

Roles of Adrenergic and Cholinergic Stimulation in Spontaneous Atrial Fibrillation in Dogs

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OBJECTIVES	We studied the effects of beta-adrenergic and cholinergic stimulation and blockade on spontaneous atrial fibrillation (AF) in the intact dog heart.
BACKGROUND	Paroxysmal AF is often preceded by changes in autonomic tone, but the relative roles of adrenergic and cholinergic influences on AF induction are not well known.
METHODS	Perfusion of catecholamines and acetylcholine (ACh), as well as their combination, through the sinus node artery was used to induce AF in 20 anesthetized open-chest dogs without electrical stimulation of atria.
RESULTS	Isoproterenol and adrenaline (10 to 100 $\mu\text{mol/l}$) induced AF in 21% (3 of 14) and 17% (1 of 6) of dogs, respectively. Atropine (1 to 2 mg) treatment prevented catecholamine-mediated AF, indicating a critical role of cholinergic tone in these AF episodes. Acetylcholine ($2.8 \pm 0.3 \mu\text{mol/l}$) induced AF in all dogs. Beta-blockade by propranolol (1 mg/kg) did not prevent ACh-induced AF, but increased the threshold ACh concentration for AF induction to $23.5 \pm 3.4 \mu\text{mol/l}$ ($p < 0.05$). Acetylcholine-mediated AF was facilitated by isoproterenol (1 to 2 and 10 $\mu\text{mol/l}$), which decreased the threshold ACh concentration for AF induction to 0.5 ± 0.1 and $0.4 \pm 0.1 \mu\text{mol/l}$, respectively ($p < 0.05$) and increased the AF duration (from 25 ± 7 to 141 ± 54 and 233 ± 60 s, respectively; $p < 0.05$). Epicardial mapping of the right atrium (112 unipolar electrodes) demonstrated similar activation patterns during arrhythmias induced by ACh and catecholamines.
CONCLUSIONS	These data indicate that although both autonomic systems play a role in AF, cholinergic stimulation is likely the main factor for spontaneous AF initiation in this animal model. Adrenergic tone modulates the initiation and maintenance of cholinergically mediated AF. (J Am Coll Cardiol 2004;43:483–90) © 2004 by the American College of Cardiology Foundation

Neural mechanisms are believed to play a critical role in paroxysmal atrial fibrillation (AF) (1,2). However, the underlying mechanisms of neural paroxysmal AF have not yet been clearly demonstrated. Although both vagal stimulation and ACh application can result in AF in experimental animals without electrical stimulation of atria (3–6), it is not known whether beta-adrenergic stimulation produces the same effect. Recent clinical studies have shown conflicting patterns of autonomic tone fluctuations before AF onset in different groups of patients (2,7,8). It was suggested that autonomic imbalance is probably more important for AF initiation than enhanced vagal or sympathetic drive alone (7), but the relative role of adrenergic and cholinergic influences on AF induction is not well known. Therefore, this work was designed to study the effects of beta-adrenergic and cholinergic stimulation and blockade on spontaneous AF in the intact dog heart.

METHODS

Surgical procedures. Normal mongrel dogs ($n = 20$) weighing 16 to 28 kg were anesthetized with pentobarbital

(30 mg/kg intravenously [IV]) and maintained under physiologic conditions, as described elsewhere (5,6). Both vagi were ligated and decentralized in the cervical region. A right thoracotomy was performed at the fourth intercostal space, and the sinus node artery (SNA) was cannulated, as described elsewhere (5,9–11). Two bipolar electrodes and a multi-electrode epicardial template (Fig. 1) were sewn to the posterior right atrium (RA).

Experimental protocol. The SNA perfusions with oxygenated Tyrode's solution were delivered by an infusion pump (model 22, Harvard Apparatus Ltd., South Natick, Massachusetts) at a flow rate of 1.2 ml/min for 3 min and 9 ml/min for 1 min. The ionic composition of Tyrode's solution was (in mmol/l): NaCl 131, KCl 4, CaCl₂ 2.7, MgCl₂ 0.5, NaHCO₃ 18, NaH₂PO₄ 1.8; glucose 5.5. The temperature of the perfusate was at $37 \pm 1^\circ\text{C}$, and the pH was 7.35 ± 0.05 . The perfusion at 1.2 ml/min had no effect on the sinus rate (Fig. 2A, left), whereas the perfusion at 9 ml/min induced a slight sinus rate slowing (Fig. 2A, right).

Then we perfused the SNA with acetylcholine (ACh) at the flow rate of 9 ml/min. This perfusion rate was used to provide a rapid and reliable effect of neurotransmitters on the sinus node region that is usually well supplied by collateral circulation (9,10). The perfusion was stopped after 1 min if no AF occurred or immediately after AF onset. We determined the threshold (minimum) arrhythmogenic concentration of ACh for reproducibility (3 times) of AF

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Abbreviations and Acronyms

- ACh = acetylcholine
- AF = atrial fibrillation
- AFCL = atrial fibrillation cycle length
- APB = atrial premature beat
- FWRA = free wall of right atrium
- IVC = inferior vena cava
- RA = right atrium
- RAA = right atrial appendage
- SNA = sinus node artery
- SVC = superior vena cava

induction by perfusion with incremental ACh concentrations of 1, 3, 6, and 10 $\mu\text{mol/l}$. Intervals between perfusions were 5 to 10 min. The threshold for arrhythmogenic ACh concentration, as defined in this manner, did not change during the sham experiments (11).

To study the effect of adrenergic stimulation, we then perfused the SNA with incremental concentrations (1 to 2 and 10 $\mu\text{mol/l}$) of adrenaline (alpha- and beta-adrenergic stimulation, $n = 6$) and isoproterenol (beta-adrenergic stimulation, $n = 14$). In addition, perfusions with 100 $\mu\text{mol/l}$ isoproterenol ($n = 2$) and adrenaline ($n = 6$) were carried out in eight dogs. We used two perfusion patterns, repeated up to three times, at a flow rate of 1.2 ml/min for 3 min and 9 ml/min for 1 min in 10 to 25 min for restoration. Perfusions with 1 to 2 $\mu\text{mol/l}$ catecholamines did not change the arterial blood pressure. Perfusion at 9 ml/min with 10 $\mu\text{mol/l}$ catecholamines transiently increased systolic and diastolic blood pressure to 154 ± 8 and 108 ± 5 mm Hg, respectively ($p < 0.05$ vs. control: 135 ± 5 and 93 ± 3 mm Hg), as did 100 $\mu\text{mol/l}$ catecholamines (211 ± 18 and 149 ± 15 mm Hg [1.2 ml/min] and 245 ± 18 and 173 ± 15 mm Hg [9 ml/min]; $p < 0.001$ vs. control for both).

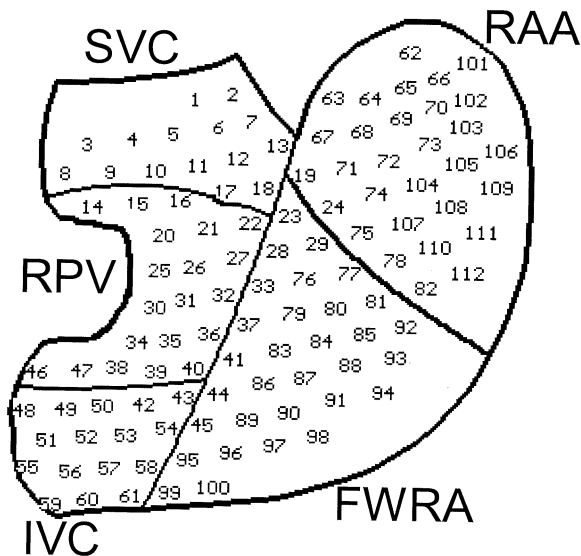


Figure 1. Tracings of the electrode array with main anatomic landmarks. Numbers indicate sites of unipolar electrodes. FWRA = free wall of right atrium; IVC = inferior vena cava; RAA = right atrial appendage; RPV = right pulmonary vein; SVC = superior vena cava.

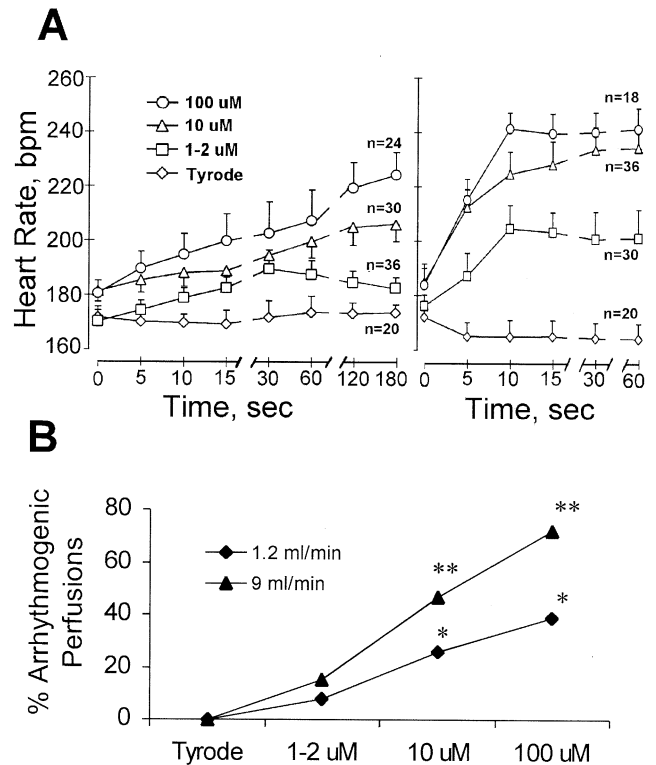


Figure 2. (A) Effects of sinus node artery perfusion with 1 to 2, 10, and 100 $\mu\text{mol/l}$ ($\mu\text{mol/l}$) catecholamines at a flow rate of 1.2 ml/min for 3 min (left) and 9 ml/min for 1 min (right) on heart rate. “n” indicates the number of perfusions. All curves differ substantially from baseline. $p < 0.05$ for all individual comparisons. (B) Percentage of perfusions that induced at least one atrial tachyarrhythmia. * $p < 0.05$ vs. Tyrode’s solution. ** $p < 0.05$ vs. 1.2 ml/min.

If AF or multiple atrial premature beats (APBs) occurred during catecholamine application, that animal (after spontaneous tachyarrhythmia termination) was treated with atropine (1 to 2 mg IV and 1 $\mu\text{mol/l}$ in perfusate), and the perfusions with the same and higher catecholamine concentration were repeated. In total, atropine was administered in six dogs.

In 12 dogs, we studied the effects of simultaneous beta-adrenergic and cholinergic stimulation. After the perfusions with 1 to 2 and 10 $\mu\text{mol/l}$ isoproterenol were performed, a new threshold concentration of ACh for AF induction was determined in the presence of isoproterenol (1 to 2 and 10 $\mu\text{mol/l}$, respectively), as described earlier. Finally, propranolol was administered in eight dogs (1 mg/kg IV and 1 $\mu\text{mol/l}$ in perfusate), and again the threshold for the arrhythmogenic ACh concentration was determined.

We analyzed only APBs with a coupling interval < 200 ms. Atrial fibrillation was defined as rapid ($> 500 \text{ min}^{-1}$) atrial tachyarrhythmias (> 10 APBs) with varying morphology on the atrial electrogram and with an irregular ventricular rhythm.

Electrodes, mapping, and monitoring techniques. The Silastic sheet containing 112 unipolar silver electrodes (diameter of 1 mm, interelectrode distance of 4 to 6 mm)

was sewn to the atrial epicardial surface to cover the intercaval region, posterior RA appendage (RAA), and free wall of the RA (FWRA).

The computer system employed for acquisition and analysis of the atrial electrographic data has been described previously (6). Signals from 112 unipolar electrograms were recorded at a frequency response of 0.05 to 500 Hz and digitized at a sampling rate of 1.0 kHz/channel with 10-bit resolution. Segments of data recorded were used to automatically determine activation moments on the electrogram ($-dV/dT_{max}$) and to generate isochronal activation maps. All data were inspected to verify and correct the computer-picked activation moments. The activation time at sites at which multiple component electrograms or two discrete deflections for one atrial complex were recorded was assigned as described elsewhere (12). Electrocardiographic lead II, arterial blood pressure (measured in a femoral artery), and two RA bipolar electrograms were continuously monitored (Polysystem-EP/H, Astrocard, Meditek, Moscow, Russia).

Spatial distribution analysis. We analyzed the impulse origin during regular rhythm and APBs. The intercaval region was divided into three local regions relative to the superior vena cava (SVC), right pulmonary vein, and inferior vena cava (IVC) (Fig. 1). Beats originating in the intercaval region were considered "normotopic," because they corresponded to the impulse origin in the control study (5,6), whereas beats originating in the RAA and FWRA were regarded as "ectopic." We analyzed activation patterns of AF onset. Then, 0.5 to 1 s after AF onset, we determined the mean AF cycle length (AFCL) for a 1-s period at 16 recording sites in each of the five atrial regions selected (Fig. 1). The standard deviation in AFCL was used as an index of the heterogeneity of AFCL over the RA.

Statistical analysis. Data are presented as the mean value \pm SEM. All analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, Illinois). The statistical evaluation of the data was performed by using the Student *t* test or analysis of variance, as appropriate. Changes within a group were analyzed by using a univariate general linear model procedure with mixed effects. Chi-square analysis with Yates correction was performed to evaluate the incidence of APBs and AF and the statistical significance of a change in impulse origins. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Perfusion of SNA with catecholamines. The effects of SNA perfusion with isoproterenol and adrenaline were similar and are presented together. Perfusion of SNA with catecholamines (1 to 2, 10, and 100 $\mu\text{mol/l}$) resulted in a concentration- and flow rate-dependent increase in both background regular heart rate (Fig. 2A) and arrhythmia occurrence (Fig. 2B). At a flow rate of 1.2 ml/min, catecholamine application induced APBs in all dogs ($n = 20$).

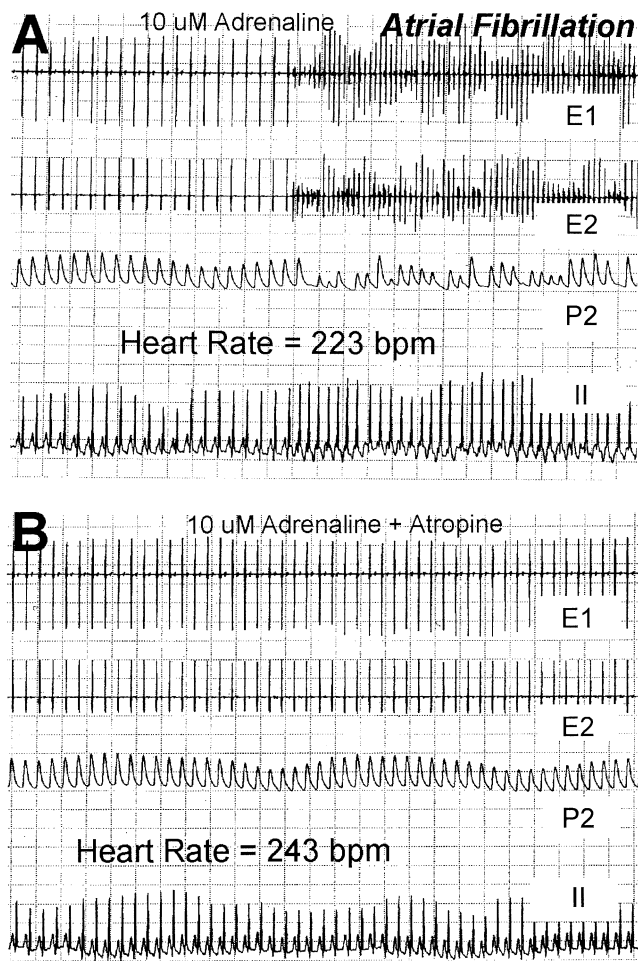


Figure 3. Catecholamine-mediated atrial fibrillation (A) and its prevention by atropine (B). The sinus node artery perfusion at 9 ml/min with 10 $\mu\text{mol/l}$ (μM) adrenaline repeatedly induced atrial fibrillation in ~ 25 s after the start of perfusion. With atropine, no tachyarrhythmic events were induced by adrenaline perfusion, despite the enhanced effect on heart rate. E1 and E2 = atrial bipolar electrograms; P2 = blood pressure recording; II = standard electrocardiographic lead II.

The perfusion at 9 ml/min resulted in both APBs in all dogs and AF episodes in four dogs, as illustrated in Figure 3A. Isoproterenol concentrations inducing AF were 10 $\mu\text{mol/l}$ (2 dogs) and 100 $\mu\text{mol/l}$ (1 dog), and the adrenaline concentration was 10 $\mu\text{mol/l}$ (1 dog). These AF episodes ($n = 7$) arose 23.6 ± 2.8 s after perfusion onset and lasted for 64 ± 19 s.

Generation of catecholamine-mediated AF was completely suppressed by pretreatment with atropine (Fig. 3B). In addition, atropine reduced the occurrence and number of catecholamine-mediated APBs in all experiments ($n = 6$) (Table 1). Catecholamine perfusions resulted in a higher background regular heart rate with atropine than without atropine (Table 1).

Perfusion of SNA with ACh: effects of isoproterenol and propranolol. Acetylcholine induced AF in all dogs ($n = 20$), with a threshold ACh concentration of 2.8 ± 0.3 $\mu\text{mol/l}$. Figure 4A demonstrates an episode of ACh-

Table 1. Tachyarrhythmias During Sinus Node Artery Perfusion With Catecholamines Before and After Atropine

Drugs	Concentration ($\mu\text{mol/l}$)	Background Heart Rate (beats/min)	Arrhythmogenic Perfusions (n)	Total Arrhythmias (n)	Single APB (n)	Two APBs (n)	<10 APBs (n)	AF (n)
Catecholamines	28 ± 10	228 ± 10	15	33	13	8	5	7
Catecholamines and atropine	46 ± 12	$245 \pm 9^*$	8*	15*	11	3	1	0*

* $p < 0.05$ vs. catecholamines. Data from six dogs (adrenaline, $n = 2$; isoproterenol, $n = 4$; total of 15 perfusions). Concentration and background heart rate values are presented as the mean \pm SEM.

APB = atrial premature beat.

mediated AF. The AF onset was preceded by slowing of the sinus rate, which was similar to slowing caused by perfusion with Tyrode's solution alone (Fig. 2A). The AF paroxysms started ~ 10 s after perfusion onset and lasted ~ 25 s. Acetylcholine-induced AF was modulated by background beta-adrenergic tone (Table 2). With adrenergic tone enhanced by isoproterenol, ACh-induced AF occurred later after perfusion onset (~ 20 to 25 s) and either without heart rate slowing or at a faster rate than that before perfusion

(Fig. 4B). The threshold ACh concentration for AF induction became significantly smaller, so that AF could be induced by ACh in concentrations ranging from 1 nmol/l to 1 $\mu\text{mol/l}$. Isoproterenol also resulted in significant AF prolongation (Table 2).

Inhibition of adrenergic tone by propranolol did not prevent ACh-mediated AF but resulted in increasing threshold ACh concentrations necessary for AF induction and a shortening duration of AF paroxysms. There was

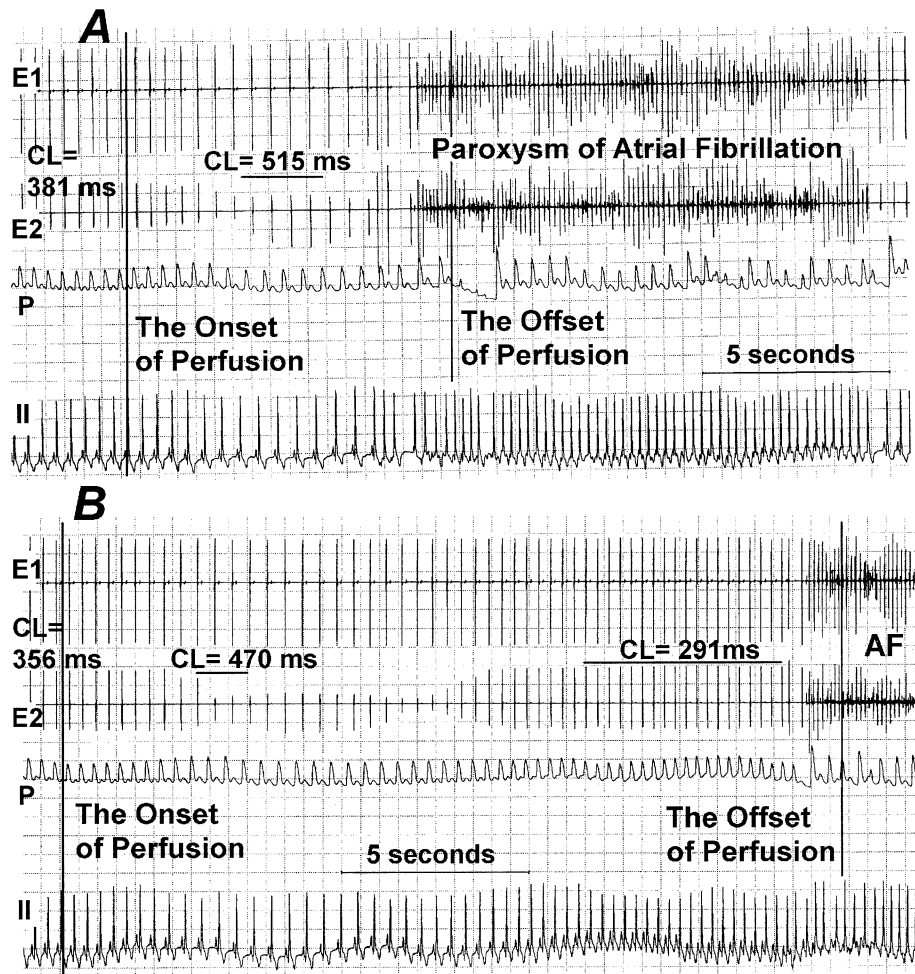


Figure 4. Atrial fibrillation induced by 1 $\mu\text{mol/l}$ acetylcholine (ACh) (A) and 0.1 $\mu\text{mol/l}$ ACh and 2 $\mu\text{mol/l}$ isoproterenol (B). In the control experiment, an ACh concentration of 1 $\mu\text{mol/l}$ was the threshold that repeatedly induced atrial fibrillation (AF) in that dog. The perfusion onset led to sinus rhythm slowing. Seven seconds after the start of perfusion, the atrial premature beat triggered AF. The perfusion was immediately stopped, and the paroxysm lasted for 12 s. Perfusion with ACh and isoproterenol resulted in less sinus rhythm slowing, and the paroxysm arose from a stable and faster rhythm than that before perfusion. Notably, the ACh concentration used to induce AF was 10 times less than that without isoproterenol. This paroxysm started with some delay and lasted for 90 s (not shown). CL = cycle length; E1 and E2 = atrial bipolar electrograms; P2 = blood pressure recording; II = standard electrocardiographic lead II.

Table 2. Effects of Isoproterenol and Propranolol on Acetylcholine-Mediated Atrial Fibrillation

Variables	Heart Rate Before Perfusion (beats/min)	Heart Rate Before AF (beats/min)	Threshold ACh ($\mu\text{mol/l}$)	Time of AF Onset (s)	AF Duration (s)
Control (n = 12)	157.4 \pm 4.1	141.5 \pm 6.8	2.8 \pm 0.3	9.7 \pm 1.7	25.4 \pm 6.9
1-2 $\mu\text{mol/l}$ isoproterenol (n = 12)	186.2 \pm 4.6*	184 \pm 9.8*	0.5 \pm 0.1*	19.9 \pm 4.2*	141.2 \pm 53.5*
10 $\mu\text{mol/l}$ isoproterenol (n = 9)	197.2 \pm 4.4*	200.6 \pm 8.2*	0.4 \pm 0.1*	26.5 \pm 7.8*	232.8 \pm 59.2*
Propranolol (n = 8)	115.4 \pm 7.3*†	79.9 \pm 10.7*††	23.5 \pm 3.4*†	21.2 \pm 6.9	74.7 \pm 40.9†

*p < 0.5 vs. control. †p < 0.05 vs. isoproterenol. ††p < 0.05 vs. heart rate before perfusion. Data are presented as the mean value \pm SEM. n = number of experiments. ACh = acetylcholine; AF = atrial fibrillation.

significant slowing of the heart rate preceding AF onset (Table 2).

Activation mapping data. In this work, we mapped the RA area because it corresponds to the distribution of SNA flow (10). In addition, our previous study (5) showed that ACh administration into the SNA produced focal or reen-

trant rapid activity only in the RA, predominantly in the intercaval region. In the control study, mapping revealed normal activation patterns (5,6), with the excitation starting in the sinoatrial node region and spreading smoothly over the RA for 35 to 45 ms. During ACh infusion, 100% impulses of regular rhythm arose in the intercaval region

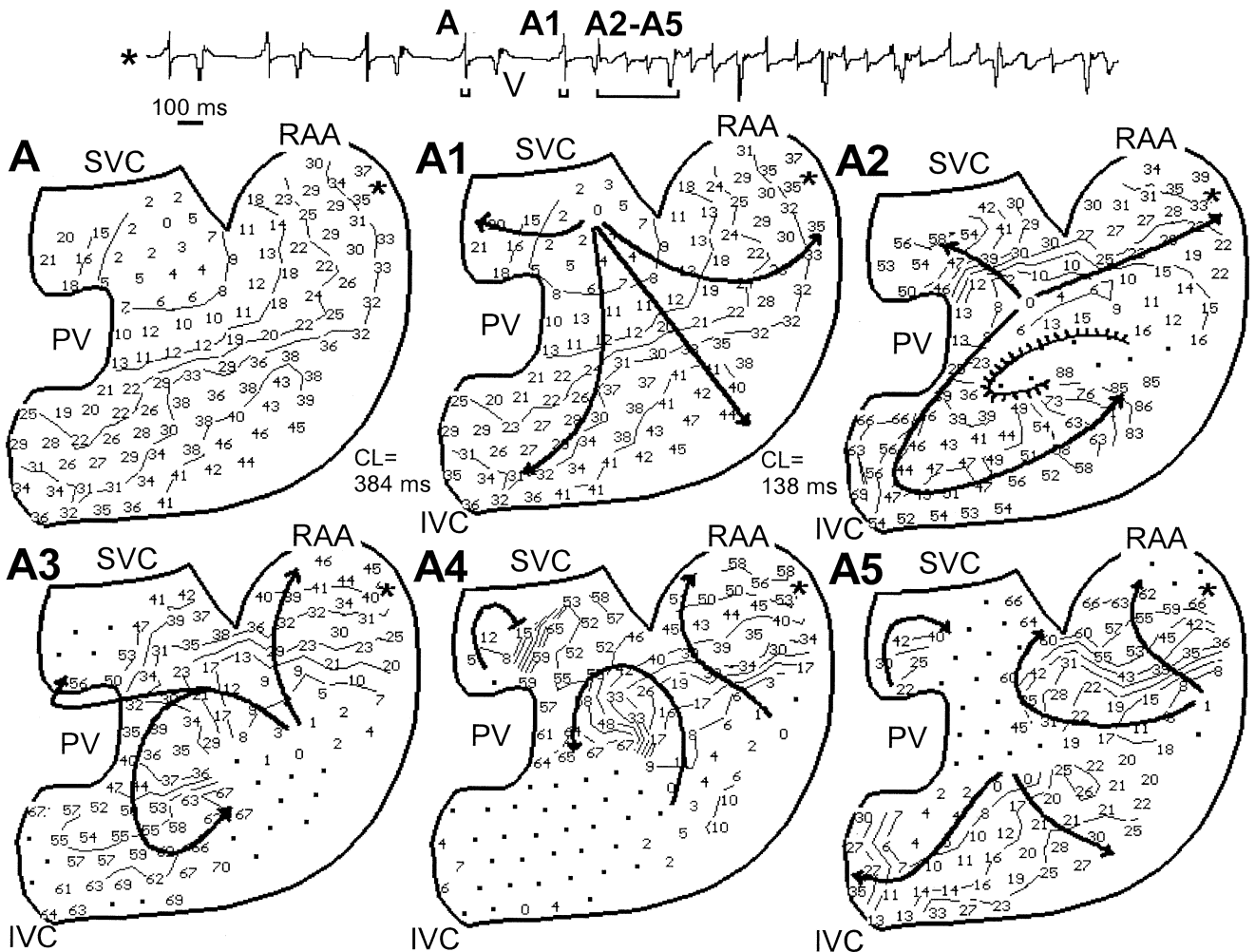


Figure 5. Atrial fibrillation induced by 1 $\mu\text{mol/l}$ acetylcholine. On the atrial unipolar electrogram at the top, A indicates atrial signal and V indicates ventricular signal. The impulses A to A5 are those in the subsequent maps. The impulses A and A1 are shown as isolated beats, and the impulses A2 to A5 are shown as consecutive cycles. Small squares indicate sites of unipolar electrodes; numbers indicate the activation time. Lines show isochrones. Arrows show the spread of excitation over the right atrium. This paroxysm started from a stable sinus rhythm with a cycle length (CL) of 384 ms. Beats A and A1 had a normal activation sequence similar to that in control (not shown). The earliest activation site was in the sinoatrial node region. The right atrium was activated for 45 ms. An atrial premature beat (A1) following beat A1 at a CL of 138 ms arose from the crista terminalis. In the free wall of right atrium, the conduction was blocked, and the impulse turned around an area of block counterclockwise. The area distal to the initial block was then activated retrogradely, and the impulse A3 re-entered the sites proximal to the initial block. Beats A4 and A5 were also re-entrant with a shortening revolution time. PV = pulmonary vein. Other abbreviations as in Figure 1.

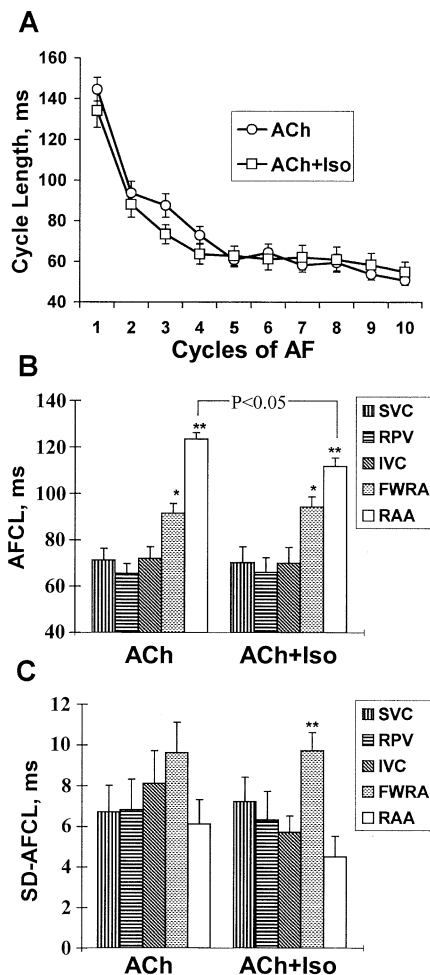


Figure 6. (A) The atrial fibrillation cycle length (AFCL) during the first 10 cycles of atrial fibrillation (AF) induced by acetylcholine (ACh) alone and ACh with isoproterenol (Iso). (B) The AFCL in different regions of the right atrium. * $p < 0.05$ vs. SVC, RPV, and IVC. ** $p < 0.001$ vs. all regions. (C) The standard deviation (SD)-AFCL in different regions of the right atrium. Abbreviations as in Figure 1.

and had a normal activation pattern, as illustrated in Figure 5 (map A). During arrhythmias induced by ACh infusion, the impulses tended to start from the ectopic regions of the RA. Although only 8% of ACh-mediated arrhythmia A1 beats (usually being part of regular rhythm) were ectopic, whereas the occurrence of ectopic A2 beats increased to 23% ($p < 0.05$ vs. Tyrode's solution). Figure 5 shows the activation sequence of AF induced by 1 $\mu\text{mol/l}$ of ACh. In this dog, APB (beat A2) from the crista terminalis with a short coupling interval of 138 ms resulted in conduction block and reentrant excitation in the FWRA. Arrhythmias induced by ACh with isoproterenol had a similar spatial distribution of beats A1 and A2 (13% and 26% of ectopic beats, respectively; $p < 0.05$ vs. Tyrode's solution) and similar activation patterns during AF onset.

There was no difference in the time-course dynamics during the first 10 cycles of these AF episodes (Fig. 6A). Although focal patterns occurred mostly in the intercaval region, reentrant excitation (macroreentry) mostly formed

in the RAA and FWRA regions, with a significant increase in AFCL, as compared with the intercaval regions (Fig. 6B), due to the SNA flow distribution (10). Although the AFCL in the RAA decreased with isoproterenol (Fig. 6B), the average AFCL over the RA did not change (82.4 ± 5 vs. 84.8 ± 3.3 ms in control; $p = \text{NS}$). The AFCL heterogeneity (standard deviation in AFCL) over the RA also did not change (22.7 ± 1.6 vs. 26.5 ± 1.4 ms in control; $p = \text{NS}$) (Fig. 6C). There was no difference in the average interval of ventricular responses (247.5 ± 8.5 vs. 248.9 ± 8.8 ms in control; $p = \text{NS}$). During both the regular rhythm and arrhythmias induced by SNA perfusion with catecholamines, impulses mostly started from the intercaval region of the RA (data not shown).

DISCUSSION

Adrenergically mediated arrhythmias. Although catecholamine infusion is often used in clinical practice to provoke paroxysmal AF, the present study is the first to demonstrate such an effect in intact animals without electrical stimulation of atria. Because both adrenaline and isoproterenol produced similar arrhythmogenic effects, beta-adrenergic stimulation might be responsible for catecholamine-mediated arrhythmias in this study. However, AF could not be induced by even very intense and yet nonphysiologic beta-adrenergic stimulation (up to 100 $\mu\text{mol/l}$ isoproterenol or adrenaline) in this model when background cholinergic tone was suppressed by atropine, indicating a critical role of cholinergic tone in these AF episodes. In addition, atropine resulted in a more than twofold decrease in APB occurrence (Table 1). Because both cervical vagi were decentralized, this result suggests that some background cholinergic tone contributed to the arrhythmogenic effect of catecholamines. The presence of background cholinergic tone is confirmed by the fact that catecholamine perfusions increased the regular heart rate to a higher extent with atropine than without it (Fig. 3).

Cholinergically mediated arrhythmias. It was shown that vagally (6,11) or ACh-mediated (4,5,9,11) APBs can trigger reentrant excitation and AF in dogs. Participation of adrenergic mechanisms in AF induction by cholinergic stimulation was previously proposed (3). In our study, isoproterenol (1 to 2 or 10 $\mu\text{mol/l}$) facilitated both the initiation and maintenance (Table 2) of ACh-mediated AF, and propranolol (1 mg/kg) produced the opposite effects. However, a full blockade of beta-adrenoreceptors did not prevent AF.

The mechanism of cholinergically mediated APBs remains debatable. Isoproterenol did not change the spatial distribution of ACh-mediated APBs or the activation sequence patterns during AF onset. So, the same mechanisms probably underlie ACh-mediated APBs and AF with and without isoproterenol in this study.

The synergistic effect of adrenergic and cholinergic stimulation for APB initiation was shown in human atrial tissues (13), where delayed afterdepolarizations of the rebound effect of ACh were induced only with adrenaline and

blocked by atropine. Because this triggered activity is related to intracellular Ca^{2+} overload, we recently tested the effects of ryanodine, a specific blocker of the sarcoplasmic Ca^{2+} release channels, in a canine model of cholinergic AF (11). It was shown that ryanodine administration (5 μ g/kg IV) did not affect the spontaneous initiation of ACh-mediated AF. In the present study, arrhythmias occurred during continuous ACh perfusion, indicating that they were not due to atrial triggered activity elicited by ACh withdrawal.

Another possible arrhythmogenic mechanism is based on the phenomenon of transient inexcitability observed in the dominant pacemaker region of the rabbit sinoatrial node under either ACh superfusion or postganglionic vagal stimulation (14). Although the hyperpolarized sinus node cells remain unexcitable, cells from surrounding subsidiary pacemaker areas, as well as the atrium, remain fully excitable. These and other results (14,15) suggest that such transiently unexcitable cells act as functional obstacles in the sinoatrial node and also in latent pacemaker areas. A propagating wave colliding with such obstacles may fragment and result in microreentrant arrhythmia. Beta-adrenergic tone may facilitate this process. For example, vagally mediated transient inexcitability in the rabbit sinus node was significantly larger without than with propranolol and was accompanied with a higher incidence of tachyarrhythmias (Dr. Fedorov, unpublished data, 1997).

Transient inexcitability might explain the initiation of ACh-induced APBs during regular atrial rhythm. It could create unidirectional entrance block in the latent pacemakers, preventing them from discharging by dominant rhythm. The importance of entrance block for ectopic pacemaker activity was shown by Jalife and Moe (16). The occurrence of entrance block and APBs in atria, due to cholinergic stimulation, was suggested (4,17). In addition, local shortening of atrial refractory periods by ACh could facilitate the breakthrough of concealed pacemaker activity toward the atrium.

Prolongation of AF by isoproterenol can be explained by several reasons. First, it was shown that the effect of simultaneous sympathetic and vagal stimulation on RA refractoriness is not only additive but also synergistic (18). Simultaneous perfusion of isoproterenol and ACh might efficaciously shorten the RA refractoriness. Despite a lower ACh concentration used to induce AF with isoproterenol, the average AFCL over the RA became somewhat smaller. Second, a high atrial rate induced by isoproterenol, by itself, could also result in atrial refractoriness shortening, as observed after even a short period of rapid atrial pacing in dogs (19), thus facilitating ACh-mediated AF. This possibly explains why the AFCL decreased in the RAA, situated mainly outside of the SNA flow (10) and, thus, the accessibility of ACh. In addition, isoproterenol infused into the SNA could accumulate and affect the atrial myocardium. Differences in heart hemodynamics and other variables that can accompany beta-adrenergic stimulation might also be significant in promoting ACh-mediated AF. The effects of

isoproterenol perfusion on ACh-mediated AF were reversible. Subsequent full blockade of beta-adrenoreceptors by propranolol (1 mg/kg IV) resulted in a shortening AF duration and increasing threshold ACh concentrations necessary for AF induction episodes, but did not prevent ACh-mediated AF.

Study limitations. The results of this study were obtained in normal dog hearts and might not necessary apply to structurally abnormal hearts, and a model of acute AF cannot fully reproduce the clinical situation. The autonomic neural pathways were not intact in our experiments. We performed cervical vagotomy to prevent vagal reflexes in response to neurotransmitters infusion; however, the intrinsic cardiac nervous system (20,21) could significantly contribute to our results.

Although the action of infused neurotransmitters was limited to the SNA flow distribution (10), it is known that sympathetic and parasympathetic effects spread throughout the atria. Although the pulmonary vein area (considered clinically the most significant for atrial arrhythmogenesis) was not a target of this study, it has probably been influenced by neurotransmitters. The APBs and focal AF near the right pulmonary vein, as well as the SVC or IVC, were often registered in this study, as in previous studies in which tachyarrhythmias were induced by intensive vagal stimulation or ACh administration in dogs treated with propranolol (5,6). A recent study (22) showed that the occurrence of APBs and AF in the canine pulmonary vein induced by stimulating autonomic nerves via the left pulmonary artery was fully abolished by atropine, whereas beta-receptor blockade only increased the voltage of high-frequency electrical stimulation for arrhythmia induction. Because data (23) indicate that latent pacemakers present in the pulmonary vein, the mechanism of these APBs could also be considered in terms of pacemaker hypotheses (4,15) of arrhythmogenesis.

We used RA mapping in our experiments. A previous study (5) showed that ACh administration into the SNA initially produced focal or reentrant rapid activity only in the RA, mainly in the intercaval region or septum. Although some impulses revealing the intercaval origin in the present study could actually be the activation breakthroughs from the septum, this cannot affect the main results of this work.

Possible clinical implications. Our results indicate that the increased level of adrenergic activity facilitates both the initiation and maintenance of cholinergically mediated AF in this model. Depending on beta-adrenergic tone and the corresponding background heart rate, ACh-mediated AF occurred during a slowing or accelerating heart rate. These data may partly explain why, in some patients (24), daytime AF paroxysms (intense adrenergic tone) are longer than nocturnal ones (intense vagal tone) and longer AF episodes are typified by heart rate acceleration, or why, in some patients (7), paroxysmal AF appears to be "vagally" and "adrenergically" mediated during different hours of the day. Increases in sympathetic tone that are sufficient to increase

blood pressure would be accompanied by reflex increases in parasympathetic tone. Thus, the development of AF under conditions of heightened sympathetic tone could actually be the result of increased atrial release of ACh. A primary role of cholinergic stimulation in AF generation is supported by the results of a recent study revealing an abrupt shift toward vagal predominance immediately before AF onset in a large group of patients with "lone" paroxysmal AF and structural heart disease (8).

Conclusions. Our data indicate that both autonomic systems contribute to AF initiation and maintenance; however, the cholinergic stimulation is likely the main factor for spontaneous AF initiation in this animal model, and adrenergic tone modulates the initiation and maintenance of cholinergically mediated AF.

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