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Electrical Stimulation to Identify Neural Elements on the Heart: Their Role in Atrial Fibrillation

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***Abstract. Experimental Studies*: Anesthetized dogs were subjected to a right then left thoracotomy. Two modes of electrical stimulation were used to activate ganglionated plexi (GP) on the epicardium of the atria: (1) Near the base of each pulmonary vein (PV), trains of high frequency stimuli (HFS) were coupled to each atrial paced beat so as to fall within the refractory period to achieve nerve stimulation without atrial excitation; and (2) Continuous HFS was applied via plaque electrodes sutured to epicardial fat pads (containing a GP) near the right superior (RS) and left superior (LS) PVs. The chest was then closed. An ablation catheter, inserted percutaneously, was positioned fluoroscopically in the right atrium across from the epicardial plaque electrode near the RSPV. Transeptal puncture was used to place an ablation catheter at the LSPV-left atrial junction. HFS applied to each of the epicardial fat pads induced atrial fibrillation (AF) and also caused high grade AV block due to a strong parasympathetic effect on the AV node. Radiofrequency ablation from the right and left atrial endocardium abolished the vagal response to HFS delivered to the plaque electrodes on the fat pads close to the RSPV and LSPV, respectively.**

***Clinical Studies*: Sixty (60) patients with paroxysmal or persistent AF underwent PV antrum isolation (27 patients) or PV antrum isolation plus left atrial GP ablation (33 patients). Endocardial HFS at the border of the PV antra near the 4 GPs produced AF and high grade AV block (vagal response) during AF. RFA at these sites abolished the vagal response. Testing in a small number of patients with very short follow-up suggests that adding GP ablation to PV antrum isolation may increase ablation success (absence of AF recurrence) from 70% to 91%.**

***Conclusions*: These basic and clinical studies suggest that localized cardiac autonomic ganglia (GPs) may play a critical role in the initiation and maintenance of AF.**

***Key Words.* atrial fibrillation, autonomic nervous system, neurotransmitters, radiofrequency ablation**

Introduction

Long before the advent of intracardiac recordings and ablation of cardiac arrhythmias, basic studies had shown that electrical stimulation of autonomic nerves innervating the heart could induce atrial fibrillation (AF) [1,2]. In 1972, Armour et al. [3] were able to induce both atrial and ventricular arrhythmias by stimulation of nerves on the heart itself. It was the astute clinical observations of Coumel and his associates [4–6] which allowed the identification of patients who developed AF predominantly during sleep ("vagal AF") and those that had AF induced predominantly by exercise or emotion. The impetus for targeting the pulmonary veins (PVs) for ablation of AF came in 1998 when Haissaguerre and associates found AF was induced by triggering beats from the PVs in the majority of patients with paroxysmal AF [7].

Experimental Studies

Several basic questions arise from the findings of Haissaguerre and co-workers. For example, how does a premature depolarization from a PV become transduced into AF? Using a modification of the technique reported by Armour et al. [3] Schauerte and co-workers electrically stimulated neural elements on the heart without exciting the adjacent myocardium [8]. Figure 1 shows the application of short trains of high frequency current (5 ms cycle length, square wave pulses 0.1 ms in duration) near the base of the right inferior (RI)PV during left atrial pacing 2 mm from that site (electrode RIPV D-2). The high frequency train was initiated two milliseconds after each atrial pacing stimulus so that it occurred within the atrial refractory period. Thus, atrial excitation was avoided while

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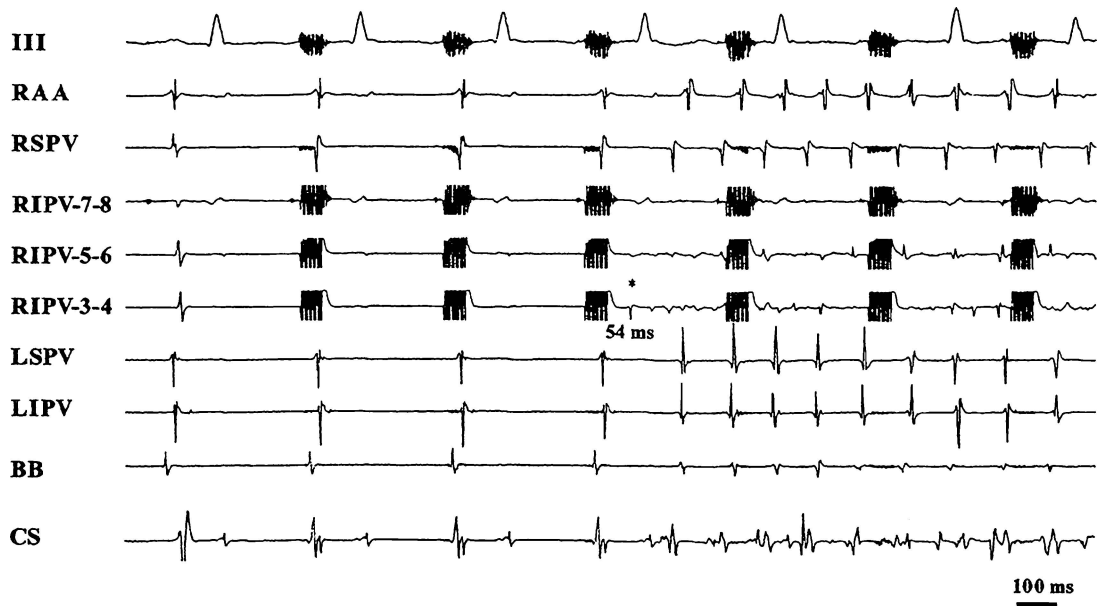

Fig. 1. *Tracings from the top: ECG lead III and electrograms from the right atrial appendage (RAA), sites adjacent to the right superior pulmonary vein (RSPV), the right inferior pulmonary vein (RIPV), left superior pulmonary vein (LSPV), left inferior pulmonary vein (LIPV), Bachman's bundle (BB) and coronary sinus (CS). A train of high frequency electrical stimuli (cycle length = 5 ms; each stimulus 0.1 ms in duration, train duration = 50 ms) was applied near the base of the RIPV at the site of RIPV D-2 (not shown) electrogram, 2 ms after each pacing stimulus starting at the second beat. At the fourth beat a single, spontaneous extrasystole (asterisk) occurs, arising 54 ms after the high frequency train. This extrasystole preceded the onset of atrial fibrillation (AF). Note the atrial potentials closest to the "arrhythmogenic" site (RIPV 3-4) are at a higher frequency (firing pattern) than those at the other electrode recording sites. See text for further details. (Modified from reference 8, Fig. 4).*

local nerve stimulation was achieved. AF was initiated after the 4th beat with high frequency stimulation (HFS). Earliest activation at the onset of AF was recorded by the RIPV electrodes (asterisk) closest to the site of HFS. The atrial cycle length recorded in the other atrial and PV electrograms was considerably longer than in the RIPV electrograms, suggesting that a region of LA close to the site of HFS (probably including the RIPV) was "driving" the AF. Schauerte et al. [8] demonstrated that these responses were blunted or prevented by beta-blocking agents and consistently abolished by atropine indicating an underlying etiology based on autonomic nerve stimulation. Thus, these studies implicated local autonomic stimulation in the initiation of the PV trigger (asterisk, Fig. 1) and the ability of the trigger to induce AF.

In order to establish a more definitive link between autonomic nerves and the initiation of AF, we electrically stimulated clusters of autonomic neurons on the heart which reside in fat pads near the PV-atrial junctions in both canine and human hearts [9–11]. In a series of canine experiments, the heart was exposed using a right and then a left thoracotomy [12]. Electrode plaques were sutured to the epicardial fat pads containing the clusters of autonomic ganglia (ganglionated plexi, GP) adjacent to the right superior (RS)PV and left superior (LS)PV. Figure 2 is a schematic of the posterior aspect of the heart. The filled circles represent sites at which autonomic ganglia were anatomically found in canine [10] and human [11] hearts. The diagonal-lined squares show the location of the plaque electrodes situated on the fat pads containing the GPs.

HFS (50 ms cycle length, square wave pulses 0.1 ms in duration, voltage 0.6 to 2.4) applied epicardially to the GP without exciting the atrium caused slowing of the sinus rate (not shown but previously demonstrated) [9,13,14]. The same response was obtained by HFS delivered endocardially from an electrode catheter located beneath the epicardial fat pad using the same stimulation parameters. An ablation catheter, introduced from a femoral vein, was positioned in the right atrium (RA) across from the epicardial plaque electrode (fat pad) adjacent to the RSPV. HFS was delivered at this site on the RA endocardium. When the voltage was increased, direct atrial activation occurred and AF was induced. This was accompanied by a vagal response, manifested by high grade AV block with marked slowing of the ventricular response (Fig. 3A). The results of these studies suggested that neuronal stimulation of the GP caused neurotransmitter (acetylcholine) release from axons innervating the sinus node

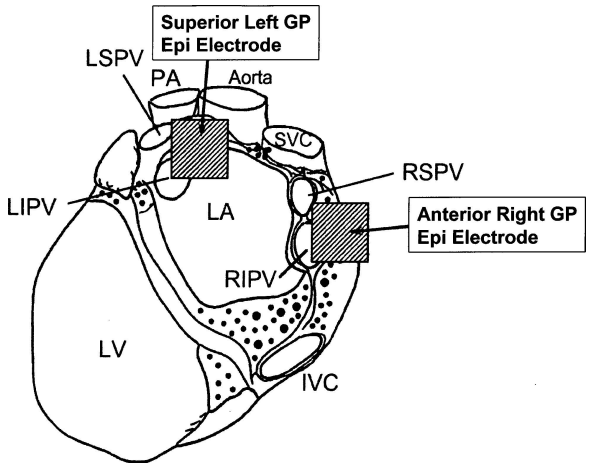

Fig. 2. *Schematic diagram showing the instrumentation applied to various atrial sites in the dog heart (posterior-anterior view). The diagonal-lined squares represent plaque electrodes sutured to two epicardial (Epi) fat pads containing ganglionated plexi (GP). The fat pads are located on the antrum adjacent to the RSPV (anterior right GP) and LSPV (superior left GP). The filled circles represent the distribution of ganglia on the atria and ventricles mainly in the vicinity of the PVs and the vena cavae. Modified from reference [10] Fig. 1).*

(sinus slowing evident during sinus rhythm) and the AV node (slowing of the ventricular response during AF).

Radiofrequency energy (35 watts, 55°C, 90 sec) was then delivered to sites on the RA endocardium producing a vagal response. These sites were located opposite the plaque electrode on the fat pad containing the GP. Following ablation, epicardial stimulation of the fat pad, using the same stimulation parameters, failed to decrease the ventricular response (Fig. 3B). In another series of experimental studies, GP stimulation was used to slow the heart rate. In addition, single atrial extrastimuli delivered close to the PVs resulted in AF only when the adjacent GP was stimulated. Suppression of both heart rate slowing as well as AF induction was achieved by chemical "ablation" of the GP, obtained by injecting lidocaine into the GP [15].

Clinical Studies

The results of the experimental studies [8,12,15] provided the basis for clinical studies which are just beginning. One study is testing the value of adding endocardial GP ablation to conventional AF ablation in patients with paroxysmal or persistent AF. The protocol for conventional AF ablation consists of complete isolation of the right and left PV antra, with additional linear lesions and/or focal ablation if atrial tachycardia is induced after PV antrum isolation.

Prior to GP ablation, the location of each of the four GPs accessible from the left atrium was identified by delivering continuous applications of HFS (cycle length 50 ms, 12V) to sites within and around the left and right PVs and PV antra. A greater stimulus duration of 1–10 ms was required (compared to 0.1 ms in animal studies) to activate these epicardial GPs by endocardial HFS. Figure 4A shows a typical response to high frequency stimulation (during AF) applied to the LA endocardium beneath a GP, in this case the GP was located anterior to the right PVs. Complete AV block occurred one second after the onset of HFS, characteristic of the short time constant for parasympathetic stimulation. As in the animal studies (Fig. 3) continuous HFS (not trains within the atrial refractory period) consistently produced AF. Therefore, a positive (vagal) response to HFS, suggesting stimulation close to a GP, was defined as a $\geq$50% increase in mean R-R interval during AF. HFS was terminated within a few seconds to minimize any heating of the endocardium. For "GP ablation", RF energy was delivered to all sites exhibiting a positive response to HFS. The endpoint of GP ablation was elimination of the vagal response at these sites (Fig. 4B).

Left atrial HFS produced a positive (vagal) response at four discrete locations just outside the right and left PV antra. The four sites were: (1) anterior to the right pulmonary veins ("anterior right GP"); (2) inferior to the RIPV ("inferior right GP"); (3) superior and medial to the LSPV near the insertion of the ligament of Marshall into the pericardium ("superior left GP"); (4) and inferior to the LIPV ("inferior right GP"). The sites exhibiting a positive response to HFS were located within areas showing complex fractionated electrograms during AF [15,16].

We are currently comparing the recurrence of AF between 33 patients receiving conventional AF ablation plus GP ablation (paroxysmal AF in 17 patients and persistent AF in 16 patients) and 27 patients receiving conventional ablation alone (paroxysmal AF in 14 patients and persistent AF in 13 patients). GP ablation (prior to antrum isolation) abolished focal firing from the PVs in all but 3 of the 33 patients (95%) but did not decrease the ability to induce sustained AF (>3 min). With the addition of antrum ablation, none of the patients exhibited PV firing. At the end of GP ablation and antrum isolation the inducibility of sustained AF (8/33 patients, 24%) was similar to the group with just conventional ablation (7/27 patients, 26%).

Thus far into the study, with only a small number of patients and short length of follow-up, there is a suggestion that freedom from AF may be greater following conventional AF ablation plus GP ablation than conventional AF ablation alone in patients with either paroxysmal or persistent

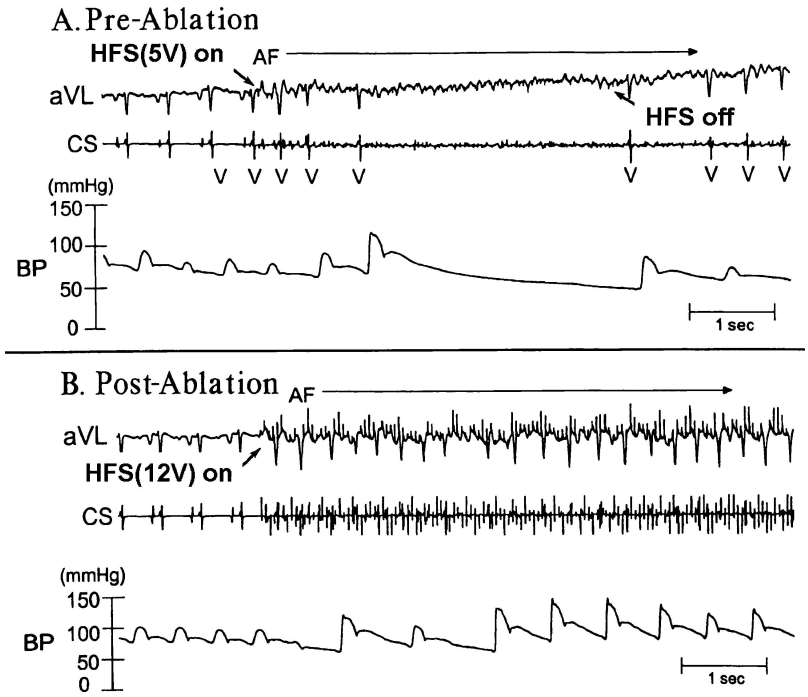

Fig. 3. *In a dog heart, recordings were made of ECG lead aVL, an electrogram from the coronary sinus (CS) and blood pressure (BP). During sinus rhythm, continuous HFS (cycle length 50 ms, square wave duration 0.1 ms, 5 volts) was applied to the RA endocardium across from the GP located close to the RSPV. Panel A Pre-ablation. AF was induced at the onset of HFS (HFS, 5V On) and was followed by a period of AV block until the HFS was discontinued (HFS Off↓). Panel B Post-ablation. After RF ablation at this right atrial endocardial site, HFS at higher voltage (HFS, 12V On) failed to induce slowing of the ventricular response during AF. See text for further details.*

AF [12]. Thirty (30) of the 33 patients (91%) are free of AF following the combination of GP ablation and conventional AF ablation (all 17 patients with paroxysmal AF and 13 of 16 [81%] patients with persistent AF) compared to 19 of 27 patients (70%) following conventional ablation alone (10 of 14 [71%] patients with paroxysmal AF and 9 of 13 [69%] patients with persistent AF). However, the duration of follow-up was only 1–10 (median 5) months for the group with added GP ablation and 1–15 (median 12) months for the group with conventional ablation alone.

GP ablation (defined as complete elimination of the vagal response to HFS) required only 1–8 (median 4) RF applications for the anterior right GP; 1–8 (median 4) RF applications for the inferior right GP; 1–7 (median 2) RF applications for the superior left GP; and 1–11 (median 6) RF applications for the inferior left GP. This was a smaller number of RF applications than required for PV antrum isolation. In addition, the energy was applied further from the PVs (usually just outside the PV antrum). This suggests that GP ablation should have a low risk of thromboembolic events and PV stenosis, two of the most severe complications of AF ablation.

These preliminary findings corroborate other recent studies in which the GPs were specifically [17] or inadvertently [18] targeted in patients with paroxysmal or persistent AF. Platt et al. [17] reported a 96% success rate during a short follow-up (median 6 months). Pappone et al. [18] found sites where high power RF applications produced a vagal response (marked bradycardia) in 34% of 297 patients undergoing circumferential ablation around the PVs. Ablation of these "hot spots" by continued RF applications resulted in loss of the vagal response. A 12 month follow-up showed a 99% freedom from AF recurrence in those patients compared to 74% in patients not showing a vagal response during RF ablation [18]. Interestingly, these "hot spots" were located at sites similar to those where HFS produced a vagal response in our clinical studies.

Mechanisms of Autonomically Induced AF

Previous basic studies [8,9,19–21] have identified efferent parasympathetic and sympathetic neurons in the GPs and implicated their involvement

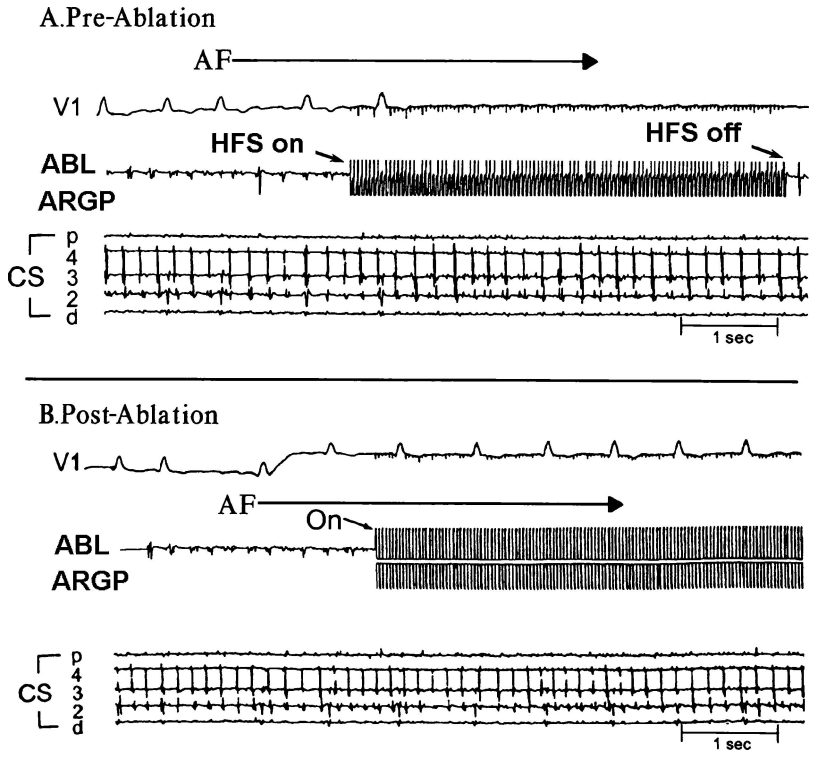

Fig. 4. *HFS in a patient with persistent AF. Panel A, Pre-ablation. In a clinical study we recorded ECG lead V_1, electrograms from the left atrial endocardial ablation catheter (ABL) positioned close to the anterior right GP (ARGP) and CS, and blood pressure. HFS (cycle length 50 ms, each square wave 10 ms in duration, 12 volts) was applied endocardially underneath the ARGP. Complete AV block occurred with the onset of HFS (HFS On) and persisted for several seconds after HFS was discontinued (HFS Off↓). Panel B, Post-ablation. As in the experimental studies, radiofrequency application to this endocardial site prevented the bradycardic response to high frequency stimulation. See text for further details.*

in the initiation and maintenance of AF [8,19]. We postulated that the local release of these neurotransmitters in the vicinity of the PV-atrial junctions would facilitate the development of extrasystoles in the PVs and provide the substrate for conversion of these premature beats into AF. Both acetylcholine and adrenergic agonists are known to shorten refractory periods in atrial myocardium [22]. In addition, the adrenergic neurotransmitters have been shown to induce enhanced automaticity [22] as well as triggered firing [23].

More recent data from our laboratory has demonstrated marked shortening of action potential duration and formation of early afterdepolarizations in superfused pulmonary vein sleeves when exposed to acetylcholine and norepinephrine or with local electrical stimulation [24]. These corroborating *in vitro* studies have led to the postulation that the release of local adrenergic agonists in conjunction with the marked shortening of the action potential by acetylcholine results in excess loading of Ca^{++} within the sarcoplasmic reticulum, increasing the magnitude and duration of Ca^{++} transients. Thus, cytosolic Ca^{++} remains high after repolarization, increasing the Na^{+}- Ca^{++} exchange current. This inward current, persisting after repolarization, can produce early after-depolarizations and triggered firing in the PV sleeves. The shortened action potential duration also allows the rapid sustained triggered firing in the PV myocardial sleeve and enhanced conduction to the adjacent atrial myocardium which can serve as the source of AF initiation and maintenance, respectively.

Conclusions

These basic and clinical studies support the hypothesis that local cardiac autonomic ganglia clustered in the fat pads (GP) at the margins of the PV antra, innervate PV myocardial sleeves and adjacent atrial myocardium, and can play a critical role in the initiation and maintenance of AF.

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