Comparison between intracellular and extracellular direct current recordings of sinus node activity for evaluation of sinoatrial conduction time

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ABSTRACT For evaluation of sinoatrial conduction time in humans, study of extracellular direct current (DC) electrograms from the sinus node has been proposed. To validate this method, we compared transmembrane potentials from multiple sites (40 to 60, "mapping" of sinoatrial activation by microelectrode technique) and extracellular DC electrograms of the sinus node in 12 isolated rabbit atria. Sinoatrial conduction time, measured by microelectrodes and by extracellular electrograms, was essentially the same if the DC electrode was positioned over the pacemaker center (35 ± 15 and 33 ± 15 msec, respectively; deviation ≤2 msec). While in all experiments phase 4 and phase 0 depolarization of dominant pacemaker fibers was reflected in the DC electrogram, its shape was influenced by pacemaker location and duration of sinoatrial impulse propagation. If sinoatrial conduction time was long (>25 msec) the transition from the diastolic to the upstroke slope was smooth and the sinus node potential was clearly separated from atrial activity. If sinoatrial conduction time was short (≤25 msec) the onset of the upstroke slope was well defined and the upstroke slope directly merged into atrial activity. Extracellular recordings 0.2 mm away from the pacemaker center were fairly unchanged in shape; however, sinoatrial conduction time was significantly underestimated. Underestimation also occurred when the tip size of the extracellular electrode was increased from 0.2 to 0.5 and 1.0 mm. Thus sinus node activity is reflected in extracellular DC recordings; however, measurement of sinoatrial conduction time by this technique requires exact localization of the electrode over the pacemaker center, which cannot be controlled in humans.


IN 1977, Cramer et al. identified low-frequency extracellular potential changes associated with electrical activity of the sinoatrial pacemaker of the rabbit by direct coupled amplification. These potential changes consist of a slow diastolic and a more rapid upstroke slope that correspond to phase 4 and phase 0 depolarization of dominant pacemaker fibers of the sinus node. Based on these observations, the technique for recording extracellular direct current (DC) electrograms was applied in human beings during cardiac surgery and cardiac catheterization to allow direct assessment of sinus node function, including sinoatrial conduction time, in an attempt to overcome the inadequacies inherent in atrial pacing methods.

In this study we compare extracellular and intracellular DC signals recorded simultaneously from multiple sites of the sinus node to determine whether extracellular DC electrograms can accurately measure sinoatrial conduction time under clinical conditions.

Methods Young rabbits (2.0 to 2.5 kg) were killed by a blow to the neck. The hearts were rapidly excised and transferred to cool Tyrode’s solution. The right atrium was isolated and the interatrial septum and atrioventricular nodal region were removed. The preparation was pinned in an incubation bath with the endocardial surface facing up and was allowed to beat spontaneously. The composition of the perfusion fluid was (mM): NaCl, 130; KCl, 4.7; CaCl2, 2.5; MgCl2, 2.6; NaHCO3, 24.9; NaH2PO4, 1.3; glucose, 11. The solution was bubbled with 95% O2, 5% CO2. The temperature was kept at 37° ± 0.1°C and the pH at 7.35 ± 0.05.

Transmembrane potentials of sinus node fibers were recorded by glass microelectrodes filled with 3M KCl (resistance 10 to 20 MΩ). The exploring electrode was mounted on a micromanipulator (Brinkmann micromanipulator, model MH), which allowed horizontal movements of the electrode with an accuracy of 0.01 mm. Unipolar extracellular recordings were obtained by a thin
silver wire (0.2 mm in diameter) that was coated with Teflon except for the tip and positioned about 0.1 mm above the tissue surface. The indifferent electrode was placed at the periphery of the incubation bath, 1.5 cm away from the exploring electrode. The electrodes were carefully chlorinated before each experiment to minimize liquid junction potentials. The recordings were free of drift at gains of 50 µV/cm.

The electrodes were directly coupled to a Tectronix AM 502 differential amplifier. The upper frequency response was limited to 300 Hz and the amplitude of the signal ranged from 150 to 400 µV.

For better comparison between intracellular and extracellular recordings, the polarity of the extracellular signal is reversed in all figures.

A bipolar surface electrogram of crista terminalis was recorded with a pair of Teflon-coated silver wires; another pair of electrodes placed on the atrial appendage served for stimulation. A programmable stimulator (Arrhythmia Investigation System Type 4279, Digitimer Ltd.) provided a train of stimuli with variable pacing intervals and duration of pacing. Stimuli were constant 2 msec voltage pulses, twice threshold. All signals were displayed on a storage oscilloscope (Tektronix 5103 N), stored on magnetic tape (Amplex PR 2200), and recorded on a Mingograf 804 (Siemens).

The location of the pacemaker and spread of sinoatrial activation were determined by 40 to 60 intracellular impalements. From 10 to 15 sites the transmembrane and extracellular activity was measured simultaneously by advancing the glass micro-electrode in an oblique direction under the tip of the extracellular electrode to record from cells directly subjacent to the extracellular electrode. Sinoatrial conduction time was defined as the interval between the activation of a dominant pacemaker fiber and atrial activation. Moment of activation was defined as the 50% amplitude of the transmembrane potential of a fiber during phase 0 and the intrinsic deflection of the surface electrogram.

For estimation of sinoatrial conduction time by constant atrial pacing techniques, the atrial return cycle after a train of eight consecutive stimuli was measured, subtracted by the spontaneous cycle length before stimulation and divided by two (unidirectional sinoatrial conduction time).

Statistical evaluation was made by linear regression analysis and the Wilcoxon rank test for paired or unpaired data.

**Results**

**Comparison of intracellular and extracellular recordings.** In 12 experiments the transmembrane potentials from multiple locations in the sinus node were compared with extracellular DC electrograms recorded simultaneously from the same sites.

Figure 1 depicts one representative experiment. For the intracellular mapping during spontaneous rhythm the earliest activity of a fiber was taken as zero reference. The isochronic lines indicate the spread of sinoatrial activity. The transmembrane potential of a dominant pacemaker fiber (upper trace) and the DC electrogram exactly over the pacemaker center (lower trace) are shown in panel A.

A slow negative-going diastolic slope and a more rapid upstroke slope of the DC electrogram closely correspond to phase 4 and phase 0 depolarization of the dominant pacemaker fiber. The smooth potential course is followed by a short positive-going and a brief negative-going deflection (polarity is reversed in the figures) before the occurrence of a sharp deflection correlated to the activation of crista terminalis.

Fiber B (0.2 mm away from the pacemaker center) and fiber C (0.4 mm away) are close to the pacemaker center. The transition from phase 4 to phase 0 of these fibers is more distinct but still gradual; the DC electrogram conforms in shape to the dominant pacemaker signal.

Fiber D is a latent pacemaker fiber. The DC electrogram shows little diastolic slope, a sharp transition into a steep upstroke slope, and a high amplitude of the extracellular sinus potential. At crista terminalis (panel E) no diastolic slope and no upstroke slope could be recorded.

A consistent finding of the cranial part of the node is an initial, positive-going deflection (panel F) of the DC electrogram.

In figure 2 the consecutive recordings of the fibers A to E are superimposed (left) and correlated to the DC electrogram from the same sites (right). Phase 4 depolarization is steepest at the dominant pacemaker fiber (A), which is the first to start phase 0 depolarization (first dashed line in left panel). The DC electrogram from the pacemaker site shows a marked diastolic slope and a gradual transition into a more rapid upstroke slope. A few milliseconds later the latent pacemaker fibers B and C depolarize, exhibiting a more distinct transition between phase 4 and phase 0 depolarization and a more rapid upstroke velocity (second dashed line in left panel). Accordingly, the diastolic slopes of the DC electrograms from these sites smoothly merge into the upstroke slopes, which, however, start later and are steeper.

The sharp onset of phase 0 depolarization of the latent pacemaker fiber D is well reflected in the DC electrogram from this site. Activation of the crista terminalis fiber (E) coincides with a sharp deflection of the DC electrogram (third dashed line).

In seven of 12 experiments — all with long sinoatrial conduction time (>25 msec) — this characteristic form of the DC electrogram could be found if the extracellular electrode was positioned over the pacemaker center.

In five of 12 preparations — all with short sinoatrial conduction time (≤25 msec) — the DC electrogram over the pacemaker center was different. Figure 3 shows an example. The microelectrode mapping reveals a true sinoatrial conduction time of 18 msec. If the extracellular electrode is located over the pacemaker center (A), phase 4 depolarization of dominant fi-
FIGURE 1. Comparison of intracellular and extracellular recordings of sinus node activity in a preparation with long sinoatrial conduction time (52 msec). The sketch of the preparation (with the pacemaker area of the sinus node on the right, superior vena cava above, inferior vena cava below, and the crista terminalis) shows the result of the microelectrode mapping during spontaneous rhythm (cycle length 500 msec). The dots indicate the position of the bipolar surface electrode. The moment of the earliest discharge of a fiber is taken as zero reference. Isochronic lines are constructed on the basis of 45 activation times. From sites A to F the transmembrane potential (upper trace), the extracellular DC electrogram (lower trace), and the bipolar surface electrogram of crista terminalis (below) have been recorded simultaneously. For better comparison, the polarity of the DC electrogram is reversed. Site A is the pacemaker center.

bers is reflected in only a small diastolic slope of the DC electrogram, and the upstroke slope of the electrogram is closely correlated in time to phase 0 depolarization of the dominant pacemaker fiber. However, the transition between diastolic and upstroke slope is not smooth, but sharp. The upstroke slope is interrupted by short deflections and directly merges into atrial activity.

Only 0.2 mm away from the pacemaker center (B) the DC electrogram shows no diastolic slope, but an initial positive-going deflection and a very fast negative-going deflection that parallels phase 0 depolarization of the latent pacemaker fiber.

Fibers C and D near crista terminalis do not exhibit any upstroke slope preceding atrial activity.

Fiber E in the blocked conduction zone of the caval region shows a double-component transmembrane potential. In the DC electrogram a slow positive-going potential wave coincides with the first component, and a sharp deflection 56 msec after atrial activity coincides with the second component of the transmembrane potential. In the cranial part of the node (F) the DC electrogram does not demonstrate a diastolic slope; the earliest extracellular potential change occurs 12 msec before atrial activity.

In all five preparations with a short sinoatrial conduction time the transition between diastolic slope and upstroke slope of the DC electrogram above the pacemaker center was sharp and the upstroke slope was not clearly separated from atrial activity. In these experiments the steepness of the diastolic slope was less marked compared with that of preparations with long sinoatrial conduction times (170 ± 32 and 358 ± 27 µV/sec, respectively; p < .05, n = 12).

In three experiments (Nos. 6, 11, and 12) we additionally studied the effects of different electrode tip sizes (0.2, 0.5, and 1.0 mm in diameter) for extracellular recording of sinus node activity. Increase of the size of the exploring electrode uniformly resulted in an attenuation of the sinus node potential. Although the
FIGURE 2. Correlation of the transmembrane potentials of a dominant pacemaker fiber (A), latent pacemaker fibers (B, C, and D), and a crista terminalis fiber (E) (left) with the DC electrograms from the same sites (right). The surface electrogram of crista terminalis is given below. Dashed lines (same moment of the atrial cycle in both panels) are drawn through the points discussed in the text. The preparation is the same as in figure 1.

FIGURE 3. Comparison of intracellular and extracellular recordings of sinus node activity in a preparation with short sinoatrial conduction time (18 msec) during spontaneous rhythm (cycle length 400 msec). The DC electrogram over the pacemaker center (A) shows a small diastolic slope and a sharp transition into an upstroke slope, which directly merges into atrial activity.
electrode was placed over the pacemaker center, the upstroke slope of the DC electrogram became small and occurred later than phase 0 depolarization of dominant pacemaker fibers (figure 4).

Pacemaker shift. Figure 5 depicts a preparation with a spontaneous pacemaker shift. For the first 2 beats the impaled fiber (upper trace) exhibits the characteristics of a dominant pacemaker fiber: smooth transition from phase 4 to phase 0 and a long period of latency (28 msec) between the activation of the fiber and of the atrium. A spontaneous pacemaker shift then occurs, paralleled by a decrease of cycle length from 416 to 364 msec. The transition between phase 4 and phase 0 depolarization becomes sharp; activation of the atrium precedes that of the fiber.

Accordingly, there is a diastolic slope and an upstroke slope in the DC electrogram (lower trace) for the first 2 beats, which are absent for the following 2 beats and reappear in the last beat.

Thus a pacemaker shift is reflected in the DC electrogram, if the extracellular electrode is positioned over the pacemaker center.

Determination of sinoatrial conduction time. In 12 experiments we compared sinoatrial conduction time determined by extensive intracellular and extracellular mapping of the sinus node and estimated by constant atrial pacing (1) (table 1).

If the onset of the upstroke slope of the DC electrogram could not precisely be determined because of a smooth transition between diastolic slope and upstroke slope (sinoatrial conduction time > 25 msec, Nos. 1 to 7), we measured sinoatrial conduction time from a point halfway between the start of the diastolic slope and the top of the upstroke slope until atrial deflection. If the transition from the diastolic slope to the upstroke slope was sharp (sinoatrial conduction time ≤ 25 msec, Nos. 8 to 12), we measured sinoatrial conduction time from the onset of the upstroke slope until atrial deflection.

As table 1 indicates, determination of sinoatrial conduction time by intracellular and extracellular recording is essentially the same if the extracellular electrode is positioned exactly over the pacemaker center. However, a short distance away from the dominant pacemaker area, true sinoatrial conduction time is underestimated. In experiment 3 (figure 1) the underestimation amounted to 8 msec (15% of the true value) and 20 msec (38%) when the DC electrode was positioned over site B (0.2 mm away from the pacemaker center) and site C (0.4 mm away), respectively. Thus underestimation of true sinoatrial conduction time occurred, although the shape of the DC electrogram was similar.

<table>
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<tr>
<th>Experiment</th>
<th>Cycle length (msec)</th>
<th>Microelectrode mapping (msec)</th>
<th>Extracellular recording (msec)</th>
<th>Sinoatrial conduction time (msec)</th>
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<td>35 ± 15</td>
<td>33 ± 15</td>
<td>25 ± 6</td>
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</table>

a By the method of Narula et al. (10)

b p < .05 compared with true sinoatrial conduction time.
to that of the electrogram from the pacemaker center. In experiment 8 (figure 3) the DC electrogram recorded over site B, close to the pacemaker center, had a different configuration compared with that of site A; true sinoatrial conduction time (18 msec) was underestimated by 6 msec. Underestimation of true sinoatrial conduction time was a consistent finding in all experiments when the exploring electrode was moved away from the pacemaker center. Even a dislocation of as small as 0.2 mm (tip size of the extracellular electrode) caused an underestimation of sinoatrial conduction time (7 ± 5 msec; p < .01, n = 12). If the exploring electrode was positioned 0.4 and 0.8 mm away from the pacemaker center toward crista terminalis, underestimation amounted to 15 ± 8 and 20 ± 10 msec, respectively (p < .01, n = 12). Measurement of sinoatrial conduction time by constant atrial pacing was quite accurate in experiments with a short sinoatrial conduction time (Nos. 8 to 12) but tended to underestimate the true values in experiments with a long sinoatrial conduction time (Nos. 1 to 7). Thus underestimation is more pronounced with longer true sinoatrial conduction time.

However, in mapping the sinoatrial spread of activation in 12 experiments we found a large spectrum of variations of the extracellular sinus node potential depending on the length of sinoatrial conduction time (long vs short, see figures 1 and 3). These results suggest the possibility that the DC electrogram over the pacemaker center is not only determined by impulse formation of the dominant pacemaker fibers but also by the direction and conduction velocity of sinoatrial impulse propagation. If sinoatrial conduction time is long and the pacemaker fibers are embedded in very slowly conducting tissue, the electrical resistance and the potential difference caused by different levels of transmembrane potentials between dominant and latent pacemaker fibers are large. As a consequence, phase 4 depolarization (which is steepest in dominant pacemaker fibers) and phase 0 depolarization (which occurs first in these fibers) are clearly reflected in the DC electrogram. A few milliseconds later the latent pacemaker fibers depolarize in the vicinity of the pacemaker center, exhibiting a smooth but more distinct transition from phase 4 to phase 0 depolarization that coincides with a more pronounced upstroke slope of the DC electrogram.

Conduction toward crista terminalis causes a short positive-going deflection of the DC electrogram due to a reversal of the potential difference within the pacemaker center. Hariman et al.11 speculated that this positive-going deflection is caused by the repolarization of dominant pacemaker fibers, while our results clearly show that at this moment the dominant pacemaker fibers are still depolarizing. The dominant fibers are the first to depolarize and the last to repolarize (figure 2).

If sinoatrial conduction time is short but the pacemaker center is at a typical site, the potential differences are small between the pacemaker center and the surrounding tissue. As a consequence, the diastolic

![Image](https://example.com/image.png)

**FIGURE 5.** Pacemaker shift during spontaneous rhythm. The shift is paralleled by a change in cycle length, a change in configuration and activation time of the transmembrane potential (upper trace), and the disappearance and reappearance of a diastolic and upstroke slope of the DC electrogram (lower trace).
slope of the DC electrogram is less prominent and the transition between the diastolic and the upstroke slope is sharp. Because of fast conduction toward crista terminals, the upstroke slope is not clearly separated from atrial activity. To better define the onset of the atrial excitation in these preparations, the extracellular sinus node signal has been subtracted from the atrial signal to get an extracellular potential determined by sinus node activity only. Thus the length of sinoatrial conduction time correlates with the shape of the DC electrogram. However, the upstroke slope of the DC electrogram coincided with phase 0 depolarization of dominant pacemaker fibers in all our experiments if the extracellular electrode was positioned over the pacemaker center, confirming the results of previous studies.1, 11, 13

Determination of sinoatrial conduction time. The existence of extracellular potential changes associated with sinus node activity in humans gave rise to the assumption that sinoatrial conduction time might be evaluated by extracellular DC recordings.2, 3 However, experimental studies that precisely compare intracellular and extracellular mapping of sinoatrial spread of activation and sinoatrial conduction time determined by atrial pacing have not been performed.

In clinical studies, failure to record sinus node potentials has been common (up to 50% of all patients3). During cardiac surgery2 and cardiac catheterization1 Hariman and colleagues reported sinoatrial conduction time values (32.4 ± 2.8 and 34.9 ± 2.9 msec, respectively) that were significantly lower than those obtained previously by premature atrial stimulation14 and constant atrial pacing.10 The authors explained this discrepancy by the errors inherent in the atrial pacing methods. Results of later studies, however, showed longer values of sinoatrial conduction time measured by DC electrograms and a good correlation with values obtained by atrial pacing methods.4, 6

Our study is the first to systematically map sinoatrial spread of activation with intracellular and extracellular electrodes. A major problem for measurement of sinoatrial conduction time in previous studies was the difficulty to precisely determine the onset of the upstroke slope in the DC electrogram. The point of departure of the upstroke slope from the trajectory of the diastolic slope, as used by Hariman et al.,2, 3 is not well defined, since the diastolic slope smoothly merges into the upstroke slope. Reiffel et al.4 and Gomes et al.6 did not define the onset of the upstroke slope for measurement of sinoatrial conduction time in their clinical studies. In this work we give an arbitrary definition of the}

onset of impulse formation in the sinus node, depending on the shape of the DC electrogram that allowed exact determination of sinoatrial conduction time. In preparations with long sinoatrial conduction times (>25 msec) the onset of impulse formation is not precisely determined in the DC electrogram, since the transition from the diastolic slope into the upstroke slope is smooth. During this period the DC electrogram conforms in shape to that of the transmembrane potential of dominant fibers. Thus we defined the moment of activation in a similar manner, halfway between the start of the diastolic slope and the top of the upstroke slope. When sinoatrial conduction time was short (<25 msec) the sharp transition from the diastolic slope into the upstroke slope coincided with the activation of dominant pacemaker fibers; therefore, the onset of the upstroke slope was taken for measurement of sinoatrial conduction time. Sinoatrial conduction time determined by extracellular recordings with these criteria and by microelectrode implanments proved to be essentially the same if the extracellular electrode was positioned over the pacemaker center (table 1). However, even a small distance away from the primary pacemaker, true values of sinoatrial conduction time were seriously underestimated, although the DC electrogram was unchanged in shape. The presence of an upstroke slope is therefore not restricted to the dominant pacemaker center as previously assumed,2, 11 and, hence, measurement of sinoatrial conduction time may be inaccurate even though a "characteristic" sinus node potential is recorded.

This problem cannot be solved by extracellular electrodes with larger tip sizes. With electrodes large enough to record from the dominant and latent pacemaker regions simultaneously, the upstroke slope of the DC electrogram was attenuated and occurred later in time compared with the activation of dominant pacemaker fibers (figure 4).

Therefore failure to accurately localize the DC electrode over the pacemaker center probably explains the low values of sinoatrial conduction time reported by Hariman et al.2, 3 rather than errors in the pacing methods. Even the longer values reported by Reiffel et al.,4 Rakovec et al.,3 and Gomes et al.6 may have underestimated the true values. Comparison of true sinoatrial conduction time obtained by intracellular and extracellular recordings (given a precise location of the electrode) and indirect evaluation of sinoatrial conduction time revealed that the constant atrial pacing technique underestimates true sinoatrial conduction time. This has been reported previously for constant atrial pacing8 and the premature stimulation technique.7
Clinical implications. Sinus node activity is accurately reflected in extracellular DC electrograms. While this method is technically more difficult, it offers several advantages over atrial stimulation studies. Spontaneous sinus node activity is recorded directly, thereby eliminating the inadequacies of pacing studies; furthermore, beat-to-beat changes of sinoatrial conduction time or intranodal pacemaker shifts may be picked up.

However, a major prerequisite for the validity of sinoatrial conduction time measurements, as shown in our experimental study, is that the extracellular electrode be positioned over the pacemaker center, which cannot be controlled in humans. A small distance away from the primary pacemaker, true values of sinoatrial conduction time can be underestimated, while the shape of the signal may exhibit typical diastolic and upstroke slopes. Therefore absolute values of sinoatrial conduction time in milliseconds, derived by this technique in humans, have to be interpreted with caution and may be shorter than true sinoatrial conduction time.

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