Original Article

Effects of Stimulation with Pulsatile Continuous Electrical Current on Atrial Electrophysiological Properties. Experimental Study of Atrial Fibrillation in Dogs

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Objective

To assess whether atrial stimulation with pulsatile continuous electrical current induces atrial fibrillation and to evaluate its effects on atrial electrophysiological properties as well as the atrial histological alterations.

Methods

Twenty-two dogs underwent right lateral thoracotomy and implantation of pacemaker electrodes in the sulcus terminalis (ST), in the right atrial appendix (RAb), and in the posteroinferior region of the left atrium (LA). A pair of electrodes was sutured to the right atrial appendix for stimulation with a 9-volt alkaline battery connected to a system (LM 555) that transforms the linear continuous energy of the battery into a pulsatile continuous current for 60 minutes. Atrial epicardial biopsy was performed before and after atrial stimulation.

Results

No differences were observed in the durations of the effective atrial refractory periods. The times of intra-atrial and interatrial conduction, as well as that of atrial extrastimuli, were prolonged. The duration of atrial electrograms was prolonged during sinus rhythm and programmed atrial stimulation; in 68% of the dogs, atrial fibrillation was induced and was sustained. Interstitial edema and bands of cellular contraction were observed in the subepicardium on optical microscopy, as were intense myofibrillar disorganization and an increase in the size of the mitochondria on electron microscopy.

Conclusion

That technique of atrial stimulation induces atrial fibrillation and causes atrial changes that increase atrial vulnerability to the appearance of atrial fibrillation.

Keywords

cardiac arrhythmia, atrial fibrillation, electrophysiology

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The interest in atrial fibrillation has currently risen not only because it is a frequent arrhythmia, but also due to its high risk of complications [1]. Due to its high prevalence, the increasing knowledge about its complications, and also the great progress of the percutaneous treatment of some arrhythmias, the interest in the therapeutic approach of atrial fibrillation has increased in recent years. The recent knowledge about the electrophysiological mechanisms involving its genesis has allowed better understanding of the triggering factors and their maintenance. For this knowledge to reach its current stage and to be able to evolve, several experimental models have been described, whose results are similar to data obtained in human hearts in the electrophysiology laboratory [2-4]. Despite all progress, so far, the literature has not had a simple, rapid, practical, and inexpensive experimental technique for artificial induction and maintenance of atrial fibrillation in dogs. That is why a simple and reproducible experimental model is useful. The previously cited experimental models used special and sophisticated pacemakers, controlled by computerized systems, capable of rapid stimulation for prolonged periods [2,3]. Those systems, however, are expensive and not always available, which is a limiting factor that delays the evolution of the knowledge involved in the appearance of that arrhythmia.

Based on the manner in which ventricular stimulation with continuous electrical current causes ventricular fibrillation, experimental stimulation of the atria with 9-volt alkaline batteries was hypothesized to have the same effect. Preliminary experiments in our laboratory showed that the direct contact of the battery with the atrial epicardium produced important lesions that could eventually influence the results. Because of this, we chose to couple the battery to an electronic system for atrial stimulation, called LM555, which transforms the linear continuous current released by the battery into a pulsatile continuous current, causing lower risk of impairing the atrial musculature. This study was planed considering the hypothesis that atrial stimulation with pulsatile continuous electrical current induces atrial fibrillation and can cause alterations in the atrial electrophysiological properties. This facilitates maintenance or the spontaneous appearance of atrial fibrillation and enables the research of new therapeutic modalities in experimental laboratories. Therefore, the objectives of this study were as follows: a) to assess the efficacy of atrial stimulation with a pulsatile continuous electrical current for inducing atrial fibrillation in dog

hearts; b) to assess the effects of that modality of stimulation on atrial electrophysiological properties (effective refractory period; intra-atrial and interatrial conduction times; duration of atrial electrograms in sinus rhythm and during programmed atrial stimulation; the wave length index of the atrial impulse); c) to assess atrial vulnerability to artificial induction or spontaneous appearance of atrial fibrillation after the stimulation period; d) to analyze the histological alterations in the atrial tissue caused by atrial stimulation (morphological or anatomical remodeling).

Methods

This study comprised healthy dogs of no defined breed, of both sexes, and weighing between 15 and 30kg. Dogs with the following characteristics were excluded: old dogs (age estimated based on dentition), in which the appearance of spontaneous atrial arrhythmias is common; animals with frequent atrial extrasystoles (above 10/min), atrial tachycardia, or paroxysmal or persistent atrial fibrillation; animals with pericardial thickening, alterations in atrial epicardial color, presence of atrial fibrosis or any other alteration suggesting previous cardiac impairment through direct inspection after chest opening. After preanesthetic medication (fentanyl, 1.0 mg/kg, intramuscular), thiopental sodium (12.5 mg/kg) was intravenously administered for anesthetic induction. After endotracheal intubation, anesthesia was maintained with 100% oxygen with a Takaoka respirator, model 670 (Takaoka, São Paulo, SP, Brazil) in association with continuous administration of halothane. The frequency of the respirator was adjusted for maintaining arterial blood gases within the physiological limits (pH between 7.3 and 7.4; arterial pO_2 greater than 90 mmHg; pCO_2 between 35 and 40 mmHg and plasma bicarbonate between 20 and 30 mmol/L). After being anesthetized, the animals underwent right lateral thoracotomy in the fourth intercostal space, followed by exposure of the heart in the pericardial sac. On the atrial epicardium, temporary pacemaker threads were implanted in the following regions: a) high right atrium, close to the sulcus terminalis (identified as ST); b) anterior and inferior right atrium, close to the atrioventricular ring, right below the atrial appendix (identified as RAb); c) posteroinferior left atrium, close to the atrioventricular ring and the atrial appendix (identified as LA); d) one pair of electrodes were implanted in the right atrial appendix, serving for atrial stimulation. The external extremities of the epicardial electrodes were connected to a multiple-channel polygraph (FEAS Eletronica, Argentina), for recording bipolar electrograms of the respective atrial regions, along with the electrocardiographic leads D1, D2, aVF. All recordings were performed at the velocity of 100 mm/s and the recording gain of 1 cm equivalent to 1.0 mV.

After preparation of the animal and through direct view, biopsies of the right atrial epicardium were performed in the anterolateral region for histological analysis (control sample).

The effective atrial refractory period was determined by programmed atrial stimulation (stimulator FEAS, Argentina) with stimulation cycles (S1-S1 interval) of 300 and 200 ms. Stimulation was performed through epicardial electrodes in the ST, RAb, and LA regions, using an intensity of 5.0 mAmperes (mA) and duration of pulse width of 2.0 ms. The intensity of 5 mA was adopted to guarantee the constant and uniform atrial tissue command during the entire experiment. For measuring the effective refractory period, the classical technique of programmed atrial stimulation was used, and the following protocol was followed: after introduction of 8 pulses (S1-S1 stimulation cycle of 300 and 200 ms), extrastimuli (S2) were applied at progressively decreasing coupling intervals (S1-S2) of 5.0 ms, until no more atrial capture was observed [5].

The intervals for determining the times of intra-atrial and interatrial conduction were obtained through continuous atrial stimulation for 10s from ST, with an intensity of 5.0 mA and pulse width of 2.0 ms. With pulse sequence at intervals of 300 and 200 ms, the times of conduction between ST and RAb (time of intra-atrial conduction) and between ST and LA (time of interatrial conduction) were determined. The time of atrial conduction of the early stimuli, based on the stimulation in ST for the RAb and LA regions, was obtained by measuring the S2-A2 interval (interval between the spike released by the stimulator and the respective atrial electrogram of the early beat in the RAb and LA regions) with stimulation cycles of 300 and 200 ms.

Dispersion of atrial refractoriness was obtained based on the knowledge about the durations of the atrial refractory periods in sinus rhythm.

Atrial vulnerability was determined with continuous and rapid atrial stimulation at S1-S1 intervals of 100 ms [equivalent to 600 pulses per minute (ppm)], with intensity of 5.0 mA, pulse width of 2.0 ms per periods of 10s, for 3 consecutive times, in the ST region before and after atrial stimulation. Atrial fibrillation induced by that technique was considered sustained if the duration was longer than or equal to 30 s, or nonsustained when its duration was shorter than 30 seconds.

The effects of the pulsatile continuous electrical current were analyzed after the atrial stimulation performed when the positive and negative poles of a 9-volt battery (Duracel) were connected to a commercially available electronic circuit called LM555. That circuit is a stable system that generates previously programmed intervals of stimulation or oscillations in a very simple and highly precise manner. The form of electrical current release from the battery is no longer continuous and linear, but of the continuous and pulsatile type. Therefore, the energy of the battery is transformed into preprogrammed frequencies of stimulation with variations in pulse width and intensity of the stimulation considered adequate for the procedure. In that protocol, the system was programmed to perform stimulations from the right atrial appendix using the current with an intensity of 5.0 mA and frequency of 600 ppm, with a pulse width of 2.0 ms for a one-hour period. After the atrial stimulation period, the electrophysiological variables obtained in the control condition were reassessed immediately. At the end of the study, another right atrial epicardial biopsy was performed for histological analyses with optical and electron microscopies. Those analyses were programmed to be performed in at least one in every 2 dogs on optical microscopy, and in one in every 4 dogs on electron microscopy. Then, the animals were sacrificed with an intravenous injection of potassium chloride for inducing ventricular fibrillation, preceded by deepening of anesthesia.

Definitions - a) Atrial fibrillation: irregular cardiac rhythm in the absence of ordered atrial electrical activity (no P wave), which is replaced by irregular waves in the baseline; b) effective atrial refractory period: the shortest S1-S2 interval, in which S2 is not followed by atrial activation [5]; overall effective atrial refractory period is the mean of the durations obtained in the ST, RAb, and

LA regions; c) atrial refractoriness dispersion: defined by the coefficient of variation of the effective atrial refractory period as the ratio between the standard deviation of the mean and the mean of the refractory periods multiplied by 100 in each cycle of stimulation [6]; d) times of intra-atrial and interatrial conduction: time required for activation of the RAb and LA from ST stimulation, measured between the spike of stimulation (S) and the corresponding local bipolar electrograms (A1); time of atrial conduction of the atrial extrastimulus is the interval measured between the spike of stimulation corresponding to the early impulse (S2) and the respective atrial electrogram (A2)[5,7]; e) atrial anatomic remodeling: histologic modifications observed in cells and cell structures (myofibrils and mitochondria) and in the interstitial medium caused by atrial stimulation; f) atrial vulnerability: induction of atrial fibrillation after rapid atrial stimulation with a cycle of 100 ms performed in 3 consecutive attempts. The time of duration of induced atrial fibrillation corresponded to the greatest episode documented after the 3 periods of atrial stimulation at 600 ppm and not to the summation of the duration of short episodes of arrhythmia after each stimulation phase.

The management of the animals conformed to the national and international guidelines for experimental laboratory study using animals. The protocol of the study was previously approved by the Research Ethics Committee of the institution.

Statistical Analysis - The means of the electrophysiological variables were obtained before atrial stimulation (control) and after it and compared. The mean duration of the effective refractory period was compared within the same cycle of stimulation (S1-S1 interval of 300 ms and S1-S1 interval of 200 ms, separately) for each ST, RAb, and LA region, as well as for the 3 regions (overall effective refractory atrial period). The same was performed with the mean durations of the functional refractory period of the ST and RAb regions. Those variables were also analyzed by comparing the values of the means obtained in the 300-ms stimulation cycles and those obtained in the 200-ms stimulation cycles, both before and after induction of atrial fibrillation. The times of intra-atrial and interatrial conduction, the time of atrial conduction of the early impulses, index of wave length of the atrial impulse and the dispersion of the atrial refractoriness were compared within the same stimulation cycle before and after atrial stimulation. The results for comparative analysis are presented as mean and standard error of the mean, while the descriptive results are shown as mean and standard deviation. The values of the continuous variables (duration of the refractory periods, times of atrial conduction, index of the wave length of the atrial impulse and dispersion of the refractory atrial period were compared by using the paired Student *t* test. The significance level of $P < 0.05$ was adopted.

Results

The study comprised 22 dogs, 17 (77.27%) of the male sex, and 5 (22.73%) of the female sex, with no defined breed and a mean weight of 23±12 kg (ranging from 16 to 27 kg). Atrial fibrillation was induced in 100% of the animals during application of a pulsatile continuous electrical current on atrial tissue (fig.1). After the appearance of the arrhythmia, observation of the surgical field showed intense dilation of the atrial chambers with an increase in the tension of the atrial walls, which was perceived on palpation. Periods of one atrial response to each 2 stimuli (atrial capture with a 2:1 relation), sometimes 3:1 capture, were observed at the beginning of stimulation. Those periods gradually transformed into 1:1 capture, being followed by degeneration of the atrial rhythm to atrial fibrillation, with variable atrioventricular conduction. In 19/22 dogs (86.36%), occasional interruptions of stimulation for approximately 3 to 5 seconds, around 30 minutes of stimulation, confirmed the presence of atrial fibrillation as the underlying rhythm, indicating that arrhythmia was present in that period. After interruption of the atrial stimulation, atrial fibrillation was spontaneously maintained in 12/22 dogs (54.54%), for 155.17±212.69 seconds (range: 10 seconds to 780 seconds). In the other 10 animals (45.46%), interruption of stimulation was followed by reestablishment of sinus rhythm.

When comparing the conditions before and after atrial stimulation, no significant differences were observed in the durations of the effective refractory periods in the ST, RAb, and LA regions with 300- and 200-ms stimulation cycles. The mean duration of the overall effective atrial refractory period significantly decreased when comparing the values obtained with cycles of 300 and 200 ms, both before and after atrial stimulation (tab. I). Comparing the mean durations of the refractory periods within the same stimulation cycle (tab. I), the differences observed were not significant.

The dispersion of duration of the effective atrial refractory period, defined as the coefficient of variation of the refractory period, was similar in both situations, before and after atrial fibrillation induction (tab. II). However, in determining the effective

Table I - Comparison of the means of the overall effective atrial refractory period of the 300- and 200-ms cycles obtained before and after atrial stimulation with continuous electrical current.

	S1-S1 (300 ms)	S1-S1 (200 ms)	p†
Before	119.60±4.06	102.90±4.08	0.006
After	122.06±4.07	105.31±3.51	0.004
p*	0.671	0.660	

* P value for comparing the mean durations of the effective atrial refractory period before and after atrial stimulation with 300- and 200-ms cycles; † P values for comparing the mean durations of the effective atrial refractory period for the 300- and 200-ms cycles.

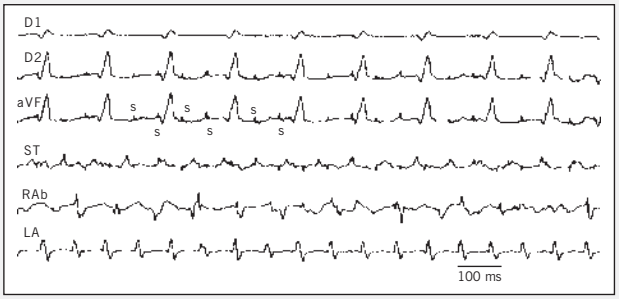


Fig. 1 – Electrocardiographic recording of dog 2 obtained in the peripheral leads D1, D2, and aVF and of the atrial epicardial bipolar electrograms corresponding to the ST, RAb, and LA regions, in that order from top to bottom, during induction of atrial fibrillation with atrial stimulation with pulsatile continuous current. During atrial fibrillation, disorganization of the atrial electrical activity is observed in the channels corresponding to ST and RAb. The stimulation spikes (S) caused by the artificial stimulator are present at 100-ms intervals. Recording at 100 mm/s.

atrial refractory period, an increase in the coefficient of variation was observed as follows: 1) in 14/21 dogs (67%) with 300-ms stimulation cycle, which passed from 7.00±1.24 ms before atrial stimulation to 12.06±1.64 ms after that (a 72% increase); and 2) in 12/20 (60%) dogs with 200-ms stimulation cycle, which passed from 7.19±1.08 ms to 11.98±1.81 ms (a 67% increase). Of the 12 dogs with an increase in the coefficient of variation of the refractory period with a 200-ms cycle after atrial stimulation, 9 (75%) also had an increase in dispersion with a 300-ms cycle. Those findings indicate that, with the stimulation protocol used in this study, the dispersion of the duration of the effective refractory period tends to be greater after atrial fibrillation is triggered.

The intra-atrial conduction time was similar before and after atrial stimulation when compared within the same stimulation cycle (S1-S1 intervals of 300 ms and 200 ms) (tab. III). The interatrial conduction time was prolonged both in the 300-ms and 200-ms cycles, however, the difference was statistically significant only in the 200-ms stimulation cycle.

In comparison with the control situation, a significant prolongation was observed in the mean values of the conduction time of the atrial extra-stimuli applied to ST and measured in RAb and LA, with 300-ms and 200-ms stimulation cycles, after atrial fibrillation induction (fig. 2). The prolongation of the ST2-LA2 and ST2-RAb2 intervals was marked after atrial stimulation as can be seen in figure 3.

Duration of the atrial electrograms in ST, RAb, and LA during sinus rhythm, before and immediately after atrial stimulation, are shown in table IV. An increase in duration of the electrograms in ST and RAb and a tendency towards an increase in the duration in LA were observed after the artificially induced period of atrial fibrillation. Figure 4 shows an example of an increase in duration of the local electrograms of the 3 regions after atrial stimulation.

Before and after atrial stimulation, inducibility of atrial fibrillation was tested with atrial stimulation at 600 ppm. In the control situation, induction of sustained atrial fibrillation was obtained in no animal; the nonsustained form was observed in only 3 dogs (13.60%; with mean duration of 18.67±11 seconds); atrial flutter was induced in one animal (4.40%) with a duration of 23s. No other type of atrial arrhythmia was induced in the other 18 (82%) dogs. However, after 60 minutes of atrial stimulation, sustained atrial fibrillation was triggered in 15/22 dogs (68.18%), with a mean duration of 148.00±123 seconds (range: from 35 to 420 seconds). Nonsustained atrial fibrillation was induced in 4 animals (mean duration, 17.00±8.72 seconds). In only 3 (13.63%) dogs, no arrhythmia was induced during the atrial vulnerability test. Programmed stimulation of ST and RAb showed that those sites were the most vulnerable to atrial fibrillation induction. Therefore, with stimulation in ST, atrial fibrillation was induced in 5/8 (62.5%) dogs; stimulation in RAb induced atrial fibrillation in 6/8 dogs (75%); and stimulation in LA induced atrial fibrillation in only 2/8 dogs (25%).

Epicardial atrial biopsy was performed in 10 (45.5%) dogs and comprised removal of tissue of the anterior region of the right atrium, 2 cm away from the site where atrial stimulation was performed with continuous current. In the control situation, no histological alteration was observed. However, in 100% of the animals undergoing biopsy after atrial fibrillation induction, areas of interstitial edema were observed in the subepicardial region, and bands of cell contraction in the subepicardial region were observed in 5/10 (50%) animals. Electron microscopy, performed in 5 animals, showed intense myofibrillar disorganization, diffuse interstitial edema, in addition to significant hypertrophy of the mitochondria, which showed diffuse lamellar disarray, in addition to areas of edema and lamellar fragmentation (fig. 5).

Discussion

This study showed that atrial stimulation with pulsatile continuous electrical current induces atrial fibrillation in a constant and reproducible manner. In more than half of the animals (54.54%), the arrhythmia persisted spontaneously for up to 13 minutes, even after interruption of stimulation. In addition, induced atrial fibrillation caused structural cell alterations, characterizing anatomical atrial remodeling, as shown in epicardial biopsy. The major alteration in atrial electrophysiology observed in this study was the prolongation of time of atrial conduction.

Stimulation of the right atrial appendix, at a frequency of 600 ppm for 60 minutes, initially causes irregular atrial capture, but approximately 15 minutes after the beginning of the procedure, atrial response in a 1:1 relation is clearly seen for some beats, followed by an intense disorganization of the electrical activity of that region. This finding suggests that, over time, the progressive shortening of atrial refractoriness with persistence of rapid stimulation allows more frequent atrial captures up to the effective refractory period, in which the wave fronts begin to coincide with the repolarization phase (period of atrial vulnerability). In addition,

Table II – Coefficient of variation of the effective atrial refractory periods (in ms) before and after atrial stimulation with 300- and 200-ms stimulation cycles (S1-S1).

	S1-S1 (300 ms)	S1-S1 (200 ms)
Before	8.71 ± 1.53*	11.12 ± 1.96†
After	10.53 ± 1.37*	11.56 ± 1.49†

* p = 0.195; † p = 0.804

Table III - Mean times of intra-atrial (ST-RAb) and interatrial (ST-LA) conduction compared within the same 300- and 200-ms stimulation cycle (S1-S1interval), before and after atrial stimulation with continuous current.

	Before		After	
	300 ms	200 ms	300 ms	200 ms
ST-RAb	14.95±0.57*	15.47±0.64†	15.86±0.34*	15.52±0.57†
ST-LA	41.90±1.50‡	39.90±1.52§	45.59±1.88‡	46.47±1.63§

* P = 0.178; † P = 0.954; ‡ P = 0.132; § P = 0.005.

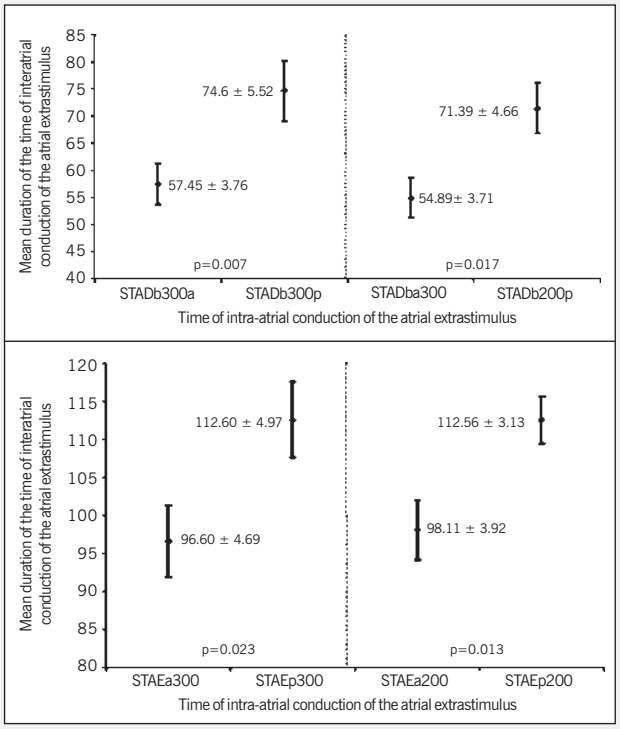


Fig. 2 - Effects of atrial stimulation with pulsatile continuous electrical current on the time of conduction of the early atrial stimulus (S2) applied to the ST region and the time it takes to be conducted until the RAb region (upper part) and the LA region (lower part). After the induction period of artificially induced atrial fibrillation, the delay of S2 observed in the ST-LA interval is greater than the same interval recorded before atrial stimulation, for the 300- and 200-ms cycles. The same was observed in regard to the time of intra-atrial conduction).

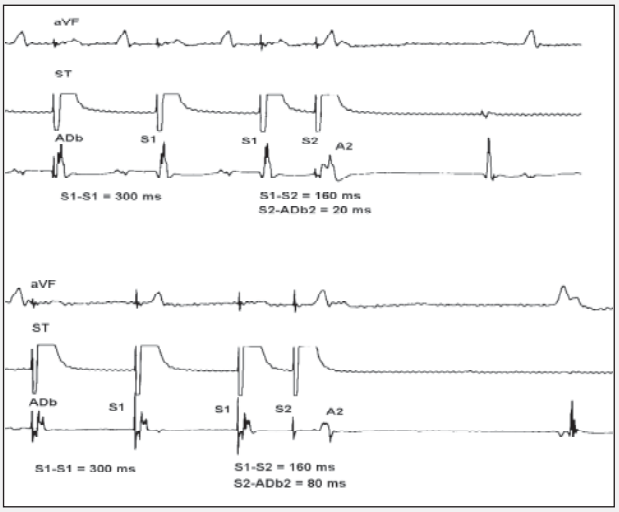


Fig. 3 - Two distinct situations recorded in the same animal (dog 25). In the upper tracing, before atrial stimulation with electrical current, and below, immediately after atrial stimulation. In both recordings, programmed atrial stimulation was performed with 300-ms stimulation cycle and coupling interval (S1-S2) of 160 ms. In the lower tracing, a greater delay in the ST2-A2 interval (80 ms) is observed as compared with that in the upper tracing (ST2-A2 of 20 ms).

one may assume that recovery of atrial excitability is heterogeneous when the atria are activated in rapid sequence, which makes the impulse travel slowly in a little excitable tissue. According to Prinzmetal, rapid atrial stimulation close to the threshold of atrial fibrillation induction causes gradual delays in local conduction, which become increasingly intense, a fact that intensifies the breaking of electrical depolarization waves [8].

Atrial fibrillation was induced in all animals during the period

Table IV - Mean duration of atrial electrograms (in ms) recorded in ST, RAb, and LA during sinus rhythm, before and after atrial stimulation.

	Before	After	% Increase	p
ST	58.77 ± 2.19	66.60 ± 1.80	13	0.001
RAb	60.18 ± 2.06	71.77 ± 2.07	19	<0.0001
LA	65.86 ± 5.16	76.04 ± 2.70	15	0.065

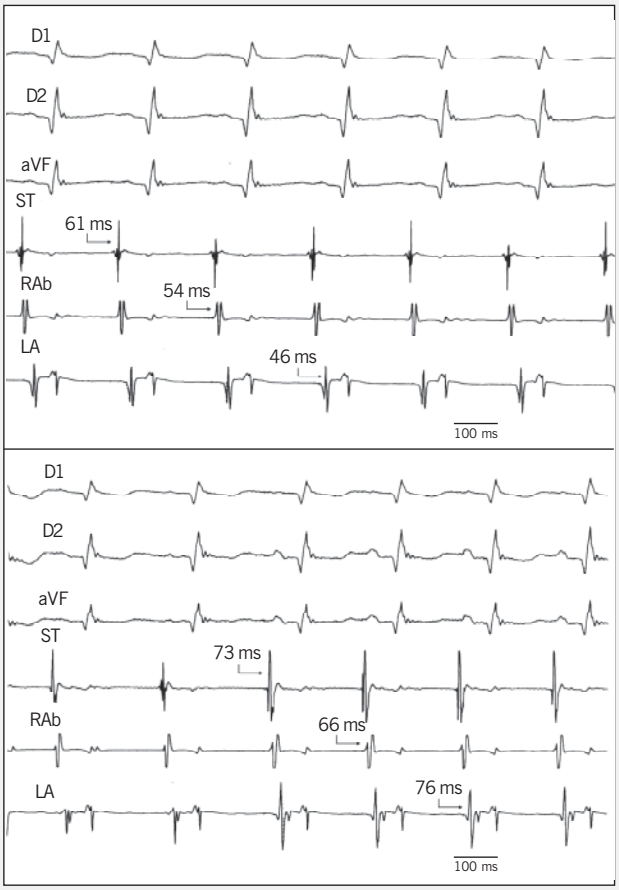
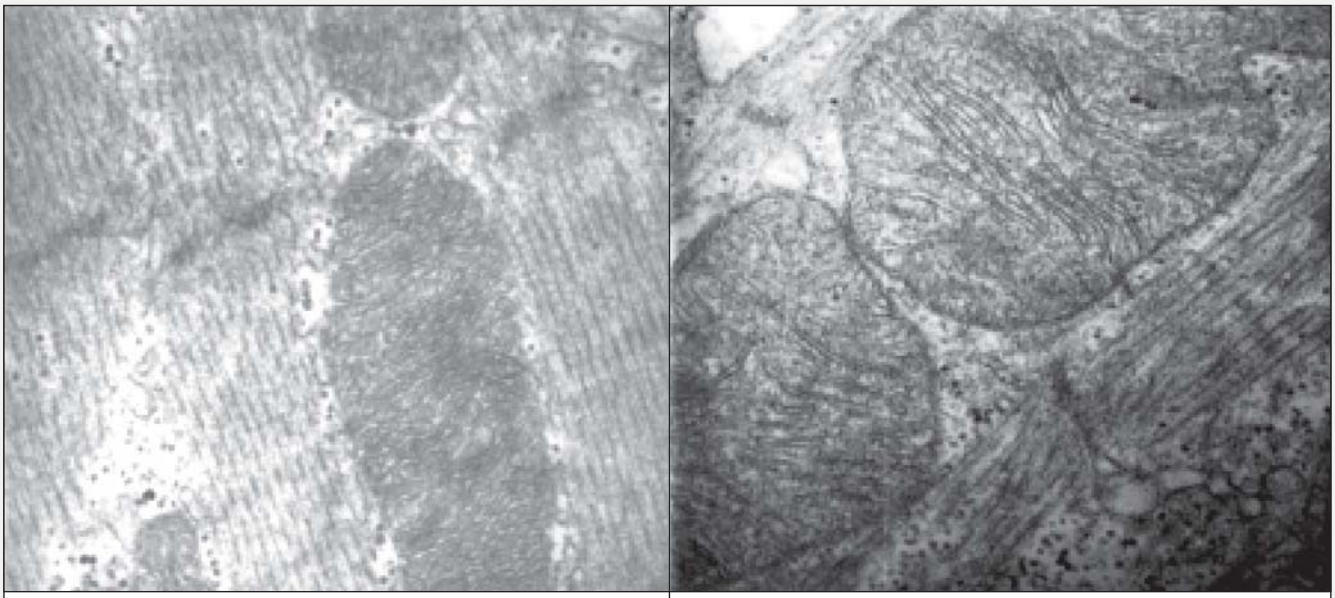


Fig. 4 - D1, D2, and aVF electrocardiographic leads and ST, RAb, and LA bipolar epicardial atrial electrograms recorded in dog 11. The upper and lower tracings show the situations before and after atrial stimulation with continuous pulsatile current, respectively. Both tracings are sinus rhythm. An increase in duration of bipolar atrial electrograms is seen after atrial stimulation.

of atrial stimulation, and in 54% of the animals the arrhythmia persisted spontaneously even after interruption of the stimulation. This may be due to the electrophysiological and structural cell modifications that facilitate the maintenance of arrhythmia. Most studies related to artificial induction of atrial fibrillation in the laboratory reported progressively longer periods of that arrhythmia as the time of stimulation increased [2-4]. Those observations indicate the occurrence of structural changes in the cells in association with electrophysiological alterations. Short periods of stimulation, as those used in our study, may cause only transient and reversible modifications in atrial electrophysiology in response to the rapid frequency of stimulation, differently from that which occurs when stimulation extends for 3 or 4 weeks. In other words, with short periods of atrial stimulation, the alterations observed would be only functional and primary, related to stimulation itself, indicating a phase of cell adaptation to artificially induced tachycardia. On the other hand, longer periods of stimulation would induce more intense structural alterations, hindering the separation of the



Control em 16000 X	After atrial stimulation EM 16000 X

Fig. 5 - Electron microscopy (EM) of atrial tissue with a 16000-fold magnification, showing the effects of atrial stimulation with pulsatile continuous electrical current for 60 minutes on the myocytes. To the left, the control situation, and, to the right, after atrial stimulation. After atrial fibrillation induction, an intense disarray of myofibrils is seen. It is worth noting the great increase in mitochondria and disorganization of their lamellae as compared with those in the control situation.

primary effect from the secondary effect of atrial stimulation in the appearance of atrial fibrillation [9].

Atrial stimulation with pulsatile continuous current caused modifications in cell electrophysiology. In other experimental models, which include atrial stimulation for more than 24 hours, sometimes for weeks, enlargement of the time of atrial conduction is frequently observed, mainly in experiments in which heart failure is developed [2]. This is due to the presence of tissue fibrosis and also the intense structural disorganization of the cells [10]. The greatest delay in atrial conduction found in our study occurred in the ST-LA interval, with atrial stimulation at 200-ms cycle. In our study, the delay occurred with the more rapid atrial stimulation between 2 distant electrodes, instead of also appearing between ST and RAb, which were close to each other. This finding indicates that the wave front finds an increasing amount of partially refractory tissue before it during rapid stimulations, as the time of recovery of local excitability is more prolonged in that condition. The degree of greater delay between the ST and LA regions with 200-ms cycle and not with 300-ms cycle could indicate inversion in adaptation of atrial conduction to more rapid frequencies, according to Manios et al [11], considering the monophasic action potential. In the right atrium and with longer stimulation cycles (ST-RAb interval with 300-ms cycle), no prolongation in the time of atrial conduction was observed, because recovery of atrial excitability is more rapid close to the stimulating source, similarly to the stimulation in ST, which would reduce the chance of conduction of the electric impulse in a still partially refractory tissue between that region and RAb [8].

One important finding in our study was the observation that the time of atrial conduction of the early impulses was significantly prolonged after atrial stimulation. Atrial fibrillation appears and tends to perpetuate itself when an important degree of delay in the conduction of the electrical impulse exists, even when no alteration in duration of the atrial refractory period exists. It has already been experimentally demonstrated in hearts of rabbits that a critical delay in conduction is necessary to initiate the activity in a reentrant circuit [12]. Cosio et al [7] and Buxton et al [13] have already shown that patients with a clinical history of atrial fibrillation have a prolongation in the conduction of extrasystoles artificially introduced in the atria, this being a mark of atrial vulnerability for the appearance of atrial fibrillation in that population. Qin et al [14] reported that patients with a history of atrial fibrillation have a much more significant and intense increase in the gradient of the curve of atrial conduction in regard to prematurity of the atrial extrastimuli than patients with no arrhythmias do. Those authors also reported that no important difference in the duration of the atrial refractory periods existed in both groups, as observed in our study, suggesting that the alteration in atrial substrate, represented by prolongation in the time of conduction was the most important factor for triggering atrial fibrillation in patients with a clinical history of that arrhythmia.

One of the conditions for the appearance of atrial fibrillation, even in normal hearts, is the heterogeneity of duration of refractory periods. Our study showed that dispersion of refractoriness was greater in the animals more vulnerable to the appearance of atrial fibrillation. Of the 15 animals, in which sustained atrial fibrillation was induced after assessment of atrial vulnerability, 11 (73.3%) had an increase in dispersion of duration of the effective atrial refractory period. This finding suggests that atrial stimulation causes an electrophysiological disarray in the tissue, resulting in regional heterogeneity of the refractory periods. Absence of significant changes in duration of the effective atrial refractory period after atrial stimulation for 60 minutes and the increase in its heterogeneity (ie, greater dispersion) in dogs in which atrial fibrillation was induced after that period were observed. These results were similar to those reported by Fareh et al [15], who observed greater atrial vulnerability in animals undergoing atrial stimulation at 400 ppm for 24 hours. In those dogs, dispersion of refractoriness was the major factor related to greater vulnerability to artificial induction of atrial fibrillation, while the effective refractory period per se and wave length showed no relation.

Immediately after interrupting induced atrial fibrillation, the duration of the local electrograms prolonged significantly during sinus rhythm in the 3 regions studied, particularly in ST and RAb. This finding indicates a local delay in conduction with slower heart rates, ie, reverse heart-rate dependent effect, and may be an important factor for the appearance of new episodes of arrhythmia, particularly in the presence of triggers, such as atrial extrasystoles. In a study by Tanigawa et al [16], right atrial mapping in patients with a history of atrial fibrillation, with or without associated sinus dysfunction, showed long-term fractioned atrial electrograms heterogeneously distributed. Tai et al [17], performing endocardial mapping in patients with and without atrial arrhythmias, showed that patients with paroxysmal atrial fibrillation or flutter had more fractioned right atrial electrograms and of longer duration than individuals with no documented atrial arrhythmia. In our dogs, structural cell alterations characterized by the presence of myofibril disarray, degeneration of myocytes, interposition of collagen tissue among myofibrils and interstitial edema may justify local delays in conduction and the consequent increase in duration of atrial electrograms [18].

In assessing atrial vulnerability, atrial fibrillation was easily induced in most animals (86.36%) after the period of atrial stimulation with continuous current. Sustenance of arrhythmia for more than 30 seconds was observed in 68.18% of them, a value similar to the 67% of dogs of the study by Gaspo et al [19], whose right atria were stimulated for 7 days [2,5]. Based on the study by Wijffels et al [3], it is currently known that long times of stimulation are required for the periods of atrial fibrillation to be sustained for 24 hours or more [3]. In our animals, however, dispersion of refractoriness and local delay in conduction of the impulse were sufficient for arrhythmia to be induced and sustained in 73.30% of them, despite the stimulation period of 60 minutes.

In our study, persistent atrial stimulation causes changes in atrial electrophysiology and architecture in the hearts of dogs with no heart disease. The changes should increase atrial vulnerability to the spontaneous appearance or triggering of arrhythmia through programmed atrial stimulation. One of the major difficulties for the experimental study of atrial fibrillation relates to its induction and sustenance for long periods, so that nonpharmacological treatments can be tested. With the greater vulnerability of the atrial tissue, both for the artificial induction and spontaneous and sustained appearance of arrhythmia, techniques of intracavitary electrical cardioversion and percutaneous ablation with catheters may also be tested, considering that it is a simple and rapid model for obtaining that arrhythmia.

Study limitations - The results of that study were obtained after atrial stimulation for only one hour. In addition, the animals were stimulated while being under general anesthesia and after chest opening, conditions different from those of the other models already reported on. Therefore, our results should not be compared with those of these studies or extrapolated to situations in which atrial stimulation is performed in the endocardium for a longer period than that used in our study.

The recordings of atrial electrograms were obtained in 3 regions, not allowing, however, that conclusions about changes in atrial frequency and morphologies of atrial electrograms of other nonrecorded areas could be established. One could formulate the hypothesis that the duration of the atrial electrograms was altered after induction of atrial fibrillation due to the occasional local edema caused by suture of the pacemaker threads. Against this possibility is the fact that observation for up to one hour after ending the experiment showed a gradual return of duration of those electrograms to the control condition. If those alterations were of traumatic origin, they might tend to persist. In addition, in a pilot study using endocardial registration with multipolar catheters, widening of the electrograms was also identified, but only after the protocol of atrial stimulation.

Our study determined neither left atrial size nor intracavitary pressure; therefore, the influence of those variables in the results could not be defined. However, direct observation of the surgical field showed an important enlargement of both atrial chambers, in addition to detecting the greater tension in the atrial wall, which was subjectively assessed on palpation of the muscular mass, at the moments when atrial fibrillation was induced. With interruption of stimulation, those alterations disappeared. During atrial fibrillation induction, significant drops in mean systemic blood pressure that could impair the hemodynamic status of the animals and influence the results were not observed.

The possibility of influences of the autonomous nervous system on our results could not be ruled out, because complete autonomous block was not performed. It is worth emphasizing, however, that the heart rates in sinus rhythm, before and after the period of atrial stimulation, were not significantly different, which may be an indicator suggesting that the autonomic conditions were similar. The nonuse of the routine practice of autonomous block was justified because the primary objective of this study was to assess a rapid and effective technique for the artificial induction of atrial fibrillation, with no concern about describing the electrophysiological mechanisms. On the other hand, the histological remodeling that we observed may have been the major cause of the electrophysiological alterations here reported, regardless of occasional changes in the autonomous nervous system.

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