Atrio-Sinus Interaction Demonstrated by Blockade of the Rapid Delayed Rectifier Current

E. Etienne Verheijck, PhD; Ronald Wilders, PhD; Lennart N. Bouman, PhD

Background—Proper pacemaking of the heart requires a specific organization of the sinoatrial (SA) node. The SA node drives the surrounding atrium but needs to be protected from its hyperpolarizing influence, which tends to suppress pacemaker activity. It has been suggested that the hyperpolarizing atrial influence is minimal at the site of the central nodal area.

Methods and Results—Atrio-sinus interaction was assessed by specific depolarization of the SA node by blocking the HERG-encoded rapid delayed rectifier current (I_{K,R}) with the drug E-4031. In the SA node, E-4031 (1 μmol/L) changed action potential configuration drastically but never resulted in pacemaker arrest. In the atrium, E-4031 did not affect the membrane resting potential, thereby leaving the normal hyperpolarizing load on the SA node intact. When the SA node was sectioned into strips and subsequently separated from the atrium, spontaneous electrical activity of the strip containing the primary pacemaker ceased on I_{K,R} blockade. When not separated from the atrium, I_{K,R} blockade never resulted in pacemaker arrest. A similar effective atrio-sinus interaction was demonstrated in computer simulations.

Conclusions—Our results demonstrate that the atrium provides an effective hyperpolarizing load on the central SA nodal area and is at least one of the controlling mechanisms for normal pacemaking function. The present study can be of help in understanding why patients with long-QT2 syndrome secondary to a mutation in HERG do not show sinus arrest.

Key Words: sinoatrial node ♦ potassium ♦ computers

The sinoatrial (SA) node forms a physiological sink in the potential profile of the right atrium because in its center maximum diastolic potential is ≈20 mV less negative than in the fibers of the surrounding atrium. Several observations indicate that this potential difference causes a hyperpolarizing load from the atrium on the SA node that may have functional implications. In rabbit heart, separation of atrial and nodal tissue induced an increase in both diastolic depolarization rate and firing rate in the nodal fibers.

When the rabbit SA node is fragmented into small isolated pieces, fragments from the tissue close to the crista terminalis (CT) show the highest beating rate and the fastest diastolic depolarization. In contrast, in the intact node, fibers close to the CT have a much slower diastolic depolarization than fibers in the center of the node. It is assumed that the center of the node is too far from the atrium for the hyperpolarizing current to be effective. Joyner and van Capelle argued that the relatively low degree of coupling, a remarkable characteristic of nodal tissue, is in fact a prerequisite for normal pacemaking: If coupling would be as high as in working myocardium, sinoatrial pacemaker fibers would be electrotonically “clamped” at a more negative membrane potential. Until now, a direct functional role of the hyperpolarizing atrio-sinus interaction in the intact SA node has not been taken into account.

In the present study, we investigated the existence and functional role of an effective atrio-sinus interaction through a different approach. Previously, we found that the rapid delayed rectifier potassium current (I_{K,R}), which has the human ether-a-go-go–related gene (HERG) as its molecular basis, could be blocked by E-4031, thereby depolarizing isolated nodal cells and causing complete arrest of spontaneous activity. In the atrium, diastolic membrane potential is maintained by the inward rectifying potassium current I_{K1}, which is not affected by E-4031. Thus, E-4031 provides a means to selectively depolarize the nodal area while maintaining the normal hyperpolarizing load of the atrium on the nodal area. We present evidence that in the intact SA node, the hyperpolarizing load of the atrium effectively contributes to pacemaker function in the intact SA node. Our data help to explain why the expected bradycardia in patients with familial long-QT syndrome secondary to a mutation in HERG (LQT2) is only mild or even absent.

Methods

Electrophysiology

SA node preparations were made as described previously. Briefly, New Zealand White rabbits (ENKI, Someren, The Netherlands;
n=17) (either sex, 1.8 to 2.5 kg) were anesthetized (intramuscular injection) with Hypnorm (Janssen). Excised hearts were immersed in a modified salt solution12 containing (mmol/L): NaCl 130.6; KCl 5.6; CaCl₂ 2.2; MgCl₂ 0.6; NaHCO₃ 24.2; glucose 11.1; and sucrose 13.2, saturated with 95% O₂ and 5% CO₂ (37±0.3°C, pH=7.4). The isolated right atrium preparation was mounted on a perforated silicon rubber block with the endocardial side up.

Activation maps were made as described previously.4 Transmembrane potentials were recorded with 15- to 40-MΩ glass microelectrodes filled with 2.7 mol/L KCl and 2 mmol/L potassium citrate. Distance between successive impalements was 0.2 mm within the primary pacemaker area and 0.4 outside. A bipolar surface electrogram of the CT provided a reference time. The sinoatrial conduction time (SACT) was used to map SA nodal activation. The mapping procedure lasted 1 to 1.5 hours. In one of the experiments we subsequently isolated SA node segments. To avoid possible effects on action potential configuration caused by activation of stretch-activated channels during the isolation procedure,13 we left the isolated segments for at least 30 minutes before starting to record electrical activity.

The preparation was allowed to beat spontaneously or was stimulated by a pair of Teflon-coated platinum wires, mounted along the atrial bipolar surface electrode (350-ms interval, constant voltage: twice threshold, 3-ms pulse duration). In records from nodal fibers, we measured action potential amplitude (APA), maximum diastolic potential (MDP), diastolic depolarization rate (DDR, measured over the first 100 ms after the MDP), maximum upstroke velocity (dV/dtmax), and action potential duration at 50% and 100% repolarization (APD₅₀ and APD₉₀). For atrial fibers, we measured APA, resting potential (Vrest), dV/dt max, APD₅₀, and APD₉₀.

Computer Simulations
For rabbit atrial cells, we used the comprehensive model by Lindblad et al.14 For rabbit SA nodal cells, we used our previously published model15 but replaced the original I_{Kr} with its components I_{k_r} and I_{k_s} by using the Zhang et al.16 equations, with the conductance tentatively set to 73.8 and 48.0 pS/pF, both between the Zhang et al.16 central and peripheral cell model values (39.9 and 25.9, and 246 and 160 pS/pF, respectively). Gap junctional current (Ij) was calculated as Ij=εg×ΔVj, where g is gap junctional conductance and ΔVj is difference in membrane potential between cells. Models were coded with the use of Compaq Visual Fortran 6.5 and ran on an Alpha NT 4.0 workstation (Microway Screamer), applying a Euler integration scheme with a 5-μs time step.

Statistics
For statistical analysis, we used mean parameter values of 20 subsequent action potentials. Results are presented as mean±SEM. Statistical significance was determined by Student’s t test for paired observations. A value of P≤0.05 was considered significant.

Results
Effect of I_{Kr} Blockade
To investigate existence and functional role of a hyperpolarizing load of the atrium on the nodal area, we aimed at specifically depolarizing the SA node without affecting membrane resting potential and thus the hyperpolarizing load of surrounding atrial tissue. Therefore, we pharmacologically depolarized the nodal area by blocking I_{kr} with 1 μmol/L E-4031. We previously demonstrated in rabbit nodal cells that 1 μmol/L E-4031 selectively blocked I_{kr} for ~85% without affecting hyperpolarizing activated current (I_{h}) and L-type Ca²⁺ current (I_{Ca,L}).9 Similar observations were made by others in rabbit SA node cells.17,18

Figure 1 shows simultaneously recorded action potentials during continuous impalements of a leading pacemaker cell in the nodal area (Figure 1A) and a cell from the atrial area in the same preparation (Figure 1B) before and during administration of E-4031. Effects were completely reversible on washout. The Table summarizes the effects of E-4031 on action potential parameters from leading pacemaker cells, that is, cells discharging earliest under control conditions (n=6). E-4031 increased cycle length, prolonged APD₅₀ and APD₉₀, strongly reduced DDR, depolarized MDP, depressed dV/dtmax, and reduced APA (Table, middle). The effects on action potential parameters were similar in preparations driven at 350 ms, excluding significant effects of pacemaker shifts or mere rate reduction (Table, right). Driving intervals <350 ms could not be used with E-4031 because atrio-sinus block then occurred. Atrial cells (Figure 1B) showed prolonged repolarization, resulting in a significant increase in APD₅₀ from 33±3 to 49±6 ms and APD₉₀ from 70±5 to 131±18 ms (n=6). Membrane resting potential (control, −74±2 mV versus E-4031 to 73±2 mV) and maximum upstroke velocity (control, 156±8 V/s versus E-4031 152±7 V/s) did not change, suggesting that the drug did not alter I_{K1} and fast sodium current (I_{Na}).19

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<th>Effect of 1 μmol/L E-4031 on Action Potential Parameters of Leading Pacemaker Cells in Intact SA Nodal Preparations</th>
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Values are mean±SEM (n=6). DDR could not be measured when the preparation was driven. *P≤0.05 vs control.
**I_{K_r} Blockade Induces Pacemaker Shift**

In 6 experiments, we mapped the primary pacemaker site before and during application of E-4031. Figure 2A (left) shows an activation map of the central portion of the SA node under control conditions. The sequence of activation is visualized by 10-ms isochrones. An asterisk indicates the primary pacemaker site. Figure 2B shows the activation map of the same preparation during administration of E-4031. Figure 2 further shows that action potentials from 3 different sites within this SA node measured before and after a pacemaker shift was induced by E-4031. Under control conditions, the cell in the central part discharged earliest (−25 ms). E-4031 induced a prominent prolongation of sinoatrial conduction time and a shift along the path of preferential conduction to cranial as well as toward the CT. The magnitude of the pacemaker shift ranged from 1 to 1.5 mm into the cranial and 0.5 to 0.8 mm into the CT direction.

**Spatial Distribution of Effects of I_{K_r} Blockade**

The shift in pacemaker dominance implicates regional differences in response to I_{K_r} blockade, which are either due to regional differences in I_{K_r} density or to atrio-sinus interaction, or both. To explore an effective hyperpolarizing load of the atrium on the central nodal area, we performed a series of experiments on strips of SA node tissue (n=9).

After locating the primary pacemaker, we divided the nodal area into 5 parts, separated by incisions. Some strips were isolated completely from the surrounding tissue to fully remove the atrial influence, whereas others remained attached to the CT. Figure 3 shows the results of 2 different experiments. In the configuration shown in the left panel, the strip marked C is completely isolated from the surrounding tissue. The other areas, marked A, B, D, and E, are separated from each other but not from the CT. The right side shows a preparation in which strips B and D were completely isolated. Strip B always contained the primary pacemaker (asterisk). The central panels of Figure 3, show spontaneous activity recorded from the cells in the different strips. Control action potentials (dashed) were recorded at the same distance from the CT as where originally the primary pacemaker was found. The impulse always originated from strip B if connected to the CT (n=3). Only when strip B was isolated from the CT, the dominant pacemaker shifted to inferior (strip A) in 4 preparations to superior (strip C) in one preparation and into the CT in one other preparation. Furthermore, the intrinsic interbeat interval of strip B was always shorter after isolation (368±10 versus 413±14 ms, n=6), and the MDP became...
slightly but not significantly more positive (−55±3 versus −57±3 ms, \( n = 6 \)).

In strips B, C, and D isolated from the CT, E-4031 (solid lines) increased cycle length, prolonged APD_{100}, depressed dV/dt_{max}, reduced APA, depolarized MDP, and reduced DDR. In strip E, no change in APA and MDP was observed. Changes in APA, dV/dt_{max}, MDP, and DDR were most prominent in strips B and C. Strip B, comprising the primary pacemaker area, appeared to be the most sensitive to E-4031; when isolated, we always (\( n = 6 \)) observed a complete cessation of activity (Figure 3, right). The resulting membrane resting potential (V_{rest}) of 28±3 mV was comparable to 33±6 (\( n = 5 \)), found in isolated nodal cells, and 34 mV, found in central small SA node balls. Cessation of spontaneous activity was never observed in any of the other strips attached to or isolated from the CT.

**Computer Simulations**

To further test the effects of atrio-sinus interactions, we carried out computer simulations in which we coupled a rabbit SA nodal cell model to a rabbit atrial cell model and selectively blocked \( I_{K_r} \) by 85%, similar to the amount of block by 1 \( \mu \text{mol/L} \) E-4031 observed in isolated nodal cells. Figure 4, A and B, shows control action potentials for the 2 models when uncoupled. Spontaneous activity of the nodal cell model ceases on \( I_{K_r} \) blockade, and the model cell depolarizes to −38 mV, in accordance with the experimental data (Figure 4A), whereas the atrial cell only shows a slight increase in APD (Figure 4B). When coupled at \( g_J = 50 \) nS (Figure 4C), the SA nodal cell drives its neighbor atrial cells at 2.1 Hz (Figure 4D). On \( I_{K_r} \) blockade, the hyperpolarizing load of the atrial cells, representing atrio-sinus interaction, enables the SA nodal cell to sustain its pace-and-drive activity at 1.7 Hz (Figure 4E). Similar results were obtained at other values of \( g_J \).

**Discussion**

In the present study, we investigated the effect of the hyperpolarizing influence of the atrium on the central nodal area. Until now, atrio-sinus interaction has been studied by isolating the nodal area from the atrium. Our approach was to selectively depolarize the SA node without affecting the hyperpolarizing load of the atrium. We demonstrate that the hyperpolarizing current from the atrium can effectively counteract sinus arrest of the central nodal area induced by blockade of \( I_{K_r} \). Outside the primary pacemaker area, E-4031 exerts a less dramatic effect on maximum diastolic potential. Even after isolation, spontaneous activity continued. These regional differences in responsiveness to \( I_{K_r} \) blockade may be due to regional variation in intrinsic membrane properties or varying atrio-sinus interaction or both.

**Regional Differences in Membrane Currents**

Regional variation in intrinsic membrane properties in the SA node region has been discussed previously. The regional differences in responsiveness to E-4031 suggest the presence of at least 2 cell types with respect to the ensemble of ionic currents that are fundamental to pacemaking. Several characterizations of nodal cells have been proposed on histological, ultrastructural, electrophysiological, and pharmacological bases. In a previous study, we found that isolated rabbit SA nodal cells show a dichotomy in response to partial \( I_{K_r} \) blockade. At a concentration of 0.1 \( \mu \text{mol/L} \) E-4031, half of the cells came to complete arrest followed by a steady depolarization to −33 mV. The other cells remained spontaneously active, albeit with low frequency. Difference in responsiveness to \( I_{K_r} \) blockade could not be matched with morphologically different cell types and electrophysiological characteristics. However, Lei et al showed that the density of \( I_{K_r} \) increases with increasing cell size.

Recently, Zhang et al performed computer simulations with a 1-dimensional model of SA node tissue coupled to atrial tissue. Regional differences in ionic membrane currents were used to demonstrate that a gradual change in intrinsic electrical activity of SA node cells from the center to the periphery of the node underlies the regional differences in
pacemaker activity as observed in the intact SA node. This “gradient model” is based on data of Honjo et al., who showed that the electrical activity of small single SA node cells, which they presumed to be central cells, is comparable to that of the center of the intact node, whereas the electrical activity of large cells, which were presumed to be peripheral cells, is comparable to that of the periphery of the intact node. However, our previous data suggest that the densities of major ionic currents in SA node rabbit cells are independent of cell size (membrane capacitance). In addition, accumulating data are available on the presence of (strands of) atrial cells in the SA node of rabbit, guinea pig, bovine, and mouse. In rabbit, we were able to isolate atrial cells from the primary pacemaker region. Thus, the presence of atrial cells in the nodal area is an important prerequisite in making a functional description of the SA node and form the basis of the “mosaic model.”

Conclusive evidence regarding regional variations in cellular properties can only be obtained by regional cell isolation after electrophysiological mapping of the SA node.

Varying Atrio-Sinus Interaction

Although regional differences in membrane currents are likely to be present, a second mechanism, which has been mostly neglected, is the varying atrio-sinus interaction. In rabbit SA node, moving from the primary pacemaker center to the atrium, slowing of diastolic depolarization and increase in upstroke velocity correlates with an overall increase in myofibril density and organization. In detailed immunohistochemical studies in rabbit, guinea pig, bovine, and mouse hearts, it was found that the very center of the node is surrounded by a region in which atrial strands protrude the nodal area. Furthermore, in rabbit SA node, a close apposition of typical nodal cells and cells with a high myofibril density and high degree of organization has been observed by electronmicroscopy. In our study with regional cell isolation, we observed that the relative number of atrial cells gradually increases from the center of the node toward the surrounding atrium. More evidence for the existence of intermingling of nodal and atrial cells within the rabbit SA node was found measuring the spread of electrotonic potential in the intact SA node, where areas of high and low electrotonic coupling were found over small distances.

Functional arguments for the existence of an effective atrio-sinus interaction were obtained in experiments in which the SA node was cut loose from the atrium. In these experiments, isolation of the SA node resulted in a reduction of the interbeat interval, an increase in DDR of latent pacemaker fibers, and a concomitant shift of the dominant pacemaker in the direction where originally the CT was located. These changes were explained by the removal of the hyperpolarizing load of the atrium. The reduced intermingling of atrial and sinoatrial cells in the central portion of the node may explain why only the strip containing the primary pacemaker became quiescent during the administration of E-4031. When attached, the atrium supplies enough hyperpolarizing load to the primary pacemaker area for the cells to continue firing. Outside the central zone, pacemaker activity is then maintained by the presence of atrial cells even when the connection with the CT is disrupted. These findings are substantiated by computer simulations in which we studied the interaction between model SA nodal and atrial cells (Figure 4). These experiments clearly show that the hyperpolarizing load of the atrium can play a functional role in preventing pacemaker arrest. In addition, regional variations in cellular properties will, most likely, also be part of the protective mechanism to prevent pacemaker arrest after IKr blockade. This mechanism, together with a possible increase in sympathetic tone, may help to explain why patients with the congenital LQTS2 syndrome, who show a reduced IKr caused by mutations in HERG, encoding for the α-subunit of IKr, do not or only mildly display bradycardia.

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References


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