and NA motor neuron projections to the heart differ significantly: 1) efferent axons originating from NA neurons project to the heart and diverge and innervate principal neurons three times as much as the efferent fibers arising from DmnX neurons, 2) axons arising from NA neurons are larger in caliber than those arising from DmnX neurons, and 3) fibers from DmnX neurons project to small intensely fluorescent cells (SIF; presumably interneurons) as well as to cardiac principal neurons, whereas axons from NA neurons innervate only principal neurons. Furthermore, we used domoic acid, an excitatory neurotoxin that acts on glutamate receptors, to selectively lesion the NA and DmnX. Lesions of the NA almost completely abolished the baroreflex control of the heart.
rate, whereas lesions of the DmnX did not, suggesting that the NA plays a more important role in baroreflex control of the heart rate than the DmnX (5, 31).

Morphological and physiological differences between the NA and DmnX raise the question of whether the NA and DmnX control the heart through different pathways and whether axons arising from neurons in these two nuclei target different populations of intrinsic cardiac principal neurons. We hypothesized that vagal fibers originating from neurons in the NA and DmnX project separately to cardiac ganglia, so that they innervate different populations of cardiac neurons and control different aspects of cardiac function. Alternatively, axons arising from NA and DmnX neurons might converge to innervate the same cardiac neurons because recruitment of C fibers from the DmnX does not increase the degree of cardiac slowing produced by stimulation of B fibers arising from NA (cf., Ref. 13).

To elucidate these issues, we used a double-labeling strategy and confocal microscopy to examine dual projections of the NA and DmnX neurons to the heart. Our results show that neurons in the NA and DmnX project to neurons throughout individual cardiac ganglia. Rather than innervating the same principal neurons, however, these two vagal nuclei project to separate subpopulations of principal neurons within each cardiac ganglion.

METHODS

Male Sprague-Dawley rats (300–350 g; Harlan Industries, Indianapolis, IN) were used for all experiments and were divided into four groups. The experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Louisville and are in agreement with the National Institutes of Health guidelines for the care and use of laboratory animals.

In the first tracing group, each animal received a series of unilateral DmnX injections of the tracer 1,1′,3,3′,3′,3′-tetramethylindocarbocyanine methanesulfonate, rhodamine red (DiI; catalog 3886; Molecular Probes, Eugene, OR) paired with injections of 4-[4-(di-hexadecylamino)-styril]-1-N-methylpyridinium iodide, fluorescein yellow-green (DiA; catalog 3883; Molecular Probes) in the contralateral NA (left DmnX: right NA, n = 4; right DmnX: left NA; n = 4). In the second tracing group, each rat received a series of unilateral DmnX injections of DiI paired with injections of DiA in the ipsilateral NA (left DmnX: n = 4; right DmnX: n = 4). In the third tracing group, the tracers DiI and DiA were switched at each injection site. Each rat received a series of unilateral DmnX injections of the tracer DiA paired with injections of DiI in the ipsilateral (n = 4) or contralateral (n = 4) NA to test the tracer effect. In the fourth group, each rat (n = 2) was injected with DiI in the left DmnX, and the left cervical vagal trunk was transected immediately after the DiI injection to assess the extent of DiI spread in the contralateral DmnX. Animals in these groups were also injected with Fluoro-Gold (FG; ip, 2 ml of 2 mg/ml) to counterstain cardiac ganglia. In a reference group, rats (n = 10) were injected with FG (3 mg/ml, 5 μl) in the cervical vagal trunk to identify DmnX and NA motor neurons. This group was used to ascertain the precision of DiI injections in DmnX and of DiA injections in the NA, i.e., the registration of DiI in the brain stem with FG-labeled DmnX motor neurons and the registration of DiA with NA motor neurons.

Reference animals from the fourth group were killed 5 days after injections to confirm tracer injection sites within the brain stem. In contrast, the other three experimental groups were killed 3 wk after injections, and the brain stem tissue, nodose ganglia, and atrial specimens of these animals were harvested for analysis of NA and DmnX fibers and endings in cardiac tissues. In the event that tracer injection sites were off center from the target nuclei, data were discarded from further analysis.

Tracer injection in the DmnX and NA. Each animal in the tracing groups was anesthetized with pentobarbital sodium (60 mg/kg ip), treated with atropine (1 mg/kg sc), and placed in a stereotaxic instrument equipped with a head holder adapted to permit the neck to be flexed sharply. A dorsal incision was made over the neck muscles, which were retracted to expose the atlanto-occipital membrane. The membrane was opened with an incision, exposing the cisterna magna and dorsal medulla. The occipital bone was trimmed with the bit of a dental drill until the caudal cerebellum was visible. The caudal end of the area postrema was used as a reference for stereotaxic coordinates. A glass micropipette, filled with DiI or DiA and connected to a picospritzer, was then advanced to the DmnX or NA. DiI or DiA was injected in small aliquots (2.5–12.5 nl each) either at seven different sites for DmnX (−1,200 to +1,120 μm; total volume 17.5–87.5 nl) or at nine different sites (−1,600 to +1,600 μm; total volume 22.5–112.5 nl) for NA, separated 400 μm longitudinally. All analyzed animals had well-placed injections, as confirmed by postmortem examination; medullary injection sites tended to fuse longitudinally. Injections were considered to be well placed only if 1) the injections, and their central cores, were centered in the DmnX and NA at all frontal levels, 2) the injection spheres extended minimally both ventrally to the DmnX and dorsally to the NA and 3) there was less than 10% spread in the frontal plane between injection sites (Fig. 1; also Fig. 1 in Ref. 8 and Fig. 1 of Ref. 6). Diameters of DiI and DiA injections in NA are ~300 and 150 μm. respectively, and 3) the series of injections primarily covered the bulk of the longitudinal extent of the DmnX and the NA.

Tissue preparation. Three weeks were allowed for the tracers to be transported to the heart. Animals were anesthetized with an overdose of pentobarbital sodium (100 mg/kg) and perfused through the heart with 0.9% saline (300 ml) and 10% phosphate-buffered (pH = 7.4, 600 ml) formalin. Nodose ganglia and brain stems were removed. Each brain stem containing the entire DmnX and NA was stored in 10% sucrose formalin overnight and sectioned transversely at 100 μm on the second day. Thoracic vagal trunks, with the cardiopulmonary branches attached from the level of recurrent laryngeal nerves to a level 3 mm caudal to the junction of the inferior vena cava and the right atrium, were also dissected. Atria were then separated and cut open as previously described (7). Briefly, the left atrium tissue block included the region of the junction with pulmonary veins; the right atrium had the superior vena cava, inferior vena cava, and the left precava vein attached. The interatrial septum was separated from the atria. Tissue surrounding the heart was gently removed, using extreme care to avoid peeling off the ganglionated plexuses (8) on the dorsal surface of the atria. Tissue was then dehydrated through graded concentrations (70%, 90%, and 2×100%) of glycerin. Finally, the tissue was mounted and covered with a cover slip in 100% glycerin and n-propyl gallate (5%) to prevent fading.

Examination of DiI and DiA injection sites. Brain stem slices were initially examined at ×100 magnification using an epifluorescence microscope equipped with filter cubes appropriate for DiI, DiA, and FG and were later scanned using a confocal microscope (Zeiss LSM 510) at ×250 magnification. To present the registrations of DiI and DiA injection sites in vagal motor nuclei in a complete fashion, a montage of individual confocal projection images was assembled using Adobe Photoshop 5.5. DiI injection sites were evident by labeling with orange-red to red colors; DiA injection sites were in the yellow-green or green part of the spectrum; and FG-labeled neurons appeared blue in color.

Data acquisition and analysis of dual vagal projections in intrinsic cardiac ganglia. A detailed description of the data acquisition strategy has been published previously (6, 8). Briefly, cardiac and nodose ganglia specimens were screened with a conventional epifluorescence microscope equipped with filter cubes appropriate for DiI (rhodamine), DiA (FITC), and FG (UV). When DiI and DiA nerve fibers and endings were both found in the same region of cardiac ganglia at
RESULTS

Histological verification of DiI injection in the DmnX and DiA injection in the NA. We used a triple labeling technique to verify the DiI and DiA injection sites. Figure 1 is a montage of confocal photomicrographs obtained from a cross section of the brain stem at the level of the area postrema (8). DiI injections in the left DmnX covered the whole left DmnX as well as some neurons in the medial portion of the right DmnX, whereas the DiA injections covered most of the NA. Therefore, both tracer injections were in precise registration with their targeted sites.

\( \times 200 \) magnification, their locations were recorded for subsequent detailed confocal microscopic analysis at \( \times 400 \) magnification. The digitized confocal images were stored on compact disks.

Consistent with earlier observations (6, 8), three major ganglionated plexuses labeled by FG were identified in the epicardium of the atria under UV light. Within each plexus, multiple ganglia were found (8). Using stacks of optical sections collected with the confocal microscope through filter cubes appropriate to DiI and DiA, we examined the dual innervation of DmnX and NA cardiac axons and their terminal endings in each of the three ganglionated plexuses. Dual vagal axonal innervation of the three plexuses was examined systematically and completely. Stacks of confocal optical sections that contained both DiI and DiA fibers and endings were analyzed sequentially, and each cell apposed by labeled varicosities or fiber swellings was considered as being innervated. The presence and close proximity of varicosities were the criteria for judging a labeled fiber to have contact with a cell (6, 8).

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Tracers were switched, i.e., the DmnX was injected with DiA and the NA was injected by DiI, the axons and endings arising from the NA appeared much denser and stronger, as shown in Fig. 4, D and E. Therefore, it was necessary to switch tracers to investigate fluorescence properties of these tracers.

Contralateral DmnX and NA efferent fibers innervate neurons in separate subregions of intrinsic cardiac ganglionic plexuses. Axons arising from neurons in contralateral DmnX and NA entered a cardiac ganglionated plexus via separate routes or connectives at different entry points (a connective is

Fig. 2. Confocal photomicrographs of DmnX and NA efferent axon innervation of cardiac ganglia principal neurons. In cardiac ganglia, DiI-labeled fibers and endings are red or orange-red, and DiA-labeled NA fibers and endings appear green or yellowish-green. The soma of the principal neurons labeled by FG are yellowish-brown. A: projection of a series of confocal optical sections shows that a DiI axon (arrow) forms a basket ending on a principal neuron at the center of the image (filled triangle), and a DiA fiber (arrow) innervates a principal neuron (open triangle) at the bottom left of the image. In addition, other green and red fibers are seen. *FG-labeled principal neuron next to the DiI basket ending. A: projection of the image in A showing the close contacts of a DiI fiber with the soma of the principal neuron, indicated by a filled triangle. It is apparent that DiA and DiI fibers innervate separate principal neurons in A. The somata and nuclei of the ganglion cells are clearly visible. *Same principal neuron as shown in A. Open triangle in B points to the same principal neuron as shown in A. C: projection showing that the DiI (red)- and DiA (green)-labeled basket endings occurred in close proximity to each other, as shown in the top of the image. D: cluster of small intensely fluorescent (SIF) cells (arrow) in the cardiac ganglion are innervated by DiI fibers. The morphological characteristics of SIF cells has been fully described in Ref. 4, and 5 SIF cells are indicated by a filled triangle in Fig. 4C'. A DiA fiber innervates two principal neurons below the SIF cells. E-H: from a 3-dimensional (3D) perspective, NA and DmnX fibers and endings may be tightly packed or interdigitated in the cardiac ganglion. In E, the projection of optically sectioned images shows several DiA and DiI baskets, which are intermingled and closely packed. In this projection image, some neurons appear to be dually innervated by DiI and DiA terminals, for example, the one indicated by a triangle. However, F-H, which are the 3 optical sections of E, reveal that NA and DmnX neurons actually project axons to different populations of principal neurons. Note that F-H are arranged such that all 4 panels are spatially registered. No converging projections to the same principal neuron were detected. Scale bar in B is 20 μm for A-H.

Fig. 3. A montage of confocal photomicrographs shows that the ipsilateral NA (DiA) and DmnX (DiI) project to the same cardiac ganglionated plexuses but to different populations of principal neurons. A: bundle of mixed DiA- and DiI-labeled fibers entered a ganglionated plexus from the bottom right (2 arrows), traversed the space between the ganglia, and generated basket endings around many principal neurons along its path. One arrow points to a DiI-labeled basket ending, and another arrow indicates a DiA-labeled basket ending (see Figs. 2B and 4C’ for optical sections of the basket endings; also see Refs. 6 and 8). B: top portion of A at a higher magnification. *Separation line of the upper and lower portions in A. Scale bars in A and B: 60 μm.
a mixture of extrinsic and intrinsic nerves connecting two separated cardiac ganglia in a ganglionated plexus. Each plexus contains a number of ganglia. Figure 4 includes three montages that show the right NA and left DmnX project to a cardiac ganglionated plexus (also see Fig. 5 of Ref. 8 for an FG-labeled cardiac ganglionated plexus and different connectives at low magnification). Figure 4A shows that a bundle of DiA-labeled NA fibers enters the plexus from a connective at one entry region, tending to innervate principal neurons situated near the entry region. Figure 4B shows that a bundle of DiI-labeled DmnX axons enters the same plexus through another entry point, to innervate principal neurons at the immediate entry region. Between these two opposite entry points, the DiA- and DiI-labeled basket endings intermingle and innervate principal neurons interdigitately, as shown in Fig. 4C. Therefore, neurons in the left and right sides of the brain stem appear to innervate topographically separated subdivisions of neurons in cardiac ganglionated plexuses.
Projection of DmnX and NA efferent fibers to cardiac ganglia: counterbalancing DiA and DiI. To examine whether our results were influenced by the differential fluorescence properties of DiI and DiA, we switched the tracers used to inject the DmnX and NA. In eight animals, we injected DiA unilaterally in the DmnX and DiI in the NA (either ipsilateral or contralateral). Comparable to previous labeling using alternate tracers in the brain stem nuclei, DiA-labeled baskets are among numerous DiI baskets. Individually, DiA- and DiI-labeled basket formations were evident. Labeled axons arising from the DmnX and NA innervated different populations of principal neurons in the same cardiac ganglion (Fig. 4, D–E). Therefore, our results did not depend on tracer fluorescence properties.

In contrast to the dense DiI-labeled axons and terminals projecting from the DmnX in Fig. 3, the DiA-labeled axons and basket endings projecting from the DmnX in Fig. 4D were much more sparse than the DiI-labeled axons and terminals originating from the NA, indicating that DiA is a weaker tracer.

Specificity of DiI and DiA labeling. We injected tracers to the DmnX and NA to label the cardiac motor axons and terminals in the heart. Three types of potential secondary labeling could occur. First, a tracer injection in the DmnX could also label ipsilateral NA neurons and vice versa. Second, a tracer injection in the DmnX or NA could also label the contralateral DmnX or NA. Third, a tracer injection in the DmnX or NA could label the nucleus of the solitary tract (NTS) region, and the vagal afferent terminals might pick up the tracer and transport it to the heart. For example, DiI injection in the DmnX may label NA, contralateral DmnX, and NTS; and DiA injection in the NA may label contralateral NA, DmnX, and NTS. Obviously, such potential problems could affect the interpretation of our findings.

According to our previous studies and after thorough examination of the brain stem, nodose ganglia, and atrial tissues used in the present study, we did not observe any evidence suggestive of such technical problems. Indeed, previously we have shown that DiI injections in the DmnX or NA did not label the other nucleus (6, 8). The present study confirms these findings. In addition, after examination of the atrial tissue whole mounts, no flower spray sensory terminals were found. Because flower-spray terminals are the major type of afferent terminals (7), we conclude that tracer injections did not label any vagal afferents in the heart. Examination of brain stem serial sections did not show any labeled contralateral NA neurons. In contrast, as shown in Fig. 1, tracer injections in the DmnX could label the contralateral DmnX, especially in the caudomedial portion of the nucleus, since the left and right DmnX are close to the midline and to each other at this level. Such contralateral labeling may account for the occasional DiI axons in the DiA bundle. However, this relatively limited technical issue concerning the two sides of the caudal DmnX has no bearing on our major observation that the DmnX and NA project to different populations of cardiac neurons.

It should be pointed out that it was important to make a relatively large injection of DiI in the DmnX, as shown in Fig. 1, because a major aim of the present study was to test the hypothesis that the NA and DmnX project to the same cardiac ganglia but different populations of principal neurons therein. Therefore, we aimed to label as many DmnX and NA cardiac motor neurons as possible such that more DiI- and DiA-labeled terminals colocalizing at the same region within a cardiac ganglion would be available for analysis.

Such strategy, however, could lead to a potential leak of the tracer to the contralateral DmnX, as shown in Fig. 1, and might affect our finding that neurons in the right and left brain stem topographically project to the different subdivisions of the plexus. To eliminate such possibility, we quantitatively estimated the maximal number of fibers being labeled from the contralateral DmnX and the innervation pattern of these axons. DiI was injected in the left DmnX. The left cervical vagal trunk was transected in two rats to evaluate the extent of spread in the contralateral DmnX. In these animals, 5 to 7 axons and 20–28 basket endings were found, corresponding to 7.4% of the total axons and 3.9% of the total baskets, which were estimated previously as originating from the left DmnX projection (8). Thus only a minority of fibers on the contralateral DmnX was labeled by injections we employed in our experiments. In contrast to the well-labeled DiI axons from the ipsilateral DmnX that entered the heart in large bundles (Fig. 4, A and B; also see Refs. 6 and 8), these sparse secondarily labeled axons were weakly labeled and randomly distributed in the cardiac ganglionated plexuses. Because in the majority of other animals the large DiI and DiA bundles were separated and entered ganglionated plexuses through different pathways and because the secondarily labeled fibers from the contralateral DmnX appeared weakly labeled, and sparsely and randomly distributed, the secondary labeling of the contralateral DmnX neurons should not detract from our findings.

DISCUSSION

Our results indicate that neurons in the NA and DmnX send axons that converge to the same cardiac ganglia and innervate distinctive principal neurons in these ganglia. Furthermore, we propose that neurons in the left and right sides of the brain stem vagal nuclei project axons topographically to different neuronal subdivisions within the cardiac ganglioned plexuses. Our previous findings have shown that the NA and DmnX project axons to neurons in cardiac ganglia differently with respect to the size of projection fields, axon caliber, and terminal targets (6, 8). We have also found that lesions of the NA almost completely abolish the baroreflex control of the heart rate (31) and that lesions of the DmnX do not change the baroreflex sensitivity (5, 31). Collectively, our data support the hypothesis that the NA and DmnX play different roles in the regulation of cardiac function.

Topographic representation of left and right vagus in cardiac ganglioned plexuses. As mentioned above, the left and right vagal cardiac axons enter the cardiac ganglioned plexus through different routes. This observation raises the possibility that the left and right brain stem may have a well-defined topographical representation of the heart. Furthermore, this may indicate that cardiac ganglioned plexuses are not homogeneous structures and that they may be organized hierarchically to receive functionally related inputs from the vagal brain stem nuclei in a topographical pattern. Whether our findings provide the anatomic basis for certain physiological observations indicative of left and right vagus lateralization is worthy of future investigation.

Regarding the topographic projections of the vagal motor nuclei to cardiac ganglioned plexuses, an additional intrigu-
ing issue is whether the different regions of the DmnX and NA project to different areas of cardiac ganglionated plexuses. In dogs and cats, Massari and colleagues (3, 17) found that different locations of the NA or DmnX project to different cardiac ganglia in fat pads and had differential control over the heart rate, A-V conduction, and left ventricle contractility. Such data in the rat are not currently available. Our present dual-labeling strategy, i.e., injection of different areas of the NA or DmnX with two tracers simultaneously and subsequent examination of cardiac ganglionated plexuses with the confocal microscope, may provide a novel method for future investigation of this issue.

NA and DmnX cardiac motor neurons: functional significance. Conventionally, the NA was considered the major vagal motor nucleus that controls the heart. Recently, we demonstrated that neurons in the DmnX also project substantially to the heart, implying that the DmnX might control cardiac function as well. The present finding that NA and DmnX project to the same cardiac ganglia suggests that they could both modulate the heart rate, A-V conduction, and myocardial contractility (6) because the adjacent cardiac principal neurons are thought to have similar cardiac functions. From these data, we reasoned that NA and DmnX might be both involved in baroreflex control of heart rate.

To study the functional role of the NA and DmnX, we injected domino acid to disrupt the integrity of the NA and DmnX, respectively, and studied their respective roles in baroreflex effect on the heart rate. To our surprise, lesions of the DmnX did not affect baroreflex sensitivity (5). In contrast, lesions of the NA almost completely eliminated baroreflex control of heart rate, indicating that the NA is critical for baroreflex control (31), whereas the DmnX is not.

Whether the DmnX can control the heart through some other cardiac reflexes is an interesting issue. Because the NA and DmnX innervate nonoverlapping populations of cardiac principal neurons and play different roles in baroreflex, we hypothesize that the NA and DmnX may play distinct roles in different cardiopulmonary reflexes. Actually, it has been postulated that the DmnX may mediate pulmonary C-fiber-evoked bradycardia (15, 28).

In summary, neurons in two spatially separated medullary nuclei, the NA and DmnX, project axons to separate nonoverlapping populations of interspersed cardiac neurons. These findings provide the anatomic framework to account for the possibility that the NA and DmnX may have different functional roles in controlling the heart. The exact nature of such potential functional differences of the two nuclei and the property of the two populations of cardiac principal neurons await further studies.

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