The Isolated Sinoatrial Node Cell in Primary Culture from the Newborn Rat


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SUMMARY. We prepared primary cell cultures of the sinus node region from newborn rat hearts. Sinoatrial node cells were easily distinguished from the other cardiac muscle cells and nonmuscle cells in culture by size, configuration, and rapid, attenuated spontaneous contractions (185.0 ± 8/min, mean ± SEM). The spontaneously contracting sinoatrial node cells were extremely sensitive to acetylcholine and norepinephrine, responding to concentrations at least 1000-fold less than other cardiac muscle cells. These same sinoatrial node cells in culture were fixed and precisely relocated by either subsequent scanning or transmission electron microscopy. The ultrastructural features of these sinoatrial node cells in culture were similar to those observed in the cells of intact sinus node sections from the source hearts. This study is the first to present single, spontaneously active, neonatal sinoatrial node cells maintained in vitro with morphological and functional properties desirable for physiological investigations. (Circ Res 55: 253-260, 1984)

Both morphologists and electrophysiologists agree that the sinus node is far from homogeneous. Ultrastructural studies in multiple species have revealed at least three distinct cardiac cells in the sinus node: typical nodal cells, transitional cells, and intercalated clear cells (James et al., 1966; Viragh and Porte, 1973; Tranum-Jensen, 1976). Electrophysiological heterogeneity is reflected by the presence of both dominant and subsidiary pacemaker cells and the shifting origin of the initial pacemaker impulse, within the sinus node itself (Trautwein and Uchizono, 1963; Brooks and Lu, 1972; Bouman et al., 1978). This heterogeneity of the sinus node can be expected to produce difficulty with demonstrating direct structural and functional correlates at the cell level.

Intricate experiments designed to correlate sinus node structure and function have utilized electrophoretic dye injections through microelectrodes (Janse et al., 1978) or extensive microelectrode mapping of the sinus node in combination with electron microscopic techniques (Masson-Pévet et al., 1978; Bleecker et al., 1980). Microelectrode impalement consistently resulted with disruption of the cells within the intact sinus node, obscuring the precise cellular origin of recorded action potentials. Thus, even with such skillful studies, direct correlates at the single cell level are not consistently feasible.

We previously reported the correlation of morphological, pharmacological, and electrophysiological characteristics of single cardiac ventricular muscle cells in primary culture (Marvin et al., 1979). These isolated ventricular muscle cells maintained phenotypic expression identical to the source tissue origin. This study reports the preparation of isolated sinoatrial node cells in primary culture using similar methodology. Like the isolated ventricular muscle cells, these individual sinoatrial node cells retain in culture the functional and ultrastructural characteristics demonstrated by the intact sinus node cells. Individual sinoatrial node cells, pharmacologically manipulated in culture, can be relocated subsequently by microscopic technique and provide direct correlates of function and structure at the single cell level.

Methods

Cell Cultures

Newborn rats from the Kyoto-Wistar colony at the University of Iowa were used to develop primary cell cultures of sinoatrial node cells. Ventricular muscle cells were also cultured, as previously reported (Marvin et al., 1979), for purposes of comparison. Under the stereomicroscope and aseptic conditions, the sinus node region of the right atrium, approximately 1–2 mm in diameter, was surgically removed from 10 to 22 rats. The margins of these sinoatrial node portions would unavoidably include cells from the proximal superior vena cava and the crista terminalis of the right atrium. The tissue fragments were soaked in a petri dish containing CV3M culture medium. The CV3M medium consisted of 85% MEM-Earle’s salts, 15% horse serum, 4 mM L-glutamine, 20 μg/ml gentamicin, 20 mM Hepes buffer (pH 7.3), and 16 mM NaHCO3. After the completion of dissection, the tissue fragments were...
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The second group of spontaneously contracting
cardiac cells was morphologically distinct from the
atrial muscle cells. The cardiac cells in this second
subset were the smallest contracting cells, 3–8 μm
in diameter and 20–35 μm long. These cells invariably
had an elongated or spindle configuration, and
lacked the radial projections of the contracting atrial
muscle cells. The distal ends of these spindle-shaped
cells flattened to a thickness of less than 2 μm, and

Sinoatrial Node Cells in Culture

A heterogeneous population of cardiac cells was
found in all cultures prepared from the sinus node
region. At least two distinctly different types of
spontaneously contracting cardiac muscle cells were
observed, as well as numerous noncontractile fibro-
cytes, endothelial cells, and vascular muscle cells,
presumably from the superior vena cava. The greater
proportion of contracting cardiac muscle cells were
relatively large atrial muscle cells, 50–90 μm in
diameter, with polyhedral central bodies and four
to five cytoplasmic radial projections. These projec-
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Electron Microscopic Observations

Following observation in the living state, the coverslips
were fixed, dehydrated, dried, and mounted for scanning
electron microscopy. A standard technique was followed
(Anderson, 1951), with the exception that the glutaralde-
hyde fixative was preheated to 37°C. For transmission
electron microscopy, cell suspensions were plated directly
on the petri dishes rather than on glass coverslips. The
underside of the petri dishes were permanently marked
to indicate the location of selected cells. Photomicrographs
of increasing magnification (10× to 320×) facilitated cell
observation by utilizing both culture topography and the
single cell configuration. In addition to photomicrographs,
a sketch of the culture topography was essential for ori-
entation, since later preparation separated the cells from
the marked petri dish. Cells selected for transmission
electron microscopy were rinsed twice with warm ISM
buffer solution prior to fixation. The ISM buffer solution
consisted of 143 mM Na+, 4.7 mM K+, 1.8 mM Ca++, 0.8
mM Mg++, 139 mM Cl−, 16 mM NaHCO3, 0.4 mM SO4−
and 17 mM Heps (pH 7.6). After being rinsed, the cells
were fixed in glutaraldehyde buffer with cacodylate,
soaked in osmium tetroxide, dehydrated in alcohol, and
embedded in Epon. Curing of the epon preparation at
50°C was interrupted at 12 hours to replicate the petri
dish cell location marks directly on the Epon. The petri
dish was carefully peeled away and the remaining cell
monolayer was returned to the oven for another 36 hours
at 60°C. After curing, the embedded cell monolayer was
examined under the light microscope. Utilizing the previ-
ously made photomicrographs and topographic sketch,
we relocated the desired cells in the embedded monolayer.
The selected monolayer area was appropriately oriented,
mounted, trimmed to less then 0.5 mm in diameter, and
sectioned between 80 and 100 nm with an American
Optical/Reichert Ultracut E microtome. These ultra-thin
sections were stained with uranyl acetate and Reynold’s
lead citrate, and photographed with a Hitachi H600 trans-
mition electron microscope. For comparison with these
cultured cells, the intact sinus node region was also pre-
ared for transmission electron microscopic observation
by standard techniques.

Statistical Analysis

Where possible, statistical comparisons were made by
Student’s group t-test of cell cultures. The 0.05 confidence
level was accepted as significant.

Results

Sinoatrial Node Cells in Culture

A heterogeneous population of cardiac cells was
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region. At least two distinctly different types of
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FIGURE 1. Scanning electron photomicrographs of isolated cardiac muscle cells in primary culture. Part A: atrial muscle cell with polyhedral central body and radial projections anchoring the cell, 800X. Part B: large ventricular muscle cell numerous radial projections, exceeding in number that of all other cardiac muscle cells, 750X. Part C: sinoatrial node cell with spindle configuration and bipolar attachment to underlying substrate, 1500X.
TABLE 1

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Range</th>
<th>Mean ± SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinoatrial node cell</td>
<td>95-300/min</td>
<td>185 ± 8/min*</td>
<td>42</td>
</tr>
<tr>
<td>Atrial muscle cell</td>
<td>85-135/min</td>
<td>112 ± 5/min*</td>
<td>56</td>
</tr>
<tr>
<td>Ventricular muscle cell</td>
<td>75-110/min</td>
<td>89 ± 2/min*</td>
<td>146</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05 level.

were the apparent points of attachment to the underlying substrate. Since these small spindle cells were never observed in either ventricular or atrial muscle cell cultures, which excluded the sinus node region, they were presumed to be isolated sinoatrial node cells (Fig. 1C).

These presumed sinoatrial node cells had distinct contractile characteristics, as well as distinct morphological characteristics, from the atrial and ventricular muscle cells. The greatest range of spontaneous contraction frequency was observed in these presumed sinoatrial node cells, and their mean frequency was significantly higher compared to atrial and ventricular muscle cells (Table 1). The mean rates of the atrial and the ventricular muscle cells were also significantly different from each other. The presumed sinoatrial node cell contractions had very attenuated excursions which could be best described as rapid twitching, whereas the slower contractions of the atrial and ventricular muscle cells were quite vigorous. When either acetylcholine or norepinephrine was applied to individual presumed sinoatrial node cells, a respective negative or positive chronotropic response was observed (Fig. 2). These presumed sinoatrial node cells were uniquely sensitive compared to ventricular muscle cells. The addition of $10^{-13}$ g/ml acetylcholine decreased contraction frequency 55.8 ± 8.9% (n = 10) and $10^{-14}$ g/ml norepinephrine increased contraction frequency 24.4 ± 2.7% (n = 10). A comparable negative chronotropic response was observed in ventricular muscle cells at $10^{-9}$ g/ml acetylcholine, 57.2 ± 12% (n = 12). Ventricular muscle cells failed to respond at all to norepinephrine in concentrations less than $10^{-11}$ g/ml (n = 23). Thus, at least a 1000-fold difference in sensitivity is suggested between sinoatrial node and ventricular muscle cells.

Ultrastructural Confirmation of Sinus Node Origin

The spontaneously contracting sinoatrial cells exposed to acetylcholine and norepinephrine were prepared for subsequent scanning or transmission electron microscopy. These single cells could be consistently relocated on a one-to-one basis for ultrastructural examination (Fig. 3). All cells contained a single, large, lobulated, and centrally situated nucleus. Rather than uniformly dispersed throughout the nucleoplasm, the chromatin was found frequently concentrated about the nuclear envelope. Most of the intracellular organelles, except the contractile apparatus, were distributed on either side of the central nucleus parallel to the long axis of the cell. The Golgi apparatus was usually perinuclear in location opposed to mitochondria, with well-developed cristae, which were more uniformly spread throughout the sarcoplasm. Glycogen granules and lipid droplets were characteristically prominent; the latter appeared to increase in density and size as cells were maintained in culture longer than 4-5 days. The ultrastructural hallmark of these cells was the myofibrils, which without exception were poorly organized and decreased in number. The paucity of myofibrils was consistent with the very attenuated contractions observed in the living state. Thick and thin filaments could be seen randomly throughout the sarcoplasm or sporadically organized into myofibrils with irregular Z lines (Fig. 3C). Although not quantified, myofibril bundles appeared most commonly in the peripheral cytoplasm in juxtaposition to the sarcolemma and aligned with the long axis of the cells. These ultrastructural findings were ob-

FIGURE 2. Video recordings of spontaneous contractions of single sinoatrial node cell during neurotransmitter exposure. Arrows indicate point of neurotransmitter application. Recording speed was 25 mm/sec, indicated by 1-second time bar. Part A: decrease in frequency from 165/minute to 95/minute followed the application of 20 μl acetylcholine at $x10^{-13}$ g/ml concentration. This response was blocked by $10^{-8}$ g/ml atropine sulfate suffusion for 2 minutes. Part B: increase in frequency from 110/minute to 145/minute followed the application of 10 μl norepinephrine at $x10^{-13}$ g/ml concentration. This response was blocked by $10^{-8}$ g/ml propranolol suffusion for 2 minutes.
FIGURE 3. Relocation of individual sinoatrial node cell in culture. Part A: this cell was spontaneously contracting 180/min in culture when phase contrast photomicrograph taken, 320×. Part B: after fixation, the identical sinoatrial node cell was relocated under the transmission electron microscope, 15000×. Part C: higher magnification demonstrates sparse and poorly organized myofibrils with arrows indicating Z band material, 25,000×.

served in all presumed sinoatrial node cells relocated by transmission electron microscopy.

Whole mount preparations of the intact sinus node from newborn rats were comparably examined by transmission electron microscopy. The node could be oriented in sectioning so the predominant cell fibers within the central portion of the sinus node head were elongated in configuration (Fig. 4). Each of these particular sinus node cells had a central nucleus, intracellular organelles aligned along the long axis of the cell at both ends of the nucleus, and a contractile apparatus, peripheral to the nucleus and organelles, parallel to the sarcolemma. The only major difference between these particular sinus node cells and those in culture was the decreased quantity contractile apparatus adjacent to the sarcolemma of the cultured cells, not an uncommon finding after enzymatic dispersion of muscle cells. Thus, the size, configuration, and distribution of intracellular structures of these in situ sinus node cells are qualitatively similar to that observed in the presumed sinoatrial node cells in vitro (Fig. 3).

Discussion

These studies demonstrate that single rat sinoatrial node cells can be isolated and maintained in primary cell culture, with the retention of both spontaneous activity and distinctive morphological characteristics. The high sensitivity of the single sinoatrial node cell to neurotransmitters indicates that muscarinic and β-receptors are not destroyed by trypsin dissociation of the sinus node. Although dose-response curves to neurotransmitters are not yet completed, these preliminary data suggest that the sensitivity of isolated sinoatrial node cells far exceeds that reported for isolated ventricular muscle cells under identical culture conditions (Hermsmeyer and Robinson, 1977; Marvin et al., 1979; Herms-
FIGURE 4. Transmission electron photomicrograph of sinus node cells in situ, 4000X. Section of sinus node from newborn rat heart demonstrating predominant cell in sinus node head. Centrally located cell is elongated with large central nucleus, perinuclear intracellular organelles, and peripheral sparse contractile apparatus in parallel to sarcolemma. Arrows indicate cell margin. The configuration, size, and distribution of intracellular organelles depicted in this cell are similar to cultured sinoatrial node cell seen in Figure 3.

meyer et al., 1982). This report emphasizes that a direct one-to-one cell correlation can be made with ultrastructural examination following physiological observation. Such single cell preparations should eliminate the disadvantage of drug diffusion through multicellular geometry and the interference of extracellular substances within the intercellular space (Osterrieder et al., 1981), problems observed during electrophysiological sinus node studies on isolated tissue.

A Langendorff preparation of adult rabbit sinus and atrioventricular tissue has yielded cells noted to be spontaneously active (Taniguchi et al., 1981). This study importantly demonstrated the feasibility of electrophysiological investigation in cells isolated from the sinus node. The preparation, however, required acute experimentation because spontaneous activity was only present immediately after isolation. The size and the shape of the nodal cells also were not wholly characteristic of the typical nodal cells in situ. The lower sensitivity of these adult sinus node cells to neurotransmitter may be related to the preexistence of mature autonomic innervation, as well as the acuteness of the preparation. A more recent report of isolated sinus node cells from the adult rabbit heart utilized a dissociation procedure combining mechanical and enzymatic dispersion (Masson-Pévet et al., 1982). Although these isolated sinus node cells had ultrastructural morphology similar to the typical nodal and transitional cells in situ, spontaneous activity was not indicated. The only evidence presented for sinus node cell viability was the exclusion of trypan blue. Thus, despite their achievements, neither one of these investigations demonstrated direct functional and morphological correlates in a single cell. Examination of individual ion channel kinetics will require single cell voltage clamp technique. We
unfortunately encountered the same difficulty with microelectrode impalement as did Taniguchi and co-workers. The continual rapid contractions consistently injured the single cell during microelectrode penetration. In addition, impalement frequently dislodged the single cell from its fragile attachment to the underlying substrate, a problem even more pronounced than in single ventricular muscle cells (Matsumi and Hermsmeyer, 1983). Such technical difficulties might be eliminated by selecting cell clusters for microelectrode impalement (with loss of single cell advantages) or further optimizing the substrate to promote sinoatrial node cell adherence.

Our study also described only one population of sinoatrial node cells. We intentionally excluded other sinoatrial node cells from this report by our specific selection criteria of small size and rapid spontaneous contractions. The expansion of our criteria to include cells with a configuration more similar to the isolated atrial cell, a larger size (40–60 um in diameter), or slower, more vigorous contractions would presumably demonstrate other cells, such as the transitional cells of the sinus node.

Cardiovascular muscle cells in culture may with time undergo the phenomenon of cell dedifferentiation and fail to maintain characteristics of the original in vivo source (Chamley et al., 1977). Spontaneous activity and high sensitivity to neurotransmitters persisted in these cultured sinoatrial node cells for 6 days. Although small ultrastructural differences might be observed, secondary to either enzymatic dispersion or early cell dedifferentiation, the retention of these functional characteristics in vitro is most crucial to experimental interpretation.

These experiments demonstrate direct functional and morphological correlates in single sinoatrial node cells from the neonatal rat. The retention of distinct ultrastructural characteristics and spontaneous activity identical to that observed in vivo suggests that this preparation would be suitable for single cell functional investigations. Although there still are difficulties with intracellular recording techniques, we believe the isolated sinoatrial node cell will offer the same advantages as the isolated ventricular muscle cell e.g., single channel recording with the patch clamp technique (Lee and Tsien, 1983).

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