Sinoatrial Transmission and Atrial Invasion during Normal Rhythm in the Rabbit Heart

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ABSTRACT

Intracellular microelectrodes and small unipolar leads applied to the endocardial surface of the right atrium in vitro were used to study the complex extracellular wave patterns recorded from the neighborhood of the cardiac pacemaker during spontaneous activity. Sinus activity propagated slowly toward the site of atrial invasion on the venous border of the crista terminalis. Atrial activation was marked by a primary negative wave that appeared 20–40 msec after pacemaker firing. Two sources of complex multiphasic waves were found. First, potentials from transitional sinus tissue propagated toward the atrium and caused low-voltage waves that preceded and slurred the onset of the atrial initial negativity. Second, bundles and layers of the crista terminalis muscle were excited asynchronously around the invasion region, as if cross-connections were infrequent. Waves originating from this source occurred after the firing of the invasion site. No extracellular wave could be associated with the firing of the true pacemaker cells. The sinoatrial ring bundle (SARB) yielded a discrete biphasic deflection along most of its way toward the coronary sinus. This potential appeared most frequently after that of the adjoining cristal muscle, raising questions about the functional role of the SARB as an internodal preferential pathway.

In 1910, Lewis (1) and Wybauw (2) demonstrated that the earliest site of primary extracellular negativity in the epicardial surface of the dog atrium lies in the sulcus terminalis near the superior vena cava. They noted that this location is approximately coincident with the head portion of the sinoatrial node described by Keith and Flack (3) and correctly assumed that this structure is the pacemaker of the dog heart. Their findings have since been confirmed by several authors (4, 5).

Further study of the dog heart by Rijlant (6) and by Van der Kooi et al. (7) has shown that in the region of primary negativity the main deflection is preceded by several low-voltage deflections. These low-voltage deflections have been described as “presinus activity” (6) or ascribed to a tortuous route of activation within the sinoatrial node prior to atrial invasion (7). Similar multiphasic records have also been obtained from the atrioventricular nodal region (7). The origin of these waves has never been clarified.

Paes de Carvalho et al. (8) in a microelectrode study of the endocardial surface of the rabbit’s right atrium have demonstrated that the pacemaking sinus cells are located in the wall of the superior vena cava near but not at the first point excited in the atrial muscle (crista terminalis). Furthermore, Paes de Carvalho (9) has shown that small early multiphasic waves similar to those observed by Van der Kooi et al. (7) can be recorded from the endocardial surface of the crista terminalis at the site of its invasion by sinus activity.

In the present study, the spatial separation between the site of multiphasic recording and the actual pacemaker of the rabbit right atrium was used to establish a correlation between the complex extracellular recording and the activation of electrophysiologically identifiable structures in the neighborhood of the sinoatrial transitional region.

Methods

Rabbits weighing 1.5 to 2 kg were killed by a blow on the head. The heart was rapidly excised, and the entire right atrium was dissected free. The chamber was opened by a cut along the anterior border of the interatrial septum and the superior vena cava, as previously described by Paes de Carvalho et al. (8). The preparation was then pinned to a paraffin block in a tissue bath so as to expose its endocardial surface. Tyrode’s solution (NaCl 137 mm, NaHCO₃ 12 mm, KCl 2.7 mm, CaCl₂ 2.7 mm, MgCl₂ 0.5 mm, NaH₂PO₄ 1.8 mm, and dextrose 6 mm) at 35°C, equilibrated with a mixture of 95% O₂,5% CO₂, continuously flowed through the tissue bath during the experiments.

The preparation was allowed to beat spontaneously under sinus node command. Electrical activity was recorded with both extracellular and intracellular elec-
trodes. Extracellular unipolar surface recordings obtained through a concentric pencillike electrode were used to draw a map of the initiation and spread of electrical activity in atrial muscle. The electrode consisted of a steel needle through which a Teflon-coated silver wire was passed. The silver wire protruded 1 mm from the tip of the needle and was fixed in place with Araldite, a thin coat of which also covered the final 3 mm of the needle. The needle itself was used as an indifferent electrode. When the silver wire touched the preparation, the reference point was thus immersed in the bath fluid and located 4 mm above the tissue. The electrode yielded local unipolar recordings identical in practice to those that can be obtained with a truly remote reference. Another surface recording was simultaneously obtained as the potential difference between two distant Teflon-coated silver wires set 0.5–1 cm apart and kept in fixed positions on the surface of the pectinate muscle region throughout a given experiment. This bipolar record was used both for timing the unipolar recordings within a cycle and for indicating, through its shape, that any given cycle was “normal” and not extrasystolic or otherwise altered in propagation pattern. In all cases, signals were amplified and displayed on a dual-beam oscilloscope for photographic recording. The amplifier’s passband was usually set at 1–10,000 Hz. In a few cases, d-c signals were also recorded. The roving unipolar electrode used for mapping the activation was mounted on a micromanipulator provided with a calibrated x-y movement (0.05-mm accuracy) that permitted location of the recording site on a grid corresponding to the endocardial surface of the preparation. Transmembrane recordings were obtained when necessary. The intracellular electrode used for drawing a map of the activation was mounted through a concentric pencillike electrode were 3M KCl-filled glass micropipettes with a d-c resistance of 10–20 megohms and a tip diameter of 0.2–0.4 μm (10). A suitable capacity-compensated electrometer was used as a preamplifier.

Results

**MORPHOLOGY OF SURFACE RECORDINGS**

Unipolar electrograms recorded from the right atrium in the neighborhood of the crista terminalis varied from a simple biphasic configuration to more complex wave forms. The distribution of these various types of potentials over the endocardial surface of the preparation was not haphazard. A large negative deflection preceded by smaller complex waves could be recorded from the crista terminalis at the site of detection of the earliest surface potentials (Fig. 1A). This finding is similar to that reported by Van der Kooi et al. (7) in dogs. Another type of complex wave form consisting of two or more biphasic deflections was consistently recorded after the initial atrial wave in a region extending a few millimeters from the site of atrial invasion in each direction along the crista terminalis (Fig. 2A, stippled area). Figure 3 shows several examples of such complex potentials. Complex wave forms of smaller amplitude were also detected along the venous border of the crista terminalis near the sinoatrial ring bundle. A simple biphasic wave shape such as that shown in Figure 1A (middle tracing) was the most common finding over the rest of the preparation. Figure 2B shows the distribution of the different wave forms in a representative experiment.

**PACEMAKER ACTIVITY AND ATRIAL INVASION**

Slow pacemakerlike transmembrane action potentials can be recorded from a broad area of the venous wall of the rabbit’s right atrium near the crista terminalis (8). However, timing with simultaneous recordings of atrial activity shows that only a restricted cell group within this area is activated early enough to be considered the “true” pacemaker. Figure 1A correlates the transmembrane activity of a true (earliest) pacemaker cell with recordings obtained at the site of detection of the earliest waves in a surface unipolar lead. The distance between the intracellular microelectrode and the surface electrode was less than 1 mm. The surface electrogram consisted of several small complex deflections followed by a primary negative wave known to be indicative of atrial activation at the venous border of the crista terminalis. Pacemaker firing preceded atrial invasion by almost 20 msec. Pacemaker firing also preceded the small complex waves in the surface recording. It follows therefore that these early complex waves which precede the initial atrial negativity must originate in the slow transitional fibers that convey excitation from the pacemaker to the atrial muscle of the crista terminalis (8). It should be noted that efforts to record a surface unipolar potential coincident with the depolarization of the true pacemaker were unsuccessful.

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Spread of Activity in the Crista Terminalis and the Right Atrial Roof

The spread of activity in the crista terminalis and the pectinate muscle region of the right atrial roof was mapped in 17 preparations from unipolar surface recordings. Figure 2A shows one of these maps. Timing was taken at the middle of the earliest intrinsic deflection in each record. The site of detection of the earliest activity in the preparation was marked as 0. Activity spread from this site throughout the crista terminalis, the pectinate muscles, and the thin atrial wall between the pectinate muscles. Activity advanced onto the crista terminalis and the atrial roof in a roughly regular wave front, as can be seen from the isochronous lines drawn at 5-msec intervals in Figure 2A. Propagation velocities along the axes of the main muscle bundles were measured in regions where simple biphasic waves were observed. Data for both the crista terminalis and the pectinate muscles.
muscles clustered around 0.7 m/sec when this criterion was met.

The complex wave forms recorded from the neighborhood of the site of atrial invasion (Fig. 2A, stippled area) were studied in greater detail. The breakdown of multiphasic potentials into their component biphasic waves made it possible to follow the propagation of a given simple wave from point to point within the region. These component waves propagated independently and sometimes in opposite directions within the complex region. Figure 3 shows, as an example, how this analysis was carried out for the records obtained from the numbered sites in Figure 2B. Actual tracings have been redrawn on the left of the figure and numbered in accordance with the site of recording. The diagram on the right of Figure 3 shows horizontal lines corresponding to each record; on each line, the timing of the main component biphasic waves in the record is marked by a black dot. The vertical distance between the lines is proportional to the distance between the sites of recording. The diagram is thus a simple plot of distance (vertical) versus time (horizontal). Slanted solid lines join dots that appear to belong to the same wave front; the slope of any of these lines is therefore a measure of the propagation velocity of the corresponding front. The velocity values obtained were 0.8, 0.5, 0.5, 0.5, and 1.4 m/sec for wave fronts a, b, c, d, and e, respectively. Slanted broken lines project possibilities for confluence of these different paths.

The preceding interpretation of data suggests that the region of invasion of the crista terminalis by sinus activity may behave electrophysiologically as a collection of sparsely interconnected fiber bundles. It should be noted that the results of the analysis illustrated in Figure 3 were by no means casual. A similar logical correlation between waves in different records could be obtained from each of the ten surface mapping experiments performed in the complex cristal region.

Intracellular recordings were performed in nine hearts to clarify the nature of the multiphasic extracellular potentials obtained in the vicinity of the region of invasion of the crista terminalis. Transmembrane potentials were recorded within 100 μm of the unipolar surface electrode. At each site, impalements at different depths were secured by progressively lowering the microelectrode into the tissue. Figure 4 illustrates one of these experiments. The surface unipolar record shown has at least three biphasic deflections. From A through F, the microelectrode was pushed into progressively deeper layers. It is apparent that at the same site cells at different depths depolarize asynchronously, in approximate coincidence with intrinsic deflections in the surface record. This type of finding was consistent in all trials within the region of complex surface recordings. Note that the first cell layer was not necessarily the first to be invaded by activity. In fact quite a lot of variation was found in this respect, leading to the conclusion that anoxia of deeper layers is not the cause of asynchrony within this complex region. The same conclusion is suggested by the fast rates of rise observed in action potentials from deep cells. These results are in keeping with the interpretation proposed for Figure 3, where the complex surface recordings were assumed to represent asynchrony of firing in distinct independent layers or bundles showing sparsely distributed points of confluence.

However, measurements of action potential duration at 50% amplitude did show a consistent shortening in deeper layers in the crista terminalis (Fig. 5). This finding should be viewed together with similar recordings obtained from the thin muscle cover between the pectinate muscles (8). It seems that epicardial cells have shorter action
potentials than do endocardial cells in the rabbit atrium.

**ACTIVATION OF THE CRISTAL SEGMENT OF THE SINOATRIAL RING BUNDLE DURING SINUS RHYTHM**

The sinoatrial ring bundle (SARB) (8) was easily visualized under the dissecting microscope in all preparations. The segment of the SARB bordering the crista terminalis (posterior segment) was studied to determine its contribution to the complex wave forms recorded in this region. In all preparations except two, surface recordings on top of the posterior segment of the SARB over its entire length from the site of invasion of the crista terminalis to the proximity of the coronary sinus region failed to detect any activity preceding that of nearby crista terminalis fibers. In contrast, a later secondary spike was often observed.

Figure 6 shows that the cristal segment of the SARB is indeed activated later than the adjoining crista terminalis. Surface records taken over the SARB typically showed two identifiable biphasic waves. The first one was a signal of activity in nearby crista terminalis muscle, as shown by the transmembrane record in Figure 6A. The second was due to activity of the SARB, as shown by the transmembrane recording from the bundle in Figure 6B. The transmembrane records taken at low sweep speed (Fig. 6B, bottom) showed that the SARB cells exhibited a taller plateau and a considerable amount of slow diastolic depolarization, as previously described (8).

Attempts to map the spread of activity in the cristal segment of the SARB during sinus rhythm showed that propagation proceeded from the region of atrial invasion (sinoatrial junction) on toward the coronary sinus region. Near the coronary sinus, the bundle dwindles to shreds and becomes invisible under the dissecting scope. The present study did not include a systematic search of the SARB connections at its coronary sinus end.

**Discussion**

**DISSECTION PROCEDURE AND THE SPONTANEOUS EXCITATION PATTERN**

In spite of the extensive dissection employed, the invasion of the crista terminalis by sinus activity seems to be unaltered in this preparation (8). The sole route between the sinoatrial node and the atrium seems to be around 2 mm wide and lies on the venous border of the crista terminalis far from the region cut during dissection. Activation of the crista terminalis proceeds from this point of invasion downward to the coronary sinus and upward.
SPONTANEOUS SINOATRIAL TRANSMISSION

toward the cut edge. Activation of the atrial roof (the pectinate muscle region) is at least grossly preserved. Activation of the interatrial septum is importantly delayed and altered by the dissection (8), but this region was not included in the present study.

ORIGIN OF COMPLEX WAVE FORMS IN THE ATRIAL INVASION REGION

This paper shows conclusively that in the rabbit there are two main sources of complex extracellular wave patterns in the area surrounding the site of invasion of the crista terminalis by sinus activity. The first source is the electrophysiologically transitional sinus tissue (8) which conveys excitation from the pacemaker cells to the larger mass of fast-conducting fibers of the crista terminalis. On its slow (0.05-0.1 m/sec) way toward the crista terminalis, the excitatory wave front yields a number of low-voltage complex deflections that precede and slur the onset of the initial negative atrial wave at the invasion site. This complex wave pattern is suggestive of a meshwork arrangement of fibers in the sinus and the transitional tissue (7, 11, 12).

The activation of the pacemaking cell group is not involved in the genesis of these early multiphasic potentials. No activity simultaneous with "true" pacemaker firing could be recorded with our surface electrodes even when the low-frequency response of the recording system was extended to direct current. This fact is probably due to a combination of small tissue mass and feeble extracellular longitudinal current at the origin of the slowly propagating wave front in the rabbit sinus muscle. It is of interest to note that Erlanger (13), in his criticism of Lewis' work on the localization of the pacemaker, pointed out that the true pacemaker might well lie in some small remnant of sinus tissue which might be electrically silent in terms of surface recordings.

The second source of complex wave patterns is a segment of the crista terminalis that extends a few millimeters in both directions from the site of atrial invasion. Complex potentials in this region appear after the inscription of the primary negative wave at the site of atrial invasion. An analysis of the wave patterns obtained from this region of the crista terminalis allowed us to interpret this second complex wave group as the result of propagation of independent wave fronts in different layers of crista terminalis muscle. This interpretation is supported by microelectrode demonstration of asynchrony of firing in different layers of crista muscle. These findings could be easily explained if (1) the crista terminalis is invaded at right angles with respect to its main fiber direction and (2) fiber bundles within this region show infrequent interconnections. In this way, adjacent bundles may even conduct in opposite directions for short distances, as suggested by the analysis carried out in Figure 3. Also, as the excitatory wave leaves the invasion region, the wave front would tend to synchronize and follow the faster routes along the main fiber directions. Consequently, the wave pattern should convert back to a simple biphasic configuration as excitation moves from the complex region. This gradual change to a simpler configuration at farther points was indeed observed in the present experiments.

Several morphological studies have pointed out that the main fiber direction in crista terminalis muscle lies in the direction of its long axis but none have called attention to the frequency of interconnections between adjacent layers or bundles (11, 14). The cristal muscle of the rabbit in the vicinity of the invasion region shows several bundles and layers running in directions other than that of the main longitudinal axis (M. O. Masuda and V. E. Freitas, unpublished observations). This finding certainly adds further cause for the complexity of the excitatory pattern. A final explanation of the complex wave forms observed in the crista terminalis around the invasion region will have to wait for further knowledge of tissue architecture in this area.

It could be argued at this point that our unipolar records might be unable to provide exact localization of the source of extracellular potentials. Unipolar leads can pick up activity from a broad area surrounding the electrode. We checked this point and found that when the electrode was 300 μm away from a propagating bundle the recorded biphasic potential was about half the size of that recorded when the electrode was in direct contact with the bundle. The biphasic wave recorded at a distance was also broader in shape, and its positive and negative peaks were further apart. However, the intrinsic zero crossing remained constant in time, provided the electrode was moved at right angles to the bundle. The recorded voltage fell to less than 30% at a distance of 600 μm and tended to fall rather slowly for further distances. These findings are in keeping with theory and observations of others (15). It would then seem to be impossible to ascertain a priori whether a given biphasic complex is generated by a small bundle near the electrode or a larger bundle further away. In the present study, the crista terminalis origins of the second group of complex waves was ascertained.
by examining the amplitude of the recorded complexes as the unipolar electrode was displaced in a transverse direction across that structure. The amplitude was maximum when the electrode roved over the crista terminalis, and it fell sharply toward each side of this region. The same method of localization was used for the smaller complex waves of sinus origin that preceded the primary negativity in the atrium and for the discrete biphasic potentials of the SARB in its course toward the coronary sinus.

**Atrial Conduction Velocity**

The observed spread of activity over the right atrial roof and the crista terminalis confirms the results obtained by other authors in both rabbits and dogs (1, 4, 5, 8, 11, 16): there is preference for conduction along the main fiber bundles (like cristal and pectinate muscles).

Measurements of conduction velocity obtained from the unipolar leads in regions where the extracellular potential was a simple biphasic wave showed values around 0.7 m/sec for both the pectinate muscles and the lower (posterior) crista terminalis. Similar measurements obtained from the complex region of the crista terminalis through the type of analysis shown in Figure 3 yielded values between 0.4 and 1.5 m/sec, with most measurements falling between 0.5 and 0.8 m/sec. We tend to have reservations about values higher than 1 m/sec. These apparently high values could result from misjudgment of the actual direction of propagation, which in this analysis was supposed to be that of the main axis of the crista terminalis. The intrinsic deflection of a unipolar lead times the external field at the bundle surface). As a consequence, the measured time lapes correspond not to the distance between two electrode locations but to the product of this distance and the cosine of the angle between the bundle and the electrode displacement line. For an angle of 45°, this error in the calculation of conduction velocity would yield an estimate some 41% higher than the actual value. It should be pointed out that fiber asynchrony within the complex region renders microelectrode recording useless for the purpose of measuring conduction velocity. However, we cannot positively exclude the possibility of the existence of some faster bundles in this complex region of the crista terminalis.

**Functional Role of the Cristal Segment of the SARB**

Paes de Carvalho et al. (8) have stated that "the sinoatrial ring bundle, running parallel to the crista, is always excited slightly before adjacent structures" and that "it is the shortest route for an impulse to travel . . . to the AV node region." The present experiments showed that the cristal segment of the SARB outside the invasion region was excited in most cases later than the adjoining crista terminalis during sinus rhythm. The discrepancy may reside in the fact that the previous views of Paes de Carvalho et al. (8) were based on micro-electrode recordings from the SARB and the crista terminalis obtained mostly within the complex invasion region. The findings reported in this paper raise questions as to whether the SARB is in fact a preferential pathway for normal internodal transmission. As a matter of fact, it has never been demonstrated that activity in the SARB can in fact propagate from its supposed coronary sinus termination to the atrioventricular node. There is to date no clear answer to the question of what is the functional role of this structure.

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