Atrial Tachyarrhythmias and Repolarization Changes Induced by Discrete Activation of Dorsal Mediastinal Cardiac Nerves in Canines

René Cardinal, PhD; J. Andrew Armour, MD, PhD; Caroline Bouchard, AHT; Michel Vermeulen, MScA; Alain Vinet, PhD; Réginald Nadeau, MD; Pierre Pagé, MD

Background—Chronotropic “vagal responses” elicited by high-frequency stimulation have been used to identify atrial targets for ablative treatment of atrial tachyarrhythmias (AT), whereas an anatomic approach consisting of extensive ablation of the ganglionated plexus areas has been proposed as an alternative. Therefore, there is a need for precise delineation of juxtacardiac nerves involved in AT initiation and clarification of their regional influences throughout the atria in relation to AT sites of origin, beyond chronotropic effects related to sinus node modulation.

Methods and Results—Unipolar electrograms were recorded from 191 biatrial epicardial sites in 13 anesthetized canines, with concomitant left atrial endocardial recording from 63 sites in 5 of 13 animals. When electric stimuli were delivered to dorsal mediastinal nerves during the atrial refractory period, atrial premature depolarizations initiating AT were elicited in all animals, most frequently without prior sinus cycle length modification. Among 63 episodes, the sites of origin of early AT beats were localized to (1) the posterolateral left atrial wall in the pulmonary vein region (33%), (2) superior left atrial loci along the Bachmann bundle (55%), and (3) the region of Bachmann bundle insertion into the superior right atrial wall (11%). Moreover, the AT sites of origin were spatially concordant with regional waveform changes during the repolarization phase of unipolar recordings. AT induction and repolarization changes were abolished after atropine administration.

Conclusions—Activation of individual dorsal mediastinal nerves induces AT arising from distinct sites of origin which are spatially concordant with regional atrial repolarization changes. (Circ Arrhythm Electrophysiol. 2010;3:511-520.)

Key Words: arrhythmia mechanisms electrophysiology mapping | nervous system | autonomic | tachyarrhythmias | vagal stimulation

Clinical studies support the notion that targeting juxtacardiac nerves may be useful adjunct therapy to pulmonary vein (PV) isolation and ablation of select atrial muscle structures for the treatment of paroxysmal atrial fibrillation.1-3 Selective ablation of loci from which chronotropic (or dromotropic) “vagal responses” are induced by high-frequency stimulation has been used by some investigators,1,2,4 whereas an anatomic approach with extensive regional ablation of the ganglionated plexus areas has been contemplated by others.5,6 Therefore, there is a need for precise delineation of juxtacardiac nerves involved in the generation of atrial tachyarrhythmia (AT) initiation and clarification of their regional influences throughout the atria in relation to AT sites of origin, beyond chronotropic and dromotropic effects related to sinus and AV node modulation.

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The major canine cardiopulmonary nerves that arise from the middle cervical and stellate ganglia and the vagus nerves course toward the heart in the dorsal mediastinum where they form, at the base of the heart dorsal to the pulmonary artery and aorta, the dorsal mediastinal cardiac nerves.7,8 Such nerves, which can be identified in humans9-11 as well as in canines,7,8 represent a major route of autonomic neural input to the intrinsic cardiac nervous system, together with nerves coursing along the superior vena cava and the PVs.12,13

Using multielectrode atrial mapping in the canine heart—a standard model to study the cardiac nervous system that bears good correspondence to human14,15—we show that the initial beats of ATs induced when electric stimuli are applied to dorsal mediastinal nerves occur most frequently without any prior neurally induced sinus rate modification and originate from several distinct left atrial locations that include the PV region. We also investigate the proposition that the sites of origin of ATs thus induced are spatially concordant with waveform changes in the repolarization phase of local unipolar recordings.16,17
Methods

Animal Preparations
Experiments were performed in 13 adult mongrel canines (either sex, 16 to 28 kg) in accordance with the Canadian Council for Animal Care and international guidelines. Animals were anesthetized with thiopental (25 mg/kg iv, supplemented as required), intubated, and ventilated. After thoracotomy, anesthetic was changed to /\text{-chloralose (25 to 50 mg/kg iv supplemented as required). ECG and blood pressure were recorded. In 8 preparations, AV block was induced by formaldehyde (37%, 0.1 mL) injection into the AV node to separate atrial from ventricular electric events, and right ventricular pacing (60/min) was instituted.

Mediastinal Nerve Stimulation
Electric stimuli were delivered to visually identified mediastinal nerves that course intrapericardially at left, middle, and right dorsal locations cranial to the superior left atrial (LA) wall and along the transverse pericardial sinus (Figure 1A and 1B) with the use of bipolar electrodes (1.5 mm apart) mounted on a probe connected to a battery-driven current source controlled by a programmable stimulator (Bloom Associates, Philadelphia, Pa) triggered by a reference signal derived from a pair of electrodes sutured onto atrial muscle. A train of 5 stimuli (1 to 2 mA, 1-ms duration, 5-ms pulse interval) was delivered once per sinus cycle (Figure 1C, arrows) with a minimum interval between trains set at \(~50\text{ ms}\) less than sinus cycle length (trigger inhibition period). A delay from the reference signal to stimulator activation was imposed such that the stimuli were applied early within the atrial cycle of the muscle sites closest to the nerve stimulation site, as previously reported for nerves coursing along the superior vena cava to avoid muscle capture. However, this was an extra precaution because muscle capture is a rare occurrence when 1- to 2-mA electric stimuli are applied to dorsal mediastinal nerves because of their location and embedment in fatty tissues. Electrode contact with the nerve sites was interrupted immediately after tachyarrhythmia onset, usually extending to the first beat after the neurally induced atrial premature depolarization at tachycardia onset (Figure 1C). Active nerve sites were identified with reference to anatomic landmarks such that, when exposed to focal electric stimuli, changes in atrial rhythm could be elicited reproducibly.

Atropine
In 9 animals, atropine maleate (1 mg/kg iv) was administered and the same sites were restimulated.

Atrial Mapping
Silicone plaques carrying 191 unipolar recording contacts (4.6- to 5.9-mm spacing) were positioned on right and left atrial surfaces (Figure 2A). Concomitantly, LA endocardial unipolar recordings were obtained in 5 animals using an inflatable balloon array (63 electrodes) introduced via the LA appendage tip. Electrodes and...
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Color-coded isochronal maps (10-ms intervals) were converted to digital format at 1000 samples/s/channel. Activation with reference to 4 limb leads) were amplified (0.05 to 450 Hz) and cardiomap) using a PC computer. Unipolar electrograms (measured by custom-made software (Cardiomap III: www.crhsc.mtl.rts.qc.ca/2B and Figure 3).

computed automatically by linear interpolation (see below, Figure 2B and Figure 3).

The spatial distribution of neural effects on repolarization was assessed by mapping the net area (integral) subtended by the unipolar electrode inserted via the LAA was used for endocardial mapping of the LA wall, PV region, BB, and interatrial septum (IAS). The endocardial LA surface is depicted as a polar representation with the IAS, PV region, BB and mitral valve (MV) in the right-hand, left-hand, upper and lower quadrants, respectively. B, Epicardial (upper) and endocardial (lower) isochronal maps illustrate that in sinus rhythm, the impulse originated from the sinus node region with earliest LA activation along the BB and, endocardially, in the IAS.

ECG were connected to a multichannel recorder (ED112/256, Institut de génie biomédical, École Polytechnique de Montréal) controlled by custom-made software (Cardiomap III: www.crhsc.mtl.rts.qc.ca/cardiomap) using a PC computer. Unipolar electrograms (measured with reference to 4 limb leads) were amplified (0.05 to 450 Hz) and converted to digital format at 1000 samples/s/channel. Activation times were identified at maximum negative potential displacement (\(-\mathrm{dV/dt}_{\text{max}}\)). Color-coded isochronal maps (10-ms intervals) were computed automatically by linear interpolation (see below, Figure 2B and Figure 3).

The spatial distribution of neural effects on repolarization was assessed by mapping the net area (integral) subtended by the unipolar electrode at each recording site during basal sinus beats and sinus beats during mediastinal nerve stimulation just before tachyarrhythmia onset. By algebraic subtraction of the integral value for the basal beat from the integral value measured during nerve stimulation, area difference maps are plotted indicating the atrial regions that were affected by nerve stimulation,16,20,21 for example, displaying neurally mediated positive changes.17 Maps of regional atrial repolarization changes were elicited beyond a threshold of 50 mV·ms (corresponding to a maximal variation among unipolar recordings of 2×SD of changes in repeat measurements under basal conditions).

Data Analysis

Separate cumulative maps were drawn for tachyarrhythmias displaying each of the 3 regions were compared between the tachyarrhythmias originating from each of these regions by analysis of variance (mixed-effect ANOVA: region as “within” and tachyarrhythmia site of origin as “between” factor). Statistical tests were done using the arc-sine transformed proportions.22 To counter a potential bias from unequal contributions among canine preparations in terms of the numbers of tachyarrhythmia, the contributions of several tachyarrhythmias from a particular site of origin in a given preparation were averaged prior to calculation of the mixed-effect ANOVA. Likewise, descriptive statistics of the variables extracted from the arrhythmias were presented as mean±SD of averages per canine preparation. Sinus cycle length changes from basal state to nerve stimulation were analyzed by paired t test analysis. The relationships between dorsal mediastinal nerve stimulation sites (left, middle, right) and rhythm modifications (tachyarrhythmia, bradycardia, bradycardia followed by tachyarrhythmia) and between the nerve stimulation sites and tachyarrhythmia sites of origin (posterolateral LA wall, Bachmann bundle, its insertion into the superior RA wall) were analyzed by 3×3 χ² test. The level of certainty for rejecting the null hypothesis was \(P<0.05\).

Results

Among the 13 animals, 85 nerve sites were identified that, when subjected to stimuli applied for short periods of time (3.7±2.2 seconds, \(\approx7\) trains), elicited 3 types of rhythm modification. The pattern that was most frequently elicited (from 63 nerve sites in all animals) consisted of the spontaneous occurrence of an atrial premature depolarization (coupling interval of 284±68 ms from the preceding sinus beat) initiating a tachyarrhythmia (cycle length, 143±19 ms versus sinus, 446±60 ms) (Figure 1C). Although the stimulation
was interrupted at tachyarrhythmia onset, episodes lasted for a median value of 10 seconds (range, 577 ms to 365 seconds; 25% quartile, 1750 ms; 75% quartile, 34 seconds) before terminating spontaneously. Such tachyarrhythmias were elicited from the left, middle, and right dorsal mediastinal nerve sites (18, 23, and 22 sites, respectively).

A second pattern consisted of sinus cycle length prolongation (from 457/11006 to 66 ms, that is, by 23/11006 12%, \( P < 0.001 \)) elicited from 14 dorsal mediastinal nerve sites in 7 animals (left, 3; middle, 2; right, 9). In a third pattern, sinus cycle length prolongation (from 478/11006 to 77 ms, that is, by 8/11006 3%, \( P < 0.005 \)) occurred before an atrial premature depolarization initiating a tachyarrhythmia was initiated (from 4 left and 4 right dorsal mediastinal nerve sites, in 5 animals). There was a significant relationship between nerve stimulation site and pattern (\( P < 0.046 \)).

Mapping Activation: Tachyarrhythmia Sites of Origin

Sinus Rhythm

During sinus rhythm (Figure 2B), epicardial maps displayed monofocal patterns in which the region of earliest 10-ms activation was identified in the superior portion of the right atrial (RA) wall in the vicinity of the root of the superior vena cava (QS unipolar electrogram morphology). At the endocardial level, the earliest activation was detected on the LA aspect of the interatrial septum (rS unipolar electrogram morphology).

Neurally Induced Atrial Premature Depolarization and Subsequent Tachyarrhythmia Beat

The earliest atrial activation was most frequently identified at LA locations in the PV region (21 of 63 tachyarrhythmias, that is, 33%) or Bachmann bundle region (35 tachyarrhythmias, that is, 55%) and less frequently at superior RA locations (7 tachyarrhythmias, that is, 11%). In the example shown in Figure 3, electric stimuli applied to a left dorsal mediastinal nerve site induced a late-coupled atrial premature depolarization in which the areas of earliest epicardial breakthrough were identified on the lateral LA wall (5 ms) and posterior wall (2 ms) adjacent to the left PV (Figure 3A). Slightly earlier activation (0 timing) was identified at underlying endocardial sites. In the subsequent beat (Figure 3B), the earliest activity was recorded epicardially in the PV region and, as occurred typically in all tachyarrhythmia episodes, there was an increase in the total epicardial activation time. In any episode among all tachyarrhythmias, the atrial premature depolarization and subsequent tachyarrhythmia beat displayed similar sites of origin but increased total epicardial activation time. In the following tachyarrhythmia beats (not shown), disorganized and overlapping activation patterns precluded identifying sites of origin (tachyarrhythmia duration, 12.3 seconds; mean cycle length, 126 ms).

In another trial (same experiment) in which electric stimuli were reapplied to a left dorsal nerve site (Figure 3C), the atrial premature depolarization thus induced displayed a distinct pattern in which the site of origin was identified in the
Bachmann bundle region and the earliest activation was identified at an endocardial site underlying the earliest epicardial breakthrough.

Direct Stimulation of Atrial Muscle in the Vicinity of Left Dorsal Mediastinal Nerve Sites

When pacing atrial muscle (Figure 3D), the earliest site of activation was identified at the site of muscle stimulation in the superior LA wall, thereby illustrating that muscle capture yielded isochronal activation patterns distinct from the ones displayed by neurally induced beats (Figure 3A and B: PV region; Figure 3C: Bachmann bundle).

Relationship With Neurally Induced Unipolar Waveform Changes

The effect on repolarization was determined by comparing and subtracting the waveform areas subtended by unipolar electrograms recorded at each recording site under basal conditions and during nerve stimulation, as illustrated in Figure 4A. This difference corresponds to the gray shading illustrated in the superimposed traces (a, b, and c) of unipolar recordings under basal (red) and during nerve stimulation (blue). In Figure 4A, left dorsal mediastinal nerve stimulation induced a marked increase in the area subtended by unipolar electrograms recorded from endocardial sites in the posterior LA wall (arrows) eliciting an atrial tachyarrhythmia.

Figure 4. Relationship between tachyarrhythmia sites of origin in the posterolateral LA wall and neurally induced repolarization changes. The upper tracing shows a unipolar recording in the basal state and during left dorsal mediastinal nerve stimulation (arrows) eliciting an atrial tachyarrhythmia. A, Changes in the atrial repolarization waveform at each site are assessed as the difference between the waveform area in unipolar recordings during neural stimulation (blue) minus basal (red). Red and blue traces show superimposed recordings from an endocardial site in the posterolateral LA wall (a), and epicardial sites in the lateral (b) and posterior (c) LA wall. Epicardial (above) and endocardial (below) left-hand maps show the regional distribution of neurally induced waveform changes represented as color codes ranging from diffuse slight fluctuations (red, orange) to markedly positive changes (green, blue) at endocardial and epicardial loci of the posterolateral LA wall. B, Isochronal maps indicate that the sites of the early tachyarrhythmia beats (in upper tracing) are localized to the endocardial posterolateral LA wall in the region of maximal neurally induced repolarization changes.
LA wall next to the superior PV (superimposed traces “a” and map: blue color code corresponding to positive changes of $+300$ to $500 \text{ mV} \cdot \text{ms}$). Marked changes were also identified epicardially in the superior LA wall and appendage (superimposed traces “b” and map: green color code corresponding to changes of $+150$ to $300 \text{ mV} \cdot \text{ms}$, orange-yellow: $+50$ to $150 \text{ mV} \cdot \text{ms}$). The majority of epicardial sites in the posterior and inferior LA wall as well as all RA sites displayed slight fluctuations which did not reach the $+50 \text{ mV} \cdot \text{ms}$ threshold for physiological significance, as illustrated with superimposed traces “c” (map: red code). The activation maps shown in Figure 4B indicate that during the first tachyarrhythmia beat, the earliest activation was identified at endocardial sites in the region of the left superior PV. Concomitantly, marked activation delay developed in the superior LA wall and appendage (as well as in RA regions remote from the tachyarrhythmia site of origin). The endocardial tachyarrhythmia site of origin as well as superior LA epicardial sites displaying marked activation delay (blue color code, bunching isochronal lines) were localized in regions in which marked repolarization changes were identified (blue or green codes).

Figure 5A shows the cumulative incidences (among 16 tachyarrhythmias) of the sites of earliest 10-ms activation when the tachyarrhythmia origin was localized in the lateral

<table>
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<td>Bachmann’s bundle ($n=19$ tachyarrhythmias)</td>
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**Figure 5.** Relationship between the spatial distribution of repolarization changes and tachyarrhythmia sites of origin in the posterolateral LA wall (A), Bachmann bundle (BB) insertion into the RA wall (B), and BB (C). Cumulative incidences of tachyarrhythmia sites of origin (left hand epicardial maps) and regional atrial unipolar waveform changes in response to mediastinal nerve stimulation (right-hand epicardial maps with LA endocardial maps in A and C). Color-coded maps show for each recording site the proportion (%) of the tachyarrhythmias ($n$) in which the corresponding recording site was activated within the earliest 10-ms activation (left hand maps) or displayed neurally induced changes $>$ $+50 \text{ mV} \cdot \text{ms}$ (right-hand maps).
LA or posterior wall next to the left PVs (left hand diagrams). The tachyarrhythmias displaying such sites of origin were elicited most frequently by stimulation of left but also middle and right dorsal mediastinal nerve sites (12, 3, and 1 tachyarrhythmias, respectively). In such instances, marked repolarization changes were identified epicardially on the lateral LA wall as well as endocardially in the PV region (Figure 5A, right-hand maps). When tachyarrhythmias originating from Bachmann bundle insertion in the superior RA (Figure 5B, summarizing 7 tachyarrhythmias, all elicited by right dorsal mediastinal nerve stimulation), right-sided repolarization changes were identified at the epicardial level (no endocardial map). In contrast, the tachyarrhythmias originating from the Bachmann bundle region (Figure 5C summarizing 19 tachyarrhythmias elicited by left: 5, middle: 5, and right: 9 dorsal mediastinal nerve stimulation) were associated with widespread repolarization changes that were identified in the Bachmann bundle region (epicardially and endocardially) as well as the LA and RA superior wall and appendage. Overall, there was a statistically significant relationship between the nerve stimulation sites and tachyarrhythmia sites of origin (P<0.001).

Moreover, statistical analysis indicated that, depending on the localization of the tachyarrhythmia sites of origin (used as
classification criterion), the numbers of recording sites displaying neurally mediated repolarization changes varied in the 3 regions under study, that is, (1) the posterolateral LA wall, (2) the RA wall, and (3) the Bachmann bundle (P = 0.007, ANOVA). A greater number of neurally mediated repolarization changes were identified in the LA wall when the tachyarrhythmias displayed left-sided sites of origin, whereas a greater number of repolarization changes were identified in the RA wall when the tachyarrhythmias had right-sided sites of origin. However, the spatial distribution of repolarization changes did not vary between the LA wall, Bachmann bundle region, and RA wall when the tachyarrhythmias originated from the Bachmann bundle (in accordance with the data illustrated in Figure 5C).

The left-hand maps shown in Figure 6 (pretropine) show the spatial distribution of repolarization change incidences according to which dorsal mediastinal nerve was stimulated. Left dorsal mediastinal nerve stimulation (A) induced repolarization changes that were identified predominantly in the LA (green and light blue color code) but also in the RA (green), whereas right dorsal mediastinal nerve stimulation (B) induced repolarization changes that were identified predominantly but not exclusively in the RA. The incidences of repolarization changes induced by middle dorsal mediastinal nerve stimulation (C) were lower, being restricted to smaller LA and RA regions.

Atropine
Atrial tachyarrhythmias were no longer elicited from previously active nerve sites in all 9 animals after atropine administration. Note that the incidences of neurally induced atrial repolarization changes were abolished after atropine (Figure 6, right-hand maps).

Discussion
Major findings are as follows. (1) The sites of origin of early atrial tachyarrhythmia beats were identified in three distinct atrial regions: (a) the posterolateral LA wall in the PV region (33%), (b) superior LA loci along the Bachmann bundle (55%), and (c) the region of Bachmann bundle insertion into the superior RA wall (11%). (2) Unipolar waveform changes during the repolarization phase (designated herein as repolarization changes) were induced by nerve stimulation in all cases. (3) The tachyarrhythmia sites of origin were spatially concordant with the atrial repolarization changes. (4) The atrial tachyarrhythmias occurred most frequently without prior sinus cycle length modification (being preceded by sinus cycle length deceleration in a minority of cases).

The dorsal mediastinal nerves carry axons of both cholinergic and adrenergic efferent as well as afferent fibers derived from several extrinsic cardiac nerves, for example, the right recurrent and right cranial vagal cardiopulmonary nerves, medial nerves exiting from the left vagosympathetic trunk and branches from the left recurrent laryngeal nerve.7,8 These nerves are known to provide neural inputs to the intrinsic cardiac ganglionated plexuses and to cardiac muscle, and carry afferent information to the various levels of the cardiac neuronal hierarchy.23 The fact that all tachyarrhythmia responses to mediastinal nerve stimulation were completely abolished by atropine is consistent with the view that cholinergic efferent neurons play a predominant role in neurally mediated atrial tachyarrhythmia formation in normal canines.19,24–20 although adrenergic modulatory influences can also be demonstrated.19,20

Sites of Origin of Atrial Premature Depolarizations and Initial Tachyarrhythmia Beats
It is noteworthy that the sites of origin of the atrial premature depolarizations and early tachyarrhythmia beats elicited by mediastinal nerve stimulation localize to specific regions, whereas the ectopic foci elicited by cervical vagus nerve stimulation are widely distributed over the atria.26 The present study complements our previous report that electric stimuli applied to the right-sided ventral mediastinal nerves coursing along the superior vena cava elicit atrial tachyarrhythmias that were consistently associated with repolarization changes within the upper portion of the RA subsidiary pacemaker complex in proximity to the superior vena cava ostium19; the tachyarrhythmias thus induced were preceded by bradycardia in all cases.19 This is in contrast with the present findings that the tachyarrhythmia sites of origin were identified from several LA locations and that only a minority of the tachyarrhythmias induced by left-sided dorsal mediastinal nerve stimulation were preceded by sinus cycle length prolongation.

The tachyarrhythmia sites of origin induced in canines by right-sided16,19 and left-sided mediastinal nerve stimulation recapitulate clinical reports that ectopic foci initiating atrial tachyarrhythmias are not restricted to the PVs20 but can be found in other regions, including the posterior LA wall, the superior vena cava and the crista terminalis.31 The special cellular electrophysiological properties and complex fiber orientation identified in canine32–34 as well as in human35 PV tissues may contribute, together with their distinctive autonomic nerve profile, to such tachyarrhythmia generation.

Relation to Neural Influences on the Atria
The spatial distribution of repolarization changes elicited by activation of a given mediastinal nerve probably results from interactions between various factors, among which (1) the nerve’s specific effenter pattern of atrial projections as well as the atrial influences mediated as a consequence of activation of the nerve’s afferent components,36 (2) the atrial distribution of muscarinic receptors,37 and (3) local variations in sarcolemmal I_{K(ACh)} protein expression.38 Interestingly, adrenergic and cholinergic epicardial nerve and muscarinic receptor densities as well as I_{K(ACh)} heterogeneity are relatively higher in the canine posterolateral LA wall.37,38

When the early tachyarrhythmias beats originated from (1) the posterolateral LA wall next to the left PVs or (2) Bachmann bundle insertion into the superior RA wall, repolarization changes were concentrated in the atrial regions in which the tachyarrhythmias sites of origin were identified. The tachyarrhythmias sites of origin identified in the posterolateral LA wall (most frequently associated with left dorsal mediastinal nerve stimulation) were adjacent to the ligament of Marshall (which includes predominantly cholinergic neural elements in the canine heart39 as well as to the LA and dorsal...
atrial ganglionated plexuses. Likewise, the tachyarrhythmia sites of origin identified in Bachmann bundle insertion into the superior RA (all associated with right dorsal mediastinal nerve stimulation) lay next to the superior extension of the RA ganglionated plexus.

However, when the tachyarrhythmia sites of origin were identified in the Bachmann bundle (associated with all 3 nerves), widespread repolarization changes were identified therein but also in the LA and RA appendages as well as in the superior LA and RA walls. That neurally induced changes were identified well beyond the tachyarrhythmia sites of origin identified in the Bachmann bundle could be explained by (1) their association with either left, middle, or right dorsal mediastinal nerve stimulation, (2) spatially divergent interconnections within the intrinsic cardiac nervous system, and/or (3) the fact that the Bachmann bundle acts as a pathway for rapid conduction between the RA and LA and that therefore tachyarrhythmia sites of origin identified in the Bachmann bundle could represent either a local event or an early epicardial breakthrough from relatively distant endocardial sites of origin that would have been missed with the relatively limited resolution of our multielectrode arrays.

The association of tachyarrhythmia sites of origin with spatially concordant sites of neurally induced repolarization changes measured during the beats that immediately preceded tachyarrhythmia onset is a direct demonstration of the classical concept that spatial heterogeneity of atrial repolarization properties are involved in tachyarrhythmia formation. Accordingly, Schauerte et al reported that atrial tachyarrhythmias induced when high-frequency electric stimuli are applied to nerves associated with PVs are associated with local refractory period changes at adjacent atrial muscle sites.

Refractory period measurement by the extrastimulus technique, the classic approach to determine local autonomic influences on the atria, cannot be used for single-beat analyses. Herein, the atrial distribution of the neurally induced influences was identified from a single beat by measuring changes in the area subtended by the unipolar electrogram during the repolarization phase of recordings from multiple atrial sites. Such changes correlate with changes in refractory period measured by the extrastimulus technique, suggesting that this variable is a useful indicator of the spatial extent and severity of changes in repolarization from one physiological state to another. Thus, electrophysiological markers related to the repolarization properties of atrial muscle could provide further information, in addition to chronotropic and dromotropic "vagal responses," to identify LA target areas associated with tachyarrhythmia sites of origin.

Limitations
Given the relatively limited resolution of the multielectrode array used herein, the present study does not provide information concerning the electrophysiological mechanisms involved in arrhythmia generation (eg, reentrant mechanism versus abnormal impulse formation). Rather, this study addresses only the role of neural inputs in the initiation of the atrial tachyarrhythmias as only the mapping characteristics of the early tachyarrhythmia beats were determined.

While testing the proposition that the tachyarrhythmia sites of origin were associated with spatially concordant repolarization changes, the different animals contributed a variable number of atrial tachyarrhythmias (from a single tachyarrhythmia, up to 6 episodes). Moreover, tachyarrhythmia sites of origin were identified in only 1 of the 3 atrial regions in 5 animals, in 2 regions in 6 animals, and in all 3 regions in only 2 animals. Therefore, it was not possible to use the "canine preparations" as either a fixed or random "between" variable in the ANOVA. However, to counter a potential bias from unequal contributions among canine preparations in terms of tachyarrhythmia numbers, the contributions of several tachyarrhythmias from a particular site of origin in a given preparation were averaged before calculation of the mixed effect ANOVA.

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Disclosures
None.

References
It has been proposed that the negative chronotropic and dromotrophic responses elicited during radiofrequency catheter ablation of atrial fibrillation (termed “vagal reflexes”) may be useful adjunct markers of left atrial sites to be considered as preferred targets for ablative therapy. Such responses require, by necessity, inputs to the sinus node and AV node either via reflexes or through widespread neuronal interconnections within the intrinsic cardiac nervous system. The present study suggests that left-sided neuronally induced changes in the unipolar waveform can be identified at several atrial locations in association with tachyarrhythmias sites of origin, without concommitant modification of sinus rate before tachyarrhythmia onset. Thus, neural ablation procedures could be successfully achieved without ascertaining chronotropic “vagal responses” with the use of electrophysiological markers related to the repolarization properties of atrial muscle.

**CLINICAL PERSPECTIVE**
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