A New Method for the Histological Study of Aging Changes in the Sinoatrial Node

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SUMMARY

In the autopsied heart, histological observation of specialized conducting cells, especially in the sinoatrial node, is very difficult. Conducting cells were investigated and classified in electron microscopic studies which were not adapted to the examination of the autopsied heart. In this study, we aimed to obtain a clear understanding of the microenvironment in the sinoatrial node of autopsied hearts. For this purpose, we prepared 1 μ thick sections using epon embedding methods for electron microscopy. Thus, we were able to isolate human SA nodal cells from the background collagen fiber, and morphological classification of conducting cells was enhanced. We examined histologically SA nodal cells from various age groups (20–86 years of age), and calculated the mean diameter and number of nodal cells in 4 age groups. SA nodal cells increased in diameter and decreased in number with aging.

Additional Indexing Words:
Sinoatrial node SA nodal cell 1 μ thin section
Collagen fiber Epon embedding Myofilament
Senile change

PROGRESS in the electrophysiological examination of the human conduction system of the heart has been remarkable. But the criteria used in the histopathological study of autopsied hearts are still based on the ratio of muscular components and fibrosis in the conduction system. The difficulty in the morphological study of the SA node comes from the fact that tissue preparations made by conventional methods are so thick (6–8 μ) that one can barely observe the fine structure of the conducting cell itself.1,2) Sections
thinner than 3 μ are fragile and unstable in paraffin embedding. SA nodal cells have small diameters almost equal to those of blood cells or the smooth muscle of the vascular media. On the other hand, the SA node abounds with collagen fibers, especially in older subjects, and SA nodal cells, are intermingled with these fibers. To our knowledge, no detailed information has yet been reported about the morphological features of the SA nodal cells in the autopsied heart. In the present study, an attempt has been made to investigate the microenvironment of the sinoatrial node by examining 1 μ thick sections.

SUBJECTS AND METHODS

The subjects used in this study were 60 patients (from 20 to 86 years of age) who had died of non-cardiac diseases. The most frequent cause of death was malignant tumors (32 cases), followed by diseases of the blood (10 cases), pneumonia (5 cases) and others (13 cases). Eight cases were medically treated for hypertension.

ECG findings were examined in the last month of life, during which ECG recordings were performed weekly or more frequently. Almost all patients showed regular sinus rhythm. Only 2 patients showed transient atrial arrhythmias terminally. No patients showed bradycardia (less than 50 beats/min) or tachycardia (more than 100 beats/min). Non-specific ST-T changes were observed in 10 cases, left ventricular hypertrophy was suggested in 4 cases, LAH was observed in 4 cases and RBBB and a pulmonary P wave were observed in 1 case each. Patients with AV conduction disturbances were excluded from this study.

Methods: The sinoatrial junctional area was removed from the heart, fixed in formalin and then cut into 5 sections by the modified method of Lev (Fig. 1). Both ends and mid portion were embedded in paraffin, cut into 6 μ thick specimens sub-serially and stained with Elastica-van-Gieson’s stain for identification of the sinoatrial node (Figs. 1 and 2). Residual tissue sections (2 portions) were further cut into 2 minute blocks, 3×2×1 mm sized solid bodies, containing the sinoatrial node, which was histologically identified by paraffin sections. These minute tissue sections were embedded in epon and cut into semi-thin sections of 1 μ thickness, using a LKB ultra-microtome with a glass knife. We stained the specimens with toluidine blue 0 and examined them under a light microscope with or without an oil immersion lens (Fig. 3).

The 60 cases were divided into 4 groups according to age, i.e. group I (20–39 years): 13 cases; group II (40–59 years): 15 cases; group III (60–
Fig. 1. Sinoatrial junctional area was removed from a formalin fixed heart. Then it was cut into 5 sections close to the right atrial appendage. Sections A, C and E were paraffin embedded for identification of the sinoatrial node. Sections B and D were left for epon embedding.

Fig. 2. After identification of the SA node, we cut 2 additional minute blocks (3×2×1 mm) from residual sections within the framework suggested in the upper figure. (Elastica-van-Gieson's stain, ×40)

79 years): 25 cases and group IV (80–86 years): 7 cases.

Histological examination of the preparation was performed as follows; we calculated the mean transverse diameter around the nucleus in every 10 nodal cells of 4 blocks in each case. Then we counted the number of nodal cells in four visual fields under high magnification (×400) of the sinoatrial node and calculated the mean number of nodal cells in each case.
Fig. 3. A photomicrograph of the sinus node from a 33-year-old male (same subject as in Fig. 2) made from a selective 1 μ section after epon embedding. Fine structures of the nodal cells are revealed. Most typical SA nodal structures are observed around "P", which is expected to possess the highest automaticity. "N" exhibits nerve fiber, and "T" shows transitional cells, which are intermediate in character between SA nodal cells and working atrial muscle. (Methyl 0 toluidine blue stain, ×400)

Results

We compared the diameter and population of nodal cells in the above mentioned 4 groups using the unpaired t-test, and differences at a 5% level (p=0.05) or less were considered to be significant. The SA nodal cells were smallest in size in group I, and increased in size in groups II–IV (Table I). On the other hand, the population of nodal cells was most abundant in group I and considerably decreased in groups II–IV.

From the morphological aspect, myofilaments were remarkably tortuous, running in many directions in group I (Fig. 4), and collagen fibers were thick
Table I. Mean Diameter and Number of SA Nodal Cells in 60 Cases

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>mean heart weight (g)</th>
<th>mean diameter (μ)</th>
<th>mean number/visual field</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td>271±93</td>
<td>8.5±2.1</td>
<td>112±20</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>361±80 (p&lt;0.05)</td>
<td>12.7±3.7 (p&lt;0.005)</td>
<td>67±29 (p&lt;0.001)</td>
</tr>
<tr>
<td>III</td>
<td>25</td>
<td>333±65 (p&lt;0.05)</td>
<td>12.8±3.3 (p&lt;0.005)</td>
<td>60±31 (p&lt;0.0001)</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>367±145 (p&lt;0.1)</td>
<td>11.4±2.2 (p&lt;0.05)</td>
<td>54±30 (p&lt;0.001)</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

Fig. 4. A high magnification photomicrograph of the sinoatrial node of a 33-year-old male. Myofibrils (arrow) and their Z band demonstrate the complicated solid construction of the nodal cells. The cluster of nodal cells exhibit a disorganized appearance, and furthermore, in each cell the directional orientation of the myofibrils is random. (Toluidine blue 0 stain, ×1,000)

and long, containing clusters of SA nodal cells. There were abundant intercellular spaces between the SA nodal cells and collagen fibers in group I. But, in the older subjects in groups II–IV, SA nodal cells were not only increased in diameter, but were stretched and separated from each other. Myofilaments appeared straight, running in nearly the same direction (Fig. 5). Collagen fibers increased and intercalated the SA nodal cells especially in groups III and IV. Degenerated and atrophied nodal cells were also observed in group IV (Fig. 6). Mean heart weight was also increased in groups II–IV, presumably due to an increased incidence of HHD and ischemic heart disease. Postmortem examination of the heart suggested coronary atherosclerosis (>75%) in 2 cases in group II and 5 cases in group
Fig. 5. A high magnification photomicrograph of the sinoatrial node of a 63-year-old male. The sinus node cell is enlarged and its myofibrils appear straight, running in nearly the same direction (arrow). The cluster of nodal cells is pulled apart to individual cells. Fine networks of the collagen fibers disappear and the intercellular spaces are filled with slender and undulating collagen fibers (*). (Toluidine blue 0 stain, ×1,000)

Fig. 6. Sinoatrial node of very old age, taken from an 84-year-old female. Degenerated and atrophied nodal cell (arrow). The number of nodal cells is decreased, and the collagen fibers are increased (*). (Toluidine blue 0 stain, ×1,000)
III. Old myocardial infarction without cardiac aneurysm was found in 1 case in group II and 4 cases in group III. Valvular heart disease was not found. HHD (>400 g) was found in 4 cases in group II, 4 cases in group III and 1 case in group IV.

DISCUSSION

Preparations of 1 μ thick sections of the sinoatrial node have made it possible to observe individual cells of the human sinoatrial node, whose precise observation would otherwise not be possible. By this procedure, one can adequately classify specialized conducting cells in autopsy cases. Observation of nodal cells in various age groups suggested that younger subjects under 39 years of age demonstrate the typical appearance of the sinoatrial node, previously reported in electron microscopic studies.3)-8) But in older subjects, SA nodal cells are decreased in number and reveal a considerable degree of morphological variation. The sinoatrial node has been said to undergo progressive fibrosis and a reduction in the number of SA nodal cells.9)-13) Our present study showed a considerable decrease in the number of cells in group II compared with that in group I. On the other hand, the transverse diameter of nodal cells in group II was increased as compared with that in group I. Additionally, SA nodal cells were stretched and enwrapped in collagen fibers in groups III and IV. A gradual decrease in number and an atrophy of SA nodal cells seemed to occur. Concerning the cause of the stretching of nodal cells, Davies stated that a degree of dilatation occurred with aging in both atria, possibly as a consequence of atrial muscle atrophy.13),15) Davies postulated that the sinus nodal cells having very few myofilaments in their cytoplasm, had very weak resisting power against the stretching force. Therefore, we believe that thick collagen networks might play an important role in supporting the clusters of SA nodal cells in the presence of atrial wall tension. In addition to the loss of muscle fibers and the increase in fibrous tissue, we were unable to find much adipose tissue in the node itself. Rossi stated that the sinus node contained some adipose tissue, and that the node was free from significant senile changes, as it showed almost constantly, a rich myocardial component in the thick collagen meshwork acquired after birth.14) We found that the intercellular spaces were filled with newly made collagen fibers with aging, and that the residual SA nodal cells were hypertrophied and stretched.
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REFERENCES