Asynchrony of Conduction within the Canine Specialized Purkinje Fiber System

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ABSTRACT

Asynchrony of conduction may prove to be an important mechanism for reentrant arrhythmias. The purpose of these experiments was to explore asynchronous conduction in the distal branches of the canine Purkinje system. Microelectrodes were placed in Purkinje bundle preparations resembling a T configuration, thereby permitting assessment of differential conduction times induced by premature beats (S2).

Equal conduction depression was observed in the post extrasystolic beat (Sj) at wide coupling intervals (S2-Sj). Asynchrony of conduction was frequently observed in response to narrow coupling intervals. These differential conduction times induced disparities of activation times greater than 50 msec. In fibers exhibiting preferential depressed conduction, local block was observed with further decrease in the coupling interval. Disparities of activation times at very short S2-Sj coupling times could be markedly increased by minimal decrease in coupling intervals (in the order of 1 to 5 msec). Conduction depression was not clearly dependent upon the level of "take-off" potential or action potential duration. Asynchronous conduction may thus be induced by narrow coupling of premature beats and could account for reentry.

ADDITIONAL KEY WORDS

electrophysiology action potential aberrance premature impulses conduction depression reentry
that by utilizing such a T configuration, factors affecting conduction proximal to the bifurcation would alter activation times equally in each branch and uniform changes in conduction would be recorded from microelectrodes impaled into these smaller branches. On the other hand, if asynchrony of conduction was present due to altered conduction in one branch, it would then manifest as a disparity of activation times in these branches. Figure 1 is a photograph of a preparation of the configuration utilized in these experiments.

The Tyrode solution, in millimoles per liter, was composed of the following: NaCl, 137.0; KCl, 2.7; MgCl₂, 0.5; Na₂HPO₄, 0.18; NaHCO₃, 12.0; glucose, 5.5; CaCl₂, 2.7. This solution, constantly saturated with 95% oxygen and 5% CO₂, was perfused at a rate of 60 drops/min and the fluid level was maintained by constant overflow suction. The solution flowing through the tissue chamber was maintained at a constant temperature of 31°C (±0.2°C).

Stimulus pulses, at a rate of 60/min, were administered via 0.005-inch, Teflon-coated platinum wire bare at the tip. Stimuli of variable prematurity were introduced with an AEL stimulus generator (Model 104A). The basic stimulus rate (S₁) was set at 1/sec and after control conduction times (CT) were recorded, premature stimuli (S₂) were applied at varying degrees of prematurity. After paired pacing was induced, the CT of the postextrasystolic S₂ was recorded. Hence, late S₂ stimuli resulted in the subsequent S₂ falling upon the relative refractory period, thereby inducing prolonged CT. The S₂-S₁ interval (coupling interval) was continuously shortened until block of the postextrasystolic beat occurred. The CT from stimulus artefact to each intracellular microelectrode was recorded with their respective transmembrane resting potentials at the onset of phase 0 of the monophasic action potential.

Glass microelectrodes having a tip diameter of <0.5 μ. were drawn from 1 mm pyrex capillary tubing and filled with 3M KCl. Implantation of single cells, preamplification and oscilloscopic monitoring of the potential changes were accomplished in a conventional manner, as described in previous communications (2, 3). The transmembrane resting potentials and monophasic action potentials were recorded with a Precision Instrument tape recorder (F1-6294). The preparation was arranged so that the stimulus was placed at the base of the central Purkinje bundle and the
bifurcation was distal, attaching to muscle. The microelectrodes were impaled at varying distances along each limb but approximately equidistant from the bifurcation. The action potentials were displayed on a Tektronix 564 memory oscilloscope with synchronization of the sweep generator provided by the stimulus pulse generator. The conduction time from the stimulus...
Parallel conduction depression is demonstrated in this preparation. Decreasing $S_2-S_1$ coupling interval from 380 to 370 msec resulted in a greater conduction depression in one fiber (a). Subsequent shortening of $S_2-S_1$ from 370 msec to 368 msec prolonged conduction time to 73.5 msec. Further shortening of the coupling interval to 366 and 365 msec prolonged conduction in both fibers to 88 msec and 123 msec, respectively. Control action potential durations were 610 msec in each fiber.

Results

Parallel Depression of Conduction in Response to Premature Beats

Variations in conduction through Purkinje fibers were classified according to the type of response in one fiber bundle with respect to the other bundle. Preparations were obtained having a T configuration, and hence, propagation through the base of the T was assumed to be uniform. Thus prolongation of conduction time to only one microelectrode would represent depression in the respective limb of the T preparation. As depicted in Figure 2, equal prolongation of activation time to each microelectrode could represent any of the following permutations: (a) Depression of CT in the common base of the T (Fig. 2B); (b) depression equally of each branch distal to the bifurcation (Fig. 2C); and (c) uniform depression of all three portions of the T (Fig. 2D). However, any disparity in depression of conduction in one or the other of the bifurcated limbs, so long as they are unequal, would implicate preferential conduction depression irrespective of depression of the proximal trunk, since this is common to both limbs distal to the bifurcation.

It is conceivable that asynchrony of conduction may originate in separate functioning subunits of the common proximal bundle. However, we varied the stimulus intensity, or duration, or both to suprathreshold values which consistently led to identical control activation times in the distal branches.

Physiologic response to refractoriness was manifest in a number of variations and an example of near equal depression of conduction is illustrated in Figure 3. Control conduction time from stimulus to microelectrode A was 23.8 msec, and that to microelectrode B was 25.2 msec, a net difference of 1.4 msec. When a premature beat was interposed at an $S_2-S_1$ of 415 msec, stimulus-microelectrode A was prolonged to 29 msec and to microelectrode B, 31 msec. The net changes in conduction times were 5.2 and 5.8 msec, respectively. This indicates that the prolongation of CT was more severe in the limb impaled by microelectrode B such that the CT was preferentially prolonged by 0.8 msec over the other limb. Depression of conduction with decreasing $S_2-S_1$ intervals...
remained nearly equal in both fibers until an S2-S1 coupling interval of 370 msec. When CT to microelectrode A was preferentially prolonged to 37 msec, equal to that of the other branch. Further decrease in the S2-S1 interval resulted in equal depression of conduction to 123 msec in each branch (Fig. 3). This experiment demonstrated near equal or parallel depression of conduction in response to premature beats. A notable point, however, is the critical nature of the coupling interval. A decrease of the S2-S1 interval from 370 msec to 368 msec was accompanied by a 36.5-msec prolongation of conduction. It may be contended that this pronounced net change may have been secondary to a change in the transmembrane resting potential, but the data show that the transmembrane resting potential was reduced by only 1 mv following the 2-msec shortening of the S2-S1 coupling interval.

**NONUNIFORM DEPRESSION OF CONDUCTION**

However, similar bundles both in length and width (the latter determined as accurately as possible under the conditions of the experiment) may vary electrophysiologically so that depression of conduction is uniform (i.e., linear) but dissimilar between branches in response to premature beats. Figure 4 is exemplary of this variant. Under control conditions, the CT of stimulus-microelectrode A was 68 msec and of microelectrode B was 73 msec. At a coupling S2-S1 interval of 210 msec, CT to each microelectrode increased 6 msec. Perhaps this is reflective of conduction depression in the common proximal bundle, or equal depression of each branch, or both after the common bundle bifurcation. However, at an S2-S1 interval of 208 msec, stimulus-microelectrode B was prolonged to 92 msec, representative of a 13-msec prolongation. In the other limb, however, stimulus-microelectrode A was prolonged to 81 msec, a net change of 7 msec. The changes are illustrated in Table 1.

It is interesting to note that at a coupling interval of 208 msec, there was a net difference ([AS-ME2] - [AS-ME1]) in conduction prolongation of 6 msec, indicative of preferential conduction depression in the limb impaled by microelectrode B. This preferential depression of conduction was apparent at
TABLE 1
Effect of Increasing Prematurity on Activation Times

<table>
<thead>
<tr>
<th>S&lt;sub&gt;S2&lt;/sub&gt;-S&lt;sub&gt;S1&lt;/sub&gt;</th>
<th>S-ME&lt;sub&gt;A&lt;/sub&gt;</th>
<th>Net change</th>
<th>S-ME&lt;sub&gt;B&lt;/sub&gt;</th>
<th>Net change</th>
<th>(S-ME&lt;sub&gt;B&lt;/sub&gt;) - (S-ME&lt;sub&gt;A&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68</td>
<td>73</td>
<td>79</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>210</td>
<td>74</td>
<td>0</td>
<td>79</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>208</td>
<td>81</td>
<td>7</td>
<td>92</td>
<td>13</td>
<td>-6</td>
</tr>
</tbody>
</table>

S-ME<sub>A</sub> = stimulus-microelectrode A; S-ME<sub>B</sub> = stimulus-microelectrode B. All values are milliseconds.

FIGURE 5
Action potentials recorded at an S<sub>S2</sub>-S<sub>S1</sub> coupling interval of 189 msec are labeled A and A' corresponding to conduction times of 104 msec and 131 msec, respectively. Decreasing the S<sub>S2</sub>-S<sub>S1</sub> coupling interval to 188 msec induces a prolongation of conduction time to 106 msec in one limb (B) while inducing a 12-msec prolongation in the opposite limb (B'). Thus, disparity of activation times at an S<sub>S2</sub>-S<sub>S1</sub> of 189 msec was 27 msec. A 1-msec decrease in coupling interval widened this disparity to 37 msec. Calibration: 25 msec and 20 msec.

an S<sub>S2</sub>-S<sub>S1</sub> coupling duration between 210 msec and 185 msec, although more marked in the limb impaled by microelectrode B between intervals of 210 and 204 msec. Thereafter, shortening the coupling interval resulted in a near linear conduction depression. That the response of CT is near linear in response to the diminution of S<sub>S2</sub>-S<sub>S1</sub> interval is significant, and in fact, assumes more import when considering the divergence of slopes of these two responses. This divergence of the slopes indicates widely disparate times of activation of near endocardial surfaces (Fig. 4). Specifically, at a coupling interval of 185 msec, the net difference between CT was 41 msec and such great disparity in time of activation over an area of approximately 10 to 15 mm raises the potential for reentry (see Discussion).

Exemplary of this asynchrony of conduction demonstrated in Figure 4 are the conduction times recorded during the same experiment and illustrated in Figure 5. At an S<sub>S2</sub>-S<sub>S1</sub> coupling interval of 189 msec, action potentials A and A' were recorded with propagation times of 104 msec and 131 msec, respectively. Decreasing the coupling interval by 1 msec resulted in a prolongation of conduction time of 2 msec, resulting in action potential B.
Conduction depression in these Purkinje branches was quite similar but sudden prolongation of conduction occurred at different coupling intervals for each respective branch. At coupling intervals of 350 msec one limb (A) exhibits sudden prolongation of conduction, while the other limb (•) was relatively unaffected until coupling intervals of 325 msec. Control action potential durations were 490 msec in each fiber. (See text).

Having conduction of 100 msec. The conduction time to action potential B', however, prolonged an additional 12 msec to 143 msec. Thus, the asynchrony of activation times recorded at an S₂-S₁ coupling interval of 139 msec was further widened an additional 10 msec induced by a 1-msec decrease in coupling interval.

This study has shown that under conditions of a regular stimulus rate, the relative time of activation of each area of the specialized conducting system remains constant. However, in response to premature beats of diminishing S₂-S₁ intervals, conduction depression may be linear, where more horizontal slopes are indicative of more severe depression of impulse propagation (Fig. 4). If microelectrode B had been placed more proximal to the stimulating electrodes on its respective limb, the control stimulus-microelectrode B would have been shorter than control stimulus-microelectrode A and with a crossing of the two extrapolated tangent lines of conduction as the S₂-S₁ interval was decreased. We have observed a physiologic state of comparable significance in that a fiber may suddenly exhibit preferential conduction depression, so that a more anatomically distal microelectrode is activated first. This is illustrated in Figure 6. In this experiment both branches exhibit parallel depression of conduction in response to diminishing S₂-S₁ intervals to 350 msec. Subsequent reduction of the coupling interval preferentially depressed stimulus-microelec-
Hypothetical diagrams localizing conduction depression with recorded data. Control conduction times were 5.5 and 7.2 msec. At an S2-S1 coupling of 575 msec, depression may have occurred in the common bundle, proximal to the bifurcation or equal depression in each limb. At an S2-S1 coupling of 340 msec depression may, in addition, have occurred in the A limb with a total prolongation of stimulus-microelectrode A to 7.3 msec, becoming identical with the opposite limb. Further shortening the S2-S1 interval induced greater depression in both limbs, with one branch (impaled by microelectrode A) being more severely affected than the other (see Fig. 6).

An alternative interpretation to this observation might be that block occurred proximal to microelectrode A and that the apparent greater prolongation of conduction of stimulus-microelectrode A with respect to stimulus-microelectrode B was due to retrograde activation of the pathway. This concept, while tenable, is subject to question in that the maximum rising velocities of phase zeros were of such magnitude as to suggest that decrement was not severe at coupling intervals slightly greater than that which induced the reversal of conduction times.

LOCALIZED BLOCK WITHIN THE PURKINJE SYSTEM

That decremental conduction can and does occur in some bundles, and may be severe enough that propagation of the action potential fails, is evidenced by a number of experimental observations. Block in small Purkinje bundles has been induced mechanically (4), as well as by premature stimuli (4-6). However, a "normal" physiologic state of block is a function of the degree of prematurity, and hence the level of membrane potential from which the premature beat arises. In an attempt to determine whether a localized block, and hence preferential conduction disturbance, might occur, a T-type Purkinje preparation was utilized with microelectrode A impaling a small free floating false tendon which measured <0.1 mm diameter. The
Effect of decreasing $S_2-S_1$ stimulus interval on a large and small Purkinje bundle. Coupling intervals for respective action potentials are indicated; action potentials recorded from the smaller bundle are indicated by underlined coupling intervals. (See text for discussion.)

The action potentials of Figure 6 recorded at a slower sweep speed, the coupling interval was 325 msec. A better propagated action potential was recorded from the large Purkinje bundle while the smaller bundle produced a local response. The propagated potential arose from a better take-off potential and returned to a less negative resting potential. The smaller bundle demonstrates a better resting potential and the $S_2$ (regular first action potential) exhibits a longer action potential duration accounting for the stimulus ($S_1$) falling earlier on the repolarization limb.
Figure 10 illustrates the relationship between take-off potential, conduction times, and coupling interval. Control action potential durations were 620 msec in each fiber. (See text for discussion.)

Three dimensional graph illustrating the relationship between take-off potential, conduction times, and coupling interval. Control action potential durations were 620 msec in each fiber. (See text for discussion.)

Discussion

This series of experiments has demonstrated asynchrony of conduction time in response to premature beats. Depression of conduction in branches bifurcating from a common proximal Purkinje bundle may be parallel or widely disparate. Asynchronous conduction, when present, may be independent of action-potential duration or take-off potential of the specific cell studied (see Figs. 3, 4, 10). The mechanism whereby this depression of conduction is induced is, therefore, not apparent in the recorded cell. This suggests that the site of the conduction disturbance is proximal to the cell in which the microelectrodes were inserted.
ASYNCHRONY OF CONDUCTION IN PURKINJE FIBER

Impaled, which implies that impulse propagation may have been depressed at a site where resting potential was less negative for one reason or another or possibly where action-potential durations are longer.

ASYNCHRONY AND REENTRY

The physiologic basis for reentrant excitation has been subject to many hypotheses. Unidirectional block has been provided as one plausible mechanism whereby antegrade conduction is blocked in one bundle and yet permits retrograde propagation. This retrogradely conducted beat then invades some portion of the specialized conducting system to induce the coupled premature beat.

Our data provide support for another possible mechanism. Reentry per se implies that the ventricular tissue is in fact excited twice following a singular impulse of higher origin. Our experiments indicate that adjacent Purkinje bundles may show widely disparate times of activation, and with closely coupled stimuli, activation times may be disparate by as much as 50 msec or more (Fig. 4). It is not unlikely that this disparity of activation time would be greater were the bundles impaled more peripherally. This assumption may be made because the magnitude of disparity would be a function of the fiber length. Propagation through the more depressed pathway may thus reach the ventricle after it has already partially repolarized following the excitation induced by the impulse propagated through the more rapidly conducting bundle. This observed disparity in conduction would lend support to the concept that reentry of the Purkinje system is not always necessary for coupled premature beats.

Our data do not exclude the possibility, however remote, that reentry may have occurred in the preparations studied. Block could be induced in a bundle if the disparity of conduction times were great enough that a more rapidly propagating impulse could re-enter the distal nonactivated portion of the alternate pathways via an anastomosis resulting in collision of the propagating wavefronts. This would induce block of that bundle. On the other hand, reentry of the faster conducting pathway by the slower propagating impulse may also have occurred. Following a short S2-S1 coupling interval conduction times showing marked depression may in fact have represented reentry. The branch could show a linear response of conduction time to a specific S1-S2 interval, then a further reduction in the S2-S1 interval produces block proximal to the microelectrode. Thus, the action potential distal to the block would, by necessity, have to propagate down the alternate limb of the T through muscle and then retrogradely activate the remainder of the blocked limb. This would readily explain the sudden marked prolongation of conduction times induced by a small decrease in S2-S1. Figure 6 represents an example subject to the alternative explanation. At a coupling interval of 345 msec, the conduction times were 6.5 msec and 7.1 msec, respectively, to each microelectrode. Shortening of the S1-S2 coupling interval to 338 msec prolonged the former conduction time to 7.9 msec, so that the microelectrode activated first at longer coupling intervals was now activated last. Impulse propagation may have progressed down the faster conducting branch, through the endocardium and retrogradely activated the alternate limb. This explanation, while tenable, was not documented by multiple impalements along the bundles. If the recorded action potential was induced by reentrant propagation, further impalements distally in the retrogradely activated bundle should have paradoxically shortened conduction times. This was not observed. Furthermore, before reversal of activation times, we would have expected to observe some degree of decrement in the bundle at slightly longer coupling intervals than that hypothetically inducing reentry. Thus, the severe depression of conduction at critical S2-S1 intervals appeared to be an inherent physiologic characteristic of some Purkinje bundles. We conclude, then, that asynchrony of conduction may induce coupled beats with or without reentry of the specialized conducting system.

An interesting observation was the marked variation of conduction-time response to var-
ied premature stimuli between hearts. In some hearts, coupling intervals of 350 msec had no effect on conduction but in others conduction depression occurred. This physiologic variation may well have been inherent in the tissues, but other variables such as tissue age, duration in the tissue chamber, and other environmental factors may have modified the tissue response.

**RELATIONSHIP OF ASYCHRONY TO ABERRANCE**

Aberrant conduction of premature supra-ventricular systoles with short coupling has long been clinically observed, and correlated with the degree of prematurity (11). It is not unusual, however, to find very early premature beats without accompanying QRS aberrance. Early in electrocardiography, a functional mechanism was proposed for rate-dependent aberrance (12). Asynchronous conduction provides several physiologic mechanisms for functional aberrance.

Several studies (1, 13) utilizing in vivo electrograms and electrophysiologic techniques have demonstrated conduction depression and block within canine false tendons due to premature beats. Electrograms were recorded from the His bundle, left bundle branch, and Purkinje-papillary muscle junction. Following an atrial premature beat conduction velocity down the left bundle and false tendons was diminished. A slight increase in prematurity induced block in the more peripheral bundle. These observations showed that block in small bundles could occur while the larger bundles were still capable of propagating an impulse. Local differences in refractoriness may exist as an inherent electrophysiologic property of some Purkinje fibers independent of their action-potential duration similar to that observed in atrial (14) and ventricular muscle (15). Furthermore, disparities in durations of action potentials may have existed in the Purkinje bundles since they were of different diameters. It is possible that block in some bundles may not be due to decremental conduction but to collision of the slower orthograde wavefront with the retrograde reentrant wavefront. Collision of wavefronts would then occur and conduction would be blocked in that bundle.

Such disparity of conduction times may be the electrophysiologic mechanism for rate-dependent aberrance where the degree of aberrance becomes greater as the rate is accelerated.

Conduction depression due to prematurity may be severe enough to induce complete (16) or localized block within the specialized conducting system below the AV junction. This localized block may be attributed to the fact that the premature impulse falls on a less negative take-off potential than in the fiber with the better propagated response. Propagation would then proceed with decrement and eventually fail within that fiber, yielding the segment distal to the block capable of transmitting a reentrant beat. Our data do not readily support this concept. Figure 9 demonstrates a prominent local response in the impaled cell of the smaller branch. This local response, however, was transmitted distal to the site of the block. Its effects were of such magnitude as to alter the membrane potential, making it less negative, until a time when both fibers attained resting potential at comparable times. It is possible that this local response could affect the refractory period so that reentrant excitation could not occur. The question raised, but not answered, by our data is, "Can a third stimulus induce retrograde propagation in the face of this electrotonic spread?" This is dependent upon the concept of electrotonus and its relationship to local response, the latter term implying changes in sodium permeability. Since a third stimulus was not available during this study, the critical question of the effect of electrotonic spread on the refractory period and local response was not determined.

Figure 8 provides data which relates to the mechanism of asynchrony of conduction. Decreasing the S₂-S₃ coupling interval by 3-msec increments induced considerable depression of the maximum rising velocity of phase 0 and amplitudes of action potential in the smaller branch, while maintaining better maximum rising velocities in the larger branch. Such changes in the configuration of
cellular depolarization are generally associated with depressed or decremental conduction (17). These changes observed in the smaller fiber do not appear to be related directly to the take-off potential since both fibers demonstrated near identical take-off potentials (Fig. 8). On the other hand, differences in the passive cellular properties within the two branches may be responsible for the differential effects on the rising velocity favoring decremental conduction in one branch. Thus, one plausible mechanism for asynchrony of conduction may be an enhanced propensity toward decremental conduction.

The complexity of the problem(s) addressed in this paper cannot be readily resolved until a number of microelectrodes can be impaled along each bundle, so that simultaneous recordings may indicate the electrophysiologic changes in multiple sites. This problem is further compounded by the observation that deeper impalement of a Purkinje bundle was often associated with a very significant change in conduction time, suggesting that each Purkinje bundle may have separately functioning subunits whose conduction may be independent, although modified electrotonically by adjacent fiber bundles.

In support of this theory, we have observed asynchronous conduction on the lateral periphery of the common proximal bundle. It is then plausible that the asynchrony observed in the distal branches arises from "fragmentation" or some form of longitudinal dissociation in the proximal Purkinje bundle. It must be emphasized, however, that this fragmentation within the proximal bundle is an infrequent observation and that probably does not represent the primary mechanism(s) for asynchronous conduction. Rather, asynchrony is more pronounced in the distal branches of the T or Y preparation.

References
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