

Research Paper

Acute vagal modulation of electrophysiology of the atrial and pulmonary veins increases vulnerability to atrial fibrillation

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Vagal activity is thought to influence atrial electrophysiological properties and play a role in the initiation and maintenance of atrial fibrillation (AF). We evaluated the effects of acute vagal stimulation on atrial conduction, refractoriness of atrial and pulmonary veins (PVs) and inducibility of AF. An open-chest epicardial approach was performed in New Zealand White rabbits with preserved autonomic innervation. Atrial electrograms were obtained with four unipolar electrodes placed epicardially along the atria ($n = 22$) and an electrode adapted to the proximal left PV ($n = 10$). The cervical vagus nerve was stimulated with bipolar platinum electrodes (20 Hz). Epicardial activation was recorded in sinus rhythm, and effective refractory periods (ERPs), dispersion of refractoriness and conduction times from high-lateral right atrium (RA) to high-lateral left atrium (LA) and PVs assessed at baseline and during vagal stimulation. Burst pacing (50 Hz, 10 s), alone or combined with vagal stimulation, was applied to the right (RAA) and left atrial appendage (LAA) and PVs to induce AF. At baseline, ERPs were lower in PVs than in LA and LAA, but did not differ significantly from RA and RAA, and there was a significant delay in the conduction time from RA to PVs compared with the activation time from RA to LA ($P < 0.01$). During vagal stimulation, ERP decreased significantly at all sites, without significant differences in the dispersion of refractoriness, and the atrial conduction times changed from 39 ± 19 to 49 ± 9 ms (RA to PVs; n.s.) and from 14 ± 7 to 28 ± 12 ms (RA to LA; $P = 0.01$). Induction of AF was reproducible in 50% of cases with 50 Hz and in 82% with 50 Hz combined with vagal stimulation ($P < 0.05$). During vagal stimulation, AF cycle length decreased at all sites, and AF duration changed from 1.0 ± 0.9 to 14.0 ± 10.0 s ($P < 0.01$), with documentation of PV tachycardia in three cases. In 70% of the animals, AF ceased immediately after interruption of vagal stimulation. We conclude that in the intact rabbit heart, vagal activity prolongs interatrial conduction and shortens atrial and PV ERP, contributing to the vulnerability to the induction and maintenance of AF. This model may be useful in the assessment of the autonomic influence in the mechanisms underlying AF.

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Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice, with impact on both life expectancy and quality of life (Camm, 1997; Benjamin *et al.* 1998; Van Den Berg *et al.* 2001). The pathophysiology of AF remains a considerable challenge, due to our limitations in understanding the precise

mechanisms underlying the initiation and maintenance of this arrhythmia. Experimental work has provided an important contribution to improve comprehension of complex AF mechanisms. The relative contribution of the multiple wavelet hypothesis proposed by Moe *et al.* (1964) and the focal sources of electrical activity described

by Haissaguerre *et al.* (1998) in explaining AF remain unknown. Nevertheless, phenomena that are able to influence electrophysiological properties of the atria can have a role in the pathogenesis of AF (Nattel, 2003).

The autonomic nervous system (ANS) is well recognized as an important modulator in mediating AF (Tai, 2001; Chen *et al.* 2006; Chen & Tan, 2007). Variations of autonomic tone may be important in the genesis of paroxysmal AF. A number of electrophysiological properties related to atrial vulnerability to AF may change as a result of vagal or sympathetic activation. Vagal stimulation reduces the velocity of the conduction in the atrial tissue and shortens the action potential duration and atrial effective refractory periods (ERPs) non-uniformly, facilitating re-entrant arrhythmias (Nattel *et al.* 2000; Chen *et al.* 2006), and sympathetic stimulation can favour reduction of atrial refractoriness and induction of trigger activity (Nattel *et al.* 2000). However, the specific contribution of each division of the ANS to the genesis of AF remains unclear. Sharifov *et al.* (2004) reported that direct infusion of acetylcholine into the sinus node artery could induce AF in 100% of dogs, but infusion of isoprenaline and adrenaline induced AF only in 21% of the animals. Sympathetic outflow does not promote AF as much as vagal stimulation, probably because the effects of sympathetic stimulation are more spatially homogeneous in the atria than those resulting from vagal activity (Olshansky, 2005). It has been recognized that vagal activity might predispose patients to develop paroxysmal AF (Coumel *et al.* 1978), that vagal overactivity is a precipitating cause of several short daily attacks of an arrhythmia which are usually not completely nocturnal, and that vagal nerve stimulation can facilitate the induction of AF (Hirose *et al.* 2002). Characterization and quantification of ANS electrophysiological effects are complex and difficult to define. Several animal models have been developed to understand the origin and possible mechanisms underlying AF. Nevertheless, an important part of these experimental studies were carried out in isolated heart models. In the present study, we used the intact anaesthetized rabbit heart, with preserved autonomic innervation, in order to investigate the effects of vagal stimulation on atrial conduction velocity and refractoriness, and its relevance in the context of vulnerability for the induction and maintenance of AF.

Methods

Ethical issues

All the experimental procedures were performed in accordance with the Portuguese and European law on animal welfare and were also approved by the Ethics Committee of the Faculty of Medicine of Lisbon.

Anaesthesia and surgical protocol

Twentytwo New Zealand White rabbits of both sexes (weight 3.9–5.0 kg) were anaesthetized with sodium pentobarbital (60 mg kg⁻¹, i.v.). A tracheal cannula was inserted below the larynx, via a tracheostomy, to allow ventilation with O₂-enriched room air through a positive-pressure ventilator (Harvard Apparatus, UK). Neuromuscular blockade was achieved with vecuronium (0.05 mg kg⁻¹ h⁻¹, i.v.), in order to assure strict control of respiratory parameters, such as rate and volume. Ventilation was adjusted to ensure an end-tidal CO₂ of 5–7%. Adequate levels of anaesthesia were maintained with a 20% solution of the anaesthetic and assessed by observing the presence of the withdrawal reflex after pinching a paw before the injection of vecuronium and from the observed changes in arterial blood pressure (BP) and heart rate after the same type of noxious stimulation following induction of neuromuscular blockade. The right femoral artery and vein were cannulated for BP monitoring (Lectromed, Letchworth, UK) and for intravenous drug and saline administration, respectively. Rectal temperature was monitored and maintained at 38 ± 1°C throughout the experiment with a heating blanket (Harvard Apparatus). The urinary bladder was cannulated and drained.

A mid-line sternectomy was performed and the pericardium opened in order to expose the heart and prepare an epicardial approach. After thoracotomy, an end-expiratory pressure of 1–2 cmH₂O was maintained. The ECG was recorded with the use of bipolar percutaneous electrodes placed in the four limbs of the animal. Arterial blood pressure, ECG, heart rate, ventilatory rate and end-tidal CO₂ were continuously monitored (PowerLab, ADInstruments, Chalgrove, UK). At the end of the experiment, the animal was killed with an overdose of sodium pentobarbital.

Experimental protocol

Electrical stimulation of the vagus nerve. The right cervical vagus nerve was identified and isolated via a mid-line neck incision and prepared for electrical stimulation with a bipolar silver electrode. As a physiological test, the vagus nerve was stimulated (20 Hz, 0.2 ms, 150–200 µA) to evoke a sinus rate reduction of 50%.

Electrophysiological protocol. Atrial electrograms were recorded in all animals from an array of four custom-made unipolar electrodes (125 µm Ag–AgCl Teflon-isolated wire), inserted into a patch of nylon mesh at interelectrode distances of 4 mm (Fig. 1). This electrode array was placed epicardially and distributed along the atria, from the high-lateral right atrium (RA) to the high-lateral left atrium (LA). In 10 animals, a fifth electrode (twin coaxial cable) was adapted to the left pulmonary

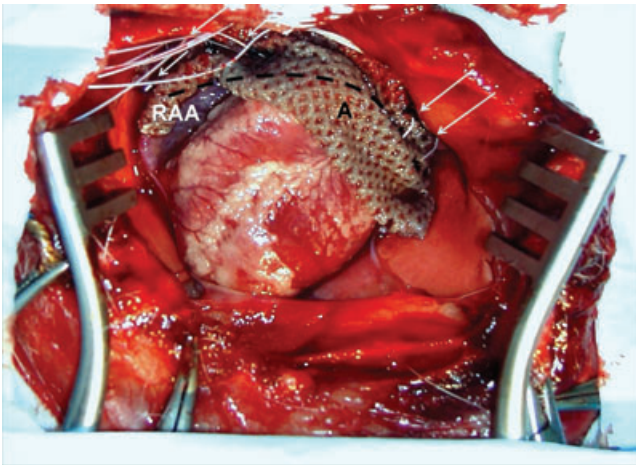


Figure 1. Open-chest preparation of the intact rabbit heart

The nylon network (A) supports four unipolar electrodes (arrows) to record electrograms along the atria (from the high-lateral right atrium to the high-lateral left atrium). Abbreviation: RAA, right atrial appendage.

veins (PVs), near the area of insertion in the LA, for recording of electrograms and pacing. Atrial epicardial activation was recorded during normal sinus rhythm, and the conduction time between RA and LA and between RA and PVs measured in baseline conditions and during vagal stimulation. The recorded signals were amplified, filtered at 0.5–300 Hz (Neurolog, Digitimer, Welwyn Garden City, UK) and digitized (PowerLab, ADInstruments; Fig. 2).

Bipolar electrical stimulation was performed with a Teflon-coated silver wire electrode (0.1 mm diameter), positioned with a micromanipulator (WPI, M330), and with a coaxial electrode adapted to the PVs for the assessment of ERPs and induction of AF. An eight-channel, programmable stimulator (Master8, AMPI, Israel) was used for pacing. As a measure of local refractoriness, ERPs were assessed in each animal at four different sites [RA, right atrial appendage (RAA), left atrial appendage (LAA) and LA]. Effective refractory periods of the PVs were also measured in 10 animals. In stable conditions, after all the surgical procedures and under

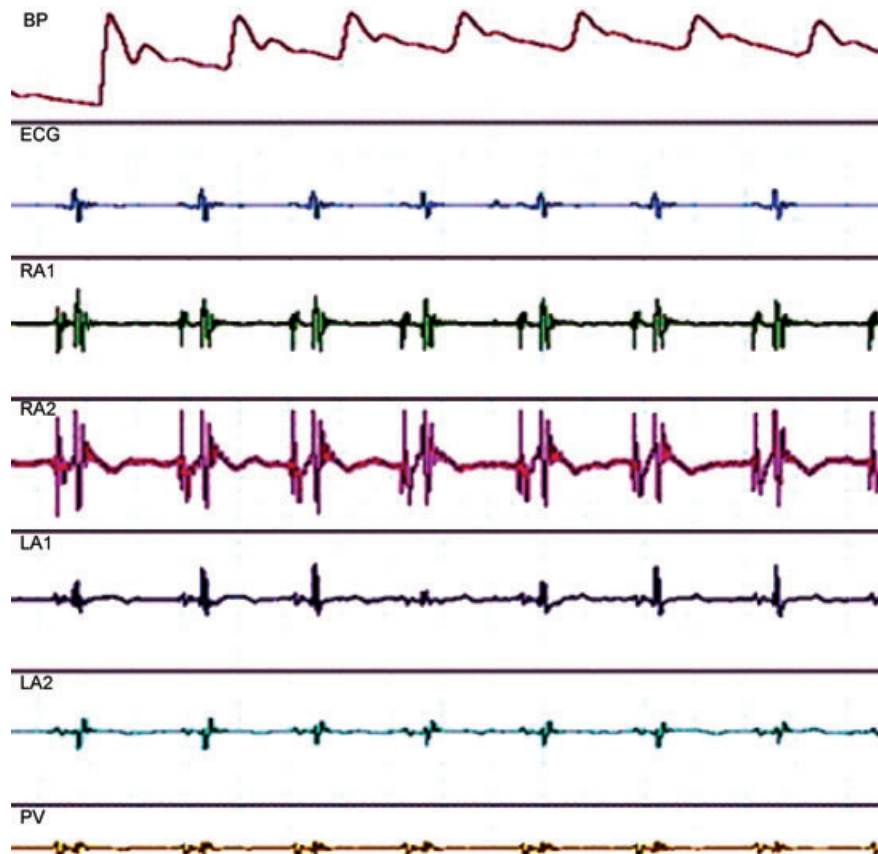


Figure 2. Continuous recording of blood pressure (BP), ECG, atrial and left pulmonary vein epicardial electrograms during sinus rhythm

Abbreviations: RA1, high-lateral right atrium; RA2, right atrial appendage; LA1, left atrial appendage; LA2, high-lateral left atrium; and PV, pulmonary veins.

neuromuscular blockade, heart rate ranged between 175 and 222 beats min^{-1} (195 ± 29 beats min^{-1} , $n = 22$), the systolic BP between 60 and 90 mmHg (82 ± 15 mmHg, $n = 22$), and the P wave between 38 and 60 ms (53 ± 8 ms, $n = 22$). Vagal stimulation at the cervical level for 5 s elicited a pronounced sinus bradycardia, with occurrence of systemic hypotension (<60 mmHg) in all animals.

Atrial and PV stimulation was performed with impulses of 2 ms duration at twice the diastolic threshold. A programmed electrical stimulation using a single premature stimulus was delivered, while pacing continuously at a basic drive cycle length of 300 ms (or 20 ms below the R–R interval in six rabbits with heart rate >200 beats min^{-1}). A premature beat was introduced in late diastole, beginning at a coupling interval of 100 ms less than the basic cycle length. The coupling interval of the premature stimulation was decreased by 10 ms steps until the ERP was reached. The ERP was defined as the longest S1–S2 interval that failed to initiate a propagation response, and measured in baseline conditions and during vagal stimulation. Dispersion of atrial refractoriness was calculated as the difference between the longest and the shortest ERP at the stimulation sites. The AF cycle length was measured in all sites with online calipers at a paper speed of 100 mm s^{-1} by averaging five consecutive cycles before the termination of AF.

The concept of vulnerability to AF induction was evaluated with bursts (50 Hz, 10 s; supraliminal intensity), delivered with a bipolar electrode placed at the right and left atrial appendages, alone or combined with vagal stimulation. Atrial fibrillation was defined as a fast atrial rhythm characterized by a fluctuating beat-to-beat

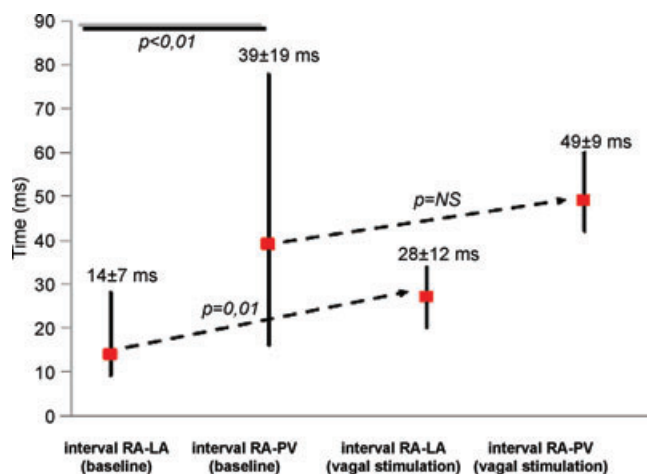


Figure 3. Atrial conduction times

Atrial conduction times between the high-lateral right atrium (RA) and the high-lateral left atrium (LA) and between the RA and left pulmonary veins (PV) near the area of insertion in the LA in baseline conditions and during right cervical vagal stimulation. * $p < 0.01$ vs. interval RA-LA; ** $p = 0.01$ vs. baseline; § $p = NS$ vs. baseline

Table 1. Effective refractory periods (ERPs) measured during baseline conditions and during vagal stimulation

	Baseline ERP (ms)	ERP during vagal stimulation (ms)	% Δ	P value
Right atrium	86 ± 17	60 ± 20	-33 ± 20%	<0.01
Right atrial appendage	79 ± 18	60 ± 22	-21 ± 33%	<0.05
Left atrial appendage	105 ± 24*	76 ± 25	-20 ± 17%	<0.05
Left atrium	102 ± 27*	76 ± 23	-26 ± 15%	<0.01
Pulmonary veins	65 ± 40	38 ± 28	-31 ± 36%	<0.01

Definition: % Δ , percentage of variation of the average ERPs. * $P < 0.01$ compared with high-lateral RA, RAA and PVs in baseline conditions.

cycle length, polarity, configuration and amplitude of the recorded atrial electrograms and lasting more than five cycles (Hirose *et al.* 2002).

Statistical analysis

Results are shown as means \pm s.d. Categorical variables are expressed as frequencies and percentages. Normality of the distributions of the continuous variables was analysed with the Kolmogorov–Smirnov test. Student’s paired t test was used to assess all paired data in the same group. Comparisons between groups were made using Student’s unpaired t test or non-parametric analysis with the Mann–Whitney U test, as appropriate. The χ^2 test, with Yates correction, was used to evaluate the differences in categorical variables. All statistical tests were two-sided, and a probability value <0.05 was required for statistical significance. Data were analysed using GraphPad Instat, version 3.05 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Effects of vagal stimulation on electrophysiological parameters of the atria

The conduction time from the RA to the LA in baseline conditions was 14 ± 7 ms (10–30 ms), which increased to 28 ± 10 ms (20–35 ms) on stimulation (20 Hz, 0.2 ms, 150–200 μA) of the right vagus nerve ($n = 22$, $P < 0.05$; Fig. 3). In baseline conditions, during normal sinus rhythm, there was a significant delay in conduction between the RA and the PVs, slightly influenced by vagal stimulation (Fig. 3).

Table 1 shows the ERPs obtained in baseline conditions and during vagal activation. In baseline conditions, there was a significant increase of atrial refractoriness from the RA, RAA and PVs to the LA and LAA. Vagal stimulation produced a reproducible reduction in the atrial ERPs assessed in all sites.

Table 2. Mean atrial fibrillation cycle lengths for atria and pulmonary veins measured in control conditions and following vagal stimulation

	High-lateral right atrium	Right atrial appendage	High-lateral left atrium	Left atrial appendage	Proximal segment of pulmonary veins
Baseline AFCL (ms)	154 ± 35	170 ± 23	173 ± 16	171 ± 16	152 ± 25
AFCL during vagal stimulation (ms)	83 ± 34*	77 ± 21**	86 ± 35**	110 ± 48§	71 ± 17**

Definition: AFCL, atrial fibrillation cycle length. * $P = 0.002$, ** $P < 0.0001$, § $P < 0.05$ for the comparison between control conditions and following vagal stimulation. Measurements obtained at different sites in the same conditions were not significantly different from each other.

The dispersion of ERPs based only on atrial measurements was significantly lower than the dispersion of ERPs calculated with atrial and PV refractoriness values (33 ± 14 versus 64 ± 25 ms, $P < 0.05$). Compared with baseline conditions, vagal stimulation did not significantly affect the dispersion of refractoriness (33 ± 14 versus 38 ± 17 ms for atrial sites, n.s.; and 64 ± 25 versus 67 ± 28 ms for all sites, n.s.).

Effects of vagal stimulation on atrial fibrillation inducibility

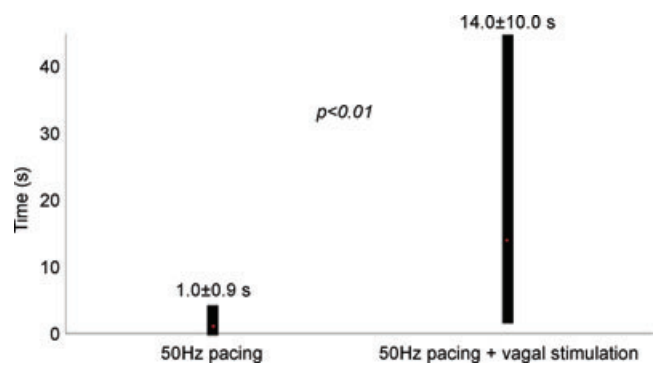
The inducibility and the duration of AF were significantly increased by vagal stimulation (Fig. 4). Atrial fibrillation was induced in 50% of the rabbits with rapid atrial bursts delivered to the RAA or PVs, but could not be induced by LAA high-rate pacing alone. During vagal activation, AF was consistently induced in 100, 60 and 20% of the rabbits by pacing the RAA, PVs and LAA, respectively (RAA versus LAA, $P < 0.05$). The mean AF cycle length measured in baseline conditions and during vagal stimulation is represented in Table 2. The cycle lengths during AF induced by 50 Hz pacing were reduced significantly by vagal stimulation in all atrial sites and PVs. On three occasions, AF was initiated by a single extrastimulus combined with vagal stimulation during ERP measurements. Pulmonary vein tachycardia (cycle length 80–90 ms) was induced by high-rate PV pacing combined with vagal stimulation in three animals (Fig. 5). The duration of the inducible AF changed from 1.0 ± 0.8 s with 50 Hz rapid stimulation alone to 14.0 ± 10.0 s in response to 50 Hz rapid stimulation combined with vagal stimulation ($P < 0.01$; Fig. 4). Atrial fibrillation lasted > 10 s in 45.5% of the rabbits (only during vagal stimulation) and terminated immediately after the cessation of vagal stimulation in seven of those 10 cases. These animals had similar interatrial and atrial to PV activation times. However, they showed the shortest ERPs in the RA, with higher dispersion of atrial refractoriness, at baseline and also during vagal stimulation (Table 3).

Discussion

The present experimental *in vivo* model allowed investigation of the effects of vagus nerve stimulation on cardiac electrophysiological properties and on the vulnerability to AF. We have characterized the influence of direct acute stimulation of the right cervical vagal trunk, the effect of which on heart rate is much larger than for identical left vagal stimulation (Loeb *et al.* 1981), on the electrical conduction and refractoriness properties of the atria and PVs. We have also confirmed a role of vagal activity in the vulnerability to the initiation of AF and its duration.

Main findings

This model shows a significant activation delay from the atria to the PVs during sinus rhythm. In fact, the mean conduction times from the RA to the PVs were about three times greater than the RA to LA interval measured in baseline conditions (Fig. 3). Also, a significant increase of baseline atrial ERPs was observed from the RA to the LA, suggesting heterogeneity of the electrophysiological properties of the atria. In addition, ERPs of the PV were significantly lower than those measured in

**Figure 4. Duration of inducible atrial fibrillation episodes**

Duration of the inducible atrial fibrillation episodes with rapid atrial stimulation alone (50 Hz pacing) and in response to rapid atrial stimulation combined with vagal activation (50 Hz pacing + vagal stimulation).

the LA and LAA. In this preparation, vagal activity was important for obtaining significant reproducible changes in atrial conduction times, atrial and PV ERPs, frequency of AF induction, mean AF cycle length and duration of the induced AF. Moreover, we demonstrated significant differences in atrial dispersion of refractoriness and RA ERPs in the group of animals with AF lasting >10 s.

Animal models and mechanisms of atrial fibrillation

Various animal models have been developed to explore the mechanisms underlying AF. The classic hypothesis of multiple circuit re-entry with variable spatial orientation, focal activity and single rotors with fibrillatory conduction, which remains the framework for our understanding of AF, was established based on experimental studies in animals. However, the development of models that allow electrophysiological studies and a contribution to the comprehension of the conditions associated with the generation of arrhythmias remains a major challenge. In dogs, microreentry,

abnormal automaticity and triggered activity have been observed as possible mechanisms for the occurrence of atrial arrhythmias (Friedman *et al.* 1996; Hocini *et al.* 2002). Hayashi *et al.* (1998) used efferent cervical vagal stimulation to induce AF by bursts of atrial pacing in anaesthetized open-chested dogs and evaluated the efficacy of class Ic and class III antiarrhythmic drugs to terminate AF. Other investigators have used versions of this model to explore the ability of different antiarrhythmics to terminate induced AF (Goldberger & Pavelec, 1986; David *et al.* 1990). Nevertheless, data regarding the use of experimental models with preserved autonomic innervation to assess the fundamental aspects of the electrophysiological properties of the atria and PVs underlying the vulnerability to development of AF are still limited. Abnormal atrial electrophysiology and higher vagal reflex activity may play an important role in the genesis of AF (Chen *et al.* 1999). In fact, it has been accepted that the ANS contributes as a modulator of the initiation, perpetuation, ventricular response rate and termination of AF, but its precise role remains controversial (Olshansky, 2005; Vos *et al.* 2008). The

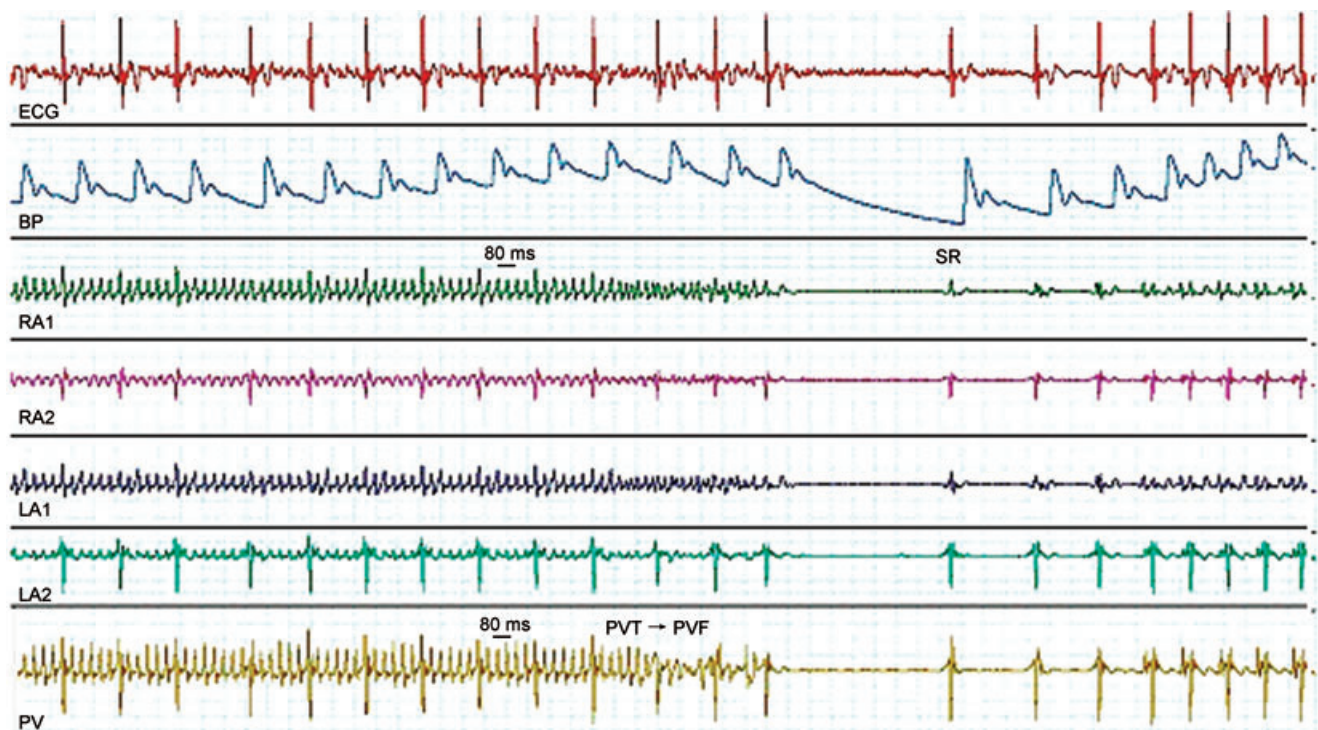


Figure 5. Example of a recording with pulmonary vein tachycardia (PVT) induced by stimulation of the pulmonary veins (50 Hz, 10 s) combined with vagal activation, showing the transition to pulmonary vein fibrillation (PVF) followed by termination of the arrhythmia

Abbreviations: BP, blood pressure; ECG, electrocardiogram; LA1, left atrial appendage; LA2, high-lateral left atrium; PV, pulmonary veins; PVF, pulmonary veins fibrillation; PVT, pulmonary veins tachycardia; RA1, high-lateral right atrium; RA2, right atrial appendage; and SR, sinus rhythm.

Table 3. Characteristics, atrial refractoriness and atrial conduction times in the rabbits with atrial fibrillation lasting <10 s and those with atrial fibrillation lasting >10 s

Parameter	Group AF <10 s	Group AF >10 s	P value
P wave duration (ms)	35 ± 9	50 ± 14	n.s. (0.08)
Baseline heart rate (beats min ⁻¹)	196 ± 13	208 ± 28	n.s.
Baseline systolic BP (mmHg)	78 ± 11	75 ± 15	n.s.
Weight (kg)	4,4 ± 0,3	4,3 ± 0,4	n.s.
Atrial dispersion of ERP baseline (ms)	22 ± 15	42 ± 5	0.03
Atrial + PVs dispersion of ERP baseline (ms)	73 ± 38	60 ± 26	n.s.
Atrial dispersion of ERP vagal (ms)	28 ± 15	48 ± 11	0.03
Atrial + PVs dispersion of ERP vagal (ms)	72 ± 40	63 ± 13	n.s.
RA ERP baseline (ms)	97 ± 12	70 ± 10	<0.05
RA ERP vagal (ms)	80 ± 20	40 ± 10	<0.05
RAA ERP baseline (ms)	67 ± 15	80 ± 11	n.s.
RAA ERP vagal (ms)	53 ± 15	64 ± 27	n.s.
LAA ERP baseline (ms)	102 ± 21	107 ± 6	n.s.
LAA ERP vagal	75 ± 24	88 ± 19	n.s.
LA ERP baseline (ms)	105 ± 35	100 ± 27	n.s.
LA ERP vagal (ms)	80 ± 56	73 ± 16	n.s.
PVs ERP baseline (ms)	75 ± 51	58 ± 38	n.s.
PVs ERP vagal (ms)	41 ± 33	34 ± 30	n.s.
Interval RA–LA baseline (ms)	10 ± 7	17 ± 4	n.s.
Interval RA–LA vagal (ms)	26 ± 9	31 ± 16	n.s.
Interval RA–PVs baseline (ms)	55 ± 50	40 ± 20	n.s.
Interval RA–PVs vagal (ms)	58 ± 20	51 ± 8	n.s.

Definitions: AF, atrial fibrillation; BP, blood pressure; ERP, effective refractory period; PVs, left pulmonary veins; and vagal, during cervical vagal nerve stimulation. Atrial + PV dispersion includes ERP measurements from high-lateral right (RA) and left atrium (LA), right (RAA) and left atrial appendage (LAA) and PVs.

importance of the ANS in AF is supported by animal experiments and recent clinical studies showing that vagal denervation enhances the efficacy of circumferential PV ablation in preventing AF recurrence (Chen & Tan, 2007).

Decreased atrial ERP and its spatial dispersion heterogeneity have been accepted to promote AF re-initiation and to provide a substrate for the re-entry of multiple wavelets to enhance the ability of the disorder to sustain itself (Nattel *et al.* 2000). In previous studies, sympathetic stimulation was much less effective than vagal stimulation in promoting AF and heterogeneity in atrial refractoriness (Liu & Nattel, 1997; Chen *et al.* 1999). Vagus nerve stimulation has been associated with atrial ERP shortening and facilitation of AF inducibility in dogs (Arora *et al.* 2008).

Our present data, using the intact rabbit heart model, support the importance of vagal activity in determining significant acute changes of atrial refractoriness and its contribution to the induction and cycle length of AF. The calculated dispersion of atrial refractoriness has not been significantly modified by vagal stimulation. This may explain the inability to sustain AF despite maintenance of vagal activation. In fact, reproducible induction of AF during vagal stimulation was obtained in all animals with a significant increase in the duration of the episodes,

but the longest duration of AF was 45 s, lasting >10 s only in 45.5% of the rabbits. Interestingly, those animals with longer AF duration showed higher dispersion of atrial refractoriness and shortest RA ERPs, despite similar conduction activation times. This emphasizes the role of refractoriness properties as important markers of vulnerability to AF. We were able to obtain conditions that decrease atrial wavelength, the product of ERP and conduction velocity, by significantly decreasing the ERPs and the conduction velocity of the atrial tissue, which permits multiple wavelets and promotes AF.

Effects of vagal stimulation on conduction times, refractoriness and vulnerability to atrial fibrillation

Electrophysiological abnormalities behind the genesis of AF include conduction disturbances, shortening of the ERP and increase in atrial dispersion of refractoriness (Fuster *et al.* 2001). Vagal tone may influence the refractoriness and conduction velocity in the atrial tissue, contributing to the vulnerability to the occurrence of AF (Chen & Tan, 2007). However, the assessment of autonomic fluctuations before the onset of paroxysmal AF, measured with heart rate variability, has shown conflicting results (Chen *et al.* 2006).

In the present study, the electrophysiological characterization of the recognized effects of vagal stimulation in conduction and refractoriness properties of the atria and PVs was clearly shown. Oh *et al.* (2006), using a canine model, demonstrated that the effects of vagal stimulation on atrial ERPs and AF inducibility were significantly changed immediately after epicardial fat pad ablation, suggesting that it is possible to achieve temporary suppression of the vagal tone in the atria. Recently, in a study with dogs, sustained AF could be induced easily during vagal nerve stimulation *in vivo* (Lemola *et al.* 2008). Zhang *et al.* (2009) also used burst stimulation of the atria and right cervical vagal stimulation to induce AF in dogs. Their study suggested that strong vagal stimulation facilitates AF inducibility. In our rabbit model, we have shown that vagal activation was able to decrease the atrial and PV cycle length during AF and significantly increase the duration of the induced AF episodes (Table 2 and Fig. 4). Vagal stimulation significantly increased the interatrial activation times, without changes in the conduction velocity between atrial and PV activation (Fig. 3). This suggests non-uniformity of vagal responses, with evidence of regional differences in conduction delay, and supports the concept that the presence of anisotropic conduction properties and slow conduction at the area of the PV–LA junction may contribute to promote re-entry formation and thus play a role as a substrate for the maintenance of AF (Kumagai *et al.* 2004; Atienza & Jalife, 2007). Also, the vagal effect on the degree of ERP reduction was slightly higher in the RA, LA and PVs.

Vagal activity seemed to influence the termination of AF directly, since in 70% of those animals with AF duration >10 s the arrhythmia stopped immediately after cessation of vagal stimulation. Therefore, despite the inability to demonstrate a significant impact on the dispersion of the ERP, vagal activation decreased the conduction velocity in the atria and evoked reductions of the ERP, contributing to the induction, duration and termination of AF.

Limitations of the study

Although programmed electrical stimulation with a single premature stimulus has been widely used for the study of atrial refractoriness properties, we believe that further studies, using different electrophysiological techniques, may be needed to confirm these findings, because the present method may be of relatively low resolution. Also, the low tendency of the rabbit atria to fibrillate and maintain the arrhythmia owing to its small size might contribute to the inability to sustain AF in this preparation. Although brief episodes of AF in the rabbit reflect an evidence of arrhythmic vulnerability, the precise mechanisms underlying AF maintenance may be different

in humans. Further experimental studies using more accurate methods may be needed to elucidate the impact of autonomic innervation on the dynamics of recurrent sustained AF episodes.

Conclusions

The acute stimulation of parasympathetic activity exerts a reduction of atrial conduction velocity and decreases ERPs in multiple atrial sites and in the PVs. This experimental preparation also displays the impact of vagal stimulation in enhancing the propensity for the inducibility of AF, demonstrating an important role in the vulnerability to the occurrence of AF and contributing to the duration of AF episodes, particularly in those animals with higher dispersion of refractoriness. Our data suggest that this experimental vagal stimulation model may provide a useful method for assessment of the influence of the ANS in the pathophysiology of AF.

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