Recording of diastolic slope from the junctional area in dogs with junctional rhythm

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ABSTRACT Stable junctional rhythm was induced in 14 of 25 anesthetized dogs by exclusion of the sinus node and surrounding atrial tissue. In 12 of the 14 dogs diastolic slope was recorded through a unipolar lead consisting of a terminal of a plunge or catheter electrode placed in the His bundle area coupled with an indifferent superior vena caval electrode. Reversed polarity, high-gain amplification (0.1 mV/cm), and low-pass filters (0.1 to 50 Hz) were used for the unipolar recording. The diastolic slope (0.15 ± 0.07 mV/sec) was recorded from a localized area close to the His bundle and preceded the bipolar His bundle deflection. Overdrive atrial stimulation was followed by slowing of the junctional rate and decrease in the diastolic slope. A strong negative correlation (r values from −.78 to −.95) was found between the junctional cycle lengths and the diastolic slopes after atrial pacing. Isoproterenol infusion increased the junctional rate and the diastolic slope, whereas vagal stimulation and verapamil injection decreased them. The effects of verapamil were partly reversed by CaCl₂ injection. The responses of the diastolic slope to overdrive pacing, vagal stimulation, and isoproterenol suggest that the deflection is indeed due to membrane current responsible for phase 4 depolarization. The responses of the junctional rhythm and the diastolic slope to verapamil and CaCl₂ suggest that AV nodal automaticity is responsible for the rhythm. Clinical application of this recording technique should help in the identification and characterization of automatic junctional rhythm.


DIASTOLIC SLOPES that accurately reflect the phase 4 depolarization of the pacemaker cells have been recorded extracellularly from the sinus node and ectopic atrial foci in dogs and humans.¹⁻³ These diastolic slopes can be used as a marker of the activity of the pacemaker, both normal and abnormal. Thus, recording of these diastolic slopes could be useful in the identification of abnormal pacemakers as a cause of the cardiac arrhythmia and in the study of the characteristics of the pacemakers responsible for the arrhythmia.

In this paper we describe the recording of diastolic slope in the junctional area in dogs with junctional rhythm and the effects of various interventions on this slope and the rate of the rhythm. Our results provide a method of recording this slope and of verification that it is indeed due to membrane current responsible for phase 4 depolarization of the cardiac pacemaker in the junctional area.

Methods
Twenty-five adult mongrel dogs weighing 15 to 23 kg and anesthetized with sodium pentobarbital (30 mg/kg) were used in this study. Under controlled ventilation, a thoracotomy through the right fourth intercostal space was performed. A plaque electrode was then sutured on the adventitia of the superior vena cava. After exposing the right atrium and right ventricle through an incision on the pericardium, a plaque electrode was sewn on the right atrial appendage. His bundle electrical activity was recorded with Teflon-coated bipolar platinum wires inserted into the region of the His bundle through the lateral right atrial wall. In several experiments His bundle activity was recorded through a bipolar electrode catheter inserted through the carotid artery and placed in the noncoronary cusp of the aorta. The right or left vagal nerve was isolated and a Palmer electrode was placed around it. Decentralization of the nerve was achieved by severing the nerve proximal to the electrode. The sinus nodes in these dogs were crushed or excised to induce a stable junctional rhythm.⁴

Diastolic slope was recorded through a unipolar lead consisting of the electrode in the His bundle area paired with an indifferent electrode on the superior vena cava. Reversed polarity and high amplification in the range of 0.1 mV/cm were used to record the unipolar His bundle electrogram. Electrocardiograms, bipolar right atrial electrograms recorded through the plaque electrode on the right atrial appendage, and bipolar and unipolar His bundle electrograms were displayed on an Electronics for Medicine DR 12 oscilloscope and recorded at paper
speeds between 25 to 100 mm/sec. Filters were set at 0.1 to 500 Hz for electrocardiograms, 40 to 500 Hz for bipolar atrial and bipolar His bundle electrograms, and 0.1 to 50 Hz for unipolar His bundle electrograms.

To evaluate the effects of overdrive suppression on the junctional rhythm and the diastolic slope, electrical stimuli of twice diastolic threshold and 2 msec width were delivered through the atrial plaque electrode with a constant-current stimulator at rates from 100 to 200 beats/min for 30 sec. Vagal stimuli consisting of 2 msec pulses of 2 V at rates varying from 1.5 to 100 Hz were used to evaluate the effects of vagal stimulation on the rhythm and the diastolic slope. The effects of verapamil on the junctional rhythm and the diastolic slope were evaluated by infusing verapamil intravenously (1.5 mg/min) for 10 min. Ten minutes after verapamil injection, CaCl₂ (15 mg/kg) was infused over 15 min to determine the reversibility of the changes induced by verapamil. The effect of isoproterenol on the junctional rhythm was evaluated by infusing isoproterenol intravenously at rates of 0.5 and 2 μg/min. The cycle lengths and diastolic slopes were averaged from 10 consecutive measurements made during control and during or after the above interventions.

Statistical analysis. We used analysis of variance for single-factor experiments with repeated measures to analyze the age values of 10 consecutive junctional cycle lengths and diastolic slopes at various treatment points. When significant differences were detected among the treatment groups, Duncan’s multiple-range test was applied to compare individual means. The differences were considered significant at p ≤ .05. All data are reported as mean ± SD.

Results

Junctional rhythm and diastolic slope. Stable junctional rhythm was successfully induced in 14 dogs by crushing or excising the sinus node and the neighboring atrial tissue. In the other 11 dogs ectopic atrial rhythm persisted or only intermittent junctional rhythm was observed. The junctional rhythm was characterized by a His bundle deflection preceding atrial activity and ventricular activity by an HV interval identical to that during sinus rhythm or atrial pacing and a QRS complex identical to that during sinus rhythm or atrial pacing (figure 1, A). The cycle length of the junctional rhythm in these 14 dogs averaged 665 ± 47 msec. In all but two dogs with junctional rhythm a negative diastolic slope (sloping upward because of reversed polarity) preceding each bipolar His bundle deflection was recorded through the unipolar lead (figure 1, A). The slope of this diastolic deflection, calculated as the tangent of the angle between the deflection and a horizontal line, was expressed in millivolts per second. In these 12 dogs the diastolic slope averaged 0.15 ± 0.07 mV/sec. Atrial pacing resulted in overdrive suppression of the rhythm in all dogs and a decrease in the slope of the diastolic deflection. As illustrated in figure 1, B atrial pacing at a rate of 200 beats/min was followed by prolongation of junctional cycle length from 740 to 750 msec at control (figure 1, A) to 870 msec. The prolongation of the junctional cycle length was accompanied by a decrease in the diastolic slope from 0.13 mV/sec at control to 0.06 mV/sec after pacing. The junctional cycle length and diastolic slope returned to control levels after 2 beats. Figure 2 shows plots correlating various junctional cycle lengths as a result of overdrive suppression by atrial pacing and the accompanying diastolic slopes measured in one dog. A strong negative correlation (r = −.89) was found between the junctional cycle length and the diastolic slope. Similar findings were observed in all seven other dogs studied (r values from −.78 to −.95).

In all dogs the diastolic slope was only recorded through plunge and catheter electrode terminals in a rather localized area from which a His bundle deflection was recorded. Figure 3 shows an example of this phenomenon in one dog. During junctional rhythm with a cycle length of 760 msec, a bipolar His bundle electrogram was recorded through a plunge electrode. The His bundle deflection preceded atrial and ventricular deflections (figure 3, A). Two unipolar electrograms (UE 1 and 2) were recorded through the two terminals of the plunge electrode. A large His bundle deflection could be recorded in one unipolar electrogram (UE 2) and a strong diastolic slope could be seen preceding this His bundle deflection. In the other unipolar electrogram (UE 1) a much smaller or no His bundle deflection was recorded and no diastolic slope could be recorded. Examination of the heart performed after the experiments showed that the distance between the two terminals of the plunge electrode was 2 mm. Thus, the diastolic slope was recorded from a localized area close to the His bundle since the deflection was observed in UE 2.

Effects of isoproterenol infusion, vagal stimulation, and verapamil injection on junctional rate and diastolic slope. Isoproterenol infusion (0.5 to 2.0 μg/min) resulted in an increase in diastolic slope and in junctional rate. This effect of isoproterenol is shown in figure 3, B and C. As we have discussed above, diastolic slope was recorded only in UE 2, in which a large His bundle deflection was observed, but not in UE 1, in which His bundle deflection was small or not recorded. As illustrated in figure 3, B, a small infusion of isoproterenol (0.5 μg/min) resulted in shortening of the junctional cycle length from 760 to 630 msec and in an increase in the slope of the diastolic deflection in UE 2 from 0.16 to 0.28 mV/sec at control to 0.36 to 0.44 mV/sec during isoproterenol infusion. A larger dose of isoproterenol (2 μg/min) resulted in further shortening of junctional cycle length to 420 msec and in a further increase in diastolic slope in UE 2 to 0.58 to 0.70 mV/sec. No diastolic slope could be recorded in UE 1 throughout the isoproterenol infusion. In six dogs in
which a low dose of isoproterenol (0.5 μg/min) was administered, junctional cycle length was significantly shortened and diastolic slope was significantly increased (table 1). A higher dose of isoproterenol caused further shortening of junctional cycle length and further increase in diastolic slope (table 1).

Vagal stimulation performed in five dogs resulted in slowing in junctional rate accompanied by a decrease

**FIGURE 1.** Junctional rhythm and diastolic slope and the effect of overdrive suppression. In each panel, tracings are arranged from top to bottom as follows: lead II of electrocardiogram (ECG), right atrial electrogram (RAE), bipolar His bundle electrogram (HBE), unipolar electrogram of the His bundle (UE), and time lines (T). A, H and V are electrograms of the atria, His bundle, and ventricles; S = stimulus artifact. Numbers on HBE indicate junctional cycle lengths, and on UE, the magnitudes of the diastolic slopes. Voltage calibration is for unipolar electrogram. During control (A) junctional cycle length varied from 740 to 750 msec. His bundle deflection preceded each atrial and ventricular deflection, with an HV interval of 40 msec. A diastolic slope of 0.12 to 0.16 mV/sec preceded the His bundle deflection. During atrial pacing at a rate of 200 beats/min (B) there was one-to-one AV conduction with a similar HV interval of 40 msec. After pacing the junctional cycle length was prolonged to 870 msec and diastolic slope decreased to 0.06 mV/sec. The junctional cycle length and diastolic slope returned to control values after 2 beats.
in the diastolic slope. Figure 4, obtained in a dog with junctional rhythm, shows that junctional cycle length was prolonged from 630 msec before vagal stimulation to 2810 msec during and immediately after right vagal stimulation and that the diastolic slope decreased from 0.24 mV/sec before vagal stimulation to 0.05 mV/sec during and immediately after vagal stimulation. The junctional cycle length and diastolic slope gradually returned to control values after a few beats. Similar findings were observed in all five dogs, two of which underwent right and left vagal stimulation (the other three underwent right vagal stimulation only).

Intravenous verapamil injection (1.5 mg/min for 10 min) also resulted in slowing of the junctional rate in five other dogs and in decrease in the diastolic slope. The effects of verapamil could be partly reversed by intravenous administration of CaCl₂ (15 mg/kg) given

![Graph](image)

**FIGURE 2.** Correlation between various postbltrial pacing junctional cycle lengths and the corresponding diastolic slopes. Data were obtained from the same dog from which tracings in figure 1 were obtained.

![Tracings](image)

**FIGURE 3.** Effect of isoproterenol infusion on junctional rhythm and two unipolar electrograms recorded from the junctional area. A, Control. UE 1 and UE 2 were obtained through two terminals of a plunge electrode placed in the His bundle area paired with an indifferent electrode on the superior vena cava. Diastolic slope was noted only in UE 2, which showed a big His bundle deflection, but not in UE 1, which showed a small or no His bundle deflection. Isoproterenol infusion (0.5 μg/minute (B) and 2 μg/min (C) decreased the junctional cycle length (CL) and increased the diastolic slope (DS) in UE 2 (arrows). No diastolic slope (arrows) was observed in UE 1 throughout the isoproterenol infusion. Other abbreviations, symbols, and calibrations are as in figure 1.
TABLE 1
Effect of infusion of two doses of isoproterenol (0.5, or low dose, and 2 μg/min, or high dose) on junctional cycle length and diastolic slope in six dogs

<table>
<thead>
<tr>
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<th>Junctional cycle length (msec)</th>
<th>Diastolic slope (mV/sec)</th>
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<tbody>
<tr>
<td>Control</td>
<td>678 ± 50</td>
<td>0.13 ± 0.04</td>
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<tr>
<td>Low-dose isoproterenol</td>
<td>552 ± 58</td>
<td>0.29 ± 0.08</td>
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<tr>
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<td>(p &lt; .05 compared with control)</td>
<td>(p &lt; .05 compared with control)</td>
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<tr>
<td>High-dose isoproterenol</td>
<td>437 ± 55</td>
<td>0.48 ± 0.12</td>
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<tr>
<td></td>
<td>(p &lt; .05 compared with control and p &lt; .05 compared with low-dose isoproterenol)</td>
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over 15 min. Figure 5 shows the effects of verapamil and CaCl₂ in one dog. During control conditions (figure 5, A) junctional cycle length was 630 msec and diastolic slope, which preceded bipolar His bundle deflection, ranged from 0.28 to 0.45 mV/sec. One-to-one retrograde atrial activation was noted at control. Immediately after verapamil injection (figure 5, B) the junctional cycle length increased to 1710 msec, the diastolic slope decreased to 0.05 to 0.10 mV/sec, and high-degree retrograde block to the atria occurred. After CaCl₂ injection (figure 5, C) the junctional cycle length shortened to 810 msec and the diastolic slope increased to 0.12 to 0.22 mV/sec. Retrograde conduction from the junction to the atria was restored, although HA interval (measured as the interval from the bipolar His bundle deflection to the atrial deflection recorded from right atrial appendage) was 75 msec at control and 140 msec after verapamil and CaCl₂. Similar high-degree retrograde block induced by verapamil and partial restoration of retrograde conduction by CaCl₂ were observed in all five dogs given verapamil. Table 2 lists the results in five dogs in which verapamil injection followed by CaCl₂ injection was administered. Verapamil significantly prolonged the junctional cycle length and reduced the diastolic slope, whereas CaCl₂ partly reversed verapamil effects but did not restore the values to control levels.

Discussion

The method we used to induce stable junctional rhythm in dogs is identical to the method described by Scherlag et al. We have shown in this study that in the majority of dogs with junctional rhythm it is possible to record diastolic slope from the junctional area. The diastolic slope could be recorded from a rather localized area, presumably the area close to the junctional pacemaker. This pacemaker is close to the His bundle but, as we will discuss, it may not necessarily be the His bundle per se. The responses of the diastolic slope to interventions like atrial pacing, vagal stimulation, and isoproterenol infusion are identical to the expected responses of phase 4 depolarization to those interventions. These
results, together with our previous results on recording of diastolic slope from the sinus node and ectopic atrial pacemakers,1-3 suggest that the diastolic slope recorded from the AV junction indeed reflects the phase 4 activity of the junctional pacemaker.

The fact that we recorded the His bundle activity and the diastolic slope from the same electrode should not be perceived as evidence that the pacemaker is located in the His bundle region. The ability to record His bundle activity is a function of amplification and does not require direct contact of the electrode with the His bundle. In fact, His bundle activity can be recorded from the body surface. A question then may be raised as to the exact origin of the impulse formation in the junctional area responsible for the automatic rhythm. Review of the literature indicates that microelectrode studies performed in the junctional area were mostly performed in the rabbit heart6-12; only a few were performed in the canine heart.13,14 The size of the plunge and catheter electrodes, the lower specificity of extracellular recording compared with intracellular recording for identification of structures, and the complex anatomy of the AV junction15,16 prohibit precise anatomic identification of the pacemaker site. Because of the above limitations, the conclusions of the following analysis for our study results in dogs should be construed only as hypotheses; further anatomic study in the canine heart would be in order. Data from microelectrode studies by Hoffman7 and by Watanabe and Dreifus8 suggest that the AV node (the N region) is not automatic and that automaticity originates from the AN region (coronary sinus ostium) or the NH region. Results of other microelectrode studies, however, have suggested that the AV node might be automatic, especially if the connection between the node and the adjoining atrium is severed.12,14 In other studies,6,9,10 however, transmembrane action potential recordings obtained from the AV node with the atrial connection

FIGURE 5. Effect of verapamil followed by CaCl₂ injection on junctional cycle length and diastolic slope. A. Control; B. after 15 mg of verapamil; C. after 15 mg/kg CaCl₂ injection. Arrowheads indicate diastolic slopes. Note the prolongation of junctional cycle length and the decrease in diastolic slope after verapamil and partial reversal of those verapamil effects by CaCl₂. Note also the high-degree retrograde block (atrial activation low to high) to the atria after verapamil and restoration of one-to-one retrograde conduction by CaCl₂. HA interval (measured from bipolar His bundle deflection to atrial deflection in right atrium) is 140 msec after verapamil and CaCl₂, still longer than control HA interval of 75 msec. Symbols, abbreviations, and calibrations are as in other figures.
intact frequently also show phase 4 depolarization (figures in references 6, 9, and 10). It has been argued that the possible damage to the AV node that may occur in such studies may induce automaticity of the node. The catheter electrode in our study would not be expected to cause damage to the AV junction.

It is highly unlikely that the AN region (coronary sinus ostium) was the site of the automatic focus in our dogs since in the rhythm that results from automaticity in this region a P wave precedes ventricular activity. Furthermore, such rhythm is not suppressed by verapamil, which can be understood since resting membrane potential of coronary sinus ostium is high (−75 to −85 mV) and beyond the range of the slow inward current. Further evidence against the AN region as the site of automaticity was the high-degree retrograde block that resulted from verapamil administration; atrial conduction from the coronary sinus is not expected to be impaired by verapamil. Further argument against a cranial location and for a more caudal location of the pacemaker is the fact that we have not seen antegrade block manifested by progressive shortening and prolongation of HH intervals (Wenckebach antegrade block from the pacemaker to the His bundle) or sudden doubling of HH intervals (2:1 antegrade block).

The remaining possible sites of the automatic focus are then the N and the NH regions. Results of most studies show that the maximum diastolic potential of fibers in the NH region is high (in the range of −70 to −80 mV). Therefore, little slow inward current can contribute to the upstroke of these fibers. The findings of Wit and Cranefield showed that high concentrations of verapamil (up to 1 mg/l) produced only a slight decrease in maximum diastolic potential and action potential amplitude and caused an increase in the slope of phase 4 depolarization of the fibers in this area. These data showing insensitivity of the lower AV node and upper His bundle regions to verapamil are evidence against NH or upper His bundle regions as the site of automaticity since the described rhythm is very sensitive to verapamil.

If the pacemaker is in the N region, why did retrograde block occur in all dogs treated with verapamil, whereas antegrade block did not occur? This can again be explained by the comparatively higher sensitivity of the upper and midnodal fibers to verapamil in contrast to the lower nodal fibers. Comparison of the AH interval during atrial pacing with the HA interval during spontaneous junctional rhythm always showed an AH interval longer than the HA interval (figure 1). This finding is more consistent with the N origin theory of the junctional rhythms, since Damato et al. have shown in dogs that during ventricular or His bundle pacing the HA interval is always longer than the AH interval. Thus, the shorter HA interval in our dogs would suggest a higher pacemaker location than the His bundle. The validity of our comparison, however, could be argued against on the basis of the fact that the junctional rate is always slower than the atrial pacing rate and, therefore, AH prolongation might occur as a result of the higher atrial pacing rate. Pending a more confirmative anatomic study, we can summarize that the pacemaker location is most likely caudal to the coronary sinus ostium (AN region), but cranial to the verapamil-insensitive lower AV node and the His bundle. Such location is most likely in the N region.

This hypothesis is in agreement with the opinions of other investigators. Scherlag et al. postulated from their study evaluating the response of junctional rhythm to ouabain that such junctional rhythm originates from automaticity within the AV node. Urrahler et al. produced stable AV junctional rhythm in dogs by suppressing sinus nodal activity with the injection of ephrine into the sinus node artery. This rhythm, they believe, originated from P cells located deep within the AV node. Since these cells are located deep within the node, they believe that it is difficult to impale them with microelectrodes and to demonstrate automaticity in the node. However, the possibility that P cells in the AV node are artifactual has been put forth. Whatever the case may be, the exact nature and site of the AV nodal pacemaker in the N region is at present not known.

In our study we were able to record only the diastolic slope but not the upstroke slope representing AV nodal potential like that we have recorded from the sinus node. In the sinus node the upstroke slope can be

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**TABLE 2**

Effect of 15 mg verapamil followed by 15 mg/kg CaCl₂ on junctional cycle length and diastolic slope in five dogs

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<thead>
<tr>
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<th>Junctional cycle length (msec)</th>
<th>Diastolic slope (mV/sec)</th>
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<tbody>
<tr>
<td>Control</td>
<td>652 ± 46</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>Verapamil</td>
<td>1617 ± 166</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>(p &lt; .05 compared with control)</td>
<td>(p &lt; .05 compared with control)</td>
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<tr>
<td>Verapamil and CaCl₂</td>
<td>962 ± 92</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>(p &lt; .05 compared with control and p &lt; .05 compared with after verapamil)</td>
<td>(p &lt; .05 compared with after verapamil)</td>
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recorded only from an area of 4 mm², whereas the diastolic slope can be recorded from a limited but a much larger area. If the same situation applies to the AV node, the reason for our failure to record the upstroke slope might be related to the inability to exactly place the electrode close to the pacemaking area. Since diastolic slope is recordable from a limited but a larger area, the problem in recording diastolic slope is significantly less. However, since diastolic slope correlates well with the junctional rate, we feel that the diastolic slope is still a reliable indicator of the pacemaker activity. Another possible explanation of the failure to record the upstroke slope is that the AV node—His interval might be too short for complete recognition of the AV nodal upstroke slope. An analogous situation is that in the sinus node, where longer sinoatrial interval permits recognition of complete sinus potential and shorter interval permits recognition of only the beginning of sinus potential. The beginning of the His bundle potentials recorded on the unipolar electrograms (figures 1 and 3 through 5) frequently precedes the recording of the His bundle potentials on bipolar electrograms by a few milliseconds. However, we believe that this phenomenon is an artifact related to different filters and amplifications used for unipolar and bipolar recordings. When the same filters and amplifications were used, the beginning of the His bundle deflection was observed at the same time on unipolar and bipolar electrograms. The effects of unipolar vs bipolar recording and of the use of different filters in His bundle recording have been reported before.

In conclusion, in this study we demonstrated that it is possible to record diastolic slopes that accurately reflect phase 4 depolarization of the pacemaker in the junctional area in dogs with junctional rhythm. Clinical application of such techniques should be helpful in identification and characterization of automatic junctional pacemakers in patients. Our results showing suppressibility of junctional rhythm by verapamil suggest that some clinical AV junctional rhythms may originate from automaticity within the AV node.

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