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Effects of ajmaline on rate-dependent atrioventricular node properties Potential role in experimental atrioventricular re-entrant tachycardia

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Abstract

Ajmaline is a well-known atrioventricular (AV) node depressant agent, but its effects on functional properties of the AV node and on experimental AV re-entrant tachycardia have not been explored. The aims of the present study were (1) to determine whether ajmaline administration modifies the rate-dependent properties of the AV node and (2) to correlate these changes with the actions of ajmaline on an in vitro model of AV re-entrant tachycardia. Selective stimulation protocols and mathematical formulations were used to quantify independently AV node recovery, facilitation, and fatigue in 10 isolated rabbit AV nodes. Ajmaline decreased facilitation and fatigue and had no significant effect on AV node recovery. The most important effect of ajmaline was rate-induced prolongation of AV node effective refractory period, resulting in a greater increase in tachycardia cycle length. AV re-entrant tachycardia was sustained when AV effective refractory period divided to tachycardia cycle length was less than 1, ajmaline suppressed AV re-entrant tachycardia by increasing the slope of the AV effective refractory period divided to tachycardia cycle length versus tachycardia rate relation, causing the critical ratio of 1 to be attained at a slower rate. A mathematical model incorporating quantitative descriptors of recovery, facilitation, and fatigue accounted for changes in nodal conduction time, AV effective refractory period, tachycardia cycle length, and AV effective refractory period divided to tachycardia cycle length under all conditions. It can be concluded that (1) ajmaline increases AV conduction time, decreases AV node fatigue, and facilitation, without altering AV node recovery. (2) Ajmaline significantly prolongs AV effective refractory period in a rate-dependent manner. (3) These changes play a role in ajmaline's actions on experimental AV re-entrant tachycardia. Ajmaline's ability to terminate re-entrant supraventricular tachycardia may be due, at least in part, to its ability to amplify the rate-induced prolongation of the nodal refractory period.

Keywords: Ajmaline; AV node; Re-entry; Electrophysiology

1. Introduction

Ajmaline, a class I antiarrhythmic drug derived from *Rauwolfia serpentina* (Siddiqui and Siddiqui, 1932) is recognized as a highly effective antiarrhythmic drug for the treatment of a wide variety of tachyarrhythmias (Wellens et al., 1980; Manz et al., 1992; Chen et al., 1994). Ajmaline prolongs the refractory periods and slows conduction in the atrial and ventricular myocardium and also prolongs atrioventricular (AV) conduction (Padrini et al., 1993).

Negative dromotropic effects of ajmaline on AV nodal conduction and refractoriness have been reported to be rate-dependent (Stark et al., 1996). On the basis of these findings, it can be hypothesized that ajmaline would have more profound effects on AV node properties during supraventricular tachyarrhythmias than during sinus rhythm.

The AV node responds to changes in input rate in characteristic ways. A number of studies have shown that the wide variety of delays that the AV node can generate in response to an increased rate are explained by dynamic interactions between the three intrinsic properties, recovery, facilitation, and fatigue (Lewis and Master, 1925; Billette, 1976; Billette et al., 1988; Shrier et al., 1987). Moreover, a mathematical model incorporating quantitative descriptors of nodal recovery, facilitation, and fatigue can account for the steady state rate-dependency of AV node conduction time (Nayebpour et al., 1991), a variety of Wenckebach block patterns (Talajic et al., 1991), and

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rate-dependent effects of cholinergic (Nayebpour et al., 1990), adrenergic stimulation (Nayebpour et al., 1992), and adenosine on AV conduction (Nayebpour et al., 1993). These mathematical descriptors can be used to quantify the effects of ajmaline on individual AV node properties and to analyze the degree to which changes in such properties affect AV node conduction at different heart rates (Nayebpour et al., 1990, 1992, 1993).

The present experiments were designed to analyze in detail ajmaline's action on tonic and rate-dependent AV node properties by means of specific pacing protocols and a mathematical model. Our specific goals were (1) to analyze the effects of ajmaline on the tonic and rate-dependent properties of the AV node, (2) to determine the extent to which changes in these rate-dependent properties account for alterations in AV node conduction and refractoriness as a function of heart rate and (3) to determine the effects of ajmaline on an experimental model of AV re-entrant tachyarrhythmias.

2. Materials and methods

2.1. General methods

Experiments were performed with isolated, superfused rabbit cardiac preparations. The preparation, perfusion system, stimulation techniques, and recording system were similar to those previously described in detail (Billette and Metayer, 1989; Nayebpour et al., 1993; Medkour et al., 1998; Lin et al., 1999). Briefly, 10 male rabbits (1.5–2 kg) were used. Anesthesia was induced with pentobarbital (35 mg/kg) injection in the ear vein and heparin (200 IU/kg) was used as anticoagulant. Lateral thoracotomy was performed and the heart was excised. The final preparation, which included the right atrium, AV node area, and upper part of the interventricular septum (approximate diameter of 2.5 cm), was mounted in a tissue bath superfused at 200 ml/min with a 5-l volume of oxygenated (95% O_2–5% CO_2) Tyrode's solution maintained at 37°C (pH 7.38). The composition of the Tyrode's solution was (mmol/l): NaCl, 128.2; KCl, 4.7; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 25; NaH_2PO_4, 0.7; and dextrose, 11.1. Only preparations that maintained stable AV node conduction for 1 h before study were used. In our experiment we measured Wenckebach cycle length before and after each experimental protocol to ensure the stability of AV node function during electrophysiological study, both under control conditions and in the presence of ajmaline. The time required for each experiment was 5–6 h, and in our study the preparation was stable for at least 8 h. Ajmaline (purchased from Sigma) was dissolved in ethanol, and then added to the superfused Tyrode's solution at a concentration of 2 and 4 μmol/l as dose 1 and dose 2, respectively. The ethanol concentration in Tyrode's solution was less than 0.01% in all experiments. The protocols were applied after a superfusion period of 20 min. Our pilot study showed that 20 min of superfusion with ajmaline is enough to obtain a steady state electrophysiological effect.

A bipolar iridium–platinum stimulating electrode with diameter 250 μm was positioned on the upper crista terminalis near the sinus node, and unipolar electrograms were recorded by silver electrodes 250 μm from the crista terminalis and His bundle. Stimulation protocols were applied using custom-made software running on a Pentium computer interfaced with a digital to analog converter and a stimulus isolator (Nihon kohden SS-202J). Electrogram signals were filtered (30 Hz to 3 kHz) and amplified (amplifier, Narcotrace 80). After analog to digital conversion the data were saved on hard disk and analyzed off-line.

2.2. Stimulation protocols used to quantify recovery, facilitation, and fatigue

Specific stimulation protocols were used, as previously described (Billette and Metayer, 1989; Nayebpour et al., 1993) to quantify the properties of AV node recovery, facilitation, and fatigue. To evaluate the basic recovery curve, the atrium was paced with a constant His-stimulus interval of 300 ms, which corresponded to a basic cycle length of 360 ± 3 ms under control conditions and 368 ± 3 and 375 ± 4 ms after dose 1 and dose 2 of ajmaline, respectively. To construct the basic recovery curve, a single premature or delayed stimulus (S_2) was introduced (Fig. 1) after every 10 basic stimuli (S_1). For consistency, the His bundle electrogram preceding any test beat designated H_1, and the atrial and His responses to the test stimulus are A_2 and H_2, respectively. The relation between the conduction time of the test beat (A_2H_2) and the preceding recovery interval (H_1A_2) was established and fitted to an exponential function as previously described (Talajic et al., 1991; Nayebpour et al., 1990, 1991, 1992, 1993).

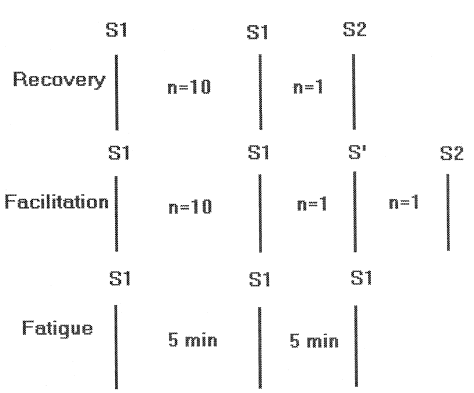

Fig. 1. Stimulation protocols used to quantify atrioventricular nodal recovery, facilitation, and fatigue. S_1 represents basic stimuli; S_2, test stimuli used to assess the atrioventricular recovery curve; and S', a conditioning stimulus used to produce facilitation. Fatigue, 5 min of slow rate alternates with 5 min of a fast rate.

To study facilitation, the recovery curve was constructed following a facilitation-inducing short cycle introduced after the last basic stimulus (Fig. 1). The facilitating stimulus (S') was inserted with a selected His-stimulus (HS′) interval to be called facilitation interval and a test S_2 stimulus was applied to establish the effect of the HS′ interval on the recovery curve relating the AV conduction time to the recovery interval. This procedure was repeated for six different values for the HS′ facilitation interval between 40 and 200 ms (40, 60, 80, 100, 150 and 200 ms).

To analyze AV node fatigue, a series of tachycardias with a constant His-stimulus interval ranging from 60 to 300 ms (60, 80, 100, 150, 200 and 300 ms) was initiated (Fig. 1), and changes in AV conduction time over 5 min at a given His-stimulus interval were observed. A recovery period of at least 5 min was allowed after each tachycardia for the dissipation of fatigue before the next tachycardia was initiated.

The functional and effective refractory periods of the AV node were measured with an extrastimulus technique. The AV functional refractory period was defined as the shortest ventricular output (known as H_1H_2 interval) resulting from premature atrial stimulation, and the AV effective refractory period was defined as the longest atrial coupling interval (known as A_1A_2) activating the atrial septum close to the AV node but failing to propagate through the bundle of His.

2.3. Induction of experimental AV re-entrant tachycardia

An experimental model of AV re-entrant tachycardia was studied, using an approach previously described (Simson et al., 1981; Talajic et al., 1990; Nayebpour et al., 1993). Briefly, each His bundle complex was detected by a signal detection unit, and a stimulus was delivered by the electrode located at the crista terminalis with a preselected His-stimulus interval delay. His-stimulus intervals studied were 60, 80, 100, 150, 200, and 300 ms. The resulting activation was conducted antegradely through the AV node, and subsequent His bundle activation was detected. Stimulation with the selected His-stimulus interval was then repeated, and the cycle recurred repetitively. A sustained tachycardia mimicking AV re-entrant tachycardia, using the AV node as the antegrade limb and the pacemaker circuit as the retrograde limb was thus initiated. Each induction of AV re-entrant tachycardia was followed by an at least 5-min recovery period. AV re-entrant tachycardias were considered to be sustained if they persisted for more than 2 min, at which time steady state values of AV conduction time and cycle length were measured, and the AV effective refractory period was determined by the extrastimulus technique.

2.4. Statistical analysis

Results are reported as the means ± S.E.M. and comparisons among multiple groups were made by two-way analysis of variance with Scheffe contrasts. Comparisons between two groups of experimental data were only made with Student's *t*-test. Two-tailed tests were used, and a probability of 5% was taken to indicate statistical significance. Non-linear curve fitting was performed with Marquardt's technique on a Pentium computer. Model-predicted values and experimental values were compared by regression analysis and deviation from unity (1) was considered statistically significant.

3. Results

3.1. General effects of ajmaline

Ajmalin caused a dose-dependent increase in AV conduction time measured as AV conduction time, Wenckebach cycle length, AV node effective and functional refractory periods and intra-atrial conduction time during slow atrial pacing (Table 1). This is in agreement with the results of a previous study (Stark et al., 1996) showing a depressant effect of ajmaline on the AV node. Drug effects, as reflected by changes in Wenckebach cycle length and AV conduction time, were stable during each experimental protocol.

3.2. Effect of ajmaline on rate-dependent AV node properties in rabbit hearts

3.2.1. AV node recovery

An example of basic nodal recovery curves in one rabbit heart preparation is shown in Fig. 2. Under control conditions AV conduction time was increased as atrial coupling intervals decreased. With further decreases in coupling interval, there was a rapid increase in AV conduction time until refractoriness was encountered. Ajmaline shifted the basic recovery curve upward in a dose-dependent fashion. Each curve was fitted by a single exponential function (r is always > 0.99) (Talajic et al., 1991; Nayebpour et al., 1990, 1991, 1992, 1993). The

Table 1
Effects of ajmaline on electrophysiological parameters

	Cycle length (ms)	SA (ms)	AH A(ms)	WBCL (ms)	AVERP (ms)	AVFRP (ms)
Control	360 ± 2.8	12 ± 0.8	44 ± 2	152 ± 4	103 ± 3	164 ± 3
Ajmaline 1	368 ± 2.9[a]	15 ± 1.1[c]	47 ± 2[b]	167 ± 4[b]	115 ± 2[b]	176 ± 3[c]
Ajmaline 2	375 ± 3.4[a]	18 ± 1.2[c]	51 ± 3[c]	194 ± 5[c]	139 ± 5[c]	196 ± 4[c]

Ajmaline 2 μmol/l (dose 1) and 4 μmol/l (dose 2) increased Wenckebach cycle length (WBCL), atrioventricular conduction time (AH), AV effective refractory period (AVERP) and functional refractory period (AVFRP), intra-atrial coduction time (SA), and cycle length in 10 rabbit preparations. Values are means ± S.E.M.

[a] $P < 0.05$ compared to control.

[b] $P < 0.001$ compared to control.

[c] $P < 0.01$ compared to control.

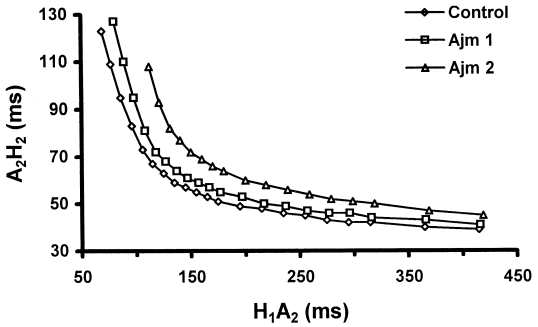

Fig. 2. Plot of ajmaline effects on the recovery curve in a representative experiment with a rabbit preparation. Ajmaline shifted the recovery curve upward without any significant effect on time constant of recovery. Values for time constant of recovery of control condition, dose 1 and dose 2 of ajmaline were 47, 52 and 56 ms, respectively. A_2H_2, AV conduction time; H_1A_2, recovery interval; Ajm_1, ajmaline dose 1; Ajm_2, ajmaline dose 2.

mean time constant of recovery averaged 47 ± 2 ms under control condition, compared with 52 ± 3 and 56 ± 3 ms after dose 1 and dose 2 of ajmaline, respectively (P = NS for each).

Ajmaline increased the AV effective refractory period from 103 ± 3 ms (control) to 115 ± 2 ms (dose 1, $P < 0.01$) and 139 ± 5 ms (dose 2, $P < 0.001$), and AV functional refractory period from 164 ± 3 ms (control) to 176 ± 3 ms (dose 1, $P < 0.001$) and 196 ± 4 ms (dose 2, $P < 0.001$). Although AV node conduction and refractoriness were changed by ajmaline, the time course of AV node recovery was not altered.

3.2.2. AV node facilitation

Single atrial premature beats shifted the recovery curve in response to a subsequent A_2 complex to the left in a fashion that depended on the facilitation interval. Fig. 3A shows raw data from a series of recovery curves obtained with varying facilitation intervals. As previously reported (Nayebpour et al., 1993), facilitation interval changed neither recovery time constant nor AV conduction time after full recovery. Therefore, the effects of facilitation are represented by a leftward shift of the AV recovery curve of a subsequent beat. Fig. 3B and C shows AV node facilitation after 2 doses of ajmaline.

Ajmaline increased the amount of leftward shift induced by facilitation. The degree of leftward shift caused by facilitation was quantified as previously described (Nayebpour et al., 1993). Briefly, we measured the AV functional refractory period, a physiologically relevant variable that can be importantly modulated by facilitation (Billette and Metayer, 1989). As shown in Fig. 4A, AV functional refractory period were significantly decreased by a short facilitation interval under all conditions. Ajmaline increased the degree of changes in AV functional refractory period, indicating a dose-dependent increase in facilitation. Fig. 4B indicates the changes in AV functional refractory period at each facilitation interval relative to values at a facilitation interval of 300 ms.

3.2.3. AV node fatigue

An example of AV conduction time changes following the initiation of tachycardia with a constant His-stimulus interval (to control the effects of recovery and facilitation) is shown in Fig. 5. When such tachycardia was initiated, AV node conduction time increased slowly over the next several minutes. The magnitude of conduction slowing was greater for tachycardias with shorter His-stimulus intervals, i.e., faster rates. Ajmaline slightly reduced the magnitude of fatigue (Fig. 6).

3.2.4. Effects of ajmaline on the steady state characteristics of AV re-entrant tachycardia

Under control conditions, AV re-entrant tachycardias at shorter His-stimulus intervals (corresponding to retrograde

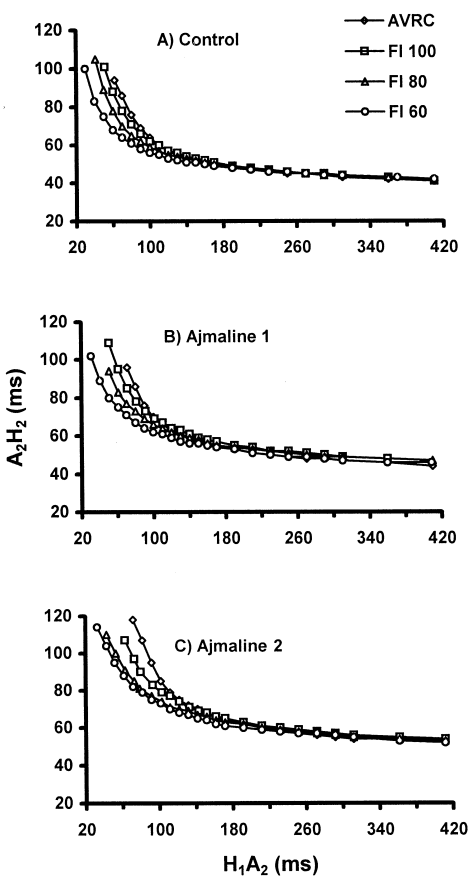

Fig. 3. Plots of effects of ajmaline on atrioventricular nodal facilitation in a representative experiment with a rabbit preparation. To avoid excessive crowding of data, results are shown for only three of six facilitation intervals (FI) studied under each set of conditions. The recovery interval (identified as H_1A_2) is the time from His bundle activation resulting from the facilitation stimulus (S') to the A_2 test response. As the facilitation interval from His bundle activation of the last basic beat to the conditioning premature stimulus (S') decreased, the AV recovery curve (AVRC) of the test beat elicited by the test S_2 stimulus shifted toward the left. Ajmaline increased the leftward shift of the facilitation curve in a dose-dependent fashion, indicating an increase in degree of facilitation. (A) Facilitation curve under control conditions, (B) after dose 1 of ajmaline and (C) after dose 2 of ajmaline. A_2H_2, AV conduction time.

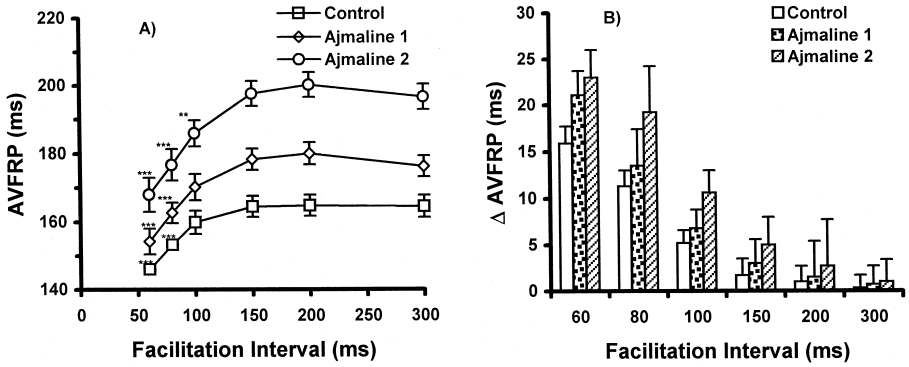

Fig. 4. Plots and bar graphs of effects of ajmaline on facilitation in 10 rabbit preparations. Panel A, effects of facilitating S' stimulus on the position of the atrioventricular nodal recovery curve associated with a subsequent S_2 test stimulus is indicated by the AV functional refractory period (AVFRP). Under control conditions AV functional refractory period decreased significantly at short facilitation intervals; ajmaline increases the degree of facilitation in a dose-dependent way. Panel B, indicates the changes in AV functional refractory period at each facilitation interval relative to values at a facilitation interval of 300 ms. ($^{**}P < 0.01$, $^{***}P < 0.001$, for value at each facilitation interval versus value at 300 ms in panel A).

pathways with faster retrograde conduction times) were faster, as shown in Fig. 7A. The relation between recovery interval and tachycardia cycle length was not linear, because of an increase in Steady state AV conduction time at shorter recovery intervals, which partially offset the decrease in retrograde conduction time (Fig. 7C). Atrial refractoriness prevented tachycardia induction at shorter recovery intervals under control conditions. Ajmaline increased tachycardia cycle length, with the increase becoming greater as the recovery interval decreased (i.e., as the tachycardia accelerated). The ajmaline-induced increase in Steady-state AV conduction time accounted for changes in cycle length of AV re-entrant tachycardia. These changes were greater for faster tachycardias. Prolonging effects of ajmaline on the effective refractory period (Fig. 7B) were also dependent on tachycardia cycle length. As the tachycardia rate increased, the tendency of ajmaline to increase AV effective refractory period was more pronounced than its effect on steady state AV conduction time.

Effects of ajmaline on the ability of AV re-entrant tachycardia to be sustained depends on the balance between two opposing actions—a tendency to increase AV effective refractory period, which makes tachycardia less likely, and a tendency to slow the tachycardia by slowing

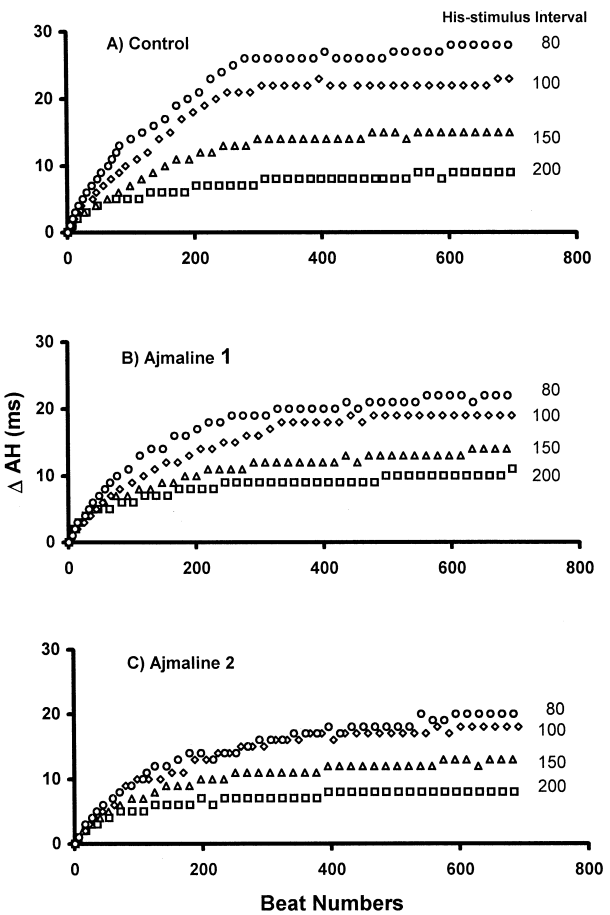

Fig. 5. Plots of effects of ajmaline on fatigue-induced changes in nodal conduction time (ΔAH) in a typical rabbit preparation. Each panel shows the time-dependent response to an abrupt and sustained increase in rate, obtained by decreasing the His-stimulus (HS) interval to each of the four values. Note that ajmaline slightly decreased the magnitude of fatigue in a dose-dependent way.

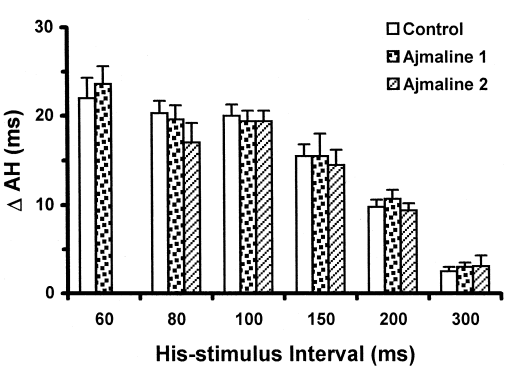

Fig. 6. Bar graph of steady state change in AV conduction time (ΔAH) resulting from fatigue induced by pacing at each His-stimulus interval on the horizontal axis. Rate-dependent fatigue was slightly decreased by ajmaline.

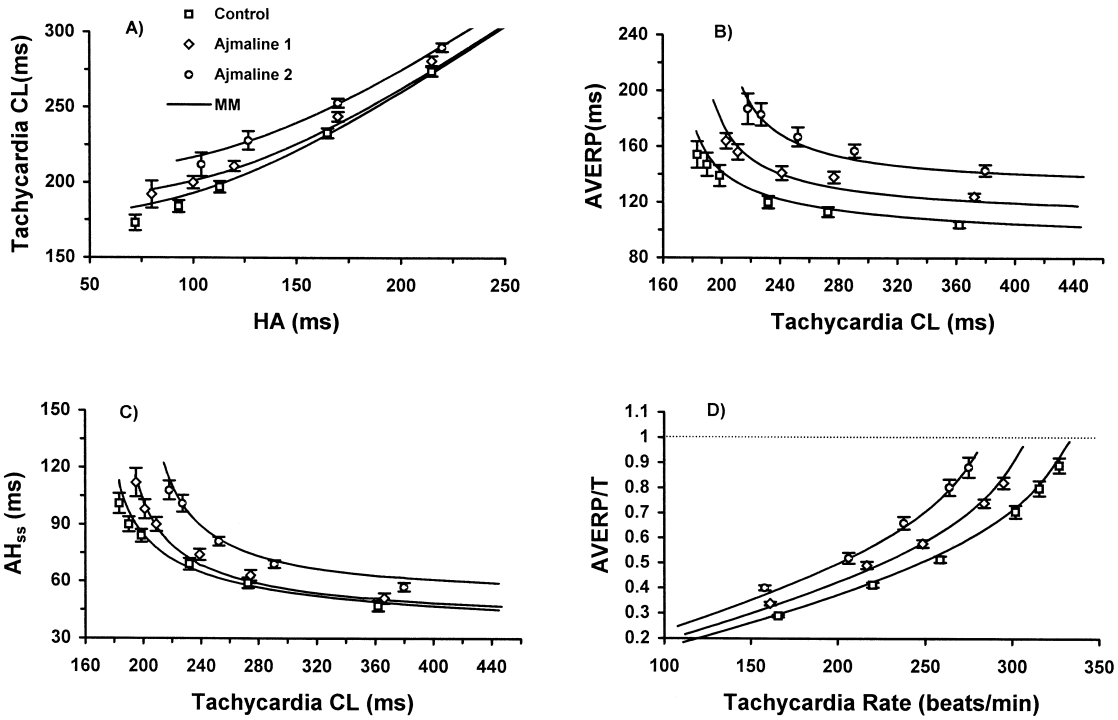

Fig. 7. Plots of observed (means ± S.E.M.) effects of ajmaline on characteristic properties of experimental AV re-entrant tachycardia in 10 rabbit preparations, along with corresponding values calculated on the basis of the mathematical model (MM) described in the text (continuous curves). Panel A, tachycardia cycle length (CL) as a function of recovery interval (HA) observed when pacing with a constant "retrograde" activation delay to mimic an accessory bypass tract. Panel B, AV effective refractory period during tachycardia, as a function of tachycardia cycle length. Panel C, AV node conduction time at steady state (AH_{ss}) during tachycardia, as a function of tachycardia cycle length. Panel D, ratio of AV effective refractory period to tachycardia cycle length (T) as a function of tachycardia rate.

AV node conduction, which makes it more likely that the tachycardia can sustain itself. The result of these opposing actions can be determined by applying the concept of wavelength, as has been explained by Mines and latter by Lewis (Mines, 1913; Lewis, 1925). The wavelength (λ) of a re-entrant circuit is equal to the product of average conduction velocity (CV) and the longest refractory period in the circuit (RP):

$$\lambda = \mathrm{CV} \cdot \mathrm{RP} \quad (1)$$

The wavelength must be shorter than the path length for the re-entrant impulse, if AV re-entrant tachycardia is to be

Table 2
Values of constants characterizing recovery, facilitation, and fatigue

Experiment	Recovery									Facilitation		
	AH_∞ (ms)			τ_{rec} (ms)			A (ms)			B (ms)		
	Con	A_1	A_2	Con	A_1	A_2	Con	A_1	A_2	Con	A_1	A_2
1	41	43	49	36	52	62	330	222	193	53	60	54
2	42	45	49	42	39	47	408	633	576	81	108	113
3	39	37	41	55	54	59	167	232	201	139	83	75
4	55	52	49	58	77	56	120	117	276	31	35	111
5	43	47	54	46	47	47	188	207	288	88	164	124
6	62	58	61	51	53	53	202	226	278	57	108	144
7	46	51	51	55	56	56	157	167	167	117	133	99
8	56	49	55	43	47	48	187	250	274	65	133	141
9	51	47	50	51	48	61	215	370	303	91	143	177
10	35	31	33	40	51	51	353	292	379	55	73	90
Mean ± S.E.M.	47 ± 3	46 ± 2	49 ± 2	47 ± 2	52 ± 3	56 ± 3	232 ± 30	271 ± 41	276 ± 41	81 ± 11	108 ± 13	112 ± 15

AH_∞ indicates AV conduction time after full recovery at control basic cycle length; Con, control; A_1, Ajmaline dose 1; A_2, Ajmaline dose 2; τ_{rec}, recovery time constant; A, constant; B, constant equal to the change in the recovery time at a facilitation interval of 0; C, rate constant of facilitation; D, maximum changes in the recovery time due to fatigue; and E, constant. Values are means ± S.E.M.

[a] $P < 0.05$ compared to control.

sustained. Mean conduction velocity is given by the length of the re-entrant circuit (L) divided by the revolution time of the tachycardia (T):

$$\mathrm{CV} = L/T \quad (2)$$

During sustained tachycardias T equals the tachycardia cycle length. After substituting Eq. (2) into Eq. (1) and rearranging terms:

$$\lambda/L = \mathrm{AVERP}/T$$

Therefore, the relation between the minimum path length to sustain re-entry (λ) and the actual anatomic path length (L) can be expressed as the ratio of AV effective refractory period divided to tachycardia cycle length (AVERP/T). Tachycardia can only be sustained if the path length is greater than the wavelength, that is, if λ/L is less than one.

Fig. 7D shows values for AV effective refractory period divided to tachycardia cycle length during various AV re-entrant tachycardias in 10 experiments. Ajmaline prevented re-entrant tachycardia by causing a frequency-dependent increase in λ during AV re-entrant tachycardia in each experiment. Under control conditions and during ajmaline administration, AV effective refractory period divided to tachycardia cycle length increased as tachycardia rate increased. However, ajmaline strongly increased the slope of this relation, resulting in larger values of AV effective refractory period divided to tachycardia cycle length for tachycardias of equal rate.

3.3. Mathematical analysis of ajmaline's rate-dependent actions

A-previously developed mathematical approach was used to define AV node conduction time, AV node refractoriness, and cycle length of experimental AV re-entrant tachycardia (Nayebpour et al., 1990, 1991, 1992). Briefly, the recovery curve of the AV node can be expressed as:

$$AH_{HA} = AH_{\infty} + A\exp(-HA/\tau_{\mathrm{rec}})$$

where AH_{HA} is the AV conduction time at a recovery interval of HA, and AH_{∞} is the AV conduction time at the longest cycle length and A, and τ_{rec}, are constants. This equation can be modified to incorporate quantitative indices of facilitation (ΔAH_{fac}) and fatigue (ΔAH_{fat}) as follows (Nayebpour et al., 1990, 1991, 1992):

$$AH_{HA} = (AH_{\infty} + \Delta AH_{\mathrm{fat}}) + A\exp\left[-(HA + \Delta HA_{\mathrm{fac}})/\tau_{\mathrm{rec}}\right] \quad (3)$$

ΔAH_{fac} and ΔAH_{fat} can be described in terms of equations fitted to the type of data shown in Figs. 4B and 6, as follows:

$$\Delta HA_{\mathrm{fac}} = \Delta\mathrm{FRP} = B\exp(-\mathrm{FI}\cdot C)$$

$$\Delta AH_{\mathrm{fat}} = \Delta AH_{\mathrm{max}}\exp(-D\cdot HA) + E$$

where estimates of ΔAH_{fac} and ΔHA_{fat} as shown in Figs. 4B and 6 are used to obtain the empirical constants B, C, ΔAH_{max}, D and E. The constants obtained from curve-fitting are shown in Table 2. Substitution of the mean constants (Table 2) into Eq. (3) allows for the calculation of the model-predicted AV conduction time during AV re-entrant tachycardia as a function of recovery interval. The tachycardia cycle length can be estimated from the model as the sum of recovery interval and the calculated AV conduction time. The effect of ajmaline in prolonging the cycle length of tachycardia was amplified as the rate increased.

The model-estimated relation between AV conduction times and cycle length of AV re-entrant tachycardia is in

			Fatigue								
C (ms^{-1})			ΔAH_{max} (ms)			D (ms^{-1})			E (ms)		
Con	A_1	A_2	Con	A_1	A_2	Con	A_1	A_2	Con	A_1	A_2
0.017	0.020	0.017	54	39	28	0.005	0.004	0.004	−11	−10	−4
0.026	0.027	0.025	89	68	48	0.006	0.007	0.005	−11	−6	−7
0.036	0.025	0.017	57	40	37	0.003	0.004	0.008	−20	−9	−1
0.036	0.031	0.023	44	40	46	0.004	0.003	0.007	−5	−6	−3
0.022	0.028	0.020	37	36	35	0.006	0.007	0.004	−5	−1	−8
0.029	0.035	0.037	59	50	44	0.004	0.003	0.003	−15	−16	−14
0.029	0.035	0.028	25	28	44	0.005	0.007	0.011	1	5	8
0.018	0.019	0.021	44	33	35	0.008	0.004	0.007	−2	−4	1
0.019	0.022	0.021	43	73	93	0.004	0.010	0.011	−9	3	−1
0.025	0.024	0.025	49	25	63	0.003	0.004	0.015	−17	−1	4
0.026 ± 0.002	0.027 ± 0.002	0.024 ± 0.002	50 ± 5	43 ± 5	46 ± 6	0.004 ± 0.0004	0.005 ± 0.0007	0.007 ± 0.001	−9.4 ± 2.1	−4.4 ± 2	−2.8 ± 1.9[a]

close agreement with experimental data as shown by model-predicted curve in Fig. 7A and C. Therefore, ajmaline's rate-dependent effects are well described by a model incorporating quantitative indices of basic recovery, facilitation and fatigue at each level of effect. This is evident from Fig. 8 where the relative contribution of the three rate-dependent properties (recovery, facilitation and fatigue) describing changes in AV conduction time are shown. Fig. 8A shows predicted rate-dependent changes in the AV conduction time due to incomplete recovery, recovery modified by facilitation, and fatigue as a function of basic cycle length. As basic cycle length decreases, incomplete recovery is expected to increase the AV conduction time to an extent indicated by the fine lines. Facilitation attenuates the effects of recovery, and when this action of facilitation is incorporated into the model, AV conduction time slowing is predicted by the dashed lines. Fatigue component alone was not able to predict the observed values (dashed–dotted line). Changes due to combined recovery and facilitation were added to those resulting from fatigue alone to obtain the total rate-dependent AV conduction time prolongation (solid heavy lines). The latter was in close agreement with experimentally observed values. In addition, regression analysis of different models based on fatigue or recovery alone, recovery plus facilitation, recovery plus fatigue, and combined recovery, facilitation and fatigue is presented in Table 30.

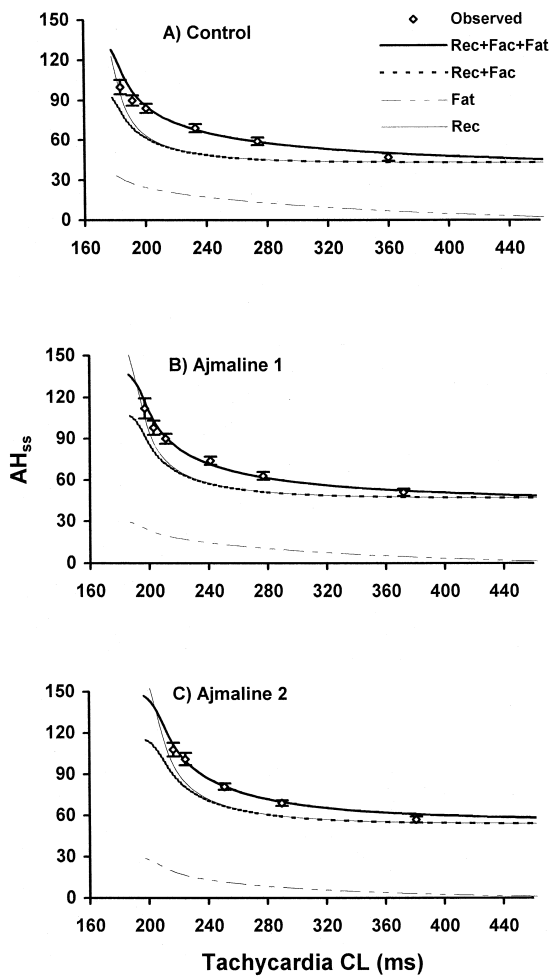

Fig. 8. Predicted rate-dependent changes in steady state AV conduction time (AH_{ss}) due to recovery, recovery modified by facilitation, and fatigue as a function of tachycardia cycle length (CL). Mean characterizing constants determined experimentally (Table 2) were substituted into equations explained in the text to obtain predictions shown. Changes due to recovery (Rec) and facilitation (Fac) were added to those resulting from fatigue (Fat) to obtain the total predicted rate-dependent AV conduction time prolongation (heavy lines). There is good agreement between model calculations and observed data.

Statistical analysis shows that the model based on the sum of all parameters (total) is in close agreement with the observed data. Effects of ajmaline on AV conduction time are shown in Fig. 8B and C, indicating that ajmaline's rate-dependent effects are well described by the same model incorporating quantitative indices of basic recovery, facilitation, and fatigue.

In order to apply the above mathematical model to explain the ajmaline effect during AV re-entrant tachycardia, one should estimate the ratio of AV effective refractory period divided to tachycardia cycle length under each set of conditions. Based on the theory explained earlier tachycardia would no longer be sustained if AV effective refractory period divided to tachycardia cycle length exceeds unity as shown in Fig. 7D. We therefore used the same model to estimate AV effective refractory period as a function of the recovery interval of a tachycardia. For all protocols under a given set of conditions, we observed that the maximum AV conduction time for conducted beats during the determination of each recovery curve was relatively constant. To estimate the shortest recovery interval permitting conduction for any set of basic recovery, facilitation and fatigue conditions, Eq. (3) was rearranged to solve for the shortest recovery interval permitting conduction ($HA_{shortest}$) as follows:

$$HA_{shortest} = -\tau_{rec}\ln\left[\left(AH_{max} - AH_{\infty} - \Delta AH_{fat}\right)/A\right] - \Delta HA_{fac} \quad (4)$$

Where τ_{rec}, AH_{∞}, ΔAH_{fat}, A, and ΔHA_{fac} have the meaning indicated above, and AH_{max} is the longest AV conduction time attainable for conducted beats during the basic recovery protocol. Addition of AV conduction time estimated from Eq. (3) to $HA_{shortest}$ estimated from Eq. (4) allows for calculation of the longest atrial coupling interval at which conduction failed (AV effective refractory period).

The solid curves in Fig. 7B show the model-determined AV effective refractory period under control conditions and during ajmaline administration, as a function of tachycardia cycle length. AV effective refractory period is prolonged by ajmaline to a substantially greater extent as the AV re-entrant tachycardia cycle length decreases. There is good agreement between model calculations and observed data.

Table 3
Comparison between different models used to predict AH_{ss} and AVERP

Slope	AH_{ss}					AVERP				
	Fat	Rec + Fac	Rec + Fat	Rec	Total	Fat	Rec + Fac	Rec + Fat	Rec	Total
Con	$0.452^{c} \pm 0.070$	$0.734^{c} \pm 0.063$	$1.380^{c} \pm 0.064$	$0.890^{a} \pm 0.054$	1.058 ± 0.046	$0.463^{c} \pm 0.087$	$0.742^{c} \pm 0.074$	$1.404^{c} \pm 0.080$	$0.742^{c} \pm 0.062$	1.055 ± 0.050
Ajm_1	$0.320^{c} \pm 0.082$	$0.771^{c} \pm 0.064$	$1.292^{c} \pm 0.069$	$0.850^{b} \pm 0.060$	1.074 ± 0.052	$0.340^{c} \pm 0.091$	$0.733^{c} \pm 0.101$	$1.561^{c} \pm 0.076$	$1.172^{b} \pm 0.059$	1.111 ± 0.059
Ajm_2	$0.298^{c} \pm 0.105$	$0.675^{c} \pm 0.071$	$1.180^{c} \pm 0.093$	$0.799^{c} \pm 0.076$	1.035 ± 0.063	$0.310^{c} \pm 0.088$	$0.777^{c} \pm 0.096$	$1.270^{c} \pm 0.099$	$0.875^{a} \pm 0.086$	1.084 ± 0.065

Comparison by analysis of regression to the observed values and different model-predicted values. If the best regression is assumed to be one (unity), therefore any deviation from unity is considered statistically different from observed values. AH_{ss}, steady state AV conduction time; AVERP, AV effective refractory period; Con, control; Ajm_1, ajmaline dose 1; Ajm_2, ajmaline dose 2, Rec, recovery; Fac, facilitation; Fat, fatigue; and Total, recovery + facilitation + fatigue.

[a] $P < 0.001$ comparing the slopes to unity.

[b] $P < 0.05$ comparing the slopes to unity.

[c] $P < 0.01$ comparing the slopes to unity.

The model can now be used to estimate both tachycardia cycle length (Fig. 7A) and AV effective refractory period (Fig. 7B) for experimental AV re-entrant tachycardia at any recovery interval and therefore to estimate the AV effective refractory period divided to tachycardia cycle length as a function of tachycardia rate. As tachycardia rate increases, AV effective refractory period divided to tachycardia cycle length increases under all conditions (Fig. 7D). In the presence of ajmaline, the curves are displaced upward, indicating prolonged AV node refractoriness at all cycle lengths. Furthermore, the slopes of the curves increase with increasing concentration of ajmaline, showing that ajmaline increases the rate-dependence of AV effective refractory period divided totachycardia cycle length. Experimental results agree well with model-predicted behavior, with sustained tachycardia no longer inducible at cycle lengths similar to those with a model-estimated AV effective refractory period divided to tachycardia cycle length was greater than 1.

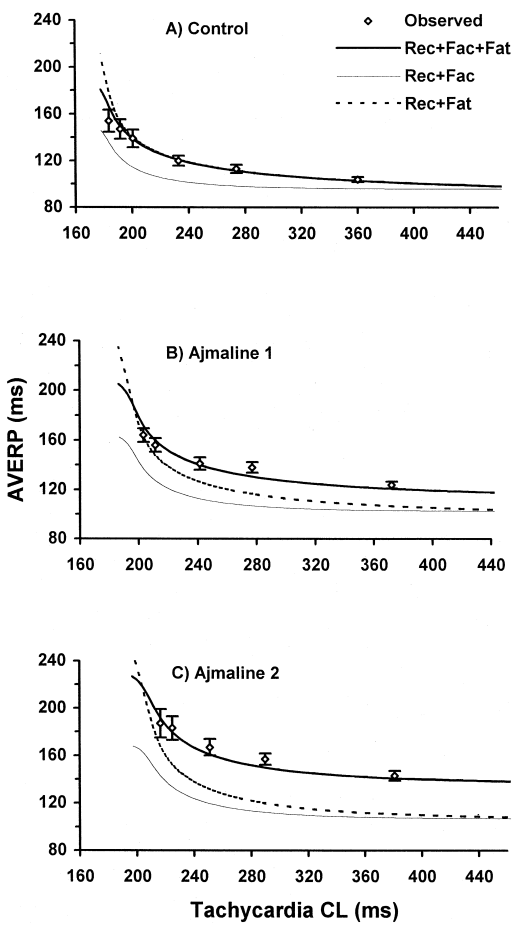

Fig. 9. Predicted rate-dependent changes in AV effective refractory period (AVERP) as a function of tachycardia cycle length. Under control conditions (panel A) predicted rate-dependent changes in AV effective refractory period due to incomplete recovery, facilitation and fatigue are in close agreement with experimentally observed values. However the other models based on recovery plus fatigue (Rec + Fat) are not in close agreement with observed data. Panels B and C show model-predicted AV effective refractory period and observed values after dose 1 and dose 2 of ajmaline respectively. The model based on combined recovery, facilitation and fatigue (Rec + Fac + Fat) predicts the values, which are in close agreement with observed data.

Fig. 9 shows the relative contribution of recovery, facilitation and fatigue to ajmaline's effects on AV effective refractory period. As is evident from the solid lines, changes due to combined recovery, facilitation and fatigue can well predict the observed values.

4. Discussion

Our study showed that ajmaline modifies the rate-dependent functional properties of rabbit AV node somewhat differently from the other pharmacological interventions (Nayebpour et al., 1990, 1993). Our results also showed that the actions of ajmaline contribute to its ability to suppress AV re-entrant tachycardia in an experimental model.

Ajmaline has been classified as a class I antiarrhythmic agent and has been used widely in clinical studies (Wellens et al., 1980; Manz and Luderitz, 1988; Luderitz and Manz, 1992; Chen et al., 1994). Ajmaline prolongs AV conduction and refractoriness. However, none of the previous studies have evaluated in detail the electrophysiological actions of ajmaline on the AV node. Stark et al. (1996) have shown that ajmaline prolongs AV conduction time and nodal refractoriness in a frequency-dependent fashion. They also noted that the effect of ajmaline on refractoriness is more pronounced than its effect on conduction time. In this study, we used for the first time a previously defined protocol to analyze the effect of ajmaline on the AV node (Billette et al.,1986, 1988; Billette and Metayer, 1989; Nayebpour et al., 1992, 1993). It is well documented that nodal delays can be explained by dynamic interactions between the three intrinsic properties of recovery, facilitation, and fatigue. The dynamics of these properties have been studied with selective stimulation protocols and have many implications for the understanding of nodal behavior in the context of supraventricular tachyarrhythmias and mechanisms of actions of many pharmacological agents.

4.1. Comparison with previous studies of ajmaline effects on the AV node

Stark et al. (1996) used isolated guinea pig hearts perfused by the method of Langendorff and found that ajmaline depresses AV conduction and refractoriness. In our study, we used isolated rabbit AV node superfused with Tyrode's solution. Using specific stimulation and recording techniques, we were also able to control and dissociate different rate-dependent properties of AV node. This enabled us to quantify the ajmaline effects on the individual rate-dependent properties of the AV node. While the previous study only showed the net effects of ajmaline

on the AV node, our study described the electrophysiological mechanism of ajmaline's effects.

4.2. Possible mechanisms of ajmaline effects on AV node properties

The mechanisms underlying rate-dependent AV node properties remain incompletely understood. It is possible that the time-dependent recovery of calcium channels from inactivation plays a major role in determining the AV node curve (Talajic and Nattel, 1986). Changes in action potential duration in the distal AV node are likely involved in facilitation (Billette, 1987; Mazgalev and Dreifus, 1991).

Our results showed that ajmaline increases the degree of facilitation. Therefore it is possible that the prolongation of AV node action potentials by ajmaline (Enomoto et al., 1995) may provide more action potential shortening, resulting from the rate increase. Fatigue effects are clearly associated with reduced excitability of nodal cells, particularly those in the central node (Meredith et al., 1968). However, the underlying mechanisms have remained elusive. Jenkins and Belardinelli (1988) and Nayebpour et al. (1993) suggested that effects of adenosine may account for a significant proportion of the fatigue-induced conduction slowing at rapid rates. Our results show that the magnitude of fatigue is slightly reduced by ajmaline. Therefore, ajmaline modified the process of rate-induced fatigue via different mechanisms. Taking into consideration the multifactorial mechanisms of fatigue (Billette et al., 1988), it is possible that ajmaline, by affecting the amount of intracellular calcium accumulation or depletion of ions, reduces the magnitude of fatigue. Further study need to be done to clarify these mechanisms.

4.3. Significant effects of ajmaline on nodal refractoriness and its ability to prevent sustained AV re-entrant tachycardia

Our results show that ajmaline had more pronounced effects on nodal refractory period than on conduction (40% prolongation in AV effective refractory period compared to 16% nodal delay by the second dose of ajmaline). It shows that ajmaline has more selective effects on AV effective refractory period than on nodal conduction. Nodal refractoriness determines the minimum cycle length with which the ventricles may be activated, and can thereby determine cardiac function in the presence of supraventricular tachyarrhythmias. Three indices of nodal refractoriness have been developed. One is the effective refractory period, which is the longest premature atrial interval that fails to generate a nodal response during a premature stimulation. Our results show that ajmaline significantly prolonged AV effective refractory period in a frequency-dependent manner. Therefore, agents such as ajmalin that increase AV node refractoriness would be expected to suppress AV re-entrant tachycardia more easily, since the AV node is already partially refractory before therapy. Furthermore, the ajmaline-induced increase in AV node refractoriness prevailed over AV node conduction slowing and led to a rate-dependent increase in wavelength. The increase in wavelength caused selective termination of faster tachycardias. The significant prolongation of nodal refractory period by ajmaline compared to its effect on nodal conduction indicates that ajmaline has a dual action. The first action could be its Na^+-blocking effect that is more prevalent in the proximal portion of the AV node (Petrecca et al., 1997). Billette (1987) has shown that atrio-nodal cells in the distal portion of the AV node have a higher resting membrane potential (more negative potential compared to mid-nodal cells in the AV node). This indicates that the Na^+-channel would be effective in AN cells, and these cells could determine the effective refractory period. It would be interesting to study the effects of other classical Na^+-channel blockers in the model described.

On the other hand, Enomoto et al. (1995) have shown that ajmaline prolongs action potential duration by inhibiting the inward portion of the inward rectifying K^+ current (I_{K1}) and decreases the delayed rectifier K^+ current (I_K). Therefore, it is possible that, by these effects, ajmaline prolongs action potential duration and refractoriness. Further study has to be done to clarify these effects.

4.4. Mathematical modeling

A mathematical model incorporating quantitative descriptors of nodal recovery, facilitation, and fatigue can account for the steady state rate-dependence of AV node conduction time (Nayebpour et al., 1991), a variety of Wenckebach block patterns (Talajic et al., 1991) and the rate-dependent effects of cholinergic, adrenergic and adenosine (Nayebpour et al., 1990, 1992, 1993). Our results and mathematical model showed that the effects of ajmaline on the AV node can be explained based on its action on the three rate-dependent properties.

4.5. Potential clinical importance of ajmaline's effects

Most tachyarrhythmias are caused by re-entry and there are several ways to subdivide re-entrant mechanisms (such as ordered and random re-entry, functional, anatomical and anisotropic re-entry). These re-entrant circuits have a short excitable gap (anatomical, functional or anisotropic mechanisms). The most obvious goal for treatment would be to prolong the refractory period (Task force of the working group on arrhythmias of the European Society of Cardiology, 1991). Our data show that ajmaline would be clinically effective to control AV re-entrant tachycadia by prolonging refractoriness.

5. Potential limitation

The isolated, superfused preparation allows for very stable, precise measurements and excellent control of the recovery variable by coupling atrial stimulation to His bundle activation (Billette, 1976, 1981; Billette et al., 1986, 1988). However, due to superfusion of the preparation, which does not allow for intra-coronary administration, high ajmalin concentrations (10-fold higher than Langendorff preparation) were required to show the same electrophysiological effect. The same limitation has been reported previously (Miyazaki et al., 1996; Henelt et al., 1996). Our experimental model of AV re-entrant tachycardia involved an electronic circuit coupling stimulation of the atrium to activation of His bundle. This is different from clinical AV re-entrant tachycardia, in that atrial activation is coupled to ventricular activation by a relatively constant ventriculo-atrial interval via an accessory pathway. Because His-ventricular interval is relatively fixed, however, the only difference between our model and one coupling atrial to ventricular activation is a constant interval. Of course, our model does not simulate other aspects of clinical AV re-entrant tachycardia, such as refractoriness of the accessory pathway. Therefore, our results should be viewed in terms of ajmaline's effect on the antegrade limb of the AV re-entrant tachycardia which is the AV node itself.

6. Conclusions

We have shown that ajmaline changes AV node rate-dependent properties in isolated rabbit preparations. These changes result in AV node conduction slowing and in prolongation of nodal refractoriness. Both of these are important factors controlling AV re-entrant tachycardia. Mathematical descriptors of recovery, facilitation, and fatigue account for the rate-dependent conduction and refractoriness properties of the in vitro rabbit AV node in both the absence and presence of ajmaline.

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