Electrophysiological evidence for specialized fiber types in rabbit atrium

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Our knowledge of the sequence of activation of the mammalian atrium has been obtained chiefly through electrophysiological studies. Such studies were initiated in 1910, when Wambauw (1) and Lewis and his collaborators (2, 3) showed that it is possible to localize the pacemaker of the heart and to map the spread of activity by recording electrical events at various points on the atrial surface. Employing initial negativity as a criterion they localized the pacemaker in the sulcus terminalis, the groove between the great veins and the atrial appendage. These observations provided definite evidence that the sino-atrial node was the pacemaker of the mammalian heart. Other observations in subsequent years have supported these results and provided a description of the spread of excitation over the atria (4-8). The same problems have been attacked repeatedly with more refined techniques (9-12). Results of these latter studies, which also locate the site of primary negativity in the outer surface in the sulcus terminalis, support the conclusion that the S-A node is the mammalian pacemaker. Most investigators have also concluded that the spread of activity from the pacemaker is more or less radial in direction and constant in velocity.

The recently developed technique of intracellular recording by means of microelectrodes has shown that the action potentials of pacemaker cells are distinctive in shape (13). Also, a variety of action potential shapes have been found to characterize different parts of the atrial muscle (14, 15). Use of the microelectrode technique thus seemed appropriate to reinvestigate the origin and spread of excitation in the right atrium.

METHODS

Rabbits weighing 3-3½ lb. were killed by a single blow on the head or were anesthetized with intravenous Nembutal. The heart was rapidly excised and the right auricle together with both venae cavae, the interatrial and upper interventricular septum and part of the base of the right ventricle was dissected free under flowing oxygenated Tyrode's solution which was equilibrated with a mixture of 95% O₂ and 5% CO₂ and maintained at 35°C. The composition of this solution was: NaCl, 137 mm; NaHCO₃, 12 mm; dextrose, 5.5 mm; CaCl₂, 1.9 mm; KCl, 2.7 mm; MgCl₂, 0.5 mm and Na₂HPO₄, 3.6 mm. In order to obtain good exposure of the internal surface of the atrium, an incision was made which extended from the free wall of the right ventricle through atro-ventricular groove and then, following the anterior border of the right appendage, terminated as a longitudinal cut in the anterior wall of the superior vena cava. After pinning the preparation to a paraffin block in the tissue bath, its appearance was like that seen in the schematic drawing of figure 1.
Mapping of the sequence of activation was done with the aid of two micromanipulators, the lateral movements of which were read with vernier scales accurate to 0.1 mm. The microelectrodes employed had an external tip diameter of less than 0.5 μ and showed a resistance of approximately 100 megohms after filling with 3 M KCl. They were connected through chlorided silver wires to the inputs of a two-channel transistorized cathode-follower and d.c. amplifier with input capacity neutralization and grid current of less than $10^{-12}$ A. Each channel was connected to one trace of a switched-beam oscilloscope (100 kc switching frequency), from which photographs were taken with a Grass camera. An enlarged two dimensional map of the preparation was made in order to plot the sequence of activation determined from the records of transmembrane action potentials. Distances on this map corresponded with the vernier scale of the micromanipulator.

RESULTS

Morphology of preparation. An extensive study of the anatomy of the auricle and its constituent muscular bands is found elsewhere (16). There are, nevertheless, some noteworthy anatomical features which should be mentioned. Figure 2 shows a drawing of a rabbit heart cut in a transverse vertical plane. The right atrium is emphasized by hatched shadowing. The orifices of the inferior vena cava (postcava) above and coronary sinus below (left precava) are readily discernible. The orifice of the superior vena cava (right precava) is hidden by a heavy bridge of muscle called the crista terminalis or right posterior crest. This muscular bundle corresponds to the sulcus terminalis seen externally between venous and atrial tissue. The crista terminalis begins in the interatrial septum and travels caudally and to the right, separating venous from atrial tissue. This muscle bundle terminates near the A-V node region below the coronary sinus. On its way the crista gives origin to a number of ramifications, known as the musculi pectinati. These features are seen in a schematic drawing of the preparation in figure 1. The musculi pectinati, subdividing and intermingling, form the skeleton of the roof of the right atrial appendage. They end close to the tricuspid valve in another solid muscle band, the right posterior crest, that surrounds the A-V orifice. In order to expose the whole internal surface of the right atrium the superior vena cava was opened by a longitudinal incision. This incision also cut the crista terminalis close to the interatrial septum.

Separating the great veins from auricular muscle there is a whitish bundle of tissue which describes an almost complete loop around the two venae cavae and the coronary sinus (fig. 1) (10). This bundle occupies the position of the embryonic venous valves and as a constant feature gives one major branch to the first pectinate muscle. Other branches are not always seen under the low magnification (X6) employed although sometimes they are quite prominent. It is noteworthy that one of the extremities of this bundle, incorporated in the eustachian valve, ends in the coronary sinus region close to the A-V node (fig. 1) (11). To facilitate reference to this structure we have called it the sino-atrial ring bundle. This term was chosen because it is likely that the bundle is derived from the embryonic venous valves which separated the sinus and atrial cavities. As seen in figure 1, the preparation employed consists of interatrial septum, venous tissue, the roof of the right

FIG. 1. Drawing of the preparation of rabbit right auricle; 1—upper interventricular septum; 2—endocardial surface of the right ventricular wall; 3—septal cusp of tricuspid valve; 4—musculi pectinati of atrial appendage; 5—upper crista terminalis; 6—interatrial septum; 7—superior vena cava; 8—inferior vena cava orifice; 9—orifice of the left precava (coronary sinus); 10—location of the S-A node; 11—A-V node; 12—His bundle. The general extent of 11 and 12 are outlined by dashes. Cut ends of the crista terminalis and sino-atrial ring bundle can be seen on either side of the superior vena cava.

FIG. 2. Transverse section through the rabbit heart. Shaded area indicates the tissue studied.
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appendage, the upper part of interventricular septum and upper part of right ventricular free wall.

Distribution of various action potential types. Since the shape of the auricular action potential is strongly influenced by factors such as temperature and K⁺/Ca⁺⁺ ratio, the action potentials shown in figure 3 were recorded at a temperature of 33°C and a concentration of 2.7 mM K⁺ and 1.9 mM Ca⁺⁺.

In figure 3B a pacemaker type action potential is shown. Noteworthy features are the marked diastolic depolarization (18 mV) and the smooth, slow transition from the diastolic slope to the action potential upstroke. The low amplitude and absence of overshoot are also characteristic of action potentials recorded from a true pacemaker. Fibers which show action potentials of this type and fire 50-60 msec. before atrial muscle (fig. 1A) are true pacemakers. These fibers are located in venous tissue and constitute the sino-atrial node.

Figure 3C shows the potential shape recorded from the segment of the S-A ring bundle running parallel to the crista terminalis. This action potential has a much higher rising velocity than that shown in 3B but still shows some slow diastolic depolarization (6 mV). In addition, the peak of the action potential is rounded and overshoot is small. One prominent feature of this record is the more abrupt transition from the diastolic prepotential to the upstroke of the action potential. The absence of a smooth transition such as is seen in figure 3B probably means that this fiber has been fired by propagated activity before its own prepotential attained threshold. The immediate corollary is that such a fiber has a slower intrinsic rhythm than the one seen in figure 3A. On the septal side of the S-A ring bundle one records a fast rising action potential with a clear plateau and steady diastolic potential (fig. 3D). In other regions of the S-A ring bundle one finds a variety of action potential shapes intermediate between the two types just described. The distribution of the action potential shapes seen in figure 3B, C and D is as follows.

The pacemaker which shows the most marked diastolic slope, the smoothest transition from this slope to the action potential upstroke and the lowest rising velocity is located in venous tissue. Propagating from this point towards auricular muscle, the shape changes gradually to one with less diastolic depolarization, greater amplitude and higher rising velocity. Finally, surrounding the vein tissue, one finds the S-A ring bundle type action potentials. The S-A ring bundle segment running parallel to the crista terminalis is the most peripheral region showing slow diastolic depolarization. The transition from the S-A ring bundle to the crista terminalis is characterized by a sudden stabilization of the diastolic level and shortening of the action potential duration (fig. 3E). The same transition is seen in the interatrial septum although here action potentials of the S-A ring bundle do not show slow diastolic depolarization under the conditions of these experiments.

When the microelectrode is moved from the crista terminalis along the musculi pectinati the action potential becomes shorter (fig. 3F) until, as the A-V ring is approached, the plateau almost disappears. If the electrode is inserted in the atrial roof in the spaces in between the musculi pectinati, one records a very sharp, rapidly rising action potential showing no plateau (fig. 3G). Figure 4 compares the rising phases of action potentials from the crista terminalis (fig. 4A), the upper His bundle (fig. 4C) and the atrial roof (fig. 4B). The higher rising velocity of the action potential from the atrial roof fiber can be appreciated by comparing the distance between two dots on the upstroke of each record. These dots are separated by an interval of .01 msec. This difference is even more impressive since the action potential shown in figure 4 has a lower resting potential than those shown in figure 4A and C.

When records are obtained from fibers progressively closer to the tricuspid valve from any point around the A-V ring the action potential shape changes as shown in figure 5. This change takes place over a short distance and is characterized by a diminution in rising velocity, disappearance of the spiked reversal and finally the appearance of a slowly rising, rounded action potential similar to that of the A-V node (17). Finally, when the recording electrode is moved towards the A-V node in the area just above the A-V valve, the action potentials gradually develop diastolic depolarization which attains its maximum in the A-V nodal region. From this point, where most of the A-V delay occurs (17) there is a transition from the rounded shape just described to action potentials showing a rapid rising phase, spiked reversal and long plateau. These are recorded from the upper His bundle (fig. 3H) and resemble action potentials of Purkinje fibers. These observations suggest that the region of A-V node in which delay occurs is derived from the lower portions of the canals auricularis rather than from the sinus venosus, as has been suggested elsewhere (18, 20).

Spread of excitation. Figure 6 shows a map based on data from five experiments. Both the distribution of action potentials of a particular type and the time of activation of each area have been constant in all experiments. The true pacemaker has always been located in nodal fibers situated within the wall of the superior vena cava. The smooth-shaped action potentials seen in figure 3B are recorded from this region. Since the
The excitation wave propagates very slowly through this tissue (less than 0.05 m/sec.), it is possible to locate points relatively close to the pacemaker which are activated as late as 70-80 msec. after the pacemaker. Although these points depolarize later than any others in the auricle, activity in the true pacemaker cells precedes depolarization of the atrial roof by as much as 60 msec. This is illustrated in figure 34.

As the excitation front progresses towards the periphery of the venous region, it gradually gains velocity and the action potentials show a faster rising phase. The front arrives at the right segment of the S-A ring bundle (the segment at the base of the right venous valve) and crista terminals considerably before it reaches the left segment of the S-A ring bundle (the septal segment, at the base of the left venous valve). As far as action potential shape is concerned, the S-A ring bundle marks the limit between S-A nodal and plain auricular tissue. Since the excitation wave travels radially from the pacemaker, the right segment of the S-A ring bundle and adjacent parts of the crista terminals are invaded by a rather extensive segment of the excitation front in a very short time interval. This probably accounts for apparently higher conduction velocities recorded from the crista terminals. Thus in figure 6 we see a latency of 42 msec for the central region of crista terminals and on each side a value of 43 msec. One other characteristic of the spread of excitation from the venous region to S-A ring bundle and auricular muscle is the abrupt change in conduction velocity observed within a space of less than 0.5 mm. This phenomenon is seen regardless of the direction of propagation and is similar in its abrupt nature to that observed in other transitional areas such as from auricle to A-V node or to A-V ring (17). The difference is that these structures are regions of low conduction velocity between two rapidly conducting segments while the opposite is true in the case of the transition from the slowly conducting venous tissue to the rapidly conducting auricle.

Since the conduction velocity in the S-A ring bundle and crista terminals is much greater than in venous tissue, the excitation wave spreads rapidly along the crista and soon reaches muscle fibers in the vein not yet depolarized by direct spread. Excitation then enters the venous area from the crista. This is seen in figure 6, latency at the upper end of the crista is 43 msec. and in the adjacent venous tissue, 50 msec.

From the first point excited in the S-A ring bundle and crista the excitation wave spreads along the crista and musculi pectinati through the whole atrial roof at approximately the same apparent velocity (around 0.45-0.6 m/sec.). The S-A ring bundle, running parallel to the crista, is always excited slightly before adjacent structures. Thus it is the shortest path for an impulse to travel from the first point excited by the venous tissue to the A-V node region. However, the difference in time is small so that a lesion involving on the S-A ring bundle does not appreciably increase the A-V interval. When the preparation is intact, i.e. when the S-A ring bundle and crista terminals have not been cut close to the septum, excitation traveling along the crista reaches the interatrial septum quite early. Once the crista terminals has been divided to open the superior vena cava, however, excitation of the septum is delayed by as much as 25-30 msec. and reaches the septum from the coronary sinus region, as is seen in figure 6.

The excitation wave takes around 26.5 msec. to go from the first point excited in the S-A ring bundle (37.5 msec.) to the area close to the A-V ring at the extreme left of figure 6 (64 msec.). As the excitation front approaches the tricuspid valve conduction velocity decreases; this change is associated with the alterations in the action potential shape described above. In this area the wave of excitation takes as long as 9 msec. to cross a distance of 1.8 mm (distance between B and F in fig. 5). The same phenomenon is observed at all points around the A-V ring. In the A-V node it assumes special features in that not only is the delay increased but also it is from this site that excitation reaches the His bundle. One outstanding point is that the propagation through the A-V node is perpendicular to the tricuspid valve as in the rest of the A-V ring, and not along the valve, as is the case in the His bundle.

![Figure 4](http://ajplegacy.physiology.org/) Rising phase of action potentials recorded at high sweep velocity. A—crista terminalis, B—atrial roof, C—upper His bundle. Time calibration below C is in intervals of 1 msec. Amplification is the same in all 3 records.

![Figure 5](http://ajplegacy.physiology.org/) Change in latency and action potential shape in fibers of the A-V ring. Top trace—action potential of an atrial fiber located in the interatrial septum, employed as a time reference. Top trace, fibers of atrial muscle (A) and fibers progressively closer to the A-V ring (B-E). Fibers shown in B and E are separated by a distance of 1.8 mm. Time calibration in C represents 50 msec. F shows the same record as E at a lower sweep velocity.
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DISCUSSION

Suitability of preparation for studies on origin and propagation of excitation. The first question to consider is whether or not the dissection of the preparation interferes with the localization of the pacemaker. This is unlikely for two reasons: a) the crista terminalis and S-A ring bundle are cut very close to the interatrial septum and the normal pacemaker has not been identified in this area. b) The pacemaker site, as determined with the microelectrode technique, is in nodal cells imbedded in venous tissue considerably removed from the cut edges of the preparation.

Another point to examine is the meaning of the latencies obtained for the interatrial septum. The data presented do not represent normal values for latencies of the interatrial septum since this region is normally excited via S-A ring bundle and crista terminalis (see RESULTS). For all other areas it is likely that excitation has traversed normal paths at almost normal velocities.

It might also be mentioned that for a point near the foramen ovale the change in latency resulting from severing the connections between crista and the first pectinate muscle on one side and the interatrial septum in the other amounts to 25 or 30 msec. This change in latency is so close to the values found by Bachmann (21) after clamping his interatrial band (30-35 msec.) that it suggests lie might have been severing the connections of crista and first musculus pectinatus with the septum.

Distribution of tissues possessing a pacemaker potentiality. By pacemaker potentiality is meant the ability of a tissue to develop pacemaker activity, i.e. to initiate the rhythm of the heart in the absence of excitation from another source. These tissues either show pacemaker-like diastolic depolarization under normal conditions or after appropriate variations in experimental conditions. Such pacemaker potentiality has only been found in tissue which shows both embryological and electrophysiological differentiation, as described below.

It may be useful to recall that the remnants of the embryonic sinus venosus are represented in the adult mammalian heart (22) by a) musculature of the superior vena cava, b) musculature of the venous valves, c) musculature imbedded in the sulcus terminalis (S-A node) and by d) musculature of the coronary sinus.

Such remnants are better developed in animals lower in the zoological scale and show clear differentiation. A comparative anatomical study by Maciezenz has shown the phylogenesis of these structures (23). In the rabbit the musculature at the base of the left venous valve (corresponding to the septal segment of the S-A ring bundle) has already lost some identifying characteristics.

Another specialized structure in the atrium is the A-V ring which is formed from the lower segment of the auricular canal (22). This A-V ring, in the embryo, connects the auricles with the ventricles all around the cardiac tube. With the development of the definitive A-V valves, this connection is interrupted in most areas, leaving only strands communicating with the ventricle.

Higher on the zoological scale the number of strands is reduced until just two or three persist in some birds. In the mammals this number has been further reduced to only one (23, 24), the His bundle.

The electrophysiological data, as far as localization of tissues with pacemaker potentiality is concerned, shows a remarkable agreement both with the ontogenetic and morphological observations. Under the experimental conditions employed, the superior vena cava, A-V node and adjacent A-V ring, and the right segment of the S-A ring bundle (right venous valve) show marked diastolic depolarization. The same slow depolarization is recorded from fibers of the His bundle and at other points around the A-V ring with appropriate modifications of the experimental conditions and has also been recorded from the septal segment of the S-A ring bundle.

Localization of pacemaker. One of the most interesting results of these experiments is the demonstration that the pacemaker is in muscle fibers located in the superior vena cava and not in the sulcus terminalis. The pacemaker is indeed the site of primary negativity. Nevertheless for the slowly rising action potentials of the pacemaker the conduction velocity is so slow and the wave length of depolarization so long that it would be difficult to record such activity with a unipolar or differential surface electrode. Nevertheless some investigators have obtained an indication of activity preceding the primary negativity in the form of high frequency oscillations (12) or displacements of the base line (25). Bosler (26) working with a unipolar d.c. recording system picked up slow variations of the base line before the start of the auricular action potential. The fast changes recorded in a.c. systems by others probably represent the fast rising potential of the region close to the S-A ring bundle.

FIG. 6. Map showing spread of excitation in the preparation of right atrium. Figures represent latency in msec. with respect to the pacemaker located in the S-A node (0.0). Action potential shape recorded from each site is indicated in the figure.
Our results are in agreement with the embryological studies which have shown that the last parts formed in the heart have the highest rhythmicity. The last of the major divisions of the heart, the sinus venosus, is generally accepted to be the pacemaker and also displays a contractile function in the lower animals. In the mammals most of the contractile function disappears but the pacemaker activity remains.

Is the S-A ring bundle a specialized conduction system? The S-A ring bundle is a specialized structure in the sense that it shows both histological and electrophysiological differentiation. Our data do not permit a conclusive statement concerning its role as a special conducting system. Nevertheless, a conducting system with the same distribution as the S-A ring bundle would present the following advantages: a) rapid excitation of crista terminalis would prevent reflux of blood into the veins during auricular contraction, b) the same rapid excitation would guarantee early excitation of the left auricle (see discussion, 1st paragraph), and c) such rapid excitation would increase the synchronization and efficacy of contraction of the atrial roof.

Our experiments have not shown a higher conduction velocity in the S-A ring bundle than in the crista terminalis in rabbits. However, if S-A ring bundle establishes syncytial connections with the tissues surrounding it, the techniques employed would be inappropriate for such a determination. It is very important that in certain cases the S-A ring bundle (in particular its right segment) is able to undertake pacemaker activity. It is interesting to note that in the rabbit the S-A node described by Keith and Flack (22) lies under this right segment of the S-A ring bundle (27).

Relationship of A-V node and A-V ring. It has been suggested elsewhere that the A-V node consists of an atrial part formed from the sinus venosus and a second part formed from the A-V ring (18-20). This description does not seem to be exact as far as the physiological behavior is concerned either in adults or in embryos.

Among other arguments are the following: a) in the A-V node the greater part of the delay occurs when the excitation wave is traveling through a region that shows the same kind of action potentials as the A-V ring (17). Also, as in the case of the A-V ring, the action potential shape recorded just above the A-V node is auricular in type (17). b) The excitation wave crosses the A-V node in the same direction as it crosses the A-V ring tissue, that is, perpendicularly to the A-V valve. After crossing the A-V node the excitation wave reaches the His bundle and again is rapidly conducted in a direction parallel to the A-V ring. c) Normal P-R intervals exist in the embryo at a time when the connections all around the A-V ring are intact, showing that the delay is a characteristic of the A-V ring tissue and not of the A-V node in particular (28). This, we think, is the final argument for ruling out any physiological participation of sinus tissue in A-V transmission.

Validity of conduction velocity measurements. It may have been noticed that any mention of accurate conduction velocity measurements has been purposely omitted. In fact, it is next to impossible to make such measurements in a syncytial muscle where we can never be sure of the exact direction of spread. If we assume that the excitation wave is propagating in a straight line, the lower a value the more accurate it will be. But if the propagation is following a circuitous pathway the same will not be true. Our figures for conduction velocity in the auricular muscle are probably reliable since similar results have been obtained both with normally conducted beats and following stimulation at different sites. The same holds for the very low velocities in venous tissue and A-V ring. In the crista terminalis the spread was quite rapid and a figure of 0.5-1 m/sec is probably an accurate range for conduction velocity in this tissue.

REFERENCES