Sinus Node Automaticity During Atrial Fibrillation in Isolated Rabbit Hearts

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Background. It is still unclear what role the sinus node may play in the genesis or perpetuation of atrial fibrillation. Therefore, we studied the electrical activity in different regions of the sinus node during atrial fibrillation.

Methods and Results. In Langendorff-perfused rabbit hearts, paroxysms of atrial fibrillation were induced by burst pacing. Standard microelectrode techniques were used to record transmembrane potentials from different regions of the sinus node. We found that during atrial fibrillation, a high degree (5:1) of sinoatrial entrance block was present that protected the pacemaker fibers in the center of the sinus node against the high rate of fibrillatory impulses. As a result, the true pacemaker fibers in the center of the node were activated with only a slightly higher average rate than during sinus rhythm. Spontaneous diastolic depolarization was still present but was modulated by electrotonic depolarizations due to intranodal conduction block of atrial fibrillatory impulses. Incidentally, phase 4 depolarization resulted in the generation of spontaneous action potentials in the sinus node. However, the high activation rate in the sinoatrial border during atrial fibrillation prevented these spontaneous impulses to exit from the sinus node. Because of the minimal degree of sinus node overdrive suppression (9%) and the presence of concealed automaticity during atrial fibrillation, spontaneous termination of atrial fibrillation was promptly followed by resumption of normal sinus rhythm.

Conclusions. During atrial fibrillation, sinus automaticity still is present in the center of the sinus node and hardly overdrive suppressed due to a high degree of sinoatrial entrance block. (Circulation 1992;86:263-271)

Key Words • overdrive suppression • sinus node • atrial fibrillation

Although atrial fibrillation is one of the earliest recognized cardiac arrhythmias, the underlying basic mechanisms still are not fully understood. In 1959, Moe and Abildskov introduced the multiple wavelet hypothesis, which postulates that atrial fibrillation was maintained by multiple reentering atrial wavelets. Later, Moe and Abildskov's multiple wavelet hypothesis was confirmed experimentally by Allessie and coworkers using high-resolution mapping techniques to reconstruct the excitation pattern of the atria during sustained atrial fibrillation in canine and human hearts.

Several studies have suggested that the sinus node may play an important role in the genesis or perpetuation of atrial fibrillation. Ablation of the sinus node by surgical or chemical techniques has been shown to reduce both the incidence and duration of atrial fibrillation. On the other hand, clinical studies have revealed a higher incidence of atrial fibrillation in patients with the sick sinus node syndrome. The goal of the present study was to study impulse conduction and impulse generation in the sinus node during electrically induced atrial fibrillation. Therefore, the cellular electrical activity in different regions of the sinus node was recorded directly by microelectrodes.

Methods

Preparation

In the present experiments, 11 Flemish Giant rabbits of either sex and weighing 4.5–5.0 kg were used. After heparinization (2,000 IU i.v.), the animals were killed by cervical dislocation. Through a midsternal thoracotomy, the heart was exposed and quickly excised. The aorta was cannulated and connected to a Langendorff-perfusion system filled with warmed oxygenated (37°C) Tyrode’s solution. The perfusion pressure was maintained at 50 mm Hg, resulting in a coronary flow of about 40 ml/min. The Tyrode’s solution was saturated with a mixture of 95% O2–5% CO2 and contained (in mmol/l) NaCl 130, KCl 5.6, NaHCO3 24.2, CaCl2 2.2, MgCl2 0.6, NaH2PO4 1.2, and glucose 12.2. pH was 7.35±0.05. If long-lasting episodes of atrial fibrillation were required, the potassium concentration of Tyrode’s solution was lowered to 2.2 mmol/l. By shortening the atrial refractory period, moderate hypokalemia promotes atrial reentry without affecting automaticity or conduction of the sinus node. The ventricles were removed by a circular incision 2 mm caudal to the atrioventricular groove. After ligation of the cut coronary arteries, the isolated, perfused atria were turned upside down and placed into a tissue bath.

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The tricuspid ring was distended by needles to expose the endocardial surface of the sinus node area, which was fixed to a silicon bar to minimize tissue movement during microelectrode recordings.

**Signal Recording and Electrical Stimulation**

Episodes of atrial fibrillation were induced by applying 2-second bursts of rectangular stimuli (duration, 2 msec; intensity, six times threshold; cycle length, 20 msec) to the left atrium. In about 35% of the cases, a burst of stimuli was followed by an episode of atrial fibrillation. If not, a second burst of stimuli was applied with an interval of 10 seconds to the previous one until atrial fibrillation was induced. For stimulation, a programmable stimulator (Janssen Scientific Instruments) was used connected to a bipolar stimulating electrode (Teflon-coated silver wire; diameter, 0.25 mm; inter-electrode distance, 1–2 mm).

Standard microelectrode techniques (glass capillaries filled with 3 mol/1 KCl and a tip resistance of 10–30 MΩ) were used for recording of sinus node transmembrane potentials during atrial fibrillation. Surface electrodes (Teflon-coated silver wire; diameter, 0.25 mm) were placed on the crista terminalis and atrial septum for recording of unipolar or bipolar atrial electrograms. The position of the microelectrode was controlled by two vernier scales (accuracy, 0.01 mm). A dissecting microscope (magnification, ×16) was used to correlate the localization of the microelectrode to the anatomic landmarks of the sinus node region. At the beginning of the experiment, the location of the central portion of the sinus node was determined by recording typical slow action potentials from true pacemaker fibers at a sino-atrial distance of at least 0.5 mm. For statistical data analysis, the Student's t test was used.

**Results**

**Characteristics of Atrial Fibrillation**

In seven experiments, multiple episodes of sustained atrial fibrillation lasting 1–35 minutes (mean, 16±11 minutes) were initiated by burst pacing. In Figure 1, three unipolar electrograms are shown recorded simultaneously during atrial fibrillation from the cranial (CTc) and caudal (CTd) parts of the crista terminalis and the atrial septum. The electrograms showed a continuous variation in both cycle length and configuration. High-amplitude single rapid negative deflections alternated with low-amplitude multiple component complexes. Besides this temporal variation in local cycle length, spatial differences in fibrillation interval are apparent. For all experiments (n=7), the average fibrillation interval at the crista terminalis was 86±7 msec.

**Intracellular Recordings from the Sinus Node During Atrial Fibrillation**

During sustained atrial fibrillation, multiple intracellular recordings were obtained from different regions of the sinus node using a single roving microelectrode. In all cases, we found a marked spatial and temporal variation in cellular responses. In Figure 2, a number of transmembrane potentials are shown that had been recorded in one of the experiments during the same episode of atrial fibrillation from different fibers in the sinus node together with two unipolar electrograms from the crista terminalis. The individual signals (A–M) are plotted at the corresponding sites of recording. As can be seen, during atrial fibrillation a marked heterogeneity in cellular activity was present in the sinus node. At the margin of the crista terminalis (cells D and M), the fibers showed rapid and irregular responses, similar to the electrograms recorded from the atrium (CT). In the border of the sinus node (cells C, F, G, H, J, and L), the average cycle length was clearly longer than in the atrial myocardium. Some fibers in the border zone (C, F, G, and L) exhibited a high incidence of electrotonic potentials, whereas others did not (H and J). In the central portion of the sinus node (cells A, B, E, and I), the cellular response rate again was lower than in the border zone and was only slightly higher than during normal sinus rhythm. This progressive reduction in
cellular response rate from the crista terminalis toward the center of the sinus node demonstrates the presence of a high degree of sinoatrial entrance block during atrial fibrillation.

**Characteristics of Sinoatrial Entrance Block**

To study the characteristics of sinoatrial entrance block during atrial fibrillation, in all experiments (n=7) and during a single episode of atrial fibrillation, a series of intracellular recordings were made in steps of 0.2 mm along a straight line perpendicular to the crista terminalis. In Figure 3, the results of one of these experiments are shown. The sites of intracellular recording (A-I) and a bipolar electrogram from the crista terminalis are indicated in the diagram at the left. The average cycle length at each recording site during at least 10 seconds of atrial fibrillation is given at the right of each tracing. Transmembrane potentials with an amplitude less than 50% of a full-blown action potential were considered electrotonic depolarizations. In this example, the average local fibrillation interval in the crista terminalis was 79±20 msec. The action potentials obtained from a fiber at the margin of the crista terminalis (A) showed all of the characteristics of atrial myocardium. Frequently, the late repolarization phase of these action potentials was disturbed by electrotonic potentials of low amplitude and short duration, indicating local conduction block at the site of recording. In the border zone of the sinus node (fibers B and C), the action potentials were of longer duration and varied widely in amplitude, duration, and steepness of depolarization. Relatively normal action potentials frequently were succeeded by short low-amplitude potentials indicative of intranodal conduction block. In addition, single or multiple electrotonic potentials occurred early in the repolarization phase, causing a marked electrotonic prolongation of the action potential. The average cycle lengths in fibers B and C were 164±53 and 188±60 msec, respectively. Thus, during atrial fibrillation, an average 2:1 sinoatrial entrance block existed in the border of the sinus node.

Deeper into the sinus node, another drastic reduction in cellular activation rate occurred. Between recording sites C and D (0.4 and 0.6 mm from the crista terminalis), the average fibrillation interval increased from 188±60 to 403±81 msec. Thus, in the center of the
node, the average fibrillation interval was fourfold to fivefold longer than in the crista terminalis. The action potentials recorded from sites D–I were characterized by a slow upstroke and a long duration. The incidence of electrotonic depolarizations was less frequent than in the sinoatrial border, but in all fibers clear electrotonic potentials were recorded that modulated the shape of the action potential and phase 4 depolarization.

In Figure 4, the intranodal cycle length during atrial fibrillation is plotted as a function of sinoatrial distance together with the shortest 1:1 intranodal response interval during atrial pacing (Wenckebach point) as obtained in another series of experiments. In the border of the sinus node (0–0.4 mm), the average fibrillation interval differed only slightly from the Wenckebach point. However, at a distance of more than 0.4 mm from the crista terminalis, the degree of sinoatrial entrance block during atrial fibrillation was significantly higher than that occurring during regular pacing. In fact, during atrial fibrillation, the average cycle length in the central pacemaker fibers was only slightly shorter than that occurring during normal sinus rhythm.

**Sinus Node Automaticity During Atrial Fibrillation**

Due to the high degree of sinoatrial entrance block, the majority of the atrial fibrillatory wavelets did not propagate into the center of the sinus node. The resulting long cycle length allowed the pacemaker fibers to develop clear spontaneous diastolic depolarization. Most of the time, however, the pacemaker fibers were discharged by one of the entering fibrillation waves before they reached their threshold of activation. Occasionally, however, if the time interval between successive penetrating atrial impulses was longer than the interval during sinus rhythm, the sinus node fibers spontaneously generated an action potential. Figure 5 is an example of this phenomenon. The upper tracing shows a bipolar electrogram recorded from the crista terminalis together with the transmembrane potential recorded from a pacemaker fiber in the center of the sinus node (sinoatrial distance, 1.5 mm). Temporarily, all atrial fibrillation wavelets were blocked in the sinus node, and the pacemaker fiber was effectively protected from being activated retrogradely. As a result, spontaneous diastolic depolarization was not interrupted and led to the generation of an action potential. This registration of the electrical activity in the center of the sinus node during atrial fibrillation cannot be distinguished from recordings of a true pacemaker fiber during normal sinus rhythm. The cycle length of this "concealed automaticity" during atrial fibrillation was 460 msec compared with 455 msec during sinus rhythm.
Electrotonic Modulation of Sinus Node Automaticity

In all experiments during atrial fibrillation, the transmembrane recordings from sinus node fibers frequently showed subnormal action potentials or electrotonic depolarizations. These potentials probably resulted from nearby sinoatrial entrance block or from dissociated activation of neighboring groups of sinus node fibers and often disturbed the course of spontaneous diastolic depolarization. Figure 6 shows several examples of electrotonic potentials from different fibers in the center of the sinus node during atrial fibrillation. Figure 6A shows two single mid-diastolic electrotonic potentials (arrowheads); the second is followed by a smooth transition into the upstroke of a spontaneously generated action potential 450 msec after the preceding response. Recording B illustrates how electrotonic sub-threshold potentials can be followed immediately by an action potential. The activation cycles were 355 and 420 msec, respectively. Figure 6C shows an early subnormal action potential interpolated between two normal action potentials (interval, 590 msec). Recording D shows a long cycle of 630 msec due to the occurrence of two successive diastolic potentials; the first probably represents a poorly developed action potential, and the second is an electrotonic depolarization. Finally, recording E shows how multiple electrotonic potentials can keep the cell depolarized during a prolonged period of time. In this example, the membrane was kept depolarized for more than 500 msec, prolonging the sinus interval to 850 msec.

During atrial fibrillation, the configuration of action potentials of fibers in the center of the sinus node changed continuously. This beat-to-beat variation is illustrated in Figure 7. Nine successive activation cycles of a pacemaker fiber 1.5 mm from the crista terminalis are superimposed. The interval between the successive action potentials varied from 330 to 452 msec (mean, 388±38 msec). The amplitude, dV/dt max, maximal diastolic potential, and duration of the action potential as well as the slope of phase 4 depolarization varied considerably during the successive cycles. The continuous tracing at the top of Figure 7 demonstrates that electrotonic potentials during different phases of spontaneous diastolic depolarization appear to modulate the discharge of the pacemaker fiber. Cycles 1 and 6 (380 and 330 msec, respectively) are shortened by an electrotonic potential late in diastole, whereas cycles 3 and 5 (452 and 420 msec, respectively) appear to be prolonged by a mid-diastolic electrotonic potential. The second cycle (345 msec) shows an abrupt transition into the fast upstroke of the action potential, indicating that this cycle was terminated by retrograde activation of the pacemaker by an entering atrial depolarization wave.

In recent studies, it was demonstrated that spontaneous action potential generation in sinus node pacemaker fibers is modulated by electrotonic subthreshold potentials according to a typical phase–response relation. In the present experiments, however, the amplitude and duration of electrotonic potentials in sinus node pacemaker fibers varied too much to allow construction of phase–response curves during atrial fibrillation.

Sinus Node Recovery Time After Termination of Atrial Fibrillation

In four experiments carried out at an extracellular potassium concentration of 5.6 mmol, the degree of sinus node overdrive suppression during atrial fibrillation was determined. The duration of the episodes of induced atrial fibrillation varied between 1 and 7 seconds (mean, 2.1±1.2 seconds). The first escape cycle after termination of fibrillation cannot be used for measurement of overdrive suppression since it is impossible to know when the escaping sinus node fiber was activated by one of the last atrial fibrillatory waves. Therefore, we used the second and following sinus...
cycles to quantify the degree of overdrive suppression of the sinus node during atrial fibrillation. In Figure 8, the average cycle length of the second through sixth sinus cycles (SC₂-₆) after spontaneous termination of atrial fibrillation is compared with the cycle length during stable sinus rhythm (362±28 msec; n=231). After spontaneous termination of fibrillation, the interval of the second sinus cycle (SC₂) was prolonged by 9±7% (p<0.001). Within the next few beats, the sinus interval gradually returned to its control value. The third sinus cycle (SC₃) still was prolonged by 4±5% (p<0.001), whereas SC₄ and the following cycles differed less than 2% from control. No statistical correlation was found between the length of SC₂ and the duration of the preceding episode of atrial fibrillation.

Discussion

Our results show that during atrial fibrillation, the center of the sinus node continues to exhibit automaticity and that spontaneous phase 4 depolarization is

Figure 6. Tracings of several examples of electrotonic potentials in the sinus node during the same episode of atrial fibrillation (arrowheads). Numbers represent activation intervals between the successive action potentials at 50% of the amplitude.

Figure 7. Tracings of beat-to-beat variation in action potential morphology and cycle length of a pacemaker fiber during atrial fibrillation. The nine consecutive cycles (top tracing) are superimposed at the bottom to enable the variation in action potential parameters to be visualized. The cycle length between the consecutive action potentials is indicated (upper tracing).
hardly overdrive suppressed. During cholinergically induced atrial fibrillation in the American bullfrog, Nelson and Smith\textsuperscript{5} found that a slow and regular automatic rhythm was maintained in the sinus venosus. In the isolated rabbit sinus node, Sano et al\textsuperscript{6} found that spontaneous sinus impulses could trigger short runs of atrial fibrillation. During these paroxysms of fibrillation, a high degree of sinoatrial entrance block was present. Recently, sinoatrial entrance block during atrial fibrillation was described in humans.\textsuperscript{21}

If spontaneously generated sinus impulses find an exit to the atrium, they might contribute to perpetuation of the fibrillatory process by increasing the number of atrial wavelets. However, our present findings show that spontaneous impulses generated in the center of the node are unlikely to activate the atrium because the sinoatrial border already is activated at its highest possible rate by atrial fibrillatory wavelets. Thus, the border of the sinus node not only protects the center of the node against incoming fibrillatory waves but also prevents spontaneous sinus impulses from exiting toward the atrium. It therefore is not until atrial fibrillation terminates that concealed sinus automaticity manifests itself. This may lead to prompt resumption of sinus rhythm or immediate reinitiation of atrial fibrillation.

**Sinoatrial Entrance Block**

Results of the present study revealed that during atrial fibrillation only 20% of the atrial impulses traversed through the sinoatrial border zone to activate the true pacemaker fibers in the center of the node. Intracellular recordings from the border of the sinus node showed well-developed action potentials alternating with potentials of short duration and reduced amplitude. Because such brief potentials have been shown to be associated with local conduction block,\textsuperscript{22} this suggests that during atrial fibrillation frequent local conduction block occurs in the sinoatrial border. Furthermore, because the fibrillatory impulses are blocked in different parts of the sinus node, this leads to a high degree of dissociated activation of different areas of the node.

In a previous study\textsuperscript{19} using the isolated right atrium of the rabbit, we investigated the conduction properties in different regions of the sinus node during atrial pacing. Because the results from these experiments were very reproducible, they can be compared with the local response rate in the sinus node during atrial fibrillation. The average response rate of the border zone during atrial fibrillation was similar to the maximal 1:1 response rate demonstrated during regular atrial pacing.\textsuperscript{19} Therefore, the occurrence of sinoatrial entrance block during fibrillation appears to be caused primarily by the longer refractory period in the sinoatrial junction.\textsuperscript{23} Because the refractory period of sinus node fibers exceeds the refractory period of the atrium, the rapid repetitive fibrillatory impulses frequently occur when the sinus node has not recovered from activation by a previous atrial impulse. By this mechanism, about 50% of the atrial impulses were blocked at the sinoatrial border zone.

In the center of the sinus node, the average rate during atrial fibrillation was markedly slower than could be explained by a longer refractory period.\textsuperscript{19} Concealed conduction of fibrillatory wavelets at the transition between border zone and center of the node can explain the additional reduction in activation rate in the central pacemaker fibers. Several studies have shown that partial penetration of early premature beats into the sinus or atrioventricular node prolongs the functional refractory period.\textsuperscript{24-30} In the present experiments, concealed intranodal conduction of fibrillatory wavelets was demonstrated by the presence of frequent electrotonic potentials that prolonged the cycle length between successive action potentials.

**Concealed Automaticity**

The high degree of entrance block to the central portion of the sinus node during atrial fibrillation resulted in an activation rate of the pacemaker fibers almost equal to normal sinus rhythm. The sinus node fibers still exhibited spontaneous diastolic potentials associated with local conduction block of fibrillatory wave fronts within the sinus node.\textsuperscript{31,32} Because electrotonic potentials can modulate the intrinsic rate of automaticity of pacemaker cells\textsuperscript{20} and the moment and amplitude of electrotonic potentials vary in different parts of the node, this may result in multiple dissociated sites of pacemaking. Even if the separate pacemakers discharge only occasionally, together they may represent a continuous source of concealed automaticity during atrial fibrillation.

**Overdrive Suppression**

Spontaneous termination of atrial fibrillation was followed by prompt resumption of sinus rhythm with only a slight degree of overdrive suppression. Several studies have demonstrated that after a period of regular atrial pacing, the sinus node recovery time lengthens with increasing pacing rates.\textsuperscript{33-35} In the isolated rabbit sinus node, Steinbeck et al\textsuperscript{35} found a maximal overdrive suppression of 32% after atrial pacing with 4.4 Hz. At higher pacing rates, the sinus node recovery time shortened again due to the occurrence of sinoatrial entrance block. A comparable situation exists during atrial fibrillation. Despite the high atrial rate, the pacemaker fibers were discharged at a rate only slightly higher than that occurring during normal sinus rhythm; consequently, they were hardly subjected to overdrive suppression. This explains why after spontaneous termination of
fibrillation the second sinus escape cycle was only slightly prolonged by the same amount (9%), which is analogous to overdrive suppression of the sinus node by pacing the atrium at a constant cycle length of 400 msec.55

Study Limitations
Investigation of the electrical activity in the sinus node during atrial fibrillation requires simultaneous recordings of multiple intracellular potentials from pacemaker fibers in the sinus node. Because simultaneous multiple intracellular recordings technically are very difficult to obtain, we used a single roving microelectrode to record successive intracellular potentials from different regions in the sinus node during a single episode of atrial fibrillation. Although in this way the various cellular phenomena during atrial fibrillation could be studied, it did not allow a detailed reconstruction of the excitation of the sinus node during atrial fibrillation. Furthermore, although the present study provides clear evidence for electrotonic modulation of sinus node automaticity, the variation in amplitudes of electrotonic potentials caused by intranodal block of fibrillatory wavelets prohibited the construction of a quantitative phase-response curve.20

Finally, we used a potassium concentration of 2.2 mmol/l to prolong the duration of atrial fibrillation. It has been shown that a moderate degree of hypokalemia results in shortening of the wavelength of the atrial impulse, thus facilitating the induction and perpetuation of fibrillation.14-36 This may have implications for the significance of our results in understanding sinus node behavior during atrial fibrillation in humans. However, it has been shown that sinus automaticity and conduction are not significantly influenced by a moderate decrease in extracellular potassium concentration.15-17

Clinical Implications
The occurrence of intranodal conduction block has been shown to be a primary prerequisite for sinoatrial echo beats.3-37 In the present study, we demonstrated a high degree of dissociated activation of the sinus node fibers during atrial fibrillation. This suggests that the atrial myocardium surrounding the sinus node may be activated frequently by sinoatrial echoes contributing to the perpetuation of atrial fibrillation.3 In addition, the presence of concealed sinus automaticity also may contribute to perpetuation of atrial fibrillation. If atrial fibrillation terminates, the early escape of a spontaneous sinus impulse may immediately reinstitute the fibrillatory process, thus prolonging the duration of atrial fibrillation.

Finally, the observation that during atrial fibrillation the sinus node is hardly overdrive suppressed explains the prompt resumption of normal sinus rhythm after spontaneous cessation of atrial fibrillation.38 On the other hand, if patients show a long sinus pause after spontaneous termination of fibrillation, this may point to the presence of sinus node dysfunction.12,13

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