His Bundle Electrograms of Dog

Correlation with Intracellular Recordings

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SUMMARY
Transmembrane potentials from the penetrating and branching portions of the bundle of His were simultaneously recorded along with bipolar extracellular electrograms. Recordings were obtained during periods of normal antegrade and retrograde conduction and also during various degrees of conduction delay and block within and distal to the bundle of His. The onset and termination of extracellular electrogram coincided with the upstrokes of the transmembrane action potentials recorded from the penetrating and branching portions respectively and the duration of the extracellular electrogram equaled the interelectrode conduction time. Thus, the extracellular electrogram accurately reflected the timing and duration of the electrical activity of the entire bundle of His (penetrating and branching portions). Intra-His bundle conduction delay as determined by an increase in interelectrode conduction time and a decrease in the upstroke velocity of phase O of the action potentials, resulted in a decrease in the amplitude and an increase in the duration of the extracellular recording. Significant intra-His bundle conduction delay resulted in two His bundle deflections (H and H') in the extracellular electrogram recordings. The onset of the two His deflections coincided with the upstrokes of the two transmembrane potential recordings. When intra-His bundle block occurred the action potential distal to the block and the corresponding deflection on the extracellular recording disappeared. The results of this study provide further evidence supporting the validity of clinical electrode-catheter recordings of His bundle activity.

FOR OVER one hundred years, electrophysiologists have used extracellular recordings to study the electrical activity of the intact heart as well as of isolated tissue preparations.\(^1\)\(^-\)\(^10\) The recent introduction of an electro catheter technique to record His bundle electrograms in man is an extension of the extracellular recording method.\(^11\)\(^-\)\(^12\) His bundle electrograms are being increasingly used in the study of A-V conduction\(^13\)\(^-\)\(^16\) and in the analysis of cardiac arrhythmias in man.\(^17\)\(^-\)\(^22\)

In these studies validation of the His bundle electrogram has been obtained either by pacing the bundle of His\(^21\) or by noting its behavior during various physiological and pharmacological interventions.\(^12\) However, since the bundle of His is anatomically divided into penetrating and branching portions, it has been questioned whether extracellular recordings, as obtained by a catheter electrode, reflect total His bundle activity or electrical activity of only a part of one of the two portions. The present study was designed to test whether the surface electrogram truly reflects the electrical events and the total activation of the His bundle. This was done by simultaneously recording transmembrane action potentials from the penetrating and branching portions of the bundle of His along with bipolar extracellular electrograms. Recordings were obtained during periods of normal antegrade and retrograde conduction and also during various degrees of conduction delay and block within and distal to the bundle of His.

Materials and Methods
Six adult mongrel dogs weighing 10–15 kg were anesthetized with intravenous sodium pentobarbital (30
mg/kg); the hearts were rapidly removed through a right lateral thoracotomy and placed in cool oxygenated Tyrode's solution of the following composition (millimolar): NaCl 137.0 KCl 3.0; NaH₂PO₄, 1.8; CaCl₂ 2.7; MgCl₂0.5; dextrose 5.5; and NaHCO₃ 12.0.

A block of tissue consisting of a portion of the right atrium, the interventricular septum, the proximal portion of the root of the aorta and the bundle branches extending to the papillary muscles, was removed and transferred to a dissection chamber. The specimen was then pinned to the wax base of the bath. The A-V conducting system, consisting of the A-V nodal region, the entire bundle of His and both the right and left bundle branches was dissected and identified using the technique of Elizari et al. During the dissection the specimen was perfused with cool oxygenated Tyrode's solution.

The dissected specimen was placed in a 40 ml tissue bath for recording of electrograms and transmembrane action potentials. The bath was perfused continuously with Tyrode's solution which was gassed with 95% O₂ and 5% CO₂ and the temperature of the perfusate was maintained between 34-36°C. Transmembrane action potentials were recorded by means of machine-pulled glass capillary microelectrodes filled with 3M KCl (tip resistance 20–40 megohms). Microelectrodes were connected by AG - AgCl wires to amplifiers with high input impedance and input capacity neutralization (Bioelectric Instrument Co., NF-1). The outputs of these amplifiers were displayed on recording and monitoring oscilloscopes (Tektronix 565 and 532).

Bipolar electrograms from the bundle of His were recorded by means of teflon coated silver wires (0.012" diameter) and D. C. amplifier, and were also displayed along with the transmembrane action potentials. 50 msec time marks were displayed on the oscilloscope. Calibration of the recording system was performed by introducing a 100 mV direct current calibration pulse into the bath via ground. Photographic records of the oscilloscope face were obtained using moving film in a Grass C₄ oscillographic camera.

The preparation was stimulated by rectangular pulses delivered from waveform and pulse generators (Tektronix 160 series) through a pair of silver wires. The stimulating pulses were 2 msec in duration and approximately twice diastolic threshold. The output of a second set of waveform and pulse generators was used to deliver premature stimuli after every eighth basic beat. The timing of the premature stimuli could be varied with an accuracy of up to ± 1 msec.

In each experiment the penetrating and the branching portions of the bundle of His were identified grossly by anatomic landmarks. Briefly, the penetrating portion of the bundle of His was that part which was closest to the A-V node and which penetrated the central fibrous body, and the branching portion was in the area where the right and left bundle branches originate. One of the silver wires of the bipolar surface electrodes was placed on the penetrating and the other on the branching portion of the bundle of His. The microelectrodes were positioned along the longitudinal axis in the same areas of the penetrating and branching portions of the His bundle. The interelectrode distances along the longitudinal axis were approximately 8 mm and 2 mm along the transverse axis. Transmembrane action potentials from the penetrating and branching portions of the bundle of His were identified by their characteristic configuration. Transmembrane action potentials from the interventricular septum were excluded by (1) their timing in reference to the stimulus artifact, i.e. they occurred much later than the surface electrogram of the bundle of His, and (2) by their characteristic morphology. Pacing to initiate antegrade conduction was performed by placing a separate pair of bipolar silver wires at the A-V junction near the coronary sinus region and to study retrograde conduction was performed by stimulating the distal portion of either the right bundle branch or a division of the left bundle branch. Any changes in the location of the stimulating and recording electrodes are identified in the text of the Results. All control recordings were obtained after an initial period of stabilization.

Results

In all figures, transmembrane action potentials recordings from the penetrating portion of the bundle of His are identified as P and those from the branching portion as B.

Normal Antegrade and Retrograde Activation of the Bundle of His

Figure 1 is a representative recording from an experiment in which the bundle of His was antegrade depolarized. As shown in this figure the penetrating portion (P) was the first to be activated, followed by the branching portion (B). The onset and termination of the surface electrogram (H) coincide with the upstrokes of the transmembrane action potentials recorded from the penetrating and branching portions respectively and the duration of the surface electrogram (9.6 msec) equals the interelectrode conduction time. Thus, the surface electrogram accurately reflected the timing and duration of the electrical activity of the entire bundle of His.

Premature Stimulation of Bundle of His

Figure 2 illustrates the effects which premature beats have on both intra and extracellular recordings. In panel A of Figure 2, a premature atrial beat introduced at an S₁-S₂ coupling interval of 306 msec resulted in activation of both the penetrating and branching portions of the His bundle. However, there was an increase in interelectrode conduction time and a significant decrease in the upstroke velocity of phase 0 of the transmembrane action potentials. These changes resulted in a marked decrease in the amplitude of the extracellular recording and an increase in its duration. In panel B, a slight decrease in S₁-S₂ interval (304 msec)
Antegrade conduction within bundle of His. "P" represents the onset of phase O of the transmembrane action potential recorded from the penetrating portion of the bundle of His and "B" the transmembrane action potential from the branching portion. "H" is the bipolar surface electrogram recording. "S" denotes the driving stimulus. Time marks are at 50 msec and the calibration is for 50 mV. Interelectrode conduction time equals the duration of the H deflection. Similar designations will be used for subsequent figures.

The first beat in each panel represents the basic drive beat (S1). The H1 deflection coincides with the interelectrode conduction time of the transmembrane action potentials from the penetrating (P) and branching (B) portions of the bundle of His. In panel A, premature activation of the atrium (S2) resulted in delayed conduction within the bundle of His. Note that H2 is decreased in amplitude and increased in duration. The duration of the H electrogram is bracketed and was measured from the onset of the P action potential to the peak of phase O of the B potential. In panel B, an earlier premature beat (S2) resulted in a local response in the penetrating portion of the bundle of His and no extracellular electrogram recording.
resulted in a "local response" in the penetrating portion and no response in the branching portion of the bundle of His. This "local response" did not produce any deflection on the surface electrogram recording.

**Intra-His Bundle Conduction Delay and Block**

Figure 3 is representative of three experiments in which intra-His bundle conduction delay and block were produced by gentle pressure applied to the bundle of His with the tip of a No. 21 gauge needle. In Figure 3 the bundle of His was activated in a retrograde direction by stimulating the distal right bundle branch. Activation proceeded from the branching to the penetrating portion. In each panel, the basic drive beat (S₁) demonstrates an increase in interelectrode conduction time and two His bundle deflections (H and H') on the surface recording. Panel A demonstrates that premature stimulation results in retrograde intra-His bundle delay as manifested by an increase in the H'-H interval. In panel B, S₂ was retrogradely blocked between the branching and penetrating portions of the bundle of His and only an H' electrogram was recorded on the surface tracing. Intra-His bundle conduction delay and block occurred during both antegrade and retrograde activation.

Figure 4 illustrates the results of an experiment in which the microelectrode from the branching portion of the bundle of His was moved 5 mm distally to record from the proximal portion of the right bundle branch. For the basic drive beat of both panels the upstroke of the right bundle branch action potential occurs beyond the duration of the surface electrogram (H₁). These findings persist when conduction delay (panel A) and block (panel B) are produced by premature stimulation.

In Figure 5, the transmembrane action potentials were recorded from the penetrating and branching portions of the bundle of His but the bipolar surface electrodes were separated to include the area from the penetrating portion of the His bundle to the proximal portion of the right bundle branch. The distance between the two poles of the surface electrodes was approximately 1.3 cm. During antegrade activation (panel A), the surface electrogram shows a His bundle deflection followed by a very sharp right bundle branch deflection. The onset of the His deflection corresponded to the upstroke of the transmembrane action potential from the penetrating portion and the action potential from the branching portion occurred coincident with the terminal portion of the His bundle electrogram. The right bundle branch electrogram occurred outside the duration of the intracellular recordings. Similar findings were obtained during retrograde conduction (panel B). This figure demonstrates that when right bundle branch activity is recorded along with the His bundle on the surface electrogram the former occurs as a separate high frequency deflection. When the microelectrode from the branching portion of the His bundle was positioned in the proximal right bundle branch region the upstroke of...
the transmembrane action potential and the right bundle branch electrogram coincided (fig. not shown).

Discussion

The results of this study demonstrate that under conditions of normal antegrade and retrograde conduction, the bipolar surface electrogram positioned to encompass the course of the common bundle accurately reflects total electrical activity (branching and penetrating portions) of the bundle of His. Our findings indicate that the single cell recordings represented an accurate sampling of the electrical activity occurring in the regions of the penetrating and branching portions of the His bundle and furthermore that the electrical activity of these cells in all likelihood contributed to the surface electrogram.

This is supported by the following: 1) during normal conduction the total duration of the His bundle electrogram almost exactly equaled the interelectrode conduction time, 2) impairment of conduction, as manifested by a decrease in the upstroke velocity of phase 0 of transmembrane action potentials and an increase in interelectrode conduction time, was faithfully reflected in the surface electrogram as an increase in duration and decrease in amplitude, 3) during intra-His bundle block both the intracellular and extracellular recordings distal to the site of block disappeared, 4) impulses which produced only a "local response" in the bundle of His (fig. 2) did not result in a deflection on the surface electrogram, 5) the surface electrogram was unaffected by conduction delay or block occurring distal to the bundle of His, and 6) when the surface electrogram recorded proximal right bundle branch activity this fast deflection was well separated from the transmembrane action potential recordings of the bundle of His.

Both Spach et al. and Myerberg, Nilsson and Zobley have demonstrated, in their studies of isolated Purkinje fibers, a direct relationship between the maximum $dv/dt$ of phase 0 of the transmembrane potentials and amplitude of the surface electrogram. Our findings during intra-His conduction delay are in agreement with their observations. In addition, our findings have demonstrated that a significant conduction delay can result in two separate His bundle deflections (H and H`). In this regard it should be noted that the extracellular electrode distance (8mm) was sufficiently wide to record two separate deflections.

Several clinical correlations can be made by extrapolating our experimental results to the clinical situation in which His bundle recordings are obtained by the electrode catheter technique. It is to be noted, however, that in making these clinical correlations, certain technical differences between

Figure 4

In panel A transmembrane action potential recordings were made of the penetrating (P) portion of the His bundle and the proximal portion of the right bundle branch (RB). The extracellular electrodes were positioned at the penetrating and branching portions of the bundle of His. Note that the RB potential occurs beyond the extracellular His bundle recording (H1) for the basic beat. Premature stimulation (second beat) resulted in conduction delay between the bundle of His and the proximal right bundle branch. The amplitude and duration of H1 was unchanged and the RB potential occurs beyond the H2 deflection.

In panel B the coupling interval of the premature beat was decreased to 296 msec. Block occurred between the bundle of His and the proximal right bundle branch. The amplitude of duration of H2 was the same as H1.
Figure 5

Transmembrane action potential recordings from the penetrating (P) and branching (B) portions of the His bundle. The extracellular electrodes were separated to include the area from the penetrating portion of the His bundle to the proximal right bundle branch.

This experimental study and the clinical situation must be borne in mind. The two most important differences are 1) in this experimental study the extracellular recording electrodes were positioned on the bundle of His and this fixed position was maintained throughout the study, while during clinical studies the recording electrodes are not in direct contact with the bundle of His and they may or may not always bear a fixed relationship to the bundle of His. In this regard we have compared recordings obtained by the catheter technique and by inserting plunge wires into the bundle of His. The timing and the duration of the His bundle electrograms were exactly the same by both techniques.27 We have also noted that in a majority of cases the electrode catheter technique provides stable recordings of His bundle activity during sinus rhythm, acceleration of the atrial rate and premature atrial stimulation. 2) In this experimental study extracellular recordings were obtained using a DC amplifier whereas in most clinical studies the His bundle electrogram is recorded between a band pass of 40-500 Hz, and thus all signals below 40 Hz and above 500 Hz will be filtered out.

In spite of these differences in the two methods one can make certain correlations between our experimental results and the electrode catheter technique of recording of His bundle activity.

1. Electrode catheter recordings of His bundle activity can reflect total activity (penetrating and branching portions) providing the catheter is properly positioned in the region of the tricuspid valve, the interelectrode distance is sufficient to encompass the course of the common bundle, and one or both electrodes are in very close proximity to the bundle of His.

2. Clinically, intra-His bundle conduction delay can be recognized when both an increase in duration and a decrease in amplitude of the His bundle deflection occurs. In addition, when pre-
mature beats block within the bundle of His, electrode catheter recordings may show only a very low amplitude deflection which may be overlooked and the site of block erroneously attributed to the A-V node. Obviously, the amplitude of His bundle deflections is dependent upon the gain and filter frequency settings at which electrode catheter recordings are made and also on the proximity of the electrodes to the bundle itself.

3. The demonstration of so-called “split” His bundle deflections (H and H’) confirms previous observations of this phenomenon made in man and experimental animals. Wenckebach type conduction within the bundle of His, in the presence of normal ventricular activation, has been demonstrated in the intact dog heart using extracellular recordings. More recently, confirmation of intra-His bundle Wenckebach phenomena have been demonstrated using transmembrane action potential recordings from the penetrating and branching portions of the common bundle.

It is apparent, as demonstrated by figure 5, that electrode catheter recordings of two deflections may also represent a combination of His bundle and right bundle branch potentials. While the distinction between H-H’ and an H-RB combination may be difficult at times, the interpretation should be made within the clinical context and consideration should be given to such factors as 1) the position of recording electrodes in the heart, 2) the presence or absence of normal ventricular activation and 3) the response of the two deflections to premature atrial stimulation and to direct stimulation of the A-V junctional region.

The results of this study provide further evidence supporting the validity of clinical electrode catheter recordings of His bundle activity.

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