Morphological and membrane characteristics of spider and spindle cells isolated from rabbit sinus node

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Wu, Jianyi, Richard B. Schuessler, Mark D. Rodefeld, Jeffrey E. Saffitz, and John P. Boineau. Morphological and membrane characteristics of spider and spindle cells isolated from rabbit sinus node. Am J Physiol Heart Circ Physiol 280: H1232–H1240, 2001.—This study reports the comparative quantitative, morphological, and electrophysiological properties of two pacemaker cell types, spider and spindle-shaped cells, isolated from the rabbit sinoatrial node. Isolated nodal cells were studied with perforated and ruptured patch whole cell recording techniques. The basic spontaneous cycle length of the spider cells was 381 ± 12 ms, and the basic spontaneous cycle length of the spindle cells was 456 ± 17 ms (n = 12, P < 0.05). The spider cells had a more positive maximum diastolic potential (−54 ± 1 mV) compared with the spindle cells (−68 ± 1 mV, P < 0.05). The overshoot and action potential amplitudes were also smaller in the spider cells. The hyperpolarization-activated inward (Ih) current density, measured from their tail currents, was 15 ± 1.3 pA/pF for the spider cells and 9 ± 0.7 pA/pF for the spindle cells (P < 0.01). Ih current activation voltage was more positive in the spider cells than the spindle cells. ISOproterenol (1 μM) decreased the spontaneous cycle length of the spider cells by 28 ± 3% and the spindle cells by 20 ± 1.5% (P < 0.05). Acetylcholine (0.5 μM) hyperpolarized the membrane potential of the spider cells to −86 ± 0.7 mV and the spindle cells to −76 ± 0.8 mV (P < 0.05). In summary, there are at least two distinct pacemaker cell types in the sinus node with different electrophysiological characteristics.

hyperpolarization-activated inward current; pacemaker current

KEITH AND FLACK (14) first described the specialized tissue of the sinus node and suggested that it was the site of impulse origin. A later study (17) confirmed that the impulse originated from the node during sinus rhythm. In a series of previous studies in both canine and human subjects, we demonstrated the multicentric origin of the atrial depolarization wavefront. Although unifocal impulse origin within the node was noted, wavefronts frequently began at two or more exit sites and dispersed over a distance of 1–5 cm (4, 17). Also, the multicentric activation patterns frequently changed, such that one or another of the points of impulse origin activated earlier than the others. These dynamic changes in the complex activation patterns were linked to changes in heart rate induced by adrenergic and cholinergic influences, including cardiac nerve stimulation or infusion of neurotransmitter substances. No matter the instantaneous pattern of activation or which site was earliest, wavefront origin was restricted to one or more of these specific locations. As a result of these studies and the work of others (17), we concluded that the changes in rate and complex activation reflected the interaction of two factors: 1) changes in the site of the dominant sinus node pacemaker, and 2) change in the site of earliest exit from the sinus node. In addition, we also observed extranodal sites of impulse origin that extended the spatial, as well as functional, range of impulse origin. We referred to this linkage between change in heart rate and activation pattern as the atrial pacemaker complex.

To evaluate the possibility that the concomitant changes in heart rate and atrial activation were related to functional differences in atrial pacemaker cells, we initiated the present study. We described two different atrial pacemaker cell types: a stellate or spiderlike multistranded cell and a spindle-shaped cell isolated first from the canine and later from the rabbit sinus node. The spider cells always demonstrated faster spontaneous depolarization than the spindle cells. In addition, we (15) previously reported on the unique distribution of connexin protein gap junctions in these cells compared with typical atrial myocytes. The purpose of the present study was to: 1) reproducibly isolate both spider and spindle pacemaker cell types from the rabbit sinoatrial node (SAN); 2) characterize and compare the quantitative electrophysiological properties and morphology of the two cell types; and 3) determine whether the spider cells represented a single cell type or actually two or more spindle cells partially superimposed, giving the erroneous impression of multiple stellate projections. Specifically, each morphological cell type was functionally characterized by its spontaneous rate of depolarization, action poten-
tial parameters, magnitude and activation of the hyperpolarization-activated inward ($I_f$) current, and its response to isoproterenol and acetylcholine. There were significant differences in intrinsic rate, diastolic potential, membrane $I_f$ currents, and responses to adrenergic and cholinergic neurotransmitter stimulation, which consistently correlated with each cell type. The data are compared with a previous report (5), which also describes spider and spindle cells, in which no significant functional differences were observed. Recently, Verheijck et al. (21) also described three morphologically distinct nodal cell types isolated from the rabbit SAN, mapped their distribution within the node, and recorded their electrical characteristics. In contrast with the present study, they did not find significant correlation between cell types and electrophysiological differences.

**METHODS**

**Isolation of SAN cells.** Adult rabbits of either sex were anesthetized with pentobarbital sodium (30 mg/kg). After a median thoracotomy, the heart was rapidly removed, mounted on a Langendorff perfusion apparatus, perfused for 3 min with a Tyrode solution [containing (in mM) 125 NaCl, 5.4 KCl, 1.8 CaCl$_2$, 1.0 MgCl$_2$, 24 NaHCO$_3$, 0.6 NaHPO$_4$, and 11 glucose], and saturated with 95% O$_2$-5% CO$_2$ at pH 7.4. The initial perfusion interval was followed by a 3-min perfusion with a Ca$^{2+}$-free Tyrode solution and a final perfusion for 15 min with a Tyrode solution containing 36 μM Ca$^{2+}$, 0.02% albumin, and 0.05% collagenase (336 μg, type II; Worthington Biochemical, Freehold, NJ). After the final collagenase perfusion, the SAN region (5 x 10 mm) was dissected from the right atrium, cut perpendicular to the crista terminals into three to four pieces, and incubated in fresh enzyme solution containing 0.1% elastase (type II-A; Sigma Chemical, St. Louis, MO) and 0.001% protease (type XIV, Sigma Chemical) for 60 min with constant shaking in a water bath at 37 ± 1°C. The SAN tissue strips were placed in Ca$^{2+}$-free Tyrode solution and transferred into a high-potassium solution [containing (in mM) 100 potassium aspartic acid, 30 KCl, 2 K$_2$ATP, and 11 glucose and 0.02% albumin titrated with KOH to pH 7.35]. After the tissue strips were incubated at 4°C for 60 min, each piece was gently triturated using a polyethylene transfer pipette (tip diameter 3 mm) in a small centrifuge tube containing 6 ml of high-potassium solution. During the trituration procedure, the tissue was teased apart, and cells became suspended in the solution. The cell suspension was stored in a 25-ml beaker at 4°C until use within 10 h after isolation.

**Patch-clamp whole cell recording.** Cells were transferred to a small (0.5 ml) bath mounted on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and allowed to settle for 20 min. Cells were superfused initially with Ca$^{2+}$-free Tyrode solution for 3 min and then superfused continuously at a rate of 2 ml/min with normal Tyrode solution [containing (in mM) 137 NaCl, 4.0 KCl, 1.0 MgCl$_2$, 1.8 CaCl$_2$, 10 HEPES, and 11 glucose titrated with NaOH to pH 7.4] at 36 ± 1°C. Whole cell recordings were obtained using glass pipettes (inner diameter 2–3 μm) made with a programmable puller (P-87/PC, Sutter Instrument, Novato, CA) and heat polished with a microforge (Narishige, Tokyo, Japan). The internal pipette solution used for the amphotericin B perforated patch recording (9, 16) contained (in mM) 100 potassium aspartic acid, 40 KCl, and 10 HEPES and was titrated with KOH to a pH of 7.2. Amphotericin B was dissolved in dimethyl sulfoxide at a concentration of 60 mg/ml and then added to the internal pipette solution to yield a final concentration of 150 μg/ml. This internal solution was ultrasonified (Branson Sonifier 250, Danbury, CT) for 5 min to fully disperse the amphotericin B. After ultrasonification, the solution was clear yellow in appearance. To prevent precipitation of amphotericin B, the solution was continuously stirred until it was used. Pipettes were first filled by putting the pipette tip into the amphotericin-containing solution for more than 30 s and then back filling with the same solution.

The perforated patch whole cell recording method was used in most experiments in which action potentials were recorded. To characterize the electrophysiological properties of $I_f$, the ruptured whole cell recording method was used (2). The blocking agents added to the extracellular solution to inhibit other membrane currents that might interfere with the analysis of $I_f$ included 2 mM NiCl$_2$, 0.5 mM BaCl$_2$, 2 mM 4-aminopyridine, and 0.2 mM lidocaine. The intracellular solution for the ruptured whole cell recordings contained (in mM) 100 potassium aspartic acid, 30 KCl, 1 MgCl$_2$, 10 HEPES, 1 EGTA, and 5 K$_2$ATP and was titrated with KOH to a pH of 7.2.

Whole cell recording procedures were performed using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) to record action potentials (bridge mode) and ionic currents (discontinuous single-electrode voltage-clamp mode), as previously described (24, 25). The sample rate was 10–12 kHz, and the signals were filtered at 0.3–1.0 kHz. Computer software (pCLAMP, Axon Instruments) was used to generate the voltage-clamp protocols and to acquire and analyze voltage and current signals. Current and voltage were sampled using a 12-bit resolution analog-to-digital (A/D) converter interfaced to a Zenith computer. Data were also stored on videotape with a PCM-4 A/D videocassette recorder adapter (Medical Systems, Greenvale, NY). During the experiments, cells were visualized by a video camera mounted to the microscope.

The liquid junctional potential between the pipette and bath solution was corrected by 10 mV. Specifically, the junction potential value was obtained by measuring the potential difference when the extracellular solution was changed to the intracellular solution. An agar-KCl bridge of 3 M was used as a reference ground. The membrane capacitance was measured by delivering a ramp pulse [change in voltage over time (dV/dt) = 4 V/s] and dividing the half-amplitude of the current jump by the rate of the ramp at the turning point of the ramp pulse (24, 25). The input resistance was measured by delivering a 0.1-nA pulse and recording the change in voltage. Two criteria were used to assess the stability of the recording: 1) a good seal configuration, which was indicated by a high-input resistance in the gigahm range and a sharp square wave during the measurements of membrane capacitance; and 2) a 2-min waiting interval after establishing the whole cell recording configuration, which allowed the pipette solution to equilibrate with the cell. Isoproterenol (Iso) (Sigma Chemical) and acetylcholine (ACh) (Sigma Chemical) were freshly prepared for each experiment. Data are presented as means ± SE and were compared using an unpaired t-test with Dunn-Sidak correction for multivariate data (Table 1) and ANOVA for data with more than two factors, cell type and Iso treatment (Table 2). Data with a P value <0.05 were considered statistically significant.

**Histology.** Previous investigators (5, 8) have suggested that isolated cells having a spider shape were actually multiple cells because they appeared to have more than one nucleus. In experiments performed to determine whether spider cells were composed of multiple or single cells, cell
suspensions were allowed to settle at 4°C for 3 h, and the suspension solution was suctioned off. The cells were rapidly fixed with Karnovsky’s fixative, and small amounts of the suspensions were spread on glass slides and allowed to air dry. The cells were stained with either hematoxylin-eosin (H-E) or toluidine blue.

Pacemaker cells were easily differentiated from myocytes. The myocytes showed clear striation, sharp edges, and a more intense H-E staining. The myocytes were also larger in both length and width. In addition, myocytes did not exhibit spontaneous contraction. The different pacemaker cell types were differentiated by the number of projections originating from the central body of the cell. Spider cells were differentiated from spindle cells by having more than two projections off the central cell body. Figure 1 shows examples of spider and spindle cells. For each of the cell type, the maximal length and the maximal width of the cell central body, the number of projections from the central cell body, and whether or not there were smaller fine branches originating from the projections off the central cell body were tabulated.

Single spider cells with multiple projections were differentiated from pairs or clusters of either spindle or multiple spider cells by the presence of a single nucleus and also by careful focusing above and below the plane of the predominant cell and its nucleus to discern the edges of other cell bodies when superimposition or clustering was present. Additionally, when cells were superimposed, it was possible to observe the independent contractions of two or more cell bodies. Although inexperience with the technique could lead to erroneous conclusions, over time it became possible to distinguish between multiple and single cells and avoid any confusion. Finally, only the data from cells that could be convincingly identified as singular and mononuclear and exhibiting spontaneous depolarizations were used for this report.

RESULTS

The initial series of experiments was performed to characterize the electrophysiological properties of the spider- and spindle-shaped cells. Although cells isolated from the rabbit SAN region exhibited a variety of morphologies and action potential configurations, they could be readily classified as pacemaker cells and myocytes (5, 25). Pacemaker cells were identified by their irregular shapes and a paucity of myofilaments, together with spontaneous contractions and rhythmic pacemaker activity. Examples of nine representative pacemaker cells are illustrated in Fig. 1. The six cells in Fig. 1, left and middle, are typical spider cells, and the cells in Fig. 1, right, represent the typical spindle cells observed in this study. Both cell types beat spontaneously in normal Tyrode solution containing 1.8 mM Ca$^{2+}$. Approximately 30–40% of the spontaneously beating cells were spider cells (atrial myocytes did not contract spontaneously). In contrast with a previous report (5), the spider cells generally had a single central nucleus. The spindle cells were elongated with tapered ends and appeared bent with faint striations, as reported by others (2, 5, 7, 20). The spider cells had an average length of 98 ± 5.8 μm (n = 14), and the length of the spindle cells was 125 ± 10.2 μm.

Fig. 1. Examples of nine different pacemaker cells. Left and middle (top, middle, and bottom): examples of single nucleated spider cells. Right (top, middle, and bottom): examples of spindle cells. Nearly all cells have some small projections. The calibration bar (bottom left) is 30 μM. All cells were from the same sample and are stained with hematoxylin-eosin.
access resistance and a good voltage clamp, cells were voltage clamped using the ruptured rather than perforated patch recording method. Results obtained from two typical experiments are shown in Fig. 2. The cells were superfused with normal Tyrode solution with no channel blocking agents. After the formation of a whole cell recording configuration, action potentials were recorded in the bridge mode, and the cells were then voltage clamped to potentials between −40 and −130 mV from the holding potential of −30 mV. \( I_r \) was clearly observed at potentials negative to −60 mV under these physiological conditions.

Figure 3 shows the activation curves of \( I_r \) in these two types of cells. Channel blocking agents were added to the extracellular solution, as described in METHODS. The voltage-clamp protocol used (Fig. 3A, top) was similar to that reported previously (25). Currents obtained from a typical spider cell are shown in Fig. 3A, bottom. Tail currents measured at +20 mV were normalized with respect to maximal tail current amplitude in each individual experiment. These data are plotted against the corresponding voltage imposed during hyperpolarization. The activation curve was obtained by fitting the data to the following Boltzmann equation: \( I_r/I_{\text{max}} = 1 + \exp[(V_t - V_{1/2})/k]^{-1} \), where \( I_r \) is the test current, \( I_{\text{max}} \) is the maximal current, \( V_t \) is the test voltage pulse for activation, \( V_{1/2} \) is the half-maximal voltage, and \( k \) is the slope factor. The half-maximal voltages for the spider and spindle cells (−84 ± 1 mV, \( n = 7 \), and −90 ± 1 mV, \( n = 7 \), respectively) demonstrated that the activation voltages in the spider cells were more positive than in the spindle cells (\( P < 0.05 \)). The slope factors of the activation curves were not significantly different between the spider (10.8 mV) and spindle (11.0 mV) cells. \( I_r \) current densities measured at −130 mV for the spider and spindle cells were 27.6 ± 5.0 pA/pF (\( n = 7 \)) and 16.4 ± 1.8 pA/pF (\( n = 6 \)), respectively (\( P < 0.01 \)). The \( I_r \) current densities measured from the tail currents at +20 mV were 14.9 ± 1.3 pA/pF for the spider cells and 8.7 ± 0.7 pA/pF for the spindle cells (\( P < 0.01 \)).

![Image](http://ajpheart.physiology.org/)

**Fig. 2.** Hyperpolarization-activated inward (\( I_r \)) current recordings obtained from the spider (A) and spindle (B) cells. The voltage-clamp protocols are shown at top. The cells were clamped at the holding potential of −30 mV and hyperpolarized to potentials between −40 and −130 mV in steps of 10 mV. Selected superimposed currents are shown at bottom. The numbers indicate the voltage-clamp potential imposed during hyperpolarization. The current in the spider cell (A) at −70 mV is 139 pA, and in the spindle cell at −70 mV, the current is 69 pA (B). Both cells had similar capacitance.

### Table 1. Cell electrophysiological characteristics

<table>
<thead>
<tr>
<th></th>
<th>Spider-Shaped Cells</th>
<th>Spindle-Shaped Cell</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Capacitance, pF</td>
<td>30.8 ± 1.2</td>
<td>35.2 ± 2.4</td>
<td>0.398</td>
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<tr>
<td>( R_{\text{in}} ), GΩ</td>
<td>1.58 ± 0.35</td>
<td>1.28 ± 0.53</td>
<td>0.559</td>
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<tr>
<td>BCL, ms</td>
<td>381.1 ± 12.0</td>
<td>456.3 ± 16.7</td>
<td>0.002</td>
</tr>
<tr>
<td>MDP, mV</td>
<td>−54.9 ± 1.0</td>
<td>−68.0 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OS, mV</td>
<td>20.2 ± 1.2</td>
<td>26.7 ± 1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>( \text{APD}_{90} ), ms</td>
<td>107.1 ± 1.8</td>
<td>118.3 ± 3.5</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 12 \) cells in each group. \( R_{\text{in}} \), input resistance; BCL, basic cycle length; MDP, maximum diastolic potential; OS, overshoot; \( \text{APD}_{90} \), action potential duration at 90% repolarization.

\( n = 8, P = 0.024 \). The maximum width of the central body of the spider cells was 7.9 ± 0.66 μm, and the width of the spindle cells was 6.0 ± 0.53 μm (\( P = 0.039 \)). The spider cells had an average of four projections off the central body (range 3–7). All the spider cells had additional fine branches originating from these larger projections, and only 25% of the spindle cells exhibited these smaller branches.

The spider cells typically contracted less vigorously than the spindle cells. In some cases, it was possible to record spontaneous action potentials associated with no observable contractions. Application of the perforated patch recording technique allowed both types of cells to maintain spontaneous activity for more than 2 h. Action potential parameters were obtained from the average of six consecutive action potentials. Three 15-s recordings were used to determine basic cycle length. Table 1 summarizes the action potential characteristics recorded from each type of cell. The spider cells were characterized by significantly faster spontaneous beating rates, more positive maximum diastolic potentials, greater overshoot potential amplitude, and shorter action potential duration compared with spindle cells.

Because the \( I_r \) current is an important ionic current associated with spontaneous pacemaker activity, a series of experiments was performed to compare the \( I_r \) currents of the spider and spindle cells. To obtain low
The effects of Iso (0.1 μM) on action potentials in these two types of cell are shown in Fig. 4. Action potentials were recorded using the perforated patch recording method. The cells were first superfused with normal Tyrode solution and then superfused for 3 min with Tyrode solution containing Iso (0.1 μM). As shown in Fig. 4 and summarized in Table 2, Iso significantly decreased the basic cycle length and increased action potential amplitudes in both types of cells. However, cycle length showed a greater decrease in spider cells (28.0 ± 3.0%) than spindle cells (20.3 ± 1.5%, P < 0.02).

Figure 5 shows the effect of ACh (0.5 μM) on a typical spider cell. ACh arrested the spontaneous activity for 30 s and hyperpolarized the membrane potential to −85 mV. Pacemaker activity resumed within 1 min of washout of ACh, and the action potential exhibited long phase 2 repolarization, similar to the action potential configuration recorded from Purkinje fibers. After 5 min of washout, the spontaneous cycle length and maximal diastolic potential fully returned to control values. Similar results were observed in a total of six experiments. ACh hyperpolarized the membrane potential to −86.2 ± 0.7 mV (n = 6).

Figure 6 shows the effect of ACh on a representative spindle cell. ACh (0.5 μM) also stopped the pacemaker activity, and the spontaneous cycle length and maximal diastolic potential fully recovered after 5 min of washout. However, the membrane potential was hyperpolarized only to −75 mV, and, during washout, the action potential did not exhibit a prolonged phase 2 repolarization. In all experiments, ACh hyperpolarized the membrane potential in the spindle cells to −76.0 ± 0.8 mV (n = 7), which was significantly more positive than the spider cell (P < 0.05).

**DISCUSSION**

*Morphological characteristics.* In their histological examinations of the sinus node, James and colleagues (12, 13) observed a pale-staining “stellate”-shaped cell similar to the example shown in Fig. 1, top left. They...
suggested that this cell type was the primary pacemaker cell in the sinus node. With the development of cell isolation and single cell recording techniques, multiple pacemaker cell types have been observed. Initially, spontaneously beating round cells were thought to be pacemaker cells (8, 19). More recent data have suggested that these cells were dying cells (25). DiFrancesco et al. (6) noted the appearance of small "spider"-like cells with three or more projections off the central cell body and observed that these cells produced the largest $I_f$ currents. However, they did not compare the functional properties of this cell type to irregular spindle-shaped cells. Denyer and Brown (5) suggested that the spider-shaped cells were actually multiple cells because they often had multiple nuclei and, along with Giles et al. and other investigators (2, 7, 20), suggested that an irregular spindle-shaped cell was the primary pacemaker cell. Subsequently, spindle cells have been studied in the greatest detail (10). In the present study, isolated spider cells usually had a single nucleus (Fig. 1), although both spider and spindle cells with multiple nuclei were observed. The presence of a single nucleus indicates a single cell; however, multiple nuclei do not necessarily imply multiple cells (22). Other observations supported the conclusion that the spider cells are generally single cells. For example, when the cells were ruptured after a recording, they formed a single ball, whereas cell pairs formed a pair of balls. In the present study, both cell types (when injured) became round and beat spontaneously for a period of time but then became quiescent. A third cell type, which spontaneously contracted, was also observed. These cells looked similar to myocytes but had a rounding of the corners, were slightly bent, and also exhibited diastolic depolarization. Because we could not exclude the possibility that these cells were injured myocytes, they were not studied. The present study does not rule out the possibility that these cells and the round cells are also unique pacemaker cell types.

In a previous study (15), we reported gap junction (connexin protein) distributions associated with both spider and spindle cells compared with atrial myocytes. In sharp contrast with the ordinary myocytes where cells were linked by broad, thick gap junctions at the ends and sides of the cells, the two pacemaker cell types demonstrated these proteins at focal points ran-

Table 2. Effects of isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>Spider-Shaped Cells ($n = 5$)</th>
<th>Spindle-Shaped Cells ($n = 6$)</th>
<th>$P$</th>
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<tbody>
<tr>
<td></td>
<td>$\pm$ SE</td>
<td>$\pm$ SE</td>
<td></td>
</tr>
<tr>
<td>BCL, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>398 ± 11.0</td>
<td>448 ± 22.6</td>
<td>0.039</td>
</tr>
<tr>
<td>Iso (0.1 μM)</td>
<td>286 ± 15.8</td>
<td>356 ± 15.2</td>
<td>0.004</td>
</tr>
<tr>
<td>%Change</td>
<td>−28 ± 3.0</td>
<td>−20 ± 1.5</td>
<td>0.005</td>
</tr>
<tr>
<td>APA, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80 ± 6.0</td>
<td>98.1 ± 1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Iso (0.1 μM)</td>
<td>83 ± 5.8</td>
<td>101 ± 0.7</td>
<td>0.007</td>
</tr>
<tr>
<td>%Change</td>
<td>4.9 ± 1.5</td>
<td>2.7 ± 0.9</td>
<td>0.120</td>
</tr>
<tr>
<td>MDP, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>−54 ± 2.0</td>
<td>−69 ± 1.0</td>
<td>0.238</td>
</tr>
<tr>
<td>Iso (0.1 μM)</td>
<td>−55 ± 2.0</td>
<td>−70 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%Change</td>
<td>2.3 ± 0.3</td>
<td>1.1 ± 0.5</td>
<td>0.035</td>
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</table>

Values are means ± SE; $n = \text{no. of cells in each group}$. APA, action potential amplitude. Values beside brackets are $P$ values.

Fig. 5. Effect of ACh on the spider cell. Top: spontaneous pacemaker activity and membrane potentials recorded under control conditions, in the presence of ACh, and after the washout of ACh are shown from left to right as indicated. The time scales in all panels are the same as shown in bottom right. The dashed lines indicate the level of 0 mV. Bottom: ACh action potentials (A, B, and C) are shown at a faster sweep speed with the same time scale.
domly dispersed over the cell surfaces, along their projections, and at their termini. This study also confirmed the presence of the consistently shaped spider and spindle cells in the canine sinus node. Thus these cells are not unique to lagomorphs and could subserve pacemaker function widely across many species, including humans.

Although the data in the present study also indicate two distinct pacemaker cell types, cells were only studied when they could be clearly classified as a spider or spindle cell. The histology actually demonstrated a continuum of cell morphologies. The cells in Fig. 1, left, and the upper two cells in Fig. 1, middle, are all clearly of the spider cell type. The cell in Fig. 1, bottom, is more ambiguous, although it appears to have three projections off the cell body and is starting to resemble a spindle cell. Similarly, the cell in the Fig. 1, top right, appears to be a spindle cell with two definite projections off the central cell body, but there is a faint trace of other projections.

It was difficult to determine the relative concentrations of spindle and spider cells within the sinus node because many of the spider cells died during the isolation procedure if they did not remain in the high-potassium solution long enough, whereas more of the spindle cells survived. When the cells were exposed to the calcium-free Tyrode solution during isolation, some of the spider cells rounded up. When the cells were exposed to normal Tyrode solution with calcium, additional spider cells rounded up. If the cells were not exposed to the calcium-free Tyrode solution for a sufficient period of time, most if not all the spider cells died when exposed to calcium. In contrast, the spindle cells appeared to be more robust. This may explain why spindle cells have been studied in greater detail.

**Electrophysiological differences.** In addition to being morphologically distinct, the spider cells also exhibited different electrophysiological characteristics compared with the spindle cells. The spider cell spontaneously beat 20% faster and exhibited a 13-mV more positive maximum diastolic potential. The overshoot was 32% greater for the spindle cells, and the action potential duration was 20% longer. The $I_f$ current density of the spider cells was 68–71% greater compared with the spindle cells and activated at a more positive potential. Furthermore, the spider cells were more responsive to Iso, which increased the spontaneous rate by 28% compared with the spindle cells, which increased by only 20%. Similarly, the spider cells were more responsive during the washout of ACh and hyperpolarized to a more negative maximum diastolic potential. At these more negative diastolic potentials, the action potentials of spider cells also exhibited a different morphology (Figs. 5 and 6) compared with the spindle cells. The data suggest that the spider cell is a distinct cell type compared with the spindle cell.

Verheijck et al. (21) identified three types of pacemaker cells, which they isolated from the rabbit SAN. They described a spider cell and two types of spindle cells differentiated by cell length, with the elongated spindle cells having a length $>50 \mu M$, and the other spindle cells having a length $<50 \mu M$. In the present study, we did not observe spindle cells $<50 \mu M$ in length. They also recorded membrane potentials but not ion channel current data. In the Verheijck et al. study, the analysis of variance did not have enough power to detect action potential characteristic differences between the three cell types, and they concluded that the variability of action potential and diastolic depolarization within one morphological cell group was larger than the difference between groups. We found that the spontaneous cycle lengths of the spider cells were shorter (faster rate) than the spindle cells. We also found that the maximum diastolic potential of the

Fig. 6. Influence of ACh on the spindle cell. Membrane potentials recorded under different conditions are shown in the same manner as in Fig. 4.
spider cells was more positive than the spindle cells, and the action potential amplitudes of the spider cells were smaller than the spindle cells. Similar to Verheijck et al., we also observed that ~40% of the pacemaker cells were of the spider type. However, this may not reflect the percentages of cell types in vivo. It is possible that one of the cell types exhibits a greater mortality during the isolation process.

In the present study, the spider cells exhibited a prolonged repolarization with washout of ACh compared with the spindle cell (Figs. 5 and 6). ACh has been shown to prolong the refractory period of the intact sinus node (3). In atrial myocytes, ACh elicits a rebound stimulation of calcium current (23). It is possible that the same mechanism occurs in pacemaker cells and is responsible for the prolonged repolarization. The spider cells also had a greater responsiveness to a single concentration of ISO, suggesting that the cells may have a greater density of $\beta_1$-receptors (1) (Fig. 4).

Relationship to changes in rate and complex patterns of atrial activation. In a previous study (17), we demonstrated changes in the patterns of atrial activation that coincided with rate changes in response to cardiac nerve stimulation and infused adrenergic and cholinergic agonists. We hypothesized two mechanisms for these complexities: 1) shift in dominance of widely dispersed pacemaker cell groups and 2) SAN-atrial wavefront exit confined to selective sites. Interactions between these two factors appear to produce the instantaneous change in rate and multicentric activation patterns. The present data, together with those of the previous studies demonstrating the unique distribution of connexin protein associated with spider and spindle cells, suggest that these complex changes in atrial rate and activation result from heterogeneities at multiple system levels, including individual pacemaker cell structure, function, and coupling. All of these factors will have to be worked out on a larger scale involving linked cell groups before the integrated effects are fully understood.

Limitations. A question raised by the present study is that when cells of different morphologies are being compared, could the various cell types respond differently due to the specific recording technique and not because of intrinsic differences in the cell? We feel this is unlikely in that high-quality clamps were made on each cell type. Furthermore, neurons that have many fine projections similar to the spider cells are routinely clamped and studied, even with multiple electrodes, without the introduction of any biasing currents or voltages (18).

Although detailed morphological observations have previously been reported regarding the multistranded characteristics of the stellate cells compared with spindle cells, including the unique distribution of connexin protein localization (15), the present electrophysiological results described here are only preliminary. To fully understand the roles of stellate and spindle cells in pacemaker activity, it will be necessary to characterize each of their ionic currents, the regulation of ionic currents by neurotransmitters, and the electrophonic interactions between clusters of pacemaker cells and between pacemakers cells and working atrial muscle. Although the spider and spindle cells were the predominant types demonstrating spontaneous depolarization, rhythmicity, and action potential characteristics of atrial pacemakers in this preparation, it is possible that these characteristics could be very different in the intact SAN due to the normal intracellular connections and electrotonic interactions (11). Therefore, it is not possible at this time to conclude primacy or subsidiary for any one cell type. Finally, the demonstration of these cell morphologies, their relative distributions, interconnections, and interactive influences must eventually be confirmed in the intact SAN-atrial preparation. Different pacemaker cell types may serve functions related to both the dominant frequency of the primary pacemaker center and transmission of the impulse from the pacemaker site into the atrial myocardium.

A previous isolated cell study (5) has suggested that the spindle-shaped cell is the principal atrial pacemaker within the sinus node. The data obtained in the present study demonstrate that there are at least two different pacemaker cell types in the rabbit sinus node. These cells are morphologically distinct and functionally different. As a result, future studies that examine the response of pacemaker cells to various drugs must evaluate them in both cell types. The unique characteristics of the different cell types also raise the question as to the role each cell type plays in the initiation and conduction of the sinus impulse.

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