Morphological and Electrophysiological Correlates of Atrioventricular Nodal Response to Increased Vagal Activity

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The mechanisms responsible for slowing cardiac impulse conduction through the atrioventricular (AV) node are not well understood but include anatomical architecture, presence of cells with diverse electrophysiological characteristics, and modulation by autonomic nervous system. The present study was designed to determine the site of vagally induced slowing of conduction through the AV node. We attempted to correlate the electrophysiological response of AV nodal cells to postganglionic vagal stimulation applied in different regions of the node with the morphological findings and patterns of acetylcholinesterase-positive staining of nodal tissue. This multifaceted approach revealed that vagal stimulation produced localized hyperpolarization of the cells from the N region of the AV node, which correlated with the strong acetylcholinesterase positive staining of the central nodal area. In contrast, the density of the acetylcholinesterase staining decreased toward both the AN and His bundle regions, whereas vagal stimulation had a negligible effect on the cells from these regions. These results suggest that vagal-induced depression of AV nodal conduction is produced by release of acetylcholine predominantly around the midnodal region and the depressive action of acetylcholine is concentrated on the cells occupying the same region (i.e., the N cells). Thus, there appears to be a close juxtaposition of nerve elements and effector cells in the midnodal region of the AV node. This unique combination of available neuromediator and responding cells with hyperpolarization and depressed action potential determines the midnodal region as the focus of vagal effect on AV nodal conduction. (Circulation 1990;82:951–964)

Since the pioneering work of Tawara,¹ the atrioventricular (AV) nodal region has been the focus of intensive morphological and electrophysiological studies designed to identify the mechanisms of AV conduction. Yet there is still no unequivocal morphological classification of AV nodal zones in relation to the electrophysiological characteristics of the corresponding cells.² In part, this is due to the absence of clear boundaries between the AV node and surrounding atrial tissue as well as between the cells in the different histological regions of the AV node. Similar difficulties exist also in the characterization of the action potentials recorded from different AV nodal and neighboring cells, and development of a three-dimensional electrophysiological picture of the node seems an even more difficult task than a three-dimensional morphological reconstruction based on serial sections of the AV node.³,⁴

It is well known that the properties of conduction through the AV node are strongly influenced by the autonomic nervous system in addition to the complex nodal architecture. Increased vagal tone or application of acetylcholine causes slowly rising action potentials with depressed amplitude that eventually leads to AV block. Electrophysiological observations suggest that these effects involve mainly the cells in the N-NH zones,⁵,⁶ although little is known about the possible morphological correlates underlying this phenomenon.

There are several detailed reports describing the nerve supply of the AV junctional region. The AV node receives a generous supply of both adrenergic and cholinergic nerves.⁷–⁹ Histochemically, James and Spence¹⁰ and Anderson¹¹ showed nonhomoge-
neous distribution of acetylcholinesterase (AchE) within the node, suggesting a nonuniform density of nerve processes as well as AchE-positive conduction fibers in different zones of the node. Close contact between nerve and muscle cells has been described in all regions of the AV nodal tissues of the rabbit, whereas an ultrastructural three-dimensional reconstruction based on electron microscopic serial sectioning in the mouse heart documented numerous neuromuscular contacts as well as differences in their regional distribution. This suggests the existence of regions with predominant vagal innervation, which could correspond to the electrophysiological focus of vagal activity. However, there is no report, to our knowledge, that links the differential depressive electrophysiological effect of localized vagal stimulation with the respective morphological substrate. Thus, the aim of this study was to correlate electrophysiological and morphological approaches in an attempt to further characterize the conduction properties of the AV node during increased vagal tone.

**Terminology**

In this study, we adhere to the terminology used in the recent review by Meijler and Janse to describe the different morphological regions of the AV node. Briefly, the term “midnodal” (or “compact”) region is reserved for the closely packed cells in the central part of the AV node that were originally described by Tawara as a spindle-shaped network of small cells called “Knoten” (“node”). The term “transitional” refers to cells enveloping the midnodal region and merging posteriorly with crista terminalis (CrT) and anteriorly with the interatrial septum, thus forming the approach to the compact node. The lower nodal cells form a bundle parallel to the AV ring that increases in size anteriorly and merges with the AV bundle (bundle of His). These lower anterior cells make contact with the midnodal cells. It appears that atrial excitation wavefronts enter the AV node through the posterior and anterior transitional cells and propagate toward and through the midnodal cells to emerge as an organized synchronous wave in the anterior lower cells before entering the bundle of His.

Electrophysiological, to describe the correlation between the morphological cell types and their respective action potentials in this study, we adopted the terminology of Paes de Carvalho and de Almeida. Thus, AN zone (or AN-type cells) are represented by several groups of cells (e.g., AN, late AN, AN with two-component-upstroke) with action potential characteristics transitional between atrial and “typical” AV nodal cells. The latter occupy the N zone of the AV node and characteristically have low resting membrane potential and slow upstroke velocity of the action potential. In contrast, the action potentials recorded from the NH zone resemble those from the bundle of His. Although never shown unequivocally, it is assumed that the AN zone includes the transitional AV nodal cells, whereas the

NH zone includes the anterior portion of the lower AV nodal cells. The N zone is assumed to represent the midnodal core, although N-type action potentials have also been recorded from the nearby transitional and lower AV nodal cells.

**Methods**

**Electrophysiology**

The experiments were performed on 20 rabbit atrial–AV nodal preparations instrumented as described in recent detail previously. Briefly, the right atrium was dissected from the surrounding tissue and fixed with the endocardial surface exposed for superfusion in a tissue chamber. The superfusate contained propranolol (5 x 10^-6 M) to exclude sympathetic effects. The preparation consisted of the sinus nodal region, CrT, interatrial septum, and AV ring (Figure 1). The preparations were spontaneously beating, and bipolar surface electrograms were recorded form the CrT input to the AV node and from the bundle of His to determine the AV nodal conduction time. A small (approximately 0.25 mm^2) platinum bipolar electrode was used for subthreshold (or AV nodal cells) postganglionic vagal stimulation (PGVS) consisting of short (100–150 msec) bursts of current impulses with 1-msec duration, 200–2,000-μA amplitude, and 150-Hz frequency. The size of the stimulating electrode and the moderate intensity of stimulation were used to produce only local release of the neuromediator, acetylcholine, limited to the tissue projecting just below the stimulating tip. The PGVS electrode was gently pressed to the AV nodal surface during stimulation and moved in steps of 0.5–1.0 mm, sequentially applying the same PGVS current at as many as 50 points in the AV nodal area. The effect of the PGVS burst on AV nodal conduction was evaluated by the prolongation of the CrT-His (CrT-H) time interval in the beat immediately after PGVS. A pause of at least 60 seconds was allowed between the subsequent stimulations to achieve full recovery of the control AV nodal conduction time. During mapping, both the amplitude and the phase of PGVS as well as the sinus cycle length were constant. Standard microelectrode techniques were used in four preparations to record action potentials from different AV nodal cells. The purpose of the microelectrode impalements was not to meticulously map the AV node but instead to identify the impaled cells related to the findings of PGVS. Thus, the microelectrodes were usually impaled near the stimulating electrode. In addition, special attention was paid to the cells located where PGVS induced maximal effect on the AV nodal conduction time. In the results presented below, vagal effects on these cells are compared with the effects on more distal cells.

The position of the PGVS electrode at which the strongest vagal effect on conduction time was elicited (i.e., the focus of the vagal effect) was marked as follows. A broken microelectrode pipette (tip diam-
Morphology

For light-microscopic observation, sections were cut parallel to the AV ring and perpendicular to the endocardial surface (i.e., transverse plane). The three stained dots were used as orientation, and the average number of sections was 330 per preparation. Every 10th section was stained with hematoxylin and eosin; in addition, some were stained with Weigert van Gieson and Holmes' silver.

For histochemical studies, different blocks of tissue were oriented on cryostat chucks to allow sectioning in one of two planes relative to the AV ring. With the right endocardial surface of the interatrial septum in the horizontal position, sections were cut at right angles to the AV nodal surface either parallel or perpendicular to the AV ring. The former sections were designated transverse, and the latter were designated coronal. The frozen sections were 8–14-μm thick, and every 10th section was mounted, except for the stained-dot area, through which virtually every section was stained and studied. For observation of AchE activity, air-dried sections were incubated in a medium containing acetylthiocholine iodide as substrate to visualize the cholinergic innervation of vagally active areas. The histochemical procedure used the following incubation medium: 5 mg acetylthiocholine iodide, 6.5 ml 0.1 M acetate buffer (pH 6.0), 0.5 ml 0.1 M sodium citrate, 1.0 ml 30 mM copper sulfate, 1.0 ml distilled water, and 0.2 ml 4 mM iso-octamethyl pyrophosphoramide. Just before use, 1.0 ml 5 mM potassium ferricyanide was

![Image of rabbit atrial-atrioventricular (AV) nodal preparation. Right atrium was opened by an incision through superior vena cava (svc). SN, sinus node area; ra, right auricle; CrT, crista terminalis; ivc, inferior vena cava; IAS, interatrial septum; fo, fossa ovalis; ocs, ostium of coronary sinus; ss, sinus septum; tcv, tricuspid valve attachment to AV ring. Dots (1–3) represent staining of AV nodal endocardial surface as described in text.](http://circ.ahajournals.org/)

FIGURE 1. Rabbit atrial-atrioventricular (AV) nodal preparation. Right atrium was opened by an incision through superior vena cava (svc). SN, sinus node area; ra, right auricle; CrT, crista terminalis; ivc, inferior vena cava; IAS, interatrial septum; fo, fossa ovalis; ocs, ostium of coronary sinus; ss, sinus septum; tcv, tricuspid valve attachment to AV ring. Dots (1–3) represent staining of AV nodal endocardial surface as described in text.
added. After incubation at 37°C for 1–2 hours in the above medium, the slides were washed gently in distilled water, lightly counterstained in Harris’ hematoxylin, and then stained in light green solution. Sections were dehydrated, cleared, and mounted. The nerve fibers and cells containing AchE were stained dark brown, the nuclei were stained light purple, and cardiac muscle and fibroconnective tissue were stained green.

Results

Localization of Focus of PGVS Effects

The maximum PGVS effect on AV conduction was consistently localized to a small (1–2 mm²) area in the central part of the AV node just above the AV ring, referred to as the “focus” (point 2 in Figure 1). Outside of this area, the effect of PGVS diminished rather sharply and with the electrode located in the early AN zone (i.e., close to the CrT input to the AV node; point 1 in Figure 1) or in the late NH zone (i.e., close to the bundle of His; point 3 in Figure 1) no appreciable prolongation of AV nodal conduction time as a result of PGVS could be demonstrated. Importantly, the use of very high amplitudes of PGVS at the focus point could produce high-grade AV nodal block persisting over several beats. Hence, in such cases, the space distribution of the PGVS effect lacked the sharpness observed at moderate amplitudes. Therefore, we used amplitudes sufficient to produce marked prolongation of the AV nodal conduction time and, eventually, AV nodal block lasting for no more than one beat.

With this technique, the focus of the maximal effect of PGVS was determined in all preparations with a resolution of approximately 0.5–1 mm. At this distance from the focus, the effect of PGVS on AV nodal conduction time diminished by at least 50%. No attempt was made to describe quantitatively the space distribution of the PGVS effect.

The results illustrated in Figure 2 were obtained with the PGVS electrode located in the focus (point 2 in Figure 1). The records on the left are during control and before PGVS. The microelectrode was consecutively impaled in fibers as close to the PGVS electrode as possible (Figure 2B), as well as posteriorly (Figure 2A; point 1 in Figure 1) and anteriorly (Figure 2C; point 3 in Figure 1). The records on the right are superimposed tracings before and during the application of PGVS (short horizontal bars, curved arrows).

The action potentials in Figure 2A were apparently recorded from an AN cell as judged by their timing in reference to the inscription of the CrT and His electrograms as well as by their shape. In addition, the microelectrode location corresponded macroscopically to the posterior transitional zone. Note that although PGVS produced clear prolongation of the CrT-H interval (filled arrow), no change in the action potential was evident (open arrow).

The action potentials in Figure 2B were apparently recorded from an N (or NH) cell in either the midnodal zone or the adjacent transitional or lower nodal zone. Note that after PGVS, there was prominent membrane hyperpolarization (filled vertical arrow) associated with decreased amplitude and dV/dt of the action potential. Thus, there was clear correlation between the depressive effects of PGVS on the conduction time (filled curved arrow) and the cellular response (open curved arrow).

The action potentials in Figure 2C were recorded from a more distal NH cell. Note that after PGVS, there was a very small hyperpolarization, and the inscription of the action potential was delayed as well as the His bundle depolarization (arrows). No depression of the action potential amplitude was evident; in some cases, even an increase in dV/dt could be observed, an effect reported previously.

These observations suggest that the focus from which the maximum PGVS effect was elicited was located in the midnodal zone. The action potentials from this zone were typical for N cells, and PGVS produced a marked hyperpolarizing effect in these cells. When applied in the focus, PGVS produced a marked depressive effect on AV nodal conduction.
long) dotted area. As in Figure 2, PGVS applied in the focus produced a marked effect on AV nodal conduction; in this case, AV nodal block was evident (filled curved arrows). N cell action potentials demonstrated marked hyperpolarization, depression, and humps (Figure 3B, open arrow). Less marked hyperpolarization and a local response were evident in the record from the more anterior N cell (Figure 3C, open arrow), which was apparently located just distal to the site of AV nodal block. PGVS did not produce a significant effect in either the AN cell, which was depolarized (Figure 3A, open arrow), or the distal NH cell, which was predictably not depolarized because of conduction block (Figure 3D, open arrow). The small hyperpolarization, seen in Figure 3A, presumably represents an electrotropic influence from the nearby depressed anterior N cells (Figures 3B and 3C).

Furthermore, when PGVS was applied either in the AN zone (Figure 3E) or the NH zone (Figure 3F), there was no effect on either the AV nodal conduction time (the CrT-H interval) or on the action potentials recorded from the respective cells located in proximity to the PGVS electrode (Figures 3E and 3F).

These electrophysiological responses suggest that there is a morphological substrate associated with the midnodal zone that was responsible for the pronounced vagal effect when PGVS was applied in this zone. Prolongation of AV nodal conduction time (or AV nodal block) was associated with marked depression of the action potentials recorded from the N-type cells. The AN and distal NH cell action potentials were not influenced by PGVS applied in the focus or in either the AN or NH region, respectively. In the latter instances, the effect of PGVS on AV nodal conduction was minimal.

**Light-Microscopy Observations**

Microscopic and histochemical studies were performed to provide information about the morphological characteristics of the AV nodal tissue immediately below the focus of maximal PGVS effect determined by electrophysiological responses and to compare this region with the nearby regions from which only a weak PGVS effect could be elicited. Thus, the aim was not to provide a full morphological portrait of the AV node (a task successfully accomplished by others) but instead to look for morphological correlates of the space distribution of vagal effects determined by electrophysiological responses in various types of AV nodal cells. The most informative sections were taken parallel to the AV nodal ring and included all three marked areas (see "Methods" and Figure 1). As seen from the longitudinal section in Figure 4A, the PGVS focus (dot 2) was in the central part of the AV node; stained dot 1 was located over the approaches to the central node, and dot 3 was located over the proximal His bundle.

The posterior area (Figure 4B) was represented by somewhat loose cells with fatty infiltrates and a
considerable amount of connective tissue. This area was considered pre-AV nodal or one of the approaches to the compact AV node. The tissue below the focus (Figure 4C) contained compact, closely packed AV nodal cells; these were distinctly smaller and stained lighter than the atrial cells. Finally, the area anterior to the compact node (Figure 4D) invaginated into the base of the pars membranacea. The summit of the ventricular septum revealed some connective tissue; as the node entered this tissue, it was designated the beginning of the penetrating portion of the bundle of His. The cells were somewhat loosely spaced with fatty infiltrates and stained lighter than the myocardial cells. These observations identified the focus of maximal PGVS effect in the compact midnodal zone of the AV node. The vagal effect dissipated markedly as the PGVS electrode was moved posteriorly toward the
transitional zone (approaches to the AV node) or anteriorly toward the AV nodal bundle.

**Histochemical Observations**

All sections shown in Figures 5–7 were cut perpendicular to the endocardial surface of the AV node, as described in “Methods.” The three stained dots (Figure 1) were along a line approximately parallel to the AV nodal ring and were used for orientation. The sections in Figure 5 were cut perpendicular to the AV ring (i.e., in the coronal plane) and were from areas in proximity to the posterior (Figure 5A),

**FIGURE 4.** Panel a: Photomicrograph of three stained areas (1, 2, and 3) illustrated in Figure 1. Transverse section, Weigert-van Gieson stain. V, ventricular septum. Calibration bar, 1 mm. Panel b: Photomicrograph depicting approaches to compact atrioventricular (AV) node in vicinity of dot 1 (arrows). Hematoxylin and eosin stain. F, fat. Calibration bar, 0.1 mm. Panel c: Photomicrograph depicting compact AV nodal area in vicinity of dot 2 (arrows). Hematoxylin and eosin stain. Calibration bar, 0.1 mm. Panel d: Photomicrograph depicting nodal-bundle junction in vicinity of dot 3 (arrows). Hematoxylin and eosin stain. FT, fibrous tissue. Calibration bar, 0.1 mm.
FIGURE 5. Photomicrograph of coronal sections from vicinity of dot 1 (panel a), dot 2 (panel b), and dot 3 (panel c). Note high density of acetylcholinesterase-positive fibers in midnodal section (panel b, arrow) just below endocardium (E). Note also paucity of AchE-containing cross-sectional fibers in E and underlying fibroadipose tissue (panel a). Section in panel c was near penetrating portion of bundle of His and revealed substantial AchE activity (arrow). AM, atrial myocardium; VM, ventricular myocardium. Calibration bar, 1 mm.

central (Figure 5B), and anterior (Figure 5C) stained dots, respectively. The sections in Figure 6 were cut parallel to the AV ring (i.e., in the transverse plane) —above (Figure 6A), along (Figure 6B), and below the line of stained dots (Figure 6C). The sections in Figure 7 are higher magnifications of the posterior (Figure 7A), central (Figure 7B), and anterior (Figure 7C) portions, respectively, of the section shown in Figure 6B.

By analyzing a large number of sections (from which those shown in Figures 5–7 are representative), it was possible to semiquantitatively construct the extent of the AchE-containing tissue. This area was an approximately flat discoid embedded just below the endocardium and occupying the midnodal zone, with extensions tapering posteriorly toward the CrT input region and anteriorly toward the bundle of His (Figures 5A, 5B, 5C, and 6B). The longer axis
was oriented parallel to the AV ring (Figure 6B), and the shorter axis was perpendicular to the endocardial surface (Figure 5). The tissue staining AchE-positive (Figure 6B) was found only a short distance superiorly and inferiorly from the focus of maximal PGVS effect (Figures 6A and 6C).

The AN zone of the AV node (Figures 5A and 7A) was characterized by loosely arranged parallel fibers and a paucity of AchE-positive fibers in the loose fibroadipose tissue between endocardium and myocardium.

The N zone of the AV node (Figures 5B, 6B, and 7B), or the compact area, was characterized by a discoid configuration of densely packed cells and AchE-positive terminal nerve endings. The latter could be distinguished as more darkly stained lines on the background of less densely stained nodal fibers. Characteristically, the center (core) of this zone (Figure 7B) had a larger number of unstained fibers compared with the enveloping peripheral layers of the discoid body. It is possible that this pale region represented the midnodal zone, from which an absence of AchE reaction has been reported. Note that the staining dot marking the focus of the PGVS effect (Figure 7B, arrow) was just above the core of the discoid.

The NH zone contained a substantial number of darkly staining nerves among the parallel and somewhat loose nodal fibers (Figure 5C, arrow, and Figure 7C), although the density of AchE-positive staining was less than in the focus (Figures 6B and 7B).

Apparently, in the coronal sections (Figure 5), the AchE-positive staining tissue contained both lower nodal fibers (caudal, lower part) and transitional fibers (upper part). Similarly, the midnodal core (Figures 6B and 7B) was presumably enveloped by transitional (top) and lower nodal cells (bottom). In Figure 6A, apparently only transitional cells were present, whereas the section in Figure 6C was presumably cut through the lower nodal cells.

Discussion

The present study attempted to correlate the electrophysiological response of AV nodal cells to postganglionic vagal stimulation with the anatomical distribution of AchE within the node. The major result is the demonstration of a clear correlation between the localization of the depressive electrophysiological effects of PGVS on AV nodal conduction and the anatomical distribution of morphological substrates (i.e., nerves and AchE-positive fibers) at the focus of the vagal effect.

Electrophysiological Findings

PGVS produced marked prolongation of the AV nodal conduction time (and ultimately even AV nodal block) when it was applied in a specific small site in the center of the AV nodal region, designated the focus. When applied outside the focus, the effect of PGVS diminished rather sharply over a 0.5–1.0-mm radial distance. The action potentials recorded from cells in the focus revealed typical N-type characteristics—vagal stimulation was associated with pronounced membrane hyperpolarization and overall depression of the cellular response. PGVS, when applied in the focus, did not cause appreciable changes in the action potentials recorded from AN or distal NH cells. Furthermore, PGVS applied in the latter two regions did not have an effect on AV nodal conduction. These findings were reproducible when both the size of the stimulating electrode and the intensity of the vagal stimulation were limited. At higher stimulus intensities, the sharpness of the space distribution of the PGVS effect usually decreased.

Morphological Findings

Morphological observations confirmed that both the focus of the PGVS effect and N-type cellular recordings originated from the compact midnodal zone. We did not attempt to further verify the position of the microelectrode tip during the cellular recordings for two reasons. First, numerous studies with a broken glass electrode tip, an endocardial mark, injected substances (e.g., dyes or cobalt), or even glutaraldehyde fixation of the preparation with the impaled micropipette as an indicator of the microelectrode position have demonstrated with reasonable confidence that N-type potentials originate from cells in or near the midnodal zone. Second, we felt that absolute precision in determining the source of a particular action potential was not needed because the aim was to correlate the position of the microelectrode (e.g., the cellular group) with a macroscopic mark such as the focus of maximal PGVS effect.

The three-dimensional ultrastructural description by Thaemert showed that regions of “neuromuscular contiguity” in the AV node exist in various forms. Vascular nerve processes can dwell in sarcolemma-lined tunnels inside the nodal cells or lie within grooves on the surface of nodal cells, individually or in small bundles. A similar relation apparently exists in the rabbit AV node as well, as evidenced by our preliminary ultrastructural studies (unpublished observations, S. Imaizumi et al). These nerve processes are likely to account for the neuroeffector relation seen with PGVS.

Histochemical Findings

Additional studies by Anderson established that the lower nodal as well as the transitional cells were AchE-positive. Moreover, the nerve fibers located between the lower nodal cells were intensely AchE-positive and formed a plexus of smaller nerves with rami around the individual conduction cells.

Our histochemical results confirm these observations and identify the focus of maximal PGVS effect as a discoidal AchE-positive body occupying the midnodal zone and extending posteriorly and anteriorly. The core, which presumably corresponds to the
location of the midnodal cells, was relatively less AchE-positive but surrounded by richly AchE-stained lower nodal and transitional cells. In addition, deeply staining cholinergic nerve elements were evident in high density throughout this region. This histochemical picture correlates well with the electrophysiogical observations. Indeed, one would expect a high density of neuromediators to be available in immediate proximity to the N cells and possibly even further accentuation of neuromediator effect due to the relatively weaker AchE reaction of the core. However, this latter point needs clarification. Somewhat less AchE-positive conduction fibers could consistently be identified in the center of the midnodal longitudinal sections (Figure 7B), which confirms previous observations by Anderson. Nevertheless, the overall intensity of staining from these midnodal sections was substantially greater than from any other area, mainly due to the presence of numerous nerves.
Furthermore, AchE staining of conduction fibers in the midnodal zone of the AV node could be species as well as age dependent. The compact node of the midterm human fetus is AchE-positive but not innervated, in contrast to the surrounding transitional zone. In the adult dog, the compact node appears both AchE-positive and richly innervated. Thus, reduced AchE staining of the cells from the center of the rabbit heart AV midnodal zone cannot unequivocally be interpreted as a substrate specifically responsible for an accentuated vagal effect on the AV nodal conduction.

Conclusion

The results from this study lead to the major conclusion that the focus of maximal PGVS effect on AV nodal conduction coincides with the midnodal zone, which is embedded in and surrounded by a rich network of nerve fibers. The N-type conduction fibers should not necessarily be identified as belonging only to the center of this midnodal zone; they may also be found in the nearby enveloping layers of transitional and lower nodal cells. In this regard, the vagally responsive region (Figure 7B) would not only include the "true" midnodal zone (i.e., the core) but also the surrounding cellular groups that form the discoid region of conduction fibers and nerves that stain strongly AchE-positive. The critical role of the so-defined focus causing the vagally induced conduction delay may be related to the unique combination of anatomical factors and presence of large quantities of neuromediator and specific cellular properties. Thus, AV nodal architecture facilitates AV conduction by following pathways inevitably leading toward and through the midnodal zone, where the combination of vagal nerves and N cells with specific ionic membrane characteristics (slow channels) could make this part of the AV node the major target of vagal stimulation.

In contrast, the AN and distal NH zones lack this unique combination. On one hand, the density of nerve fibers is less (Figure 7A and 7C) than in the midnodal zone (Figure 7B), although both AN and NH cells stain weakly AchE-positive. On the other hand, the AN and distal NH cells appear less responsive to acetylcholine and PGVS, as evidenced by the level of vagally induced hyperpolarization. In addition, PGVS has been shown to enhance rather than depress dV/dt in distal NH cells. This is apparently related to the membrane properties of NH cells, which are characterized predominantly by a fast inward current, in contrast to the N cells, in which inward current is carried exclusively through the slow channels.

Finally, differences in the distribution of muscarinic receptors among the several cell types within the AV node cannot be ruled out and could explain part of the observed differences in the cellular responses.
to vagal stimulation. However, there are no available data to substantiate such speculation.

The results of this study further define the role of vagal stimulation on AV conduction and clearly identify the existence of a specific region in the node in which vagally induced depression is most profound. This region includes N-type cells with electrophysiological characteristics apparently responsible
not only for the cycle length–dependent properties of AV nodal conduction\(^3,\)\(^6\) but also for modulation of AV nodal function by the vagus.

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**Figure 7.** Photomicrographs of higher magnification of portions of section shown in Figure 6. Note loosely arranged parallel fibers and relatively sparse acetylcholinesterase (AchE) staining in AN zone (panel a). In contrast, note dense AchE staining in midnodal compact zone (panel b) and its decrease in intensity toward bundle of His (panel c). Calibration bar, 250 μm.


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**KEY WORDS**

- atrioventricular node
- vagus
- cardiovascular electrophysiology
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