Origin, Domain, and Dynamics of Rate-Induced Variations of Functional Refractory Period in Rabbit Atrioventricular Node

Jacques Billette and Robert Métayer

Different aspects of the intrinsic regulation of rate-induced variations of functional refractory period of atrioventricular node (FRPN) were studied in isolated rabbit heart preparations. First, the hypothesis that these variations originate from the net interaction between facilitation and fatigue was tested. For a constant fast rate, selective effects of facilitation and of steady-stage fatigue were independently shown to shorten and prolong, respectively, FRPN while their combined effects were shown to result in intermediate changes corresponding to the sum of their individual effects. Second, selective and combined effects on FRPN were shown to start for rates corresponding to the upper half of the 1:1 nodal conduction range and to reach their maximums at the fastest rate tested. Third, the time-courses of fatigue-induced prolongations in nodal conduction time and FRPN were shown to be closely linked. Facilitation effects on conduction time and FRPN were confirmed, as previously shown for in situ dog hearts, to be linked, but time-independent. Fourth, FRPN was shown not to correspond to particular limits in its subintervals, but to be, nevertheless, related to nodal refractoriness. Fifth, it was demonstrated that, in conditions of combined facilitation and transient fatigue such as those prevailing in current endocavitary investigations of nodal function, FRPN could be shortened, left unchanged or prolonged by a constant fast rate depending on its duration. In conclusion, the present study demonstrates the dual origin of rate-induced FRPN variations, their rate and time dependence, their relation to changes in nodal refractoriness, and their involvements in various nodal responses. (Circulation Research 1989;65:164-175)

The functional refractory period of the atrioventricular node (FRPN), defined as the minimum interval between two consecutively propagated impulses taken at the node output, is regularly determined in cardiac electrophysiological investigations by means of a periodic premature stimulation of the atrium. Because the FRPN usually shortens at faster rates, this determination is performed at different basic rates. This FRPN shortening develops very rapidly at the beginning of the fast rate and dissipates within one cycle on return to the control rate. However, in spite of the statistical consistency of the shortening, instances of no change and even prolongation in FRPN for otherwise identical heart rates and controlled experimental conditions have been frequently mentioned and remain unexplained. Another unexplained characteristic of FRPN shortening is its greater consistency and magnitude when determined early after the beginning of a fast rate.

Seeking a solution to these and other apparent discrepancies in the intrinsic regulation of rate-induced FRPN variations, we hypothesized that the total domain of these variations includes both a negative range wider than that suggested by the previously reported shortenings and dependent on nodal facilitation and a previously undefined positive range dependent on fatigue. The facilitation that develops with the first beat of a fast rate and remains unchanged thereafter would tend to produce a constant FRPN shortening. However, as the fatigue grows with the duration of the fast rate, it progressively overcomes the facilitation, leading to a reduced shortening or even a prolongation of FRPN. In the absence of facilitation, fatigue would only prolong FRPN.
The present study was designed to test this hypothesis and to examine several related aspects of the intrinsic regulation of FRPN in isolated rabbit heart preparations. The goals of the study were 1) to delineate independently the boundaries of the facilitation-dependent negative range and of the fatigue-dependent positive range of FRPN variations with various stimulation protocols, 2) to verify that the effects on FRPN of selective facilitation and fatigue produced independently correspond to their combined effects when produced together with an independent stimulation protocol, 3) to determine the time- and rate-dependent functions of the individual and combined effects of facilitation and fatigue on FRPN and compare them with associated effects on nodal conduction time, 4) to study the effects of varying the duration of a constant fast rate on FRPN, 5) to verify the applicability of the hypothesis to other nodal responses obtained with currently used stimulation patterns, and finally, 6) to study the relation between FRPN variations and the corresponding atrial, atrial-His, and His-atrial intervals.

Materials and Methods

Preparation and Recording System

Experiments were performed in three groups of six isolated superfused rabbit heart preparations (Table 1). The preparation, thermostatic perfusion, and recording system were as recently described. Briefly, the preparation, which included the right atrium, atrioventricular node area, and the upper part of the interventricular septum was mounted in a tissue bath perfused at 200 ml/min with a 6-l volume of oxygenated (95% $\text{O}_2$, 5% $\text{CO}_2$) Tyrode's solution kept at 37°C and pH 7.38. The millimolar composition of the solution was NaCl 128.2, KCl 4.7, CaCl$_2$ 2.0, MgCl$_2$ 1.0, NaHCO$_3$ 25, NaH$_2$PO$_4$ 0.7, and dextrose 11.1. Unipolar electrograms taken with Teflon-coated silver wire were recorded from the sinus node region, the crista terminalis near the sinus node, the crista terminalis near the ostium of the coronary sinus, the nodal margin of the interatrial septum, and the His bundle. Bipolar platinum-iridium stimulation electrodes were positioned on the crista terminalis near the sinus node. Stimuli were twice threshold rectangular voltage pulses of 2 msec's duration. Electrograms, together with an analog tracing of the stimuli and a time code, were recorded on a polygraph and on a tape recorder.

Stimulation Procedures

To enable the dissociation of the respective contributions of changes in recovery time, facilitation, and fatigue induced by the different protocols to the changes in FRPN, all stimuli were applied with predefined His-stimulus intervals. Each His-bundle complex was identified and the next stimulus given at a predefined time thereafter with a stimulation algorithm run on a PDP1134A computer (Digital Equipment, Marlborough, Massachusetts). The effects on FRPN of controlling the His-stimulus instead of the stimulus intervals were studied for equivalent rates achieved with the two methods in six preliminary experiments and were negligible.

Four different driving rates corresponding to 0%, 50%, 75%, and 100% shortening of the His-stimulus interval in the 1:1 nodal conduction range (Figure 1A) were defined at the beginning of each experiment with an incremental pacing. The control rate (0%) corresponded to the His-stimulus interval (297±15.8 msec) that shortened the spontaneous atrial cycle-length by 30 msec. The fastest rate (100%) corresponded to a His-stimulus interval (43±4.9 msec) that was 30 msec longer than the one that resulted in a second-degree nodal block during incremental pacing. This 30-msec margin was necessary to maintain 1:1 nodal conduction throughout sustained stimulation at 100% fast rates. The 50% and 75% fast rates were given by the His-stimulus intervals located at the corresponding percentages in the 1:1 nodal conduction range.

For the first short His-stimulus interval of a 100% fast rate to come after a long cycle result in a conducted beat, it was frequently necessary to introduce a conditioning His-stimulus interval of intermediate length before the short one. During the various premature stimulation sequences required to determine FRPN, the reduction of the His-stimulus interval of the premature beat was 20, 10, and 2 msec in the long, intermediate, and short range of His-stimulus intervals, respectively. Unless otherwise specified, 20 basic beats separated each premature beat. Between the premature stimulation sequences, the preparation was driven at the 0% basic rate. This control period lasted 5 minutes after any stimulation protocol involving a continuous fast rate; otherwise, it lasted 2 minutes.

Stimulation Protocols

The changes in FRPN produced by selective facilitation, selective fatigue, and combined facilitation and fatigue associated with a 100% fast rate were determined in each group 1 preparation with the periodic premature stimulation protocols illustrated in Figure 1B and listed in the upper left section of Table 1. To study the rate dependence of FRPN variations, short cycles corresponding to 50%, 75%, and 100% shortening of the His-stimulus interval were tested with the same protocols (Figure 1B) in each group 2 preparation (Table 1).

To study the effects on FRPN of varying the duration of a constant (100%) fast rate, four variants of the protocol illustrated in Figure 1B4 were used in group 3 preparations (Table 1). The time at which FRPN was obtained after the beginning of the fast rate was varied by changing the number of basic beats and the order of testing of the premature beats. In version one, short coupling intervals were tested first. In the other three versions, coupling intervals were tested in decrementing order. In version two, premature beats were separated by eight basic beats, and each new coupling interval
A DRIVING RATES

B FRPN DETERMINATION

1. CONTROL

2. SELECTIVE FACILITATION

3. SELECTIVE FATIGUE

4. COMBINED FACILITATION AND FATIGUE

FIGURE 1. Stimulation rates and protocols. Panel A represents, in reference to a conducted beat (SH, stimulus-His interval), the four driving rates used as corresponding to a 0%, 50%, 75%, and 100% shortening of the His-stimulus (HS) interval in the 1:1 nodal conduction range. Panel B represents the sequences of the different periodic premature stimulation protocols used to determine functional refractory period of the atrioventricular node (FRPN). Protocol B, shows the last of a series of 20 long cycles (L) imposed at the 0% basic rate and one of the premature cycles (P) used to determine the control FRPN value. Protocol B2 shows the same events as in B1 plus a short cycle (SC) added between the long and the premature cycle to induce selective facilitation. Protocol B3, which was designed to obtain steady-state selective fatigue, shows the last of a series of 20 short cycles imposed at the 100% basic rate followed by one facilitation-dissipating long cycle and a premature cycle. Note that, as indicated at the right, the testing of premature cycles started after 5 minutes of short cycles. Protocol B4, which was used to produce combined facilitation and fatigue, differs from protocol B3 only in the absence of the facilitation-dissipating long cycle. S1 and S2 respectively identify the basic and the premature stimuli regardless of their HS intervals.

was reduced by 30 msec to cover rapidly the range of coupling intervals. In version three, premature beats were separated by 20 basic beats, and each new coupling interval was reduced by 20 msec. Version four was identical to version three except that the same coupling interval was tested three times.

Group 1 preparations were also subjected to incremental pacing, step stimulation, and ramp stimulation (Table 1), and the minimum H-H intervals reached were compared to FRPN values obtained with protocols of Figure 1B. During incremental pacing, the His-stimulus interval was reduced every twentieth beat until a second-degree nodal block occurred. During the step stimulation, 20 shortened His-stimulus intervals alternated with 20 beats at the 0% rate; the His-stimulus interval of the fast rate was reduced at each new step until a second-degree nodal block occurred. For these two stimulations, the shortening of the His-stimulus interval was initially 20 msec, but was diminished to 10 msec as it approached the block value. During ramp stimulation, the His-stimulus interval of each subsequent beat was reduced by 5 msec until a second-degree nodal block occurred.

Stability Controls

To ascertain the stability of the preparations during the experimental time required for the different FRPN determinations, the control protocol (Figure 1B1) was performed three and four times between the test protocols in group 1 and group 2 preparations, respectively. Analyses of variance performed on the resulting FRPN values gave F values that indicated no statistically significant differences among the controls. The greatest difference between mean FRPN values was 2 msec. On this basis, the preparations were considered to have remained in a stable homeostatic condition during experimental time.

Data Processing

Timing of activation complex, determined off-line with previously defined methods,17 was taken from a stable fast phase of the complex, the fast crossing of the isoelectric line, whenever possible. Reported timings of the atrial input to the node were taken from the low interatrial septum electrogram. As FRPN was obtained from His-bundle intervals, this choice had no effect on reported FRPN values. The following intervals were used in the description of the results: The H1-S1 and H2-A2 intervals measured the time from the last basic His-bundle complex to the premature stimulus and atrial complex, respectively. The A1-A2 interval was the time between the last basic and the premature atrial complex. The A1-H1 interval was the time from atrial to His-bundle complex during basic beat and A2-H2 interval during premature beat. The H1-H2 interval was the time elapsed between the last basic, and the premature His-bundle complex.

Statistical Analyses

Analyses of variance for repeated measurements studies were used to determine the significance of differences between mean FRPN values. The paired comparisons with control were made with the modified t statistic and significance established with the Dunnett's method.18 Data are given as mean±SEM.

Definitions

In this study, nodal refractory curve is defined as the curve resulting from the plotting of the H1-H2 intervals obtained during a periodic premature stimulation of the atrium against the corresponding A1-A2 intervals (Figure 2); FRPN is the minimum H1-H2 interval value reached during a periodic pre-
TABLE 1. Identification of Protocols and Experimental Groups

<table>
<thead>
<tr>
<th>Group 1*</th>
<th>Maximum effects (PPS)†</th>
<th>Effects of other stimulation patterns</th>
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<tr>
<td></td>
<td>Control (B1)‡</td>
<td>Step</td>
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<tr>
<td></td>
<td>Facilitation (B2)</td>
<td>Ramp</td>
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<td></td>
<td>Fatigue (B3)</td>
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<td></td>
<td>Fac+Fat (B4)</td>
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<tr>
<th>Group 2*</th>
<th>Rate-dependence (PPS)</th>
<th>Effects of duration of PPS</th>
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<tbody>
<tr>
<td></td>
<td>Control (B1)</td>
<td>1. Short H1:S2 tested first</td>
</tr>
<tr>
<td></td>
<td>Facilitation (B2)</td>
<td>2. Basic beats=8</td>
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<tr>
<td></td>
<td>Fatigue (B3)</td>
<td>3. Each H1:S2 tested once</td>
</tr>
<tr>
<td></td>
<td>Fac+Fat (B4)</td>
<td>4. Each H1:S2 tested three times</td>
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</table>

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<tr>
<th>Group 3</th>
<th>Effects of duration of PPS</th>
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Fac+Fat, combined facilitation and fatigue stimulation protocol
*Each group contained six preparations.
†PPS, periodic premature stimulation.
‡B1, B2, B3, and B4 refer to the four stimulation protocols of Figure 1B.
§HS, His-stimulus interval with which short cycles were imposed.

mature stimulation of the atrium; recovery time, facilitation, and fatigue are as previously defined; selective steady-state fatigue is the fatigue obtained with the selective fatigue protocol after 5 minutes of fast rate imposed with a constant His-stimulus interval (the terms steady-state fatigue and fatigue are used interchangeably in the text); and nodal refractoriness is the slow and progressive recovery of excitability of nodal cells translating into the recovery-dependent increase in conduction time.

Results
Effects of Facilitation, Fatigue, and Combined Facilitation and Fatigue on Nodal Refractory Curve and FRPN

Changes in the refractory curve and FRPN caused by short cycles corresponding to the 100% basic rate and imposed with the protocols of Figure 1B were determined in each group 1 preparation, an example of which is illustrated in Figure 2. Mean FRPN values are summarized in Table 2. Control FRPN value was 160±3.5 msec. The selective facilitation protocol systematically shifted the refractory curve to the right and down (Figure 2A), shortening FRPN by an average of 15 msec. Because one or a few beats tested with His-stimulus intervals shorter than those of the 100% fast rate can be successfully propagated, FRPN was determined while the shortest possible short cycle resulting in a conducted beat (maximum facilitation) was introduced before each premature cycle. Mean FRPN value (Table 2) was only 3 msec shorter than that obtained with one cycle of the 100% fast rate that achieved a nearly maximal FRPN shortening. The selective fatigue protocol always prolonged FRPN while the refractory curve origin remained on the identity line (Figure 2B); mean prolongation was 26 msec. The combined facilitation and fatigue protocol shifted the origin of the refractory curve to the right (Figure 2C) in the same proportion as did the selective facilitation protocol and significantly prolonged FRPN by 8 msec though an FRPN shortening was observed in one preparation. This prolongation was close to the one resulting from the addition of selective facilitation and fatigue (−15+26=11 msec). These observations show that FRPN variations include both a negative and a positive range, which are related to facilitation and

TABLE 2. Mean FRPN and Subinterval Values Observed With Different Stimulation Protocols in Group 1 Preparations

<table>
<thead>
<tr>
<th>Stimulation protocols</th>
<th>FRPN</th>
<th>H1-A1</th>
<th>A1-A2</th>
<th>A2-H2</th>
<th>A1:H1</th>
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<tbody>
<tr>
<td>Control (B1)</td>
<td>160±3.5</td>
<td>87±6.4</td>
<td>73±5.9</td>
<td>127±4.0</td>
<td>40±4.4</td>
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<td>Facilitation (B2)</td>
<td>145±3.3*</td>
<td>66±6.1*</td>
<td>80±6.0</td>
<td>141±5.0*</td>
<td>76±5.2*</td>
</tr>
<tr>
<td>Fatigue (B3)</td>
<td>186±4.9*</td>
<td>108±8.3*</td>
<td>78±6.9</td>
<td>158±5.9*</td>
<td>50±5.3*</td>
</tr>
<tr>
<td>Fac+Fat (B4)</td>
<td>168±4.5†</td>
<td>78±5.2</td>
<td>90±7.5*</td>
<td>162±5.1*</td>
<td>83±6.5*</td>
</tr>
<tr>
<td>Max Fac (B5)</td>
<td>142±4.3*</td>
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<tr>
<td>Incremental pacing</td>
<td>151±4.5*</td>
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<tr>
<td>Step</td>
<td>148±6.2*</td>
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<tr>
<td>Ramp</td>
<td>150±5.8*</td>
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</table>

Values are mean±SEM in milliseconds, n=6. FRPN, functional refractory period of the atrioventricular node. B1, B2, B3, and B4 refer to the four stimulation protocols of Figure 1B. Fac + Fat, combined facilitation and fatigue; Max Fac, maximum facilitation.*p<0.01, †p<0.05 as compared with control value.
FIGURE 2. Typical effects of selective facilitation (FAC) (Panel A), selective fatigue (FAT) (Panel B), and combined facilitation and fatigue (Panel C) caused by short cycles corresponding to the 100% fast rate on the nodal refractory curve ($H_2$ vs. $A_2$ intervals) in one group 1 preparation. Panel D is a superimposition of the same refractory curves after the facilitation, and the combined facilitation and fatigue curves were corrected for recovery-dependent changes in the $A_1$-$H_1$ interval. The control (CONT) curve and the identity line are reproduced in all four panels; for progressively shorter $A_1$-$A_2$ intervals (right to left), the control curve first overlaps the identity line, then departs increasingly from it, reaches a plateau that yields a minimum $H_1$-$H_2$ interval value functional refractory period of the atrioventricular node of 156 msec, and finally starts to rise slightly, shortly before the nodal block occurs. The facilitation curve (Panel A) is shifted to the right of the control curve by 32 msec and crosses the identity line along its lower part before reaching a shortened $H_1$-$H_2$ interval of 141 msec. The fatigue (Panel B) leaves the origin of the refractory curve on the identity line but deviates more rapidly from it and reaches a prolonged minimum $H_1$-$H_2$ interval (183 msec) at a longer $A_1$-$A_2$ interval than at control. The combined facilitation and fatigue curve (Panel C) has its origin shifted to the right by 31 msec, crosses both the identity line and the control curve in the short $A_1$-$A_2$ interval range, and has a prolonged minimum $H_1$-$H_2$ interval of 174 msec. Note that the correction of the facilitation and the combined facilitation and fatigue curves (Panel D) brings them back to a common origin on the identity line with the control and fatigue curves while affecting neither the form of the curves nor the values of the functional refractory period of the atrioventricular node.

The rightward shift of the refractory curve observed in Figures 2A (32 msec) and 2C (31 msec) corresponded to a 32 and a 33 msec increase, respectively, in the last $A_1$-$H_1$ interval preceding the premature beat. The prolonged $A_1$-$H_1$ interval that was caused by the shortened $H_1$-$S_1$ interval caused all $H_2$-$H_1$ intervals to be associated with equally longer $A_1$-$A_2$ intervals, presumably independently of changes in refractoriness. This assumption is supported by the good correspondence between the shift and recovery-dependent changes in $A_1$-$H_1$ interval, its occurrence after a single short cycle before fatigue develops and its absence from the fatigue curve for which reduced excitability has been demonstrated. The two shifted refractory curves were therefore corrected for recovery-dependent changes in the $A_1$-$H_1$ interval and plotted together with the control and the fatigue curve (Figure 2D). For the facilitation curve, the correction consisted in subtracting from each $A_1$-$A_2$ interval the mean difference between the $A_1$-$H_1$ interval of the $S_1$ beat that occurred after the short cycle and the $A_1$-$H_1$ interval of the preceding beat that occurred after a long cycle. For the combined facilitation and fatigue curve, the difference between the mean $A_1$-$H_1$ interval obtained at the last $S_1$ beat during the combined effect protocol and the mean $A_1$-$H_1$ interval obtained at the beat ending the long cycle during the selective
fatigue protocol was subtracted from each A_r-A_2 interval. Because the former A_r-H_1 interval was affected by changes in recovery time and fatigue while the latter was affected only by fatigue, their difference reflected the changes due to the shortened recovery time. These corrections completely eliminated the rightward shift, bringing the origin of the two corrected refractory curves back onto the identity line with that of the control and fatigue curves while, notably, the forms of the curves and FRPN values remained unchanged (Figure 2D). Qualitatively similar results were obtained in all group 1 preparations.

The correction also affected the relation between FRPN changes and the associated A_r-A_2 interval. Before correction (Figures 2A and 2C), the A_r-A_2 interval at which FRPN occurred with facilitation (127 msec), fatigue (145 msec), and combined facilitation and fatigue (174 msec) was systematically prolonged with respect to control (117 msec) and showed no relation to the changes in FRPN. After correction (Figure 2D), the A_r-A_2 interval at which FRPN occurred showed a relation to changes in FRPN, increasing progressively from 95 to 117, 132, and 145 msec in going from facilitation to control, combined facilitation and fatigue, and fatigue curve, respectively. As previously reported by others, changes in the effective refractory period (estimated by the minimum conducted A_r-A_2 interval) also showed a parallel relation to FRPN changes after the correction.

In conclusion, the rightward shift of the refractory curve is caused by the recovery-dependent increase in the conduction time of the basic beats and can be corrected without affecting the form of the curve or the FRPN value. Changes in the nodal effective refractory period then parallel those of FRPN.

Form of Refractory Curves

Changes in the form of the refractory curve caused by the four protocols of Figure 1B were examined in group 1 preparations using the curve classification of Hoffman et al. At control, two preparations had type I curves (flat plateau) and four had a type II curve (curvilinear plateau). No type III curve (plateau followed by a sudden increase in H_r-H_2 interval) was observed at control. Facilitation shifted the plateau downward without changing its type in five preparations. In the remaining preparation (Figure 2A), the curve was transformed from type I to type II by the facilitation. Fatigue resulted in different types of curves; types I, II, and III curves were observed in two, one, and two preparations, respectively. One curve became truncated. Fatigue also tended to narrow the plateau. Combined facilitation and fatigue resulted in curve types similar to those obtained with fatigue though the plateau tended to be wider.

Rate Dependence of FRPN Variations

To study the rate dependence of the negative and positive FRPN variations and of the interactions between facilitation and fatigue, the protocols of Figure 1B were repeated in each group 2 preparation for three different fast rates corresponding to a 50%, 75%, and 100% shortening of the His-stimulus interval (ΔHS%) in group 2 preparations. The baseline corresponds to control. Note that both the FAT and FAC effects on FRPN are small for HS interval shortenings below 50% and increase rapidly thereafter, but in opposite directions, to reach their maximums at the 100% shortening. Note also that, when the facilitation and fatigue interact (middle curve), there are only small, not statistically significant, changes in FRPN.
TABLE 3. Graded Rate-Dependent Changes in FRPN and Its Subintervals in Group 2 Preparations

<table>
<thead>
<tr>
<th>Rate</th>
<th>Control (B&lt;sub&gt;1&lt;/sub&gt;)</th>
<th>Selective facilitation (B&lt;sub&gt;2&lt;/sub&gt;)</th>
<th>Selective fatigue (B&lt;sub&gt;3&lt;/sub&gt;)</th>
<th>Combined facilitation and fatigue (B&lt;sub&gt;4&lt;/sub&gt;)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (B&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>Selective facilitation (B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Selective fatigue (B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>Combined facilitation and fatigue (B&lt;sub&gt;4&lt;/sub&gt;)</td>
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<tr>
<td>Rate</td>
<td>Control (B&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>Selective facilitation (B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Selective fatigue (B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>Combined facilitation and fatigue (B&lt;sub&gt;4&lt;/sub&gt;)</td>
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<tr>
<td></td>
<td>167±6.9</td>
<td>166±6.8</td>
<td>170±9.0</td>
<td>167±6.4</td>
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<td>Rate</td>
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<td>Selective facilitation (B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Selective fatigue (B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>Combined facilitation and fatigue (B&lt;sub&gt;4&lt;/sub&gt;)</td>
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<td>80±8.2</td>
<td>77±7.4</td>
<td>90±6.3</td>
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<td>Rate</td>
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<td>Selective fatigue (B&lt;sub&gt;3&lt;/sub&gt;)</td>
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<td>88±5.4</td>
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<td>Selective fatigue (B&lt;sub&gt;3&lt;/sub&gt;)</td>
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<td>49±4.6</td>
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<td>60±4.7</td>
<td>64±4.2*</td>
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</table>

Values are mean±SEM in milliseconds, n=6. *p<0.01, tp<0.05 as compared with control. Rates were as defined in "Materials and Methods." FRPN, functional refractory period of atrioventricular node. B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, and B<sub>4</sub> refer to the four stimulation protocols of Figure 1B.

effects. The rate independence of the combined effects is therefore only apparent as it resulted from the mutual cancellation of two opposite rate-dependent processes. Hence, FRPN shortenings and prolongations produced by selective facilitation and fatigue, respectively, showed a rate dependence that was masked in conditions of interaction between these properties.

**Time Course of Facilitation and Fatigue Effects on FRPN**

To further establish the link between rate-induced changes in FRPN and the facilitation and the fatigue effects, the temporal relation between changes in FRPN and conduction time during the induction of selective facilitation and fatigue were studied separately. In six preliminary experiments, we confirmed that maximum facilitation and shortening of FRPN occur after a single cycle of a fast rate in the isolated rabbit heart preparation just as in the in situ dog heart. Subsequent short cycles maintain the shortening until a long cycle occurs and dissipates the facilitation completely. Thus, the facilitatory effects on conduction time and FRPN coincided but were largely time-independent in that they developed and dissipated within one cycle. The temporal relation between fatigue effects on conduction time and FRPN was studied while they developed during a 5-minute period in which the 100% fast rate was imposed with a modified fatigue protocol in each group 1 preparation. To obtain the FRPN value repeatedly, testing of premature cycles started at the beginning of the fast rate with only eight, instead of 20, basic beats used, and only the H<sub>1</sub>-S<sub>2</sub> intervals in the range of those in which the FRPN occurred were tested. Time course of fatigue effects on conduction time was assessed from changes in the A<sub>1</sub>-H<sub>1</sub> interval ending the long cycle that preceded the premature cycle at which any FRPN value was obtained. As illustrated with one example obtained in one preparation (Figure 4), changes in A<sub>1</sub>-H<sub>1</sub> interval (fatigue) and FRPN were closely related in all six preparations. Mean time to reach 90% of final prolongation in FRPN and A<sub>1</sub>-H<sub>1</sub> interval was 115±29 and 112±22 seconds, respectively. However, as seen in Figure 4, the first FRPN value (177±5.6 msec) obtained during the fast rate was longer than the control value (162±4.4 msec), while the first A<sub>1</sub>-H<sub>1</sub> interval (42±4.5 msec) was only slightly prolonged as compared with control (40±4.5 msec). That is, FRPN underwent a substantial initial prolongation that was independent of...
fatigue. In spite of this initial difference, the above observations support the existence of a good temporal relation between facilitation and fatigue effects on nodal conduction time and the corresponding changes in FRPN.

**Duration of Fast Rate and FRPN**

A logical, though unreported, consequence of the growth of fatigue is that the rate-induced variations in FRPN arising from combined facilitation and transient fatigue could vary with the time at which FRPN is obtained after the beginning of the fast rate. This possibility needs to be verified; the time that FRPN is obtained is rarely controlled with protocols currently used in electrophysiological investigations of nodal function. Therefore, this time was varied for a 100% fast rate in group 3 preparations (Table 1) by changing the order of testing of the premature beats and/or the number of basic beats (see Stimulation Protocols in “Materials and Methods”). When short H1-S2 intervals were tested first after the beginning of the fast rate, FRPN obtained at 6±3.2 seconds of fast rate was shortened from a control value of 161±4.1 msec to 153±4.9 msec, p<0.05. When H1-S2 intervals were tested in rapid decrementing order while eight basic beats separated each premature beat, FRPN was slightly shortened (159±5.4 msec, p>0.05) and was obtained after 24±1.8 seconds of fast rate. When the number of basic beats was increased to 20, FRPN was prolonged (167±5.9 msec, p>0.05) and obtained at 70±6.2 seconds of fast rate. Finally, when 20 basic beats separated each premature beat and the same coupling interval was tested three times, FRPN obtained after 217±14.3 seconds of fast rate was prolonged to 169±5.3 msec, p<0.05. Thus, depending on the time at which it is obtained after the beginning of the fast rate of a constant magnitude, FRPN can be shortened, be left unchanged, or be prolonged.

**Pattern of Stimulation and FRPN**

Other stimulation patterns currently used to characterize nodal functional properties include incremental pacing, frequency step, and ramp (see Stimulation Protocols in “Materials and Methods”). The minimum H-H interval reached with each of these stimulation patterns was determined in each group 1 preparation and compared with FRPN values obtained shortly before with premature stimulation protocols (Table 2). The three stimulation patterns systematically resulted in a shorter minimum H-H interval than the control FRPN value. The minimum H-H interval reached with the step stimulation was 148±6.2 msec, which is very close to the value obtained with selective facilitation (145±3.3 msec). This similitude can be attributed to the fact that the alternation between short and long cycles during the step stimulation allowed a full facilitatory effect while leaving little opportunity for the cumulation of fatigue. With the incremental pacing and the ramp stimulation, the minimum H-H interval reached was 151±4.5 and 150±5.8 msec, respectively. This slight prolongation as compared with the value obtained with the step stimulation can be attributed to fatigue; the incremental pacing and the ramp stimulation lasted long enough for the node to develop some fatigue, but this possibility cannot be objectively verified with present protocols. These findings suggest that the changes in the minimum H-H interval obtained with various stimulation patterns can be explained with the interaction hypothesis between facilitation and fatigue effects.

**Variations of FRPN Subintervals**

FRPN occurs when the sum of its subintervals \((H1-H2=H1-A2+2 H2=A1-A2)-A1-H1+A2-H2\) reaches a minimum value. In the present study, two aspects of this relation were examined. The first concerned the problem of whether FRPN arises from absolute limits reached in its subintervals. The second aspect concerned whether changes in FRPN were associated with specific changes of these subintervals. The subintervals obtained for the four protocols of Figure 5B in group 1 (Table 2) and in group 2 (Table 3) preparations were systematically analyzed.

To examine whether FRPN was associated with an absolute limit reached in one or more of its subintervals, we compared the subintervals associated with any given FRPN with those obtained at the next shorter and next longer coupling interval. Such a comparison is illustrated in Figure 5A for the first control FRPN determination, and it can be seen that the three cycles resulted in three different subinterval relations. The \(A1-A2\) and \(H1-A2\) intervals could be shortened and the \(A2-H2\) interval could be prolonged beyond their respective value at FRPN. The same observations were made for FRPN values obtained with the other three protocols. Thus, FRPN was not associated with any absolute limit in its subintervals.

Changes in FRPN were associated with systematic changes in its subintervals, however. This can be appreciated from Tables 2 and 3 and from the ladder diagram (Figure 5B) constructed with the mean values of the subintervals obtained in group 1 preparations. The FRPN shortening and prolongation caused by the facilitation and the fatigue were associated with statistically significant shortening and prolongation of the \(H1-A2\) interval, respectively. Both the selective facilitation and the selective fatigue tended to prolong the \(A1-H2\) interval at FRPN, but this prolongation was not statistically significant. Nevertheless, this prolongation lessened the effects on FRPN of the \(H1-A2\) interval shortening caused by facilitation while it added to the \(H1-A2\) prolongation caused by fatigue. For instance, the net 15-msec shortening in FRPN seen with facilitation in group 1 preparations (Figure 5B) was associated with an 21-msec shortening in the \(H1-A2\) interval plus a 7-msec increase in the \(A2-H2\)
interval, although the latter was not statistically significant. Conversely, the 26-msec increase in FRPN induced by selective fatigue was associated with a 21-msec increase in the H2-A2 interval and a 5-msec increase in the A2-H1 interval. The combined facilitation and fatigue protocol, which resulted in a slight net prolongation of FRPN, shortened the associated H1-A2 interval while prolonging the A2-H1 interval so that these opposite effects tended to cancel each other.

Although the A2-A3 and A3-H1 intervals at which FRPN occurred (Figure 5B and Tables 2 and 3) were significantly prolonged by facilitation, fatigue, and combined facilitation and fatigue, the values resulting from their differences could be either negative or positive and were, as expected, equal to the changes in H1-A2 intervals. The increase in A1-H1 interval associated with facilitation and combined facilitation and fatigue was greater than that caused by fatigue. During the fatigue protocol, the A1-H1 interval was obtained at the end of a long cycle, and thus, reflected only fatigue effects. With facilitation and combined effects, the A1-H1 interval occurred after a short cycle and thereby underwent a marked recovery-dependent prolongation as compared with that obtained with the control and the fatigue protocols. As for the rightward shift of the refractory curve (see Correction of Nodal Refractory Curve in “Results”), changes in A1-A2 and A2-H1 intervals, therefore, have no significance in reflecting changes in nodal refractoriness.

In summary, FRPN was not associated with any absolute limit in its subintervals. Facilitation induced a shortening in the H1-A2 interval at which FRPN occurred while fatigue induced a prolongation. The A2-H1 interval associated with FRPN tended to be prolonged by all protocols but to a greater extent by the combined facilitation and fatigue protocol.

**Discussion**

**Extended Bidirectional Domain for FRPN Variations**

An important finding of the present study was that the repeatedly reported rate-induced shortening in FRPN corresponds to the net result of interactions between two opposite properties, facilitation and fatigue, and covers only a fraction of a wider potential domain for FRPN variations. Indeed, when selectively determined for a 100% fast rate, facilitation always resulted in FRPN shortening that covered a wider negative range than that obtained with combined effects, while fatigue resulted systematically in an FRPN prolongation occurring in a previously undefined positive range. Moreover, for any given rate, the sum of the independently obtained effects of facilitation and fatigue on FRPN corresponded to their combined effects also obtained independently in a separate protocol. Finally, the dynamics of FRPN variations corresponds to that of facilitation and fatigue. Hence, the domain of FRPN variations must be extended beyond that suggested by previously reported shortening to include a wider negative range that depends on facilitation and a positive range that depends on fatigue.

**Dynamics of Rate-Induced FRPN Variations**

The present findings suggest that the FRPN variations occurring during a fast rate imposed with a protocol that results in combined facilitation and fatigue, such as those used in current electrophysiological investigations of the conduction system, can be explained with the individual dynamic characteristics of these properties. For fast rates of short duration, facilitation is maximal and fatigue is minimal, so that the net changes in FRPN approximate closely those produced by facilitation alone. In the present study, as in a previous one, FRPN was maximally shortened after the first short cycle of the fast rate. This differs from that of the functional refractory period of the ventricles. It also differs from another report on the effects of successive short cycles on
FRPN; the origin of this difference is uncertain, but may be related to the fact that, in the present study, recovery-dependent changes in nodal conduction time were completed within two beats while this occurred only after many beats when the fast rate is imposed with constant stimulus intervals. Whatever the origin of the difference, as the duration of the fast rate increases, fatigue grows and cancels a progressively greater amount of facilitation effects, leading to a reduced shortening, no change or even a prolongation in FRPN. Hence, the longer the duration of the fast rate, the smaller and the less statistically significant are the changes in FRPN. Fatigue-induced prolongation in FRPN is systematic and more easily seen when facilitation is dissipated by a long cycle. These dynamic characteristics render, as shown in the present study, FRPN variations very sensitive to factors that affect the level of facilitation and fatigue such as the magnitude and the duration of the rate, the presence or absence of long cycles, and the achievement or lack of achievement of a steady-state.

Another dynamic characteristic of facilitation-induced and fatigue-induced FRPN changes was their beginning with heart rates corresponding to the upper half of the 1:1 nodal conduction range and their ensuing progressive increase so that greatest changes were observed at the fastest rate (Figure 3 and Table 3). Although this rate-dependence was apparently reduced by the mutual cancellation in conditions of interaction, it remains an important determinant of FRPN variations.

**FRPN as an Index of Nodal Refractoriness**

The present findings demonstrate that the facilitation and fatigue properties previously defined in terms of changes in the recovery curve (A₂-H₂ versus H₂-A₂ intervals) cause parallel changes in FRPN; this observation supports the existence of a link between nodal refractoriness and FRPN. However, when A₂-H₂ intervals obtained at different basic rates are compared for similar A₁-A₂ intervals, the resulting recovery curves show a rate-induced prolongation of the A₂-H₂ intervals while FRPN tends to be shortened. This discrepancy has been interpreted as evidence for a lack of correspondence between FRPN and nodal refractoriness. A similar conclusion was reached in another study in which the beat-to-beat changes in FRPN occurring during 4:3 Wenckebach cycles were compared with corresponding changes in the effective refractory period which was then taken as an index of nodal refractoriness; FRPN and refractoriness were found to be inversely related. However, when the A₁-A₂ intervals are corrected for changes in the conduction time of the A₁ beat occurring during Wenckebach cycles, beat-to-beat changes in the effective and functional refractory period are closely related. Similarly, when A₂-H₂ intervals obtained at different basic rates were compared for identical H₁-A₂ intervals to eliminate the shortening effects of the rate-induced prolongation of the A₂-H₂ interval on the recovery time of premature beats introduced with comparable A₁-A₂ intervals, they showed a rate-induced shortening of the A₂-H₂ intervals in the short H₂-A₂ interval range. This facilitatory effect agrees fully with the concomitant FRPN shortening.

FRPN did not, however, correspond to an absolute limit reached in the H₁-A₂ or A₂-H₂ interval values. This occurred because any further shortening in the H₁-A₂ interval beyond that associated with FRPN resulted in a proportional or greater A₂-H₂ interval prolongation that, in turn, resulted in a constant or prolonged H₂-H₁ interval as compared with that corresponding to FRPN. Shorter H₁-A₂ intervals than that of FRPN, therefore, does not imply a different time of occurrence of the A₁ beat in the recovery cycle of the distal node where FRPN originates. Indeed, the prolonged conduction time associated with the shortened recovery time causes the very early A₁ beats that would otherwise be blocked to arrive at the distal node at the same time, or even later, than the beat at which FRPN occurs. This matching gate process causes the resulting H₁-H₂ intervals to be constant or prolonged rather than shortened, thus, accounting for the plateau and rising left limb of the refractory curve. FRPN would start with the upstroke of the action potentials in the distal node and would end at the earliest moment at which these cells can respond to a propagating impulse. For a condition where the propagating impulse reaches the distal node early after the beginning of its recovery, FRPN and distal refractoriness would be closely bound; this may be the case at the control basic rate. However, when the delay generated exceeds what is required to overcome distal refractoriness, the excess delay adds to the duration of FRPN that may then overestimate distal refractoriness. Such a situation might have occurred for the combined facilitation and fatigue that simultaneously shortened the H₁-A₂ intervals associated with FRPN while prolonging the corresponding A₂-H₂ intervals (Table 3). In this condition, distal refractoriness was likely shortened by the facilitation while proximal refractoriness was prolonged by the addition of a delay of propagation that then shifted the origin of FRPN upward in the node. Therefore, the lack of correspondence between FRPN and absolute limits in its subintervals is not indicative of a lack of correspondence between FRPN and nodal refractoriness, but rather of limitations of the A₁-A₂ and/or H₁-A₂ intervals in assessing recovery time in those nodal cells that matter most for nodal refractory properties. Specific studies based on combined intracellular and extracellular recordings are obviously needed to elucidate further the intranodal origin of FRPN variations.

**Mechanisms of Rate-Induced Changes in FRPN**

Although the present results contribute to the understanding of the functional origin of rate-induced changes in FRPN, they do not warrant speculations about the membrane mechanisms underlying these changes. Associated changes in action
potential characteristics and in underlying ionic currents in different typical nodal cells remain largely unknown. Nevertheless, it may be worth mentioning that the marked shortening in action potential duration seen in the distal nodal cells in response to an early atrial premature beat could be responsible for the facilitatory effects on FRPN by allowing a shorter minimum cell cycle-length in the distal node. It may also be suggested that the fatigue-induced prolongation of FRPN likely occurred through a prolongation of both refractoriness and conduction time in the different sections of the node. The fatigue-induced prolongation of refractoriness has not been studied yet with intracellular recordings. Fatigue is, however, clearly associated with reduced diastolic excitability in various nodal cells.

Limitations

Although the algebraic sum of the FRPN changes produced individually by selective facilitation and selective fatigue corresponded to a large extent to FRPN changes caused by the combined effects of facilitation and fatigue (Figure 3 and Tables 2 and 3), there were small differences, the origin of which is uncertain. The assumptions that the facilitation present after 5 minutes of fast rate is the same as that at the very beginning of the rate and that it could be entirely dissipated by a single long cycle may both carry a margin of error. It is also possible that identical His-stimulus intervals introduced with the four protocols of Figure 1B resulted in slightly different cycle-lengths in the cells responsible for FRPN and for its modulations by facilitation and fatigue.

Because the rate-induced changes in FRPN were obtained in isolated preparations and did not correspond to the time constant of responses linked to changes in autonomic tone, FRPN changes are believed to be primarily intrinsic phenomena. However, because no autonomic blockade was performed in the present experiments to avoid the instability due to changes in drug effects, the possibility of autonomic involvement, albeit unlikely, cannot be ruled out completely. This possibility is also made unlikely by recent demonstration (unpublished observations) that nodal recovery, facilitation and fatigue properties persist largely unaltered after autonomic blockade. It is also obvious that, in the intact animal, regulating mechanisms of extranodal origin can occur and add an indirect contribution to the rate-induced changes in FRPN. Nevertheless, understanding the intrinsic responses remains a prerequisite for an accurate interpretation of any response, externally regulated or not.

Part of FRPN prolongation obtained with the selective fatigue protocol and perhaps, by association, with the combined facilitation and fatigue protocol might have a different origin than fatigue itself. This is suggested by the observation (Figure 4) that, during the fatigue induction protocol, a substantial fraction of FRPN prolongation developed at the very beginning of the fast rate well before the fatigue effects on conduction time had time to develop. The origin of this fatigue-independent fraction of FRPN prolongation observed with fatigue protocols is obscured. Until this origin is understood, it will be difficult to dissociate the two components of the FRPN prolongations. However, the possibility remains that a FRPN prolongation independent of fatigue may occur when a long cycle follows a few short cycles.

Implications

The determinant role of FRPN in protecting the ventricles against too early reactivation in various supraventricular tachyarrhythmias, including atrial fibrillation, warrants the current practice of determining FRPN in most electrophysiological investigations. The present findings provide a new functional background to explain the up and down swings of rate-induced FRPN variations. Important considerations in studies upon these variations are the magnitude and the duration of the fast rate, the interaction, mutual cancellation and dissociation of facilitation and fatigue effects, and the possibility of achieving a steady-state conduction. The study also implies that these considerations apply to a variety of different nodal responses obtained within a given type, or between different types, of stimulation sequences. Moreover, given the good reproducibility of the nodal functional properties obtained in a clinical setting, it should be possible to undertake functional investigations similar to those of the present study in humans. These considerations may also guide the definition of simplified stimulation protocols to shorten the investigation procedures. Finally, the findings that the refractory curves can be corrected for recovery-dependent changes in the conduction time of last basic beat without affecting FRPN value (Figure 2D) and that the curves then show, as reported also by others, a consistent relation between rate-induced changes in the nodal effective and functional refractory periods, may be useful in the graphical comparison of curves obtained in various conditions of stimulation. For instance, the corrected curves may be used for the definition of drug effects on nodal refractoriness in a context similar to that of the present study.

Conclusions

The present study supports the following conclusions: 1) Rate-induced FRPN variations originate from the interaction between facilitation and fatigue that are responsible for a wider than previously reported range of shortenings and for a previously undefined range of prolongations, respectively. 2) Selective and combined effects of facilitation and fatigue on FRPN start for rates corresponding to the upper half of the 1:1 nodal conduction range and reach a maximum at fastest rate. 3) The time-courses of fatigue-induced prolongations in nodal conduction time and FRPN are closely linked. Facilitation effects on conduction time and FRPN
also coincide temporally, but are much faster than those of fatigue. 4) FRPN is not associated to a particular limit in its subintervals, but is nevertheless related to nodal refractoriness. 5) In conditions of interaction between facilitation and fatigue, the opposite effects on FRPN are additive and may shorten, leave unchanged or prolong FRPN, depending on the duration of the fast rate that affects the fatigue produced while the facilitation remains constant. 6) Present findings have important implications for the design and interpretation of studies on nodal refractoriness.

Acknowledgments
The authors are grateful to Dr. Michael Guevara for his constructive comments and suggestions on the manuscript and to June Manson for text revision. The authors acknowledge the technical assistance of Maurice Tremblay and Lise Liamondon.

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Key Words • refractory curve • facilitation • fatigue • specialized conduction system • excitability
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Circ Res. 1989;65:164-175
doi: 10.1161/01.RES.65.1.164

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