Evaluation of Post-Repolarization Refractoriness for Conduction Block in Cardiac Muscle

— Studies in an Artificial Isthmus in the Canine Right Atrium —

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Post-repolarization refractoriness (PRR) is an important factor in determining conduction block and is the difference between the effective refractory period (ERP) and the duration of the monophasic action potential (MAPD). In the present study, conduction block in an artificial isthmus in the canine atrium was evaluated and the coupling interval of a premature beat, which caused the block, was defined as the block coupling interval (BCI). The usefulness of this value was also evaluated. Radiofrequency linear ablation was performed on the right atrial surface parallel to the atrioventricular groove in 5 mongrel dogs, and an artificial isthmus (8–10 mm wide and 25–30 mm long) was created. Fourteen simultaneous unipolar recordings were performed in the isthmus with a resolution of 1.2 mm. Single extra-stimuli with basic drive train were delivered to induce conduction block in the isthmus and when it occurred, the coupling interval at the recording site just proximal to the site of the block was defined as the BCI. At the site of the block, the ERP and MAPD at each drive cycle length were measured. The PRR was calculated using 2 different formulae: (1) $[\text{ERP} - \text{MAPD}]$, and (2) $[\text{BCI} - \text{MAPD}]$. It was found that each value was shortened in accordance with the shortening of the basic drive cycle length. In all basic drive trains, $\text{BCI} > \text{ERP} > \text{MAPD}$, and $[\text{ERP} - \text{MAPD}]$ was always shorter than $[\text{BCI} - \text{MAPD}]$. In the shorter cycle length of basic drives, the difference between $[\text{ERP} - \text{MAPD}]$ and $[\text{BCI} - \text{MAPD}]$ was more prominent. In the artificial isthmus model in the canine atrium, BCI was always longer than the ERP measured at the same site as the block. Because the ERP may not directly reflect the block phenomenon, the electrophysiologic evaluation should use the BCI instead, as in the PRR evaluation. (*Jpn Circ J* 2001; 65: 40–45)

Key Words: Conduction block; Post-repolarization refractoriness; Refractory period

Conduction block in cardiac muscle occurs when the activation wave front encounters refractoriness of the area distal to the direction of the activation conduction, an event that causes tachycardia termination when the conduction block occurs in a tachycardia reentrant circuit.^{1–6} The duration of the refractoriness is usually determined by the action potential duration of the previous beat, but the refractoriness can last beyond the end of the repolarization phase of the action potential and this is known as ‘post-repolarization refractoriness’ (PRR).^{7–10} To understand the mechanism of a drug’s ability to terminate tachycardia, it is important to evaluate the change in PRR as well as the change in action potential duration. In recent studies, PRR has been calculated as $[\text{effective refractory period (ERP)}] - [\text{duration of the monophasic action potential (MAPD)}]$,^{7,10} but because the ‘local capture’ (measurement of the ERP) and the ‘conduction block’ are different electrophysiological phenomena, PRR calculated as $[\text{ERP} - \text{MAPD}]$ may not reflect the ‘real’ electrophysiologic parameter that determines conduction block.^{5,11} In the present

study, conduction block in an artificial isthmus in the canine right atrium was evaluated to propose a novel method for evaluating the PRR in cardiac muscle.^{12,13}

Methods

In 5 mongrel dogs weighing 10–15 kg, the right atrial surface was exposed by right thoracotomy^{5,14} under intravenous pentobarbital anesthesia (30 mg/kg bodyweight) and mechanical ventilation (Harvard Inc, Boston, MA, USA) with room air. The body temperature of each dog was kept within the normal physiological range with a heating pad and a heating lamp was also used for warming the exposed cardiac surface. All studies were performed in accordance with the guidelines specified by the Institutional Animal Care and Use Committee of the Kitasato University School of Medicine.

Artificial Isthmus Creation and Electrode Placement

From the epicardial surface of the exposed right atrium, radiofrequency ablation was performed several times (Marinr MC, 4 mm tip, 7F and Atakr, Medtronic Japan Inc, Tokyo, Japan), then the linearly ablated lesion was made parallel to the atrioventricular (AV) groove. As a result, an artificial isthmus of 8–10 mm width and 25–30 mm length was created between the ablated lesion and the AV groove (Fig 1A).¹² A comb shaped, hand-held 16-polar electrode

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Fig 1. (A) Artificial isthmus on the exposed canine right atrium between the ablated area and the AV groove, 8–10 mm wide and 25–30 mm long. Letter A with a square wave indicates the pacing site of premature stimuli. Letters B–J indicate the recording sites during premature beats. (B) Schematic of the comb-shaped electrode array used for pacing and recording in the isthmus. The inter-electrode distance was 1.2 mm. Each pair of electrodes marked by letters corresponds to the site in (A). (See text for details.)

Fig 2. (A) Selected bipolar electrograms recorded during introduction of a premature beat. (B, C) Recording sites. A premature stimulus with a coupling interval of 140 ms was delivered after 8 basic drive trains with a cycle length of 300 ms. Arrows indicate the relative sequence of activation. Conduction block during the premature beat occurred between sites B and C, and the recorded coupling interval of the premature beat at site B (144 ms) was defined as the block coupling interval (BCI). (B) Schematic of the activation sequence during the basic drive train. The pacing site is represented by a square wave. (C) Schematic of the activation sequence during the premature beat. As a result of the conduction block between sites B and C, the activation sequence in the isthmus was reversed. (See text for details.) S, stimulation artifact; AV groove, atrioventricular groove.

Fig 3. Monophasic action potentials (MAP) recorded at the same electrode sites as in Fig 2 are shown with one surface ECG lead. MAP duration (MAPD) was measured as the time interval between the onset and 90% repolarization time of the MAPD trace and was 120 ms during the 300-ms basic drive train. (See text for details.) SS, time interval between stimuli during basic drive train.

array with an inter-electrode distance of 1.2 mm (Fig 1B) was placed within the isthmus and the most proximal pair of electrodes was placed just outside the isthmus (Fig 1A). The pair of electrodes outside the isthmus was used for pacing, and the remaining 7 pairs of electrodes were used for bipolar recording of the cardiac electrogram in the isthmus. An additional 2 pairs of hook-shaped electrodes were placed outside the isthmus and were used for bipolar recording of the cardiac electrogram outside the isthmus (Fig 1A).

Pacing Protocol and Measurement of Electrophysiologic Parameters

From the proximal pair of electrodes just outside the isthmus, single extra-stimuli with 8 basic drive trains of 300, 200 or 150 ms cycle length were delivered. The coupling interval of the premature stimulus was shortened by a 2-ms step until the premature stimulus caused a conduction block in the isthmus. When a conduction block occurred, the site of the block in the isthmus was determined and the recorded coupling interval of the premature beat at the recording site just proximal to the site of the block was defined as the 'block coupling interval' (BCI)^{5,11}. At the site of the block, the ERP at the 300, 200 and 150 ms drive train cycle lengths was determined by 2-ms step premature stimuli. At the same site of the block, the duration of the monophasic action potential (MAPD) was recorded during atrial pacing from the electrode pair outside the isthmus with a fixed paced cycle length of 300, 200 or 150 ms. The time interval between the onset of the depolarization and 90% repolarization time of the action potential was measured as a MAPD at each drive cycle length⁷⁻¹⁰. The reproducibility of each phenomenon was confirmed by repeating the same procedure 2 or 3 times. When the value determined was different, the mean of all values was used.

Electrical stimulation was delivered by a programmable

cardiac stimulator (BC03, Fukuda-Denshi Co, Tokyo, Japan) at twice the diastolic threshold of each pacing site with a 2-ms rectangular pulse. One surface ECG lead and the atrial electrograms were amplified using a polygraph system (Polygraph 360, NEC Inc, Tokyo, Japan). These analogue signals were converted to digital signals with a sampling frequency of 1000 Hz (Power Lab 8sp, BioResearch Co Ltd, Tokyo, Japan) and stored on a computer hard disk. The band-pass filter was set at 50–300 Hz for standard cardiac electrogram recording and at open–300 Hz for MAPD recording.

Calculation of the PRR

The PRR was calculated by 2 different formulae: (1) the difference between the ERP and the MAPD at the site of the block in the isthmus; that is, [ERP–MAPD], and (2) the difference between the BCI and the MAPD at the site of the block; that is, [BCI–MAPD]^{12,13}.

Statistics

Data are expressed as mean ± SD. Basic comparative statistical analysis was performed with the one way ANOVA test. $P < 0.05$ was considered significant.

Results

Fig 2 is an example of a conduction block in the artificial isthmus during a premature beat delivered from the proximal pair of electrodes just outside the isthmus (site A in Fig 1A). A premature stimulus with a coupling interval of 140 ms was delivered after the 8 basic drive trains with a cycle length of 300 ms. During the basic drive train, the time sequence of the recorded atrial electrograms indicates the activation conduction from site B to site G through the isthmus as well as the conduction from site B to site I outside the isthmus (Fig 2A, B). During the premature beat, atrial activation at site B could not produce activation at that site and the activation sequence in the distal portion of the isthmus was reversed as a result of the conduction block between sites B and C (Fig 2C). This was the longest coupling interval that induced a conduction block in the isthmus. Although the delivered coupling interval of the premature beat was 140 ms, the recorded coupling interval of the premature beat at site B was 144 ms because of the conduction delay between the pacing site and the recording site. This recorded coupling interval of 144 ms at site B (ie, the recording site orthodromically just proximal to the site of the block) was defined as the BCI of the isthmus during a 300 ms basic drive train because this coupling interval actually caused a block^{5,11}. The BCI during the basic drive trains of 200 and 150 ms was determined in the same manner. Although the site of the block in the isthmus was different in each dog, in an individual dog it was always the same regardless of the basic drive cycle length.

After determining the site of the block in the isthmus, the ERP of the site of block (ie, site C, Fig 3) was determined as the longest coupling interval that failed to capture the atrial tissue by delivering a premature beat at the site of the block with a 2-fold diastolic threshold (eg, the ERP was 124 ms during a 300 ms basic drive cycle length in the dog shown in Fig 3). During the basic drive train, MAPD was determined at the site of the block and was 120 ms during the 300 ms drive train (Fig 3). The ERP and MAPD were determined during the basic drive trains of 200 and 150 ms in the same manner. The PRR of the site of the block in the isthmus of

Table 1 Summary of Electrophysiologic Parameters

BCL (ms)	BCI (ms)	ERP (ms)	MAPD (ms)	BCI-MAPD (ms)	ERP-MAPD (ms)	p value
300	138±9	130±7	120±6	18±10	10±5	0.018
200	126±13	120±12	111±4	15±10	8±10	0.048
150	124±6	102±6	89±5	35±6	12±4	<0.001

BCL, basic cycle length; BCI, block coupling interval; ERP, effective refractory period; MAPD, duration of monophasic action potential. p value is the comparison between BCI-MAPD and ERP-MAPD.

this dog during the 300 ms basic drive train was calculated as 24 ms using the BCI (ie, [BCI-MAPD]=144-120 ms) and only 4 ms using the ERP (ie, [ERP-MAPD]=124-120 ms).

Table 1 is a summary of data obtained from all 5 dogs. Each value shortened in accordance with the shortening of the basic drive cycle length. In individual dogs, the BCI was always the longest, ERP the intermediate and the MAPD was the shortest regardless of the basic drive cycle length. As a result, the PRR calculated using the [BCI-MAPD] formula was significantly longer than when calculated using [ERP-MAPD] in all basic drive cycle lengths. The difference between these PRR values (ie, [BCI-MAPD] vs [ERP-MAPD]) was greater during the shorter basic drive cycle lengths (Fig 4).

Discussion

The present study evaluating conduction blocks in an artificial isthmus on the canine right atrium has revealed several important points. First, the local coupling interval of the premature beat, which actually caused the conduction block, could be determined and was defined as the BCI. Second, although the site of the block in the isthmus was different in each dog, the site of the block was always the same in individual dogs regardless of the basic cycle length. Third, the BCI shortened in a frequency dependent manner similar to ERP or MAPD. Fourth, in all isthmuses studied, the BCI was always significantly longer than ERP and MAPD regardless of the basic cycle length. Finally, the PRR calculated using the formula [BCI-MAPD] was always longer than when calculated as [ERP-MAPD].

Conduction block in cardiac muscle is determined not only from the duration of the action potential but also from the PRR, which can last beyond the end of the repolarization phase of the action potential^{7-10,12} Other studies have calculated the PRR as the difference between the local ERP and MAPD⁷⁻¹⁰ Practically, ERP is the longest coupling interval of the premature stimulus that fails to capture the local cardiac muscle, but the problem is that local capture and conduction block are electrophysiologically different phenomena. In a previous report studying premature beats in an atrial flutter reentrant circuit in the canine right atrium,^{5,11} the BCI was significantly longer than the ERP determined at the same site. Therefore, the PRR calculated as [ERP-MAPD] may not reflect the real PRR when determining conduction block in cardiac muscle. In our unique model of an artificial isthmus in the canine right atrium, the conduction block was reproducibly caused by premature beats and the BCI could be determined.

Inoue et al¹⁵ and Abe et al¹⁶ have already reported that an artificial isthmus on the canine right atrium caused conduction blocks or slowing of conduction, but it was not reproducible and the conduction property of the isthmus was not fully evaluated. In our preliminary studies, conduction in the isthmus was not reproducible when the isthmus

Fig 4. Post-repolarization refractoriness (PRR) evaluated using the effective refractory period (ERP) and the block coupling interval (BCI). PRR calculated using the 2 different formulae are shown for each cycle length of the basic drive trains. At any cycle length, PRR calculated as [BCI-MAPD] was significantly longer than that calculated from [ERP-MAPD] and the difference was more prominent at shorter cycle lengths. (See text for details.) MAPD, duration of monophasic action potential.

was made between 2 linearly ablated areas on the right atrium, probably because of the rough and irregular edge of the ablated areas.¹² In the present form of the isthmus model, conduction became reproducible and its properties easily studied. The area of the isthmus may include cardiac muscle that has a decremental property, and this may play a role in the reproducible conduction block in the artificial isthmus model used in the present study.¹⁷ In a previous report of the conduction block of premature beats in an area of slow conduction in an atrial flutter reentrant circuit,⁵ the site of the block was difficult to determine because the width of the blocked wave front was too large, and the stability of the central line of block of the reentrant circuit was unclear because the line of block was functionally determined. However, in the present study, our unique model of an artificial isthmus allowed easy determination of the site of the block because the width of the blocked wave front was limited and both edges of the wave front were anatomically determined.

Although the site of the block in the isthmus was different in each dog, in individual dogs it was always the same regardless of the basic cycle length. Because of the conduction delay of premature beats in atrial tissue, the local coupling intervals of the premature beat are different at each recording site (ie, the further from the pacing site, the longer the local coupling interval of a premature beat).⁵ If the conduction property is constant in all parts of the isthmus, the conduction block should occur at the entrance of the isthmus because the local coupling interval of the premature beat is shortest there, as shown in Fig 2. The site

of the block was variable in each dog probably because the edge of the ablated area was not smooth and the actual width of the isthmus varied, but, importantly, we have documented that the site of the block in each isthmus was always the same regardless of the basic cycle length and coupling interval of the premature beats, which indicates that conduction block always occurs at a site critical for conduction in each type of conductive tissue!⁵

In the present study, the BCI shortened in a frequency dependent manner similar to ERP or MAPD, which are major factors for determining the BCI⁷⁻¹⁰. However, it is important to note that in the present study the BCI was always significantly longer than ERP at the same site. As a result, the PRR calculated by [BCI-MAPD] was always longer than that determined from [ERP-MAPD], which indicates that the latter equation may underestimate the electrophysiologic property of the conduction block, at least in this model. However, the reason why the BCI and ERP differ at the same atrial site is unclear. As described earlier, the ERP was determined by delivering a premature stimulus directly to the estimated site, and the BCI was determined as the recorded local coupling interval at a site just proximal to the site of the block when normal propagation of activation was blocked. Therefore, determination of the BCI, but not ERP, appears to reflect the actual safety factor for conduction, which is related to local cell-to-cell uncoupling,^{18,19} anisotropic conduction,^{20,21} source-sink relationship,^{22,23} etc. Additionally, measurement of the ERP strongly depends on the strength of the premature stimuli; that is, the larger the stimulation energy, the shorter the ERP. Using a smaller stimulation output may decrease the difference between the BCI and ERP, but it should be kept in mind that the conventional measurement of ERP using a 2-fold diastolic threshold may underestimate the PRR for conduction block.

Clinical Implications

The action of antiarrhythmic drugs on the tachycardia reentrant circuit cannot be fully understood from their effect on the ERP or conduction time, especially for sodium-channel blockers (ie, class I antiarrhythmic drugs),⁷⁻¹⁰ but was clearly explained in the present study by the difference between the BCI and ERP. Because BCI is the actual and direct parameter for conduction block, evaluating the action of antiarrhythmic drugs on the BCI in this model of conduction block reflects the drug's action on the actual tachycardia reentrant circuit. Therefore, the BCI and PRR, calculated as [BCI-MAPD], is a unique and useful electrophysiologic parameter for determining tachycardia termination in the mechanism of reentry.

Study Limitations

There are several limitations to the present study. First, this was an evaluation only in an artificial and specific structure, so the relationship to a real reentrant circuit is unclear. Second, the isthmus was created in relatively normal atrial tissue, but the clinical reentrant circuit may include pathological tissue. Third, the area close to the AV groove may include cardiac muscle with a decremental property, which might have influenced the results of the present study. Finally, mainly for practical reasons, the edge of the isthmus was not smooth and the quantitative effect of the isthmus itself (ie, its width or length) on conduction property could not be evaluated. These limitations should be considered when evaluating the present findings.

Conclusions

In a conduction block model of an artificial isthmus in the canine atrium, the BCI was always longer than the ERP measured at the same site of conduction block. The PRR calculated using the BCI (ie, [BCI-MAPD]) was significantly longer than that calculated using ERP (ie, [ERP-MAPD]). Because the ERP does not directly reflect the block phenomenon during a premature beat, an electrophysiologic evaluation should be performed using BCI instead, as in the evaluation of PRR for conduction block.

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