Mechanism of atrioventricular nodal facilitation in rabbit heart: role of proximal AV node

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Mazgalev, Todor, Kent Mowrey, Igor Efimov, Gerard J. Fahy, David Van Wagoner, Yuanna Cheng, and Patrick J. Tchou. Mechanism of atrioventricular nodal facilitation in rabbit heart: role of proximal AV node. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1658–H1668, 1997.—The phenomenon of atrioventricular (AV) nodal “facilitation,” described in traditional “black box”-functional studies, implies enhanced AV nodal dromotropic function. We investigated the role of atrial prematurities in the modulation of the nodal cellular responses in the mechanism of AV nodal facilitation. Atrial and His (H) bundle electrograms and microelectrode recordings from proximal AV nodal cells were analyzed in 15 superfused rabbit AV node preparations. The pacing protocol consisted of 30 basic beats (S1; coupling interval S1-S1 = 300 ms) followed by a facilitating prematurity (S2; coupling intervals S1-S2 of 300, 200, 150, and 130 ms) followed by the test beat (S3; coupling interval S2-S3 scanned in 5-ms steps). Conduction curves (S2-H2 vs. S1-S2, S3-H3 vs. S2-S3, and S3-H3 vs. H2-S3) were constructed. Facilitation (i.e., shortening of S2-H3 when S1-S2 was shortened) was demonstrated in all preparations using the H2-S3 (P < 0.001) but not the S2-S3 format. Microelectrode recordings revealed a causal relationship between the improved proximal AV nodal cellular responses in facilitation and the prolonged S2-S3 interval. There was no evidence for enhanced nodal dromotropic function directly resulting from the introduction of the facilitating beats. Thus facilitation is based on inherent cycle-length-dependent properties of the AV node during application of a complex pacing protocol and primarily reflects the uncontrolled modulation of the proximal cellular response.

Intrinsic atrioventricular nodal properties; anterograde conduction; pacing protocol; microelectrode recording

It is well established that anterograde conduction through the atrioventricular (AV) node is dependent on the frequency and pattern of its stimulation from the atrium. The AV nodal conduction delay depends not only on the preceding coupling interval but also on the prematurity of one or more preceding beats (1, 6, 10, 14, 15, 24, 26–29). This property is of particular importance during propagation of atrial impulses with variable rhythms. In an effort to better classify the influence of the atrial rate on AV nodal function, the concept of intrinsic AV nodal rate-dependent properties composed of recovery, fatigue, and facilitation has evolved (5, 16).

The property of facilitation is best demonstrated by the use of a special stimulation protocol. It consists of basic atrial drive impulses (S1) followed by a short-coupled facilitating beat (S2) followed by a test beat (S3). Facilitation of AV nodal conduction is presumed when the conduction time of the S2-H2 (H) becomes progressively shorter with shortening of the S1-S2 prematurity. An important condition is that all S3-H3 comparisons are made for identical values of H2-S3, known as nodal recovery.

All previous studies on facilitation (5) were functional (i.e., the AV node was considered a “black box”), and the output (i.e., the conduction delay) was expressed as a function of the input under the conditions of the applied pacing protocol. The role of the intranodal organization of conduction and the inhomogeneity of the AV nodal cellular responses were not investigated. It has been proposed that the facilitating component may result from shortening of the cellular AV nodal potentials in response to the shortening of S1-S2. This would lead to a subsequently longer cellular diastole before S3. We evaluated this hypothesis in a previous study (9) and found that S1-S2-induced shortening of the action potential (AP) in the distal AV nodal region [the nodal-His (NH) region] had no appreciable effect on facilitation.

However, we have also noted (9) that, by its very design, the facilitation pacing protocol creates unequal atrial coupling intervals for the S3. That is, whenever facilitation was documented, the corresponding atrial prematurity interval (S2-S3) was longer. We hypothesize that this modulation of the atrial prematurity has a direct effect on the proximal AV nodal cells by producing an improved cellular response that manifests in a subsequently shorter conduction delay. The purpose of the present study was to evaluate this hypothesis in an effort to further elucidate the mechanism of the AV nodal facilitation.

Methods

The experimental setup and instrumental hardware were similar to those previously reported (9). Therefore, only a brief description is provided below. All studies were performed in accordance with the existing policies and the Guide for the Care and Use of Laboratory Animals established by the National Institutes of Health and were monitored by our Institutional Animal Care and Use Committee.

Atrial-AV Nodal Preparation

All experiments were performed in vitro on 15 preparations obtained from the hearts of New Zealand White rabbits of both sexes weighing 1.5–2 kg. While the rabbit was anesthetized with intravenous pentobarbital sodium, the heart was removed and an atrial-AV nodal preparation was isolated. It contained the AV node enclosed in the triangle of Koch, formed by the septal leaflet of the tricuspid valve, the tendon of Todaro, and the coronary sinus ostium (see Figs. 3, 4, and 9). The sinus node, crista terminalis (CrT), interatrial septum, the central fibrous body, and a small portion of the ventricular septum just below the bundle of His were also preserved.

The preparation was mounted on a silicon disk and immersed in a thermostatically controlled glass chamber, where...
it was superfused by oxygenated modified Tyrode solution containing (in mM) 128 NaCl, 4.7 KCl, 1.5 CaCl₂, 1.05 MgCl₂, 20 NaHCO₃, 1.19 NaH₂PO₄, and 11.1 glucose, pH range 7.25–7.35 at 35°C.

Pacing and Recordings

Custom-made quadrupolar electrodes were manufactured from 0.18-mm Teflon-insulated platinum-iridium wire, with an interelectrode distance of ~0.3 mm. The pacing bipolar was located ~1 mm from the recording bipolar. The electrodes were used to record surface bipolar electromograms from the CrT and from the bundle of His. Standard glass microelectrode techniques were utilized in six preparations to record APs from cells classified as proximal AV nodal cells from the AN (atrial nodal) and/or N (compact nodal) regions (3). In two of the experiments with microelectrodes, mechanical contraction was reduced by the addition of 10 mM 2,3-butanedione monoxime (BDM) to the superfusate after completion of the macrostudies. Cheng et al. (18) recently reported that there were no depressive effects on the AV nodal conduction at such a low concentration of BDM. Similarly, we did not see BDM effects on facilitation in the above cases.

All electrical signals were amplified to 2–5 V and filtered (low, 30 Hz; high, 3,000 Hz). They were displayed on a storage oscilloscope and simultaneously recorded on magnetic tape for off-line analysis. The electromgrams were digitized (Di/dt data 1200, Axon Instruments, Foster City, CA) at a sampling frequency of 5 kHz and analyzed with AxoScope (Axon Instruments) and Origin (Microcal).

Stimulation Protocols

Leads from the stimulating electrodes were connected to optically isolated current-stimulator units (Axon Instruments). The duration of the stimuli (2 ms) as well as the time sequence of a specific stimulation protocol were determined by a computer-controlled, eight-channel, programmable stimulator (Master-8, AMPI). Because the distance between the corresponding pacing and recording pairs was very short, no latency at the stimulating site was detectable.

Anterograde AV nodal conduction was initiated with stimulation at the CrT. The stimuli are marked as S₁, S₂, or S₃, as latency at the stimulating site was detectable. Two major pacing protocols were utilized. In both protocols, the preparation was driven at a constant rate (20 NaHCO₃, 1.19 NaH₂PO₄, and 11.1 glucose, pH range 7.25–7.35 at 35°C).

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Anterograde AV nodal conduction was initiated with stimulation at the CrT. The stimuli are marked as S₁, S₂, or S₃, as explained below. Two major pacing protocols were utilized. In both protocols, the preparation was driven at a constant rate that was periodically interrupted by one or more prematurities.

Pacing protocol 1: single prematurity protocol. The basic drive (S₁-S₂) consisted of 30 beats with a cycle length of 300 ms. The test beat S₂ was introduced with a programmed coupling interval S₂-S₃ after each basic drive. The same sequence was repeated, each time with a decreasing S₂-S₃ until atrial or AV nodal refractoriness was encountered. The S₁-S₂ intervals were named facilitating prematurities to test the expectation that shortening of S₁-S₂ for the same H₂-S₃ interval, would result in facilitation (i.e., shortening of the test conduction time (S₃-H₃); Fig. 1, A and B).

Measurements and Analysis

Time intervals were measured to the nearest millisecond using the S₁, S₂, S₃, and H (time of fastest upstroke) signals. The conduction time S₂-H₃ intervals were plotted against the recovery H₂-S₃ intervals as well as against the atrial prematurity S₂-S₃ intervals for each of the facilitating S₁-S₂ intervals. The curves representing the S₂-H₃ intervals will be referred to as conduction curves. Facilitation was considered to be present if, for comparable H₂-S₃ recovery, shortening of S₁-S₂ was associated with shortening of the test S₃-H₃ conduction time. Facilitation was manifest by a progressive shift of the conduction curves with shortening of S₁-S₂ (see RESULTS). To quantify the shortening of the conduction delay S₃-H₃ for the range of the used S₁-S₂ facilitating prematurities, the areas under the conduction curves at S₁-S₂ intervals of 300 (control), 200, 150, and 130 ms were measured. The areas were enclosed between the following boundaries: 1) bottom, the abcissa; 2) top, the curve; 3) right, a vertical line at the maximum H₂-S₃ (200 ms); and 4) left, a vertical line at the minimum common value of H₂-S₃ for the curves to be compared. The areas under the facilitation curves were expressed as percentages of the corresponding values in control and were named the index of conduction delay (CD). CD values <100% indicated facilitation. A one-way analysis of variance (ANOVA) for repeated measures was used to determine whether facilitation was induced by shortening of the S₁-S₂ interval, and post hoc analysis was performed using the Bonferroni-Dunn test.

Microelectrode recordings were performed in six preparations. Differentiation between the proximal and distal AV nodal cellular groups was aided by using the anatomical
location of the microelectrode impalements. In addition, the differences in distal cellular responses could be characterized by their AP amplitude, duration, and rate of rise as well as by their time of activation during anterograde AV nodal conduction (3). Characteristically, the proximal nodal cells were activated shortly after the atrial depolarization and, therefore, their cellular-coupling intervals were very close to the corresponding atrial prematurities. From the figures, one can see that the proximal cellular-coupling intervals differed by no more than 2–10 ms from the corresponding atrial S-S intervals. On several occasions, in addition to the stimulation procedures described above, we applied electrical stimulation directly within the AV node during recording of the intracellular responses.

RESULTS

Macroobservations: AV Nodal Conduction Curves

The phenomenon of AV nodal facilitation was easily demonstrable, as Fahy et al. (9) and others (5) previously reported. We observed facilitation in each of the 15 studied preparations. A representative example is shown in Fig. 2A. The conduction curve was generated in an S2-H2 versus H2-S3 format. Note that shortening of the facilitating prematurity (S1-S2) was associated with a progressive shortening of the test S3-H3 conduction time (ordinate) for any H2-S3 interval (abscissa). The facilitating effect was more pronounced with shorter S1-S2 intervals and in the range of short H2-S3 intervals. Thus the area under the conduction curve with an S1-S2 interval of 130 ms was 96% of control (see also Table 1), whereas the S2-H2 interval at H2-S3 of 35 ms was only 80% of control.

When the same data were plotted in an S3-H3 versus S2-S3 format, as shown in Fig. 2B, the relationship between the conduction curves was altered dramatically, leading to an opposite conclusion. Indeed, now shortening of the S1-S2 was associated with a progressive prolongation of the test S3-H3 conduction time (ordinate) for any atrial prematurity (S2-S3; abscissa).

Such an apparent contradiction is, in fact, not surprising. The formal explanation is that the data points in Fig. 2, A and B, were compared for substantially different arguments. They are connected by the simple equation S2-S3 = S2-H2 + H2-S3, which is derived from Fig. 1. It follows that each of the curves in Fig. 2A was shifted horizontally by the absolute value of S2-H2 when the data were replotted in the format shown in Fig. 2B. Furthermore, the S2-H2 conduction times were always inversely related to the corresponding S1-S2 facilitating prematurities such that the S2-H2 interval was shortest at S1-S2 of 300 ms and longest at S1-S2 of 130 ms in each preparation. This resulted in unequal shifts of the curves in Fig. 2A to their respective new positions in Fig. 2B.

From the above-mentioned formal explanation of the differences between the two formats, it is evident that, for any H2-S3 recovery interval in Fig. 2A, shortening of the S1-S2 interval is associated not only with facilitation (i.e., shorter S3-H3 conduction time) but also with a longer atrial coupling interval (S2-S3). This is illustrated in Fig. 2C. The upward shift of the S2-S3 line (vs. control), with shortening of S1-S2, was named the index of atrial prematurity (APi). We found that APi was always positive. Therefore, the facilitation observed in Fig. 2A was revealed at constant H2-S3 recovery intervals but also at consistently longer S2-S3 intervals.

The observations from all preparations are summarized in Table 1. The CDi (i.e., the measure of facilitation vs. S1-S2 = 300 ms) became <100% with shortening of the S2-S3 (facilitating) interval in each preparation (P < 0.001, ANOVA). Facilitation was observed when the S1-S2 interval was shortened from 300 ms to either 150 (P < 0.0001) or 130 ms (P < 0.0001; Bonferroni-Dunn test). Note, however, that the corresponding APi (i.e., the measure of S2-S3 interval prolongation) was always greater than zero for any S1-S2 interval in each preparation (P < 0.001).

Microobservations: Proximal Cellular Responses to Beats S2 and S3

The H2-S3 recovery interval is the time elapsed from the preceding bundle of His depolarization (the H2 electrogram) to S3 and therefore represents only a fraction of the distal nodal recovery time. In contrast,
the S1-S2 and S2-S3 intervals are the exact values of the corresponding atrial prematurities and therefore should be a close approximation of the cellular coupling interval in the proximal node. Therefore, we decided to examine the proximal cellular responses to beats S2 and S3 during the application of the two pacing protocols.

Figure 3 shows such recordings obtained with pacing protocol 1 in one preparation. Shortening of S1-S2 from 300 ms produced a monotonic increase of the S2-H2 delay and a progressive decrease of the amplitude and rate of rise of the nodal AP in response to S2 (AP2, the bold portion of the AP record). These changes became more pronounced with shorter prematurities, four of which are illustrated in Fig. 3, A–D. Initially, notch-and-hump formations were noticed (Fig. 3A) that eventually deteriorated into several AP components (Fig. 3, B–D). There were early “primary” and delayed “secondary” components. The secondary components increased in amplitude and were frequently followed by an echo beat (Fig. 3D), suggesting the initiation of a reentrant loop. On the other hand, the primary components progressively decreased in amplitude. The intervals measured between the inscription of AP1 (determined as a point of maximum first derivative) and the earliest depolarization after S2 closely followed the trend of Table 1.

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S1, basic drive beat; S2, facilitating beat; Prep, preparation no.; H, His; APi, index of atrial prematurity; CDi, index of conduction delay; S1-H1, conduction time during the basic drive with S1-S2 = 300 ms. AP shows the change of atrial prematurity S2-S3 for different facilitating intervals (S1-S2) vs. control (see illustration in Fig. 2C for additional explanation and definitions). This index was always positive and indicated a progressive prolongation of S2-S3 with shortening of S1-S2 (P < 0.001). CD represents the area under a conduction curve S3-H3 (H2-S3) at a given S1-S2 (see illustration in Fig. 2A for additional explanation and definitions) as a percentage of control. CDi < 100% was observed with shortening of S1-S2 (P < 0.001). See text for details.

Table 1. Development of facilitation during progressive shortening of facilitating S1-S2 interval from 300 (control) to 200, 150, and 130 ms

![Cellular recordings from a proximal nodal cell (inset)](https://example.com/inset.png) illustrate development of secondary components during S1-S2 interval shortening (A–D). In this and subsequent figures, simultaneously recorded traces are bipolar electrograms from site of pacing [crista terminalis (CrT)] and from bundle of His as well as action potentials (AP) of nodal cell(s). Insets show approximate location of impalement and are not drawn to scale. CS, coronary sinus; IT, tendon of Todaro; TrV, tricuspid valve. Figures contain responses to S1, S2, and S3; corresponding APs are referred to as AP1, AP2, and AP3, respectively. AP2, bold portions of AP traces in A–D, superimposed for a wide range of S1-S2 intervals in E. Numbers between CrT electrograms represent S1-S2 interval; numbers next to His electrogram represent corresponding S-H conduction time; arrows, delayed (secondary) APs; fractionated responses; curved arrow, decrease in amplitude of AP2 when S1-S2 shortens from 267 to 90 ms. See text for details.
In Fig. 4). In Fig. 4, a depressive effect of S1-S2 shortening on subsequent atrioventricular (AV) nodal conduction when S2-S3 interval was kept constant. S1-S2 was 300 ms (A) and was shortened to 130 ms (B). In both A and B, S2-S3 was 130 ms. Note delayed repolarization of AP2 and longer conduction delay (S3-H3) interval in B. Arrow, arrival of S3; dotted line, level of maximal diastolic membrane potential. See text for details.

change in S1-S2 prematurity. They were 110, 101, 99, and 97 ms in Fig. 3, A–D, respectively (numbers not shown in Fig. 3). Action potentials were recorded for a wide range of S1-S2 intervals from 300 to 90 ms, and selected traces are superimposed in Fig. 3E for ease of comparison. It is clear that shortening of S1-S2 was associated with an effective prolongation of AP2 due to the development of fractionated responses. Similar results were obtained in all preparations and confirm previously reported observations on the AV nodal premature responses (3, 20). The inhomogeneous AP2 responses differed depending on the preparation, the exact site of impalement, and the degree of prematurity. However, they were always confined to the proximal AN-N region and were most pronounced for S1-S2 prematurities shorter than 150 ms. Secondary components were never observed in the distal NH cells (9). The behavior described is consistent with prematurity-dependent fractionation of the wave front of conduction. At very short prematurity, the cellular responses were a mixture of electrotonic and active components spreading over a substantial time interval.

We further examined the effect of the S1-S2-induced modulation of the cellular response on the subsequent conduction of the S3. As previously demonstrated in Fig. 1B, shortening of S1-S2 always produced depressed S3-H3 conduction provided that the atrial prematurity S2-S3 was kept constant. Figure 4 shows that the mechanism for such depression was incomplete recovery of the proximal nodal cells. The cellular impalement was from the compact nodal region. In both panels the atrial prematurity S2-S3 was 130 ms. The cellular coupling intervals, measured between the inscription of AP2 and the nodal AP in response to S3 (AP3), were close to the atrial prematurity: 132 and 137 ms in Fig. 4, A and B, respectively (numbers not shown in Fig. 4). In Fig. 4A, with a 300-ms S1-S2 interval, the AP3 was almost fully repolarized on arrival of S3 (arrow). In Fig. 4B, however, the AP2 repolarization was delayed. Because the cellular coupling interval was almost the same when compared with Fig. 4A (137 vs. 132 ms), the delayed repolarization was responsible for the more positive membrane potential on arrival of S3 (arrow). Consequently, the AP3 amplitude was dramatically decreased along with the development of fractionated response. The S3-H3 conduction time was substantially prolonged (177 vs. 129 ms).

However, different results were obtained in the same preparation in response to the facilitating pacing protocol. Figure 5 shows the records obtained with a 300-ms S1-S2 interval, and Fig. 6 depicts the observations with a 130-ms S1-S2 interval. In both figures, A–D show electrograms and AP traces for several comparable H2-S3 intervals. A wide range of H2-S3 intervals from 200 to 1 ms was explored, and Figs. 5E and 6E show selected superimposed AP2 and AP3 (corresponding to the bold portions of the AP traces in A–D). Several striking differences between the events depicted in Figs. 5 and 6 are noted.

First, for any H2-S3 interval, the S3-H3 delay was shorter with a 130-ms S1-S2 interval (Fig. 6) than with a 300-ms S1-S2 interval (Fig. 5). The shorter S3-H3 delay correlated with the characteristics of the respective cellular response, AP3. For example, with an H2-S3 interval of 26 ms (Figs. 5B and 6B), the conduction delay of the 154-ms S3-H3 interval was associated with a marked reduction of the amplitude and rate of rise of AP3 (Fig. 5). In Fig. 6, the shorter conduction delay at the 136-ms S3-H3 was associated with an AP3 of higher amplitude and rate of rise without notching. A similar comparison applies for the other panels.

Second, with a long S1-S2 interval (Fig. 5E), shortening of the recovery interval H2-S3 led to a progressive deterioration of AP3. This included a decrease in its amplitude (curved arrow) until only a local response could be elicited for H2-S3 intervals <25 ms. Concomitantly, a secondary process caused the formation of a delayed component (filled circles in Fig. 5E) of increasing amplitude. With a 16-ms H2-S3 interval, the first component was only a small hump with an amplitude of 12 mV, the second component disappeared suddenly, and AV nodal block was documented. In contrast, with a short facilitating S1-S2 interval (Fig. 6E), shortening of the H2-S3 interval produced much less deterioration of AP3 (curved arrow). Thus, with a 16-ms H2-S3 interval, the amplitude was still 64 mV and the presence of a secondary process was hardly noticeable until extreme shortening of H2-S3.
Third, the characteristics of the transmembrane potential AP₂ were different in response to different S₁-S₂ intervals. In Fig. 6E, the dotted line represents the AP₂ seen in Fig. 5A. It is clear that shorter S₁-S₂ produced an AP₂ with reduced amplitude (70 vs. 79 mV) and rate of rise. In addition, this AP₂ returned to the resting membrane potential later (arrow).

The above analysis suggests that facilitation, observed with shorter S₁-S₂ facilitating intervals (Fig. 6), reflects an improved AP₃. However, the immediate result of the shortened S₁-S₂ prematurity was deterioration and delayed repolarization of the preceding cellular response AP₂. Careful examination of Fig. 6 shows that, although the AP₂ was prolonged, so was the subsequent S₂-S₃ interval. Indeed, comparison of the corresponding panels in Figs. 5 and 6, A–D, indicates that, for comparable H₂-H₃ intervals, the atrial prematurity S₂-S₁ was always longer during facilitation (Fig. 6). The cellular coupling intervals AP₂-AP₃ closely followed the corresponding changes in atrial prematurity S₂-S₃. Thus in Fig. 5, A–D, the intervals between the inscription of AP₂ and the first depolarization after S₃ were 128, 124, 120, and 118 ms (numbers not shown in the figure), respectively. In Fig. 6, A–D, the corresponding values were 166, 156, 150, and 147 ms (numbers not shown in the figure), respectively. That is, for comparable H₂-S₃ intervals, shortening of S₁-S₂ was associated with prolongation of the S₂-S₃ intervals and thus with prolongation of the cellular coupling intervals. This prolongation was sufficient to fully compensate and even overcompensate for the delayed AP₂ repolarization in Fig. 6. In fact, for comparable H₂-S₃ intervals, the cellular coupling intervals AP₂-AP₃ closely followed the corresponding changes in atrial prematurity S₂-S₃. Thus in Fig. 5, A–D, the intervals between the inscription of AP₂ and the first depolarization after S₃ were 128, 124, 120, and 118 ms (numbers not shown in the figure), respectively. In Fig. 6, A–D, the corresponding values were 166, 156, 150, and 147 ms (numbers not shown in the figure), respectively. That is, for comparable H₂-S₃ intervals, shortening of S₁-S₂ was associated with prolongation of the S₂-S₃ intervals and thus with prolongation of the cellular coupling intervals. This prolongation was sufficient to fully compensate and even overcompensate for the delayed AP₂ repolarization in Fig. 6. In fact, for comparable H₂-S₃ intervals, the cellular coupling intervals AP₂-AP₃ closely followed the corresponding changes in atrial prematurity S₂-S₃. Thus in Fig. 5, A–D, the intervals between the inscription of AP₂ and the first depolarization after S₃ were 128, 124, 120, and 118 ms (numbers not shown in the figure), respectively. In Fig. 6, A–D, the corresponding values were 166, 156, 150, and 147 ms (numbers not shown in the figure), respectively. That is, for comparable H₂-S₃ intervals, shortening of S₁-S₂ was associated with prolongation of the S₂-S₃ intervals and thus with prolongation of the cellular coupling intervals. This prolongation was sufficient to fully compensate and even overcompensate for the delayed AP₂ repolarization in Fig. 6. In fact, for comparable H₂-S₃ intervals, the cellular coupling intervals AP₂-AP₃ closely followed the corresponding changes in atrial prematurity S₂-S₃. Thus in Fig. 5, A–D, the intervals between the inscription of AP₂ and the first depolarization after S₃ were 128, 124, 120, and 118 ms (numbers not shown in the figure), respectively. In Fig. 6, A–D, the corresponding values were 166, 156, 150, and 147 ms (numbers not shown in the figure), respectively. That is, for comparable H₂-S₃ intervals, shortening of S₁-S₂ was associated with prolongation of the S₂-S₃ intervals and thus with prolongation of the cellular coupling intervals. This prolongation was sufficient to fully compensate and even overcompensate for the delayed AP₂ repolarization in Fig. 6. In fact, for comparable H₂-S₃ intervals, the cellular coupling intervals AP₂-AP₃ closely followed the corresponding changes in atrial prematurity S₂-S₃.
intervals, the AP3 responses in Fig. 6E were initiated from more negative membrane potentials than those in Fig. 5E and had larger amplitudes.

These observations on the opposing effects produced by the S1-S2 shortening and the concomitant S2-S3 prolongation on the conduction of the test beat S2 were consistent in all studied preparations, as evident from the combined data in Table 1.

Reversal of Facilitation

On the basis of the observations above, one might predict that an excessive shortening of the facilitating interval S1-S2 would produce strong deterioration of the cellular AP2 response. The subsequent prolongation of the S2-S3 interval may then be unable to provide enough compensation to continue to increase the degree of facilitation. We found evidence of such reversal of facilitation in five preparations. In three of them reversal was already evident at the 130-ms S1-S2 interval (Table 1, preparations 4, 10, 12, and 15). In the other two (preparations 4 and 6) the reversal of facilitation was documented at shorter S1-S2 intervals. The conduction curves from one such experiment (Fig. 7A) show that facilitation was observed during the S1-S2 interval shortening from 300 to 120 ms. When the S1-S2 interval was further shortened to 110 ms, the facilitation trend was reversed. Finally, with a 105-ms S1-S2 interval not only was facilitation not observed but also depression of AV nodal conduction occurred.

The curve in Fig. 7B was obtained from another preparation in which a wide range of S1-S2 intervals was examined. It is clear that shortening of S1-S2 between 140 and 110 ms produced little additional facilitation, whereas further shortening actually increased the S2-S3 conduction delay. The records in Fig. 8 are from the same preparation and illustrate the mechanism proposed above. In Fig. 8, A–D, there is a constant H2-S3 interval of 10 ms. The bold portions on the AP traces illustrate the progressive prolongation of the AP2 response when the S1-S2 interval was shortened from 200 (Fig. 8A) to 105 ms (Fig. 8D). This most probably resulted from fractionated wave fronts. The corresponding S2-S3 also prolonged from 95 (Fig. 8A) to 149 ms (Fig. 8D). However, this prolongation was increasingly effective only until S1-S2 was shortened to 115 ms (Fig. 8B). Indeed, in Fig. 8A, AP3 response was highly fractionated. The initial depolarization reached only 29% (arrow) of the maximum, suggesting that this particular area formed a functional “dead end,” whereas the meandering wave front reached the bundle of His in 159 ms. A very late fuller depolarization reached the impaled cell only after the His activation. In contrast, in Fig. 8B the amplitude of the AP3 response reached 81% of the maximum and facilitation was evident (S3-H3 shortened to 129 vs. 159 ms).

The events depicted in Fig. 8, A and B, were actually only two stages of a gradual process developing with the progressive shortening of S1-S2 from 300 to 115 ms, in which there was an increasing fractionation in AP2 and an exactly opposite change in AP3. Although the amplitude changes of AP3 measured in this particular impalement were indicative for the ensuing changes in the conduction delay S3-H3, they cannot be considered direct determinants of the latter, because the exact pathway and velocity are unknown.

Further shortening of S1-S2 became counterproductive (Fig. 8, C and D). With even more delayed recovery of AP2, the concomitant S2-S3 prolongation was not enough to result in improved cellular response. Consequently, the amplitude of the AP3 began to decline to 71% while the S3-H3 delay prolonged to 144 ms (Fig. 8D).

The prominent role of the atrial prematurity S2-S3 in the development of facilitation is further evident from Fig. 9. In this preparation there were two simultaneous microelectrode impalements located ~2 mm apart. Cell b, from which the AP (APb) was recorded, was located deeper and distal to the other cell a. Note that during
all manipulations of the atrial prematurity, the timing between APb and the corresponding bundle of His electrograms remained almost unchanged. On the other hand, AP of cell a (APa) was very sensitive to atrial prematurity.

In Fig. 9A (control), the S1-S2 interval was 300 ms and the recovery interval H2-S3 was set at 50 ms (the same value was kept constant for the duration of the experiment). Note that S2-S3 was 135 ms (i.e., 85 + 50). There was a reduced amplitude of APa to 48% of the maximum and an AV nodal conduction delay of 162 ms (again, the reduced amplitude in this impalement is suggestive of the fractionation of the wave front but is not a quantitative determinant of the S3-H3 conduction delay). Note also that the major portion of the increased conduction delay of S3 (162 vs. 85 ms) occurred between APa and APb. This does not imply linear conduction between cells a and b but confirms the predominant involvement of the proximal AV nodal region in the analyzed events.

In Fig. 9B, the facilitating S1-S2 interval was 200 ms. The repolarization of APa was delayed due to a small hump (encircled). However, the concomitant prolongation of S2-S3 to 167 ms (i.e., 117 + 50) produced an increase in the cellular coupling interval. Therefore, at the time of the S3 beat, the membrane was more repolarized than that in the control (Fig. 9, A and B, arrows), and the amplitude of APa reached 62% of control in beat S3. Consequently, facilitation was mainly between APa and APb.

In Fig. 9C, both S1-S2 and H2-S3 were kept the same as in Fig. 9B. However, in this case, another stimulus was delivered directly in the AV node 14 ms after S2, close to the microelectrode impalements. Note that this...
resulted in disappearance of the hump in AP\textsubscript{A} compared with Fig. 9B, possibly related to the altered pattern of nodal activation. More importantly, however, this maneuver resulted in a shorter S\textsubscript{2}-H\textsubscript{2} delay and, therefore, subsequently resulted in a shorter S\textsubscript{2}-S\textsubscript{3} atrial prematurity of 141 ms (i.e., 91 + 50). The cellular coupling interval for AP\textsubscript{A} was accordingly reduced, and the amplitude of AP\textsubscript{A} (for the S\textsubscript{2} beat) now reached only 56% of control. There was a reversal of facilitation and an increase of S\textsubscript{3}-H\textsubscript{3} from 143 to 155 ms, mainly due to the increased delay between the proximal cell a and the deeper distal cell b.

**DISCUSSION**

**Major Findings**

The major observation of the present study is that the phenomenon of AV nodal facilitation is based on a pacing protocol that produces a complex modulation of atrial prematurities. An initial short-coupled beat S\textsubscript{2} (facilitating prematurity) produces depressed conduction, associated with delayed repolarization in the proximal AV node. The facilitation protocol ensures that the subsequent test beat S\textsubscript{3} is generated with a longer S\textsubscript{2}-S\textsubscript{3} prematurity. This results in facilitation (i.e., improved S\textsubscript{3}-H\textsubscript{3} conduction) only if the S\textsubscript{2}-H\textsubscript{3} prolongation is sufficient to overcompensate for the depressing effect of the preceding S\textsubscript{2} beat. In addition, the present study describes for the first time a new feature, reversal of facilitation, and proposes a mechanism that explains both facilitation and its reversal based on the electrophysiological properties of the proximal AV nodal cells.

**Previous Studies**

Lewis and Master (16) first described the phenomenon of facilitation using the O-P interval as an index of recovery of the AV conduction system. Later, AV nodal facilitation was characterized in animals in response to pharmacological and autonomic manipulation (4, 24, 26) as well as in humans with and without dual pathway AV nodal electrophysiology (8, 11, 12). It has been shown that this property, along with the properties of recovery and fatigue (5, 24), is required to fully describe the AV nodal response to complex rhythm (28).

The explanation for AV nodal facilitation was previously unclear, and the phenomenon was ascribed to the effect of an S\textsubscript{2}-induced enhancement in the nodal recovery before the arrival of the test beat S\textsubscript{3} (16). It has been suggested that the short-coupled atrial extrastimulus (S\textsubscript{2}) may produce shortening of the AP duration of the distal nodal cells. For the same recovery interval H\textsubscript{2}-S\textsubscript{3}, these cells would enjoy a longer diastolic pause and, therefore, S\textsubscript{3} would conduct faster through the AV node (5, 24, 26, 28).

In a previous study (9), we tested this hypothesis and concluded that the contribution of the distal NH-H nodal regions cannot explain facilitation. Although the facilitation pacing protocol may be associated with a prolongation of the distal cellular diastolic interval, no causal relationship could be found between an increased distal cellular excitability and the ensuing effect of facilitation. Moreover, maneuvers such as distal nodal preexcitation that were designed to increase the distal cellular diastolic interval consistently failed to produce facilitation. However, we established in that study (9) that the failure was always associated with shortening of the atrial prematurity S\textsubscript{2}-S\textsubscript{3}.

**Mechanism of AV Nodal Facilitation**

We propose that facilitation results from the interplay of two opposing effects on the proximal AV node. The first effect is generated by the facilitating coupling interval S\textsubscript{1}-S\textsubscript{2} and causes depression of AV nodal conduction (Figs. 3 and 5–9). Thus shortening of the S\textsubscript{1}-S\textsubscript{2} interval produces a longer conduction delay S\textsubscript{2}-H\textsubscript{2}, and this is associated initially with reduction of the cellular AP amplitude in the proximal AV node. Furthermore, progressive shortening of S\textsubscript{1}-S\textsubscript{2} leads to an inhomogeneous pattern of conduction that is reflected in the fractionated responses in these cells (Figs. 3, 8, and 9), leading to a prolonged repolarization and delayed recovery. The conduction of the subsequent test beat S\textsubscript{3} will, therefore, depend on this conduction history. The second effect of the facilitation protocol is mediated by prolongation of the atrial prematurity S\textsubscript{2}-S\textsubscript{3} versus control (Fig. 2C and Table 1). It is this powerful effect that opposes and overcomes the depression of the short S\textsubscript{1}-S\textsubscript{2} interval and that causes the apparent facilitation.

Thus, as long as the S\textsubscript{2}-S\textsubscript{3} interval is sufficiently prolonged, there will be a full recovery after the deteriorated and/or fractionated proximal cellular response AP\textsubscript{2} and an improved response AP\textsubscript{3} will be generated (Figs. 5 and 6). In contrast, when the S\textsubscript{2}-S\textsubscript{3} prolongation is insufficient to compensate for a large deterioration of the AP\textsubscript{2} response, then the facilitation trend is reversed (Figs. 8 and 9) or disappears altogether (Fig. 7A). Therefore, this mechanism explains both the previously reported properties of facilitation (24) and the newly reported reversal of facilitation.

The proposed mechanism is based predominantly on the properties of the proximal AV nodal cells. The coupling intervals of these cells closely correspond to the atrial prematurities. Moreover, it is well established that the cells from the transitional AN and the compact N regions of the node play a most important role in the generation of the subsequent conduction delay during the propagation of premature beats (3, 20–22). By sensing and responding to the atrial prematurity, these proximal cells provide a variable driving force for the downstream conduction. Cells from the more distal nodal areas and from those close to the bundle of His have less impact on the cycle-length dependency of nodal conduction. The results from this (Fig. 9, cell b) and a previous study (9) confirm these observations.

The inhomogeneous response of the proximal nodal cells during conduction of premature beats has also been well documented (20). Because the recovery of the AV nodal cellular excitability lags well behind the end of an AP (22), the complex and fractionated AP\textsubscript{2} responses observed in this study could not play a facilitat-
ties, however, there were humps or fully dissociated secondary responses (humps and/or delayed APs) that were not well understood. It has been suggested that they may represent retrograde activation (reflection) over an inexcitable functional gap that divides the proximal and the more distal AV nodal regions (2, 29). Such secondary activation would be more pronounced when the primary proximal response is not a full one, as is usually the case with the short atrial prematurities S1-S2 (Figs. 3, 5, and 8). The results shown in Fig. 9 of the present study would agree with such an interpretation. During conduction at long coupling intervals, the APa responses preceded the APb responses (responses to beat S2). With short prematurities, however, there were humps or fully dissociated secondary activations in APa that followed in a retrograde manner the activation in APb (responses to beat S2). An alternative explanation for the development of secondary responses in the proximal node is the existence of multiple wave fronts that form the basis of dual pathway electrophysiology. In this case, the secondary responses could be regarded as a result of the retrograde invasion of the fast pathway domain by the later slow-pathway wave front (19). The cases in which the secondary components were associated with reentrant echo beats (Figs. 3 and 8) and disappeared in association with AV nodal block would favor such a mechanism. Regardless of the exact mechanism, the prematurity-induced deterioration of the proximal nodal cellular responses is evidence for fractionated conduction associated with delayed recovery.

Facilitation and “Supernormal” AV Nodal Conduction

The above analysis and the described mechanism for the phenomenon of facilitation indicate that the shortening of the test conduction time S3-H3, which would justify the use of the term “facilitation,” results from the complexity of the pacing protocol and can be explained by normal electrophysiological properties of the proximal AV nodal cells. Moreover, the introduction of the short-coupled prematurity S1-S2 resulted in an immediate depression of conduction so that this interval can hardly be termed “facilitating prematurity.” It was the subsequent prolonged S2-S3 that produced shortening of the test conduction time, S3-H3. Again, this latter effect was explained by the “normal” cycle-length dependency of AV nodal conduction (2). We conclude, therefore, that the facilitation pacing protocol does not produce supernormal enhancement of AV nodal conduction (23).

Although the present study challenges the suitability of the term facilitation, we do not reject the usefulness of the S1-S2-S3 pacing protocol for a detailed description of the dynamic properties of the AV nodal conduction (5). Indeed, there is experimental evidence that the conduction time of a given beat through the AV node depends not only on the current but also on the preceding prematurities in a complex way (6, 10, 14, 15, 24, 26–29). The current study confirms and further extends these observations by providing an insight into the cellular responses in the proximal AV node during facilitation. The exact design of the S1-S2-S3 protocol is of secondary importance. Thus systematic evaluation of AV nodal conduction can be performed for several prematurities, S1-S2 followed either by scanning H2-S3 (Fig. 2A) or S2-S3 (Fig. 2B) intervals. However, our results clearly demonstrate that simply modeling the AV node as a black box is insufficient for an in-depth understanding of AV nodal function. Whereas conduction curves are useful for a gross description of the functional relationship between the “input” and the “output” of the system, the precise mechanism(s) underlying the complex AV nodal conduction process can only be revealed by detailed study of the intranodal structure and cellular responses.

Study Limitations

Our results confirmed that the functional effect of the shortened S1-S2 is a delayed recovery of the proximal nodal cells and that this effect can be fully compensated by subsequent prolongation of the S2-S3. Although the kinetics of calcium currents are most likely involved in the explanation of the above effects (13), we still lack an appropriate AV nodal cellular model for quantitative description of this and other complex conduction phenomena. The task is further complicated by the fact that conduction apparently depends on the existence and interaction of more than one wave front and that mechanisms such as the electrotonic inhibition of conduction (17) may also be involved in the overall fractionated pattern of conduction at short prematurities. Therefore, whereas the mechanism proposed in the present study satisfactorily explains the phenomenon of facilitation, we cannot predict quantitatively the relationship between a certain degree of S1-S2 prematurity and the resulting degree of facilitation in a given heart. Furthermore, this study did not attempt to analyze the participation of specific proximal cellular subgroups. High-resolution mapping techniques, such as optical imaging (7), may help with such a task. Finally, we did not use pacing protocols utilizing different atrial pacing sites along with proximal cellular recordings, although such an approach may be useful for more detailed evaluation of the importance of dual-pathway electrophysiology in the phenomenon of facilitation.

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