ORIGINAL RESEARCH

Temporary Suppression of Cardiac Ganglionated Plexi Leads to Long-Term Suppression of Atrial Fibrillation: Evidence of Early Autonomic Intervention to Break the Vicious Cycle of "AF Begets AF"

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***Background*—**Botulinum toxin (BTX), temporarily suppressing cholinergic transmission (<3 weeks), has been reported to suppress atrial fibrillation (AF) for ≥1 year. We aimed to investigate the mechanism underlying long-term suppression of AF caused by injecting BTX into major atrial ganglionated plexi (GPs).

***Methods and Results*—**Bilateral thoracotomies in anesthetized dogs allowed programmed stimulation at 4 pulmonary veins, biatrial appendages, and the superior vena cava to determine the effective refractory period (ERP) in the first operation. Group 1 (n=10) received BTX injection into all GPs; group 2 (n=7) received no injection. Groups 1 and 2 received rapid atrial pacing (800 bpm) 6 days a week. Group 3 (n=7) did not undergo thoracotomy or rapid atrial pacing to serve as controls for histological studies. A second operation and the same measurements were made 3 months later. During the first operation in group 1, ERPs of 4 pulmonary veins, but not biatrial appendages or superior vena cava, increased immediately after BTX injection. AF burdens increased significantly from the fifth week after the first operation in group 2 but not in group 1. In the second operation, ERPs remained unchanged compared with ERPs before BTX injection in group 1, whereas ERPs shortened significantly at all sites except the superior vena cava in group 2. There was no difference of autonomic nerve density between group 1 and group 3. The GP choline acetyltransferase (+) and atrial tyrosine hydroxylase (+) nerve densities were higher in group 2 than in group 1 and group 3.

***Conclusions*—**Temporary suppression of major atrial GPs by BTX prevents autonomic remodeling and provides long-term suppression of AF, indicating the critical role of GPs in AF progression. **(*J Am Heart Assoc*. 2016;5:e003309 doi: 10.1161/JAHA.116.003309)**

Key Words: atrial fibrillation • autonomic • botulinum toxin • remodeling • sinus rhythm

Atrial fibrillation (AF) begets AF was first proposed by Morillo and Wijffels et al, when they found that rapid atrial pacing (RAP) caused electrical remodeling, leading to progressive shortening of the atrial effective refractory period (ERP) and increased AF duration.[1,2] Structural remodeling had also been reported with atrial enlargement and interstitial fibrosis, cellular hypertrophy, and degeneration in patients with AF.[2,3] Electrical and structural remodeling may form a vicious cycle so that one can perpetuate the other. As a result, arrhythmia burden can progress from paroxysmal to more persistent forms of AF. In addition, autonomic remodeling plays an important role in the pathogenesis of AF. Several publications demonstrated that hyperactivity or imbalance of the cardiac autonomic nervous system (ANS) facilitates the initiation and maintenance of AF.[4–11] Hyperactivity of the cardiac ANS and AF also form a vicious cycle in which the former can initiate AF and the latter further enhance the activity of the cardiac ANS to perpetuate AF.[7,9]

Botulinum toxin (BTX) is a neurotoxin produced by *Clostridium botulinum*. It can temporarily block the exocytotic release of acetylcholine stored in the synaptic vesicles and therefore inhibit the cholinergic neurotransmission crucial for the function of the postganglionic neurons.[12,13] The effect of BTX dissipates within a few weeks and had been reported to

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suppress vagally mediated AF for at least 1 week, but its electrophysiological effects dissipated within 3 weeks.[14] Recently, Pokushalov et al reported surprising results that not only injection of BTX into the epicardial fat pads containing the 4 major atrial ganglionated plexi (GPs) significantly reduced the incidence of postoperative AF but also the antiarrhythmic effects continued for at least 1 year after cardiac surgeries.[15,16] How temporary inhibition of the cardiac autonomic nervous system translates into long-term suppression of AF remains elusive. We hypothesized that suppression of the 4 major atrial GPs may break the vicious cycle of "AF begets AF" by inhibiting autonomic remodeling and subsequently prevent the progression of AF to more persistent forms. We chose BTX injection into the GP to avoid permanent injury to the autonomic neurons and atrial myocardium, thereby not introducing confounding factors such as injury-induced nerve sprouting and myocardial fibrosis.

Methods

Animal Preparation

The protocol for this study was approved by the Committee for Experiments on Animals of the Taipei Veteran General Hospital. The details were described in previous publications.[10,17,18] In brief, a total of 24 adult mongrel dogs (weight, 10–20 kg) were anesthetized with ketamine (10–20 mg/kg) and sodium pentobarbital (30 mg/kg intravenous). Group 1 (n=10) received BTX injection at all 4 left atrial (LA) GPs; group 2 (n=7) received no BTX injection. Both group 1 and group 2 animals received RAP to simulate AF (see descriptions later); group 3 (n=7) did not receive either thoracotomy or RAP. GPs and myocardia were harvested to provide baseline data. All animals received a warming blanket to maintain the core body temperature at 36.5±1.5°C. The arterial blood gas was checked hourly to keep a balanced acid-base status (pH 7.35–7.45) and oxygenation (Sao_2 >90% without hypercapnia). All dogs were ventilated with room air with a positive pressure respirator. Oxygen was administered to maintain Sao_2 >90%. Venous access was obtained by using the Seldinger technique with an 8F sheath from the right femoral vein. An arterial access was set up at the right femoral artery for blood pressure and body temperature monitoring and blood sampling.

Surgical Protocol in the First Operation

Lateral thoracotomy and recording

Group 1 and 2 dogs underwent 2 operations in this study. The chest was opened through right and left lateral thoracotomies, sequentially, at the fourth intercostal space in the first operation. Trauma was minimized to improve survival. Multielectrode catheters were sutured to multiple sites to obtain recordings and for stimulation at the right atrial (RA) appendage, right superior pulmonary vein (RSPV), and right inferior pulmonary vein (RIPV) during a right lateral thoracotomy and at the LA appendage, a left superior pulmonary vein (LSPV) and left inferior pulmonary vein (LIPV) during left lateral thoracotomy. A basket catheter introduced through the internal jugular vein was placed at the superior vena cava (SVC)–RA junction for recording the SVC electrograms. The atrial and pulmonary vein (PV) ERP was evaluated by electrical programmed stimulation (pacing cycle length 300 ms) at all of the aforementioned PVs and atrial sites. Atrial tachyarrhythmia was defined as atrial rates faster than 300 bpm for ≥5 seconds.

Identification and stimulation of the 4 major LA GPs

The detailed preparation procedures have been reported before.[10,17–19] In brief, after the lateral thoracotomy, the GPs were identified by applying high-frequency stimulation (HFS) from a Grass stimulator applied with a bipolar electrode probe (20-Hz, 0.1-ms-duration square waves, 0.6–8.0 V). The fat pad containing the anterior right GP (ARGP) was situated between the caudal end of the sinoatrial node and RSPV–atrium junction. The inferior right GP (IRGP) was located at the junction of the inferior vena cava and both atria. The superior left GP (SLGP) was located adjacent to the LSPV–atrium junction between the LA appendage and left PA. The inferior left GP (ILGP) was located on the caudal side of the LIPV–atrium junction. A bradycardic response, which showed a progressive slowing of the sinus rate by 50% or the development of second- or third-degree atrioventricular block resulting from incremental voltage levels applied to the fat pad, was used as a surrogate marker for GP stimulation.

BTX injection

BTX (Dysport [abobotulinumtoxin A]; Ipsen Biopharm Ltd) was injected into the 4 LA GPs in group 1 animals at the end of the first operation. BTX (50 μm/mL) was injected into the entire visible area of the epicardial fat pad each time (50 μm/GP), where individual GPs were identified by HFS. The needle tip was positioned manually at several points just into the epicardial fat pads under direct visualization to ensure optimal injection. Successful suppression of GP activity was defined by abolition of the heart rate response to HFS (including progressive slowing of the sinus rate by 50% or the development of second- or third-degree atrioventricular block) at the same regions that had elicited clear responses before BTX injection. In group 2 dogs, there was no BTX injected into GPs at the end of the first operation.

Pacemaker implantation and AF simulated by RAP

AF was simulated by RAP through a programmable neurostimulator (Itrel 3; Medtronic). A unipolar pacing lead (Medtronic pacing lead 6491) was fixed to the RA appendage, and a pacemaker generator was implanted subcutaneously at the right subaxillary area. After the animal recovered from the surgery (1 week after implantation), the pacemaker was programmed to pace at a rate of 800 bpm and at a pacing output twice the capturing threshold. The pacemaker was turned on for 6 continuous days per week and turned off 1 day per week to record spontaneous atrial arrhythmias. The pacing output was readjusted each week based on the capture threshold.

Another single-chamber cardiac pacemaker (Medtronic) was placed subcutaneously at the right chest wall with an epicardial unipolar lead implanted in the RA appendage (≥1 cm from the atrial pacing lead) to record the atrial events. The pacemaker was programmed to record atrial tachyarrhythmias, which was defined as an atrial rate >300 bpm for >5 seconds. Each event was also evaluated by 2 blinded investigators (L.W. Lo and H.Y. Chang) to exclude oversensing or noise artifact.

Interoperative Management and Measurements

After the first operation, intramuscular injection of antibiotics with ampicillin (20 mg/kg) and pain control with oral paracetamol (15 mg/kg) were administrated every 8 hours for 2 weeks or until the wound healed. The pacemaker was interrogated weekly to retrieve the information for the occurrence of arrhythmias. The atrial high rate events were evaluated on the seventh day when the Itrel 3 neurostimulator was turned off. Any duration of atrial tachyarrhythmias >5 seconds was recorded. The AF burden was defined as the AF duration on day 7 divided by 24 hours.

Surgical Protocol in the Second Operation

The second operation was performed 3 months after the first operation. In the second operation, a lateral thoracotomy was performed as was described for the first operation. Multielectrode catheters were sutured at the RA appendage, RSPV, RIPV, LA appendage, LSPV, and LIPV for programmed stimulation. A basket catheter was also placed in the SVC to stimulate SVC muscle sleeves. The atrial ERPs of the RSPV, RIPV, RA appendage, LSPV, LIPV, LA appendage, and SVC were evaluated as stated for the first operation.

Histological Study

Epicardial fat pad tissue and myocardium harvested from the 3 groups were used in the histological study. We previously described detailed procedures for the histological study.[17] In brief, sections were cut parallel to the plane of mitral annulus to include the epicardial and endocardial aspects of the myocardium in each slice. Masson's trichrome–stained myocardial sections were imaged, and the collagen area was calculated as a percentage of the total atrial myocardial area. For the immunohistochemistry staining, antibodies for tyrosine hydroxylase (TH) were used to stain sympathetic nerves, whereas antibodies for choline acetyltransferase (ChAT) were used to stain parasympathetic nerves. The TH- and ChAT-positive portions within GP were localized, and the cross-sectional areas of these positive portions inside the GP and myocardium were measured.

Statistical Analysis

All continuous data were presented as the mean±SE. The Mann–Whitney *U* test was used to analyze the continuous data between groups, and Wilcoxon or Friedman test was

Table 1. Pacing Thresholds at Different Sites of Both Groups

	Group 1 (n=10)			Group 2 (n=7)	
	First				
Operation	Before BTX	After BTX	Second	First	Second
RSPV, mA	1.22±0.44	1.67±0.55	2.0±0.96	0.95±0.17	0.35±0.06
RIPV, mA	1.51±0.48	1.38±0.39	2.13±0.63	1.37±0.28	0.83±0.40
RAA, mA	1.24±0.41	1.93±0.79	1.45±0.04	1.72±0.53	1.20±0.36
SVC, mA	0.47±0.09	0.55±0.11	1.38±0.46	1.09±0.31	0.91±0.08
LSPV, mA	1.67±0.59	2.19±0.68	1.76±0.14	1.28±0.58	0.75±0.17
LIPV, mA	2.39±0.75	2.01±0.57	1.93±0.70	1.93±0.64	1.33±0.88
LAA, mA	0.88±0.38	0.68±0.15	1.20±1.20	0.73±0.23	0.65±0.45

BTX indicates botulinum toxin; LAA, left atrial appendage; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RAA, right atrial appendage; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; SVC, superior vena cava.

Table 2. Changes of Heart Rate and PR Interval Between First and Second Operations

	Group 1 (n=10)			Group 2 (n=7)	
	First				
Operation	Before BTX	After BTX	Second	First	Second
Cycle length, ms	506±35	455±22*	529±38†	485±8	496±6
PR interval, ms	94±5	87±3*	96±2†	96±2	92±1

BTX indicates botulinum toxin.
*P<0.05 compared with before BTX in the first operation in group 1.
†P<0.05 compared with after BTX in the first operation in group 1.

used to analyze the continuous data within same group. Repeated measures were used for the data that collected longitudinally. In Tables 1 and 2, the statistics were adjusted for multiple comparisons. The χ^2 test with a Yates correction or Fisher's exact test was used to compare the categorical data between groups. Statistical significance was selected at a value of P<0.05.

Results

All dogs in group 1 received BTX injection into the 4 major atrial GPs without complications. The ARGP and SLGP were identified through the use of HFS in all dogs. There were no bradycardia responses during HFS at 3 of 10 IRGP fat pads and 2 of 10 ILGP fat pads in group 1 dogs. BTX was injected only into the GPs identified by HFS in group 1 dogs. All dogs in groups 1 and 2 underwent weekly follow-up for 3 months and then underwent a second operation procedure.

Electrophysiological Characteristics for the First Operation

Table 1 shows the pacing threshold before and after BTX injections in group 1. The pacing thresholds were similar at each location before and after BTX injections. In the first operation, the ERPs were checked at both 2× and 10× pacing threshold at the RSPV, RIPV, RA appendage, LSPV, LIPV, LA appendage, and SVC sites in groups 1 and 2. The baseline sinus rhythm cycle length and PR interval were similar for the 2 groups (see Table 2). Before BTX injection, the ERPs were similar for groups 1 and 2 at all sites (both 2× and 10×, Figure 1). The sinus rhythm cycle length and PR interval in group 1 were prolonged after BTX in the first procedure (Table 2). The ERPs lengthened significantly (both 2× and 10×) at RSPV (2×: 140±7 versus 148±8 ms, P<0.05; 10×: 97±6 versus 104±6 ms, P<0.05), RIPV (2×: 130±10 versus 145±9 ms, P<0.05; 10×: 85±10 versus 98±8 ms, P<0.05), LSPV (2×: 127±5 versus 133±6 ms, P<0.05, 10×: 106±6 versus 111±7 ms, P<0.05), and LIPV (2×: 131±7 versus 138±8 ms, P<005; 10×: 104±6 versus 112±7 ms, P<0.05) after BTX, compared with those before BTX in group 1 dogs. The ERPs of biatrial appendages (RAA 2×: 143±7 versus 139±8 ms, P=NS; 10×: 93±9 versus 95±7 ms, P=NS, LAA 2×: 118±7 versus 117±5 ms, P=NS; 10×: 108±7 versus 106±5 ms, P=NS) and SVC (2×: 143±7 versus 139±6 ms, P=NS; 10×: 116±5 versus 113±4, P=NS) remained unchanged after BTX compared with that without BTX in group 1 (Figure 1A).

Follow-up Data for the 2 Operation Procedures

The atrial capturing rates were similar between group 1 and group 2 dogs during RAP (188±12 versus 193±7 ms, P=NS). Electrogram recordings from the cardiac pacemaker were checked weekly on the day that RAP was discontinued in all dogs after the first operation. The atrial tachyarrhythmia burden increased significantly from the fifth week in group 2, compared with that of group 1 dogs (Figure 2). Three of 7 dogs in group 2 remained in sustained AF without sinus rhythm on the day without RAP (1 at 4 weeks, 1 at 7 weeks, and 1 at 11 weeks of RAP). Figure 3 shows an example of the stored electrogram interrogated from the cardiac pacemaker 2 months after the first operation in a group 2 dog. After RAP for 2 months, RAP was temporarily discontinued. Sustained AF was noted in the group 2 dog.

Electrophysiological Characteristics for the Second Operation

In the second operation, HFS elicited bradycardia responses in both group 1 and group 2 dogs (except those GPs without HFS response in the first procedure in group 1). There were no changes of pacing threshold at different sites compared with that in the first operation in both groups (Table 1). The sinus rhythm cycle length and PR interval returned to baseline level in group 1 and remained the same in group 2 dogs (Table 2). ERPs increased acutely after BTX injection in the first operation at RSPV, RIPV, LSPV, and LIPV in group 1 (Figure 1A and 1C), but the ERP values acquired from the second operation did not differ from the baseline values (before BTX injection) at all sites in group 1 animals (Figure 1A and 1C). In the 3 dogs that remained in sustained AF in group 2, electrical synchronized cardioversions were performed to restore to sinus rhythm. The ERPs detected in the second operation decreased significantly at all sites except SVC in group 2 dogs compared with those in the first operation (Figure 1B and 1D).

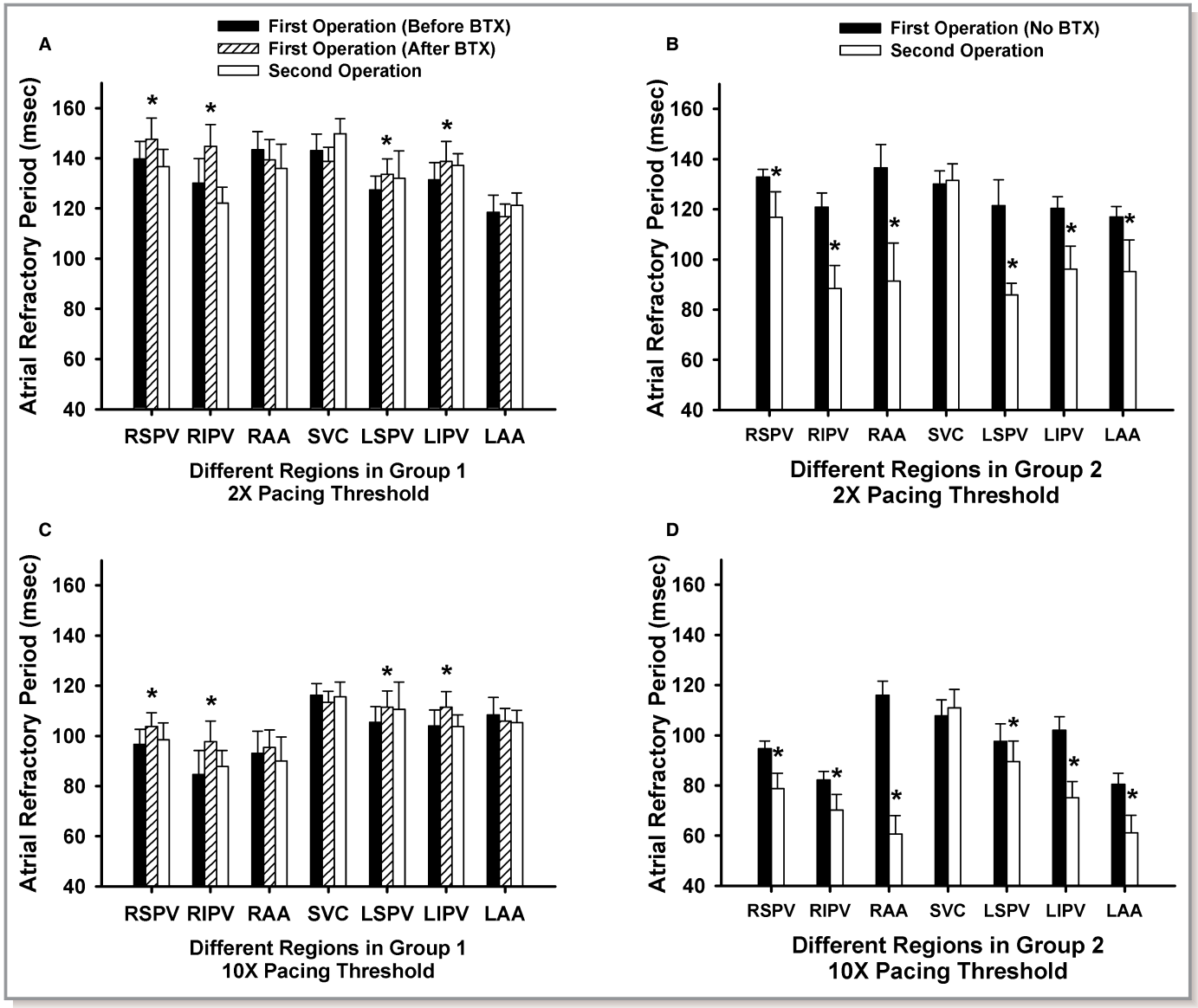

Figure 1. Atrial effective refractory period (ERP) of biatrial regions in the first and second operations. A and B, ERPs in group 1 and group 2 with 2X pacing threshold (TH). C and D, ERPs in group 1 and group 2 with 10X TH. After botulinum toxin (BTX) injection into 4 ganglionated plexi (GP), ERPs significantly prolonged in the right superior pulmonary vein (RSPV), right inferior pulmonary vein (RIPV), left superior pulmonary vein (LSPV), and left inferior pulmonary vein (LIPV) but remained comparable in right atrial appendage (RAA), left atrial appendage (LAA), and superior vena cava (SVC) in the first operation compared with those before BTX injection in group 1. In the second operation, ERPs returned to baseline levels at all sites in group 1, but ERPs shortened at RSPV, RIPV, RAA, LSPV, LIPV, and LAA but not SVC compared with those in the first operation in group 2. *P<0.05.

Histological Studies of the GPs and Atrial Myocardia

Four GPs and myocardia from the anterior wall of the LA and RA were harvested in group 1 and 2 dogs after the second operation. In group 3 dogs, without undergoing thoracotomy or RAP, animals were killed to provide baseline histological data for comparison. Figure 4 illustrates examples of the histological studies from the fat pad containing GPs with ChAT staining and myocardia with TH and trichrome staining in group 1 (A, C, and E) and group 2 (B, D, and F) dogs. In the GPs, group 1 dogs demonstrated a lower ChAT-positive nerve density (236.55±19.04 10^3 $\mu m^2/mm^2$) than that of the group 2 dogs (359.92±16.45 10^3 $\mu m^2/mm^2$, P<0.001) but similar to that of the group 3 dogs (218.76±11.02 10^3 $\mu m^2/mm^2$, P=NS). The GP TH-positive nerve density of group 1 dog was 166.02±19.59 10^3 $\mu m^2/mm^2$ and was similar to that of group 2 dogs (163.25±20.35 10^3 $\mu m^2/mm^2$, P=NS) and group 3 dogs (130.07±14.33 10^3 $\mu m^2/mm^2$, P=NS), respectively. Individual GP immunohistochemistry staining results

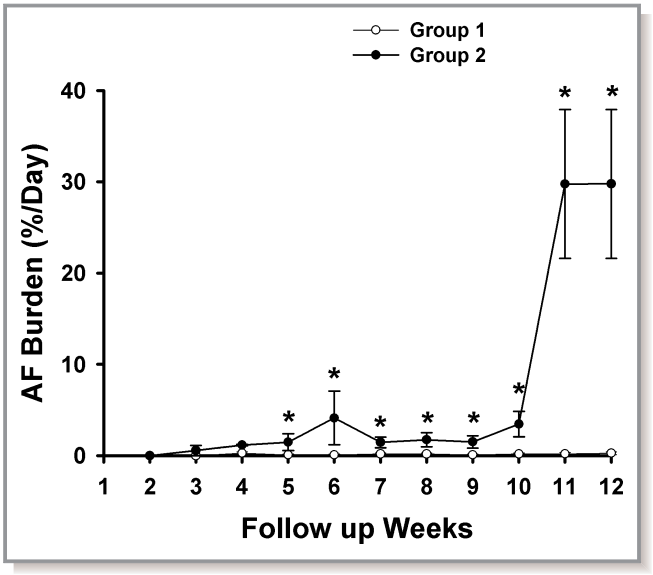

Figure 2. Serial changes of atrial fibrillation (AF) burden during weekly follow-up. The AF burden significantly increased in group 2, compared with group 1 after the fifth week of follow-ups. *P<0.05.

are shown in Figure 5. In the atrial myocardium, there was a higher TH-positive nerve density in group 2 (16.07±2.26 10^3 $\mu m^2/mm^2$) than in group 1 (1.01±0.30 10^3 $\mu m^2/mm^2$, P<0.001) and group 3 (0.96±0.28 10^3 $\mu m^2/mm^2$, P<0.001), respectively, but there was no difference between group 1 and group 3 (Figure 5D). The ChAT-positive nerve densities were comparable among 3 groups (0.44±0.15 and 0.40±0.11 versus 0.39±0.25 $\mu m^2/mm^2$, P=0.36) in the atrial myocardium (Figure 5C).

Trichrome stain was performed in right and left anterior free wall myocardial tissue in groups 1 and 2. Only limited fibrosis was demonstrated in both groups, and the connective tissue densities were similar between group 1 and group 2 in the LA (10.0±1.0% versus 9.24±0.6%, P=NS) and the RA (12.6±1.9% versus 14.4±1.3%, P=NS), respectively.

Discussions

Main Findings

In this study, we found that temporary inhibition of the intrinsic cardiac ANS by BTX led to long-term AF suppression. Acutely, there was a prolongation of the regional ERPs and loss of the bradycardia response elicited by HFS stimulation at the GP sites. The prolonged regional ERPs and the bradycardic response elicited by GP stimulation returned to the baseline level within 3 months after BTX, consistent with prior reports that the neurotoxic effects of BTX on cardiac electrophysiology dissipated in 3 weeks.[14] Notably, parasympathetic hyperinnervation at GP and sympathetic hyperinnervation at atrial myocardium were found in group 2 but not group 1 dogs, indicating that BTX prevents autonomic remodeling. Importantly, there was no ERP shortening induced by RAP and no increase in AF burden for 3 months in group 1 dogs. These findings strongly indicate that autonomic remodeling plays a crucial role in the progression of AF and that suppression of autonomic remodeling may prevent electrical remodeling and subsequently prevents AF from perpetuating itself.

AF Begets AF: How AF Perpetuates Itself

It is well recognized that both electrical remodeling and structural remodeling perpetuate AF. Electrical remodeling occurred within hours, and structural remodeling takes months to develop. Wijffels et al reported that RAP for 2 to 3 weeks led to sustained AF in healthy goats. Marked reduction was found in atrial refractoriness (−45%) and a

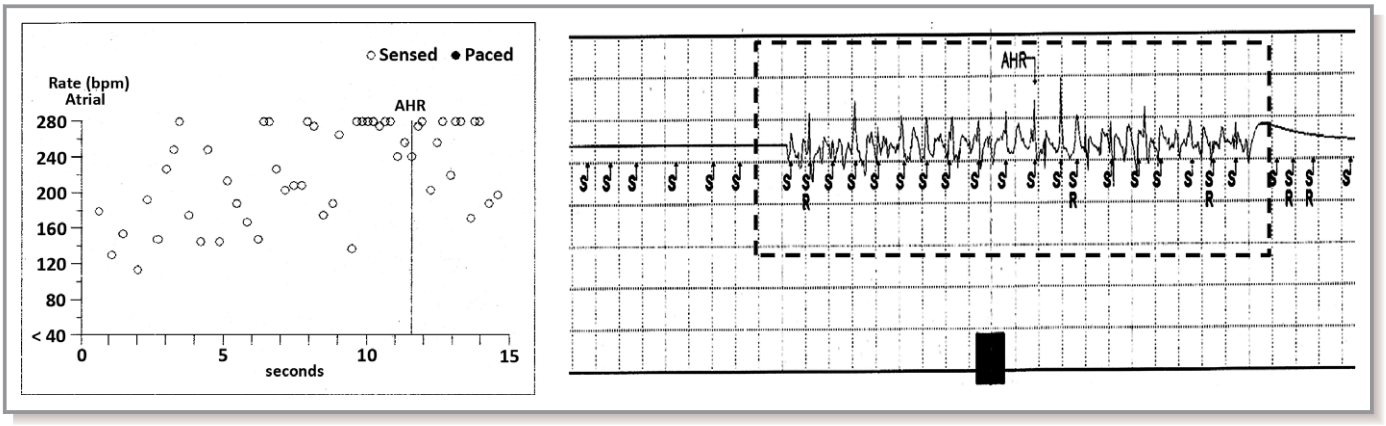

Figure 3. An example of the electrogram recordings from pacemakers interrogated 2 months after the first operation from a group 2 dog on the day without rapid atrial pacing. In the left panel, the atrial rate of >280 bpm is shown as a circle at 280 bpm due to the limited y-axis scale. Sustained AF is confirmed from the stored electrograms is denoted by the dotted box.

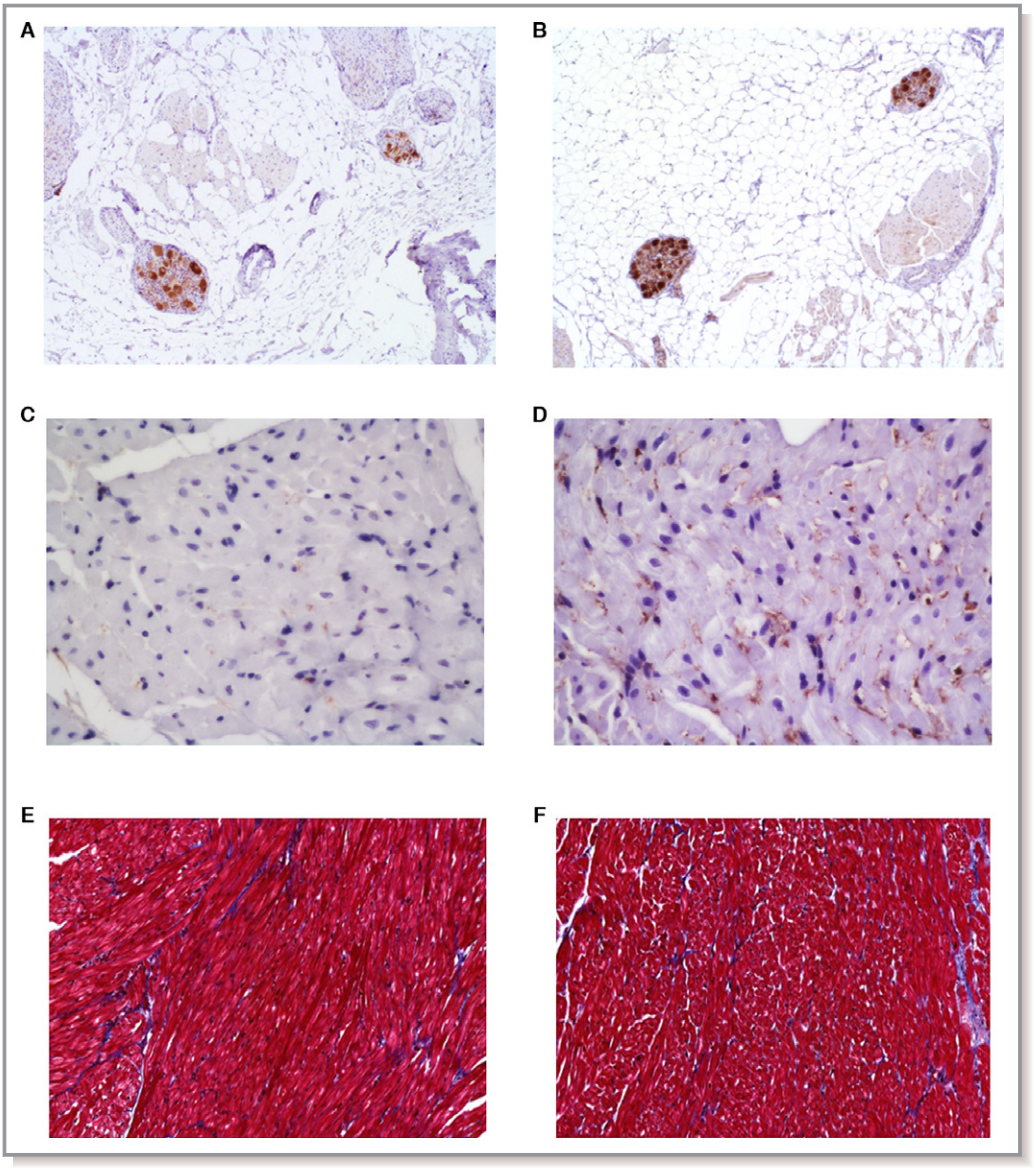

Figure 4. Examples of immunohistochemical (group 1: A and C, group 2: B and D) and trichrome staining (group 1: E, group 2: F) in cardiac ganglia and myocardia. A and B, Choline acetyltransferase (ChAT) staining showing increased cholinergic neural density within ganglia in group 2 (B) than in group 1 (A). C and D, Tyrosine hydroxylase (TH) staining showing increased sympathetic innervation in atrial myocardium in group 2 (D) than in group 1 (C). E and F, Trichrome staining demonstrating similar connective tissue distributions between group 1 (E) and group 2 (F).

reversion of the normal rate adaptation of refractory period after 24 hours to 2 weeks of AF simulated by RAP.[1] Similar findings were also demonstrated by Morillo et al with a 15% reduction in atrial ERP after sustained RAP in a canine model of sustained AF.[2] Structural remodeling is another factor that is important in the maintenance of AF. Increased myolysis and glycogen accumulation developed over 8 weeks after RAP and nearly half of myolysis happened 16 weeks after AF.[20] Atrial fibrosis typically does not occur until AF or RAP persists for several months. In the present study, the trichrome stain showed no significant difference in fibrosis between group 1 and group 2, and the result is compatible with the previous study by Wijffels et al.[1] There was no increase of atrial connective tissue in their study as well.

How AF perpetuates itself in the first few hours after AF initiation is poorly understood. Lu et al found that either GP ablation or autonomic blockade reduced ERP dispersion and prevented ERP shortening and AF inducibility in a canine model of 6-hour RAP, suggesting the presence of autonomic remodeling and its importance in facilitating atrial electrical

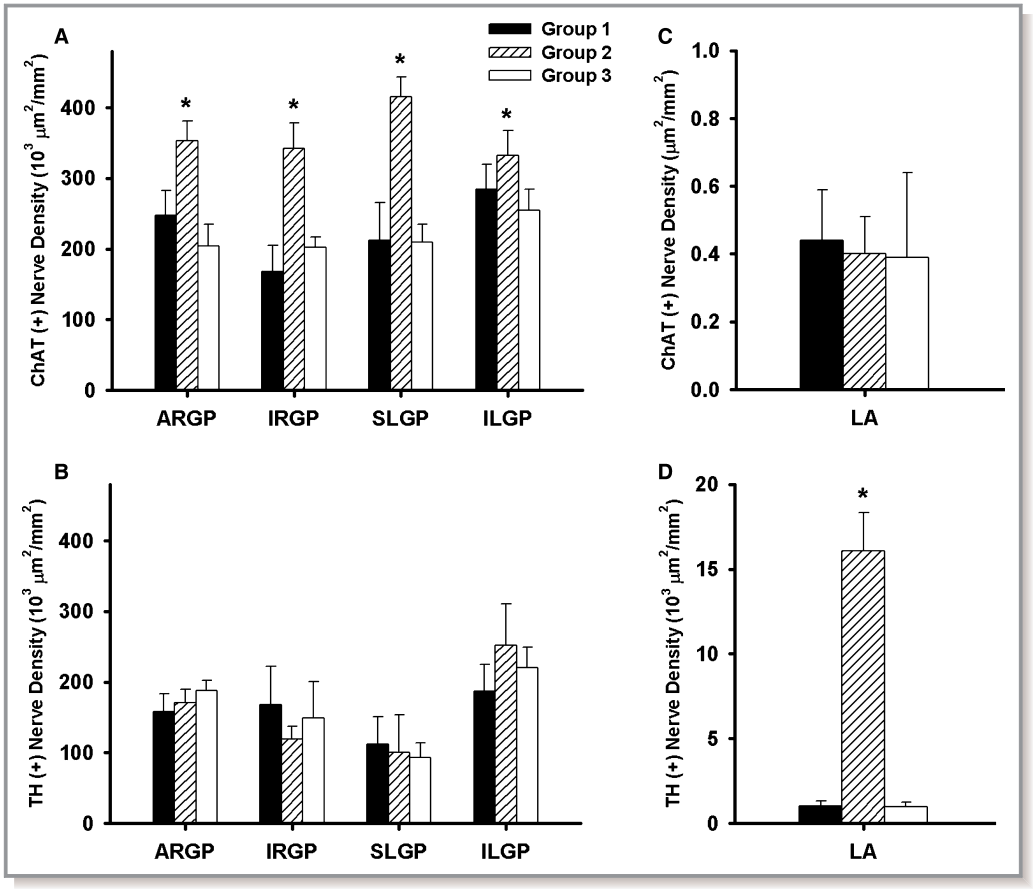

Figure 5. Parasympathetic (ChAT) and sympathetic (TH,) neural densities in individual GPs (A and B) as well as atrial myocardia (C and D) from 3 groups. A and C, Parasympathetic neuron marker density measured as total area of nerves (μm^2) per square millimeter. B and D, Sympathetic neuron marker density measured as total area of nerves (μm^2) per square millimeter. ARGP indicates anterior right ganglionated plexus; ILGP, inferior left ganglionated plexus; IRGP, inferior right ganglionated plexus; SLGP, superior left ganglionated plexus. *P<0.05, compared with group 1 and group 3, respectively.

remodeling in the acute stage.[7] Direct neural recordings from the GP also revealed that as AF continued, the activity of the major LA GP increased progressively, which helped maintain short and dispersed ERPs to sustain AF. In other word, hyperactivity of the cardiac ANS and AF form a vicious cycle.[21] The former facilitates the initiation of AF and the latter enhances the ANS activity. The important role of autonomic remodeling is later corroborated in a rabbit model of 24-hour RAP. Zhang et al demonstrated progressive nerve sprouting and sympathetic and parasympathetic hyperinnervation as evidence that autonomic remodeling may be a critical element in enabling AF to beget itself in the first 24 hours.[22] In the present study, 3 months of RAP induced hyperinnervation of the parasympathetic and sympathetic neural elements in the GP and atrial myocardia, respectively. Such autonomic remodeling was absent in group 1 animals, strongly indicating that the effects of AF suppression by BTX are mediated by preventing progressive autonomic remodeling.

Regional Neural Control of Atrial Myocardium

By blocking the acetylcholine release from the presynaptic vesicles, BTX inhibits cholinergic transmission and subsequently the function of postsynaptic autonomic neurons. It is known that autonomic innervation of the heart consists of a large neural network in which neural trafficking is usually integrated at the GP sites. Each GP contains up to thousands of autonomic neurons, mainly postsynaptic cholinergic neurons.[23] In addition to controlling the regional physiological functions such as vascular tone and electrophysiology, regional GPs may affect the function of cardiac tissues at a distance through the neural network.[23] Such regional control was demonstrated in the present study in that acute BTX injection affected only the PV sites, not the SVC muscle sleeves and atrial appendages (Figure 1). Importantly, the chronic effects of GP suppression extended beyond the adjacent regions in which shortening of ERP at the LA and RA appendage sites was eliminated as well, illustrating the

potential that modulation without destruction of this autonomic neural network can be used to treat AF.[21]

The lack of acute and chronic BTX effects on the SVC myocardial sleeve is particularly interesting. Prior studies showed that SVC firing can be induced by stimulating the SVC–Ao GP located at the junction of the aorta, SVC, and right PA. The SVC–Ao GP had been proposed to be the "head stage" for the vagal innervation to the heart.[24] Ablation of this GP not only eliminated the bradycardic responses elicited by cervical vagal stimulation but also prolonged the ERP only at the SVC site, not the RA, LA, or PV sites.[19] The observation that the chronic effects of BTX extended to the RA and LA appendages, but not the SV, suggests that the role of autonomic remodeling at the SVC myocardial sleeve may be different from that of the rest of the atria.

Clinical Perspective

GP ablation has been pursued as an additional lesion set to improve the success rate for catheter and surgical AF ablation. Recent randomized clinical trials provide clinical benefits of adding autonomic denervation, targeting the 4 major atrial GPs, to standard PV isolation.[25,26] While BTX injection into the atrial GP significantly acutely reduced the incidence of postoperative AF, it also suppressed AF during a 1-year follow-up. Our study is the first to provide a mechanistic explanation for this intriguing long-term effect of BTX, in which BTX may break the "vicious cycle" by inhibiting the major atrial GPs. As illustrated in Figure 2, spontaneous atrial tachyarrhythmia barely existed at 3 months, suggesting that the antiarrhythmic effects of BTX likely persist beyond 3 months. These findings may explain, at least partially, the suppression of AF in patients (12-month follow-up) after the acute phase of cardiac surgery.[16] As the indication for epicardial intervention continues to expand for ablation and LA appendage exclusion, epicardial injection of BTX may be an alternative to prevent AF without injuring the myocardium or neural elements and possibly provide less arrhythmogenicity than the standard catheter or surgical ablation procedures. However, whether the injection of BTX into GPs can reverse established AF substrate (eg, fibrosis or inflammation) remains to be elucidated.

Study Limitations

In this study, we did not performed placebo saline injection in group 2 in order to prevent additional trauma during first operation. It has also been reported that saline injection did not produce any additional effects to GPs.[27] The regional ERP increased immediately after BTX injection and BTX eliminated the bradycardia response elicited by HFS delivered at the GPs, indicating that BTX did suppress the cardiac ANS and that the increase in ERP represented the loss of the autonomic input from the regional GPs. The main goal of this study was to evaluate the burden of spontaneous atrial tachyarrhythmias. We did not check ERP weekly to avoid inducing sustained atrial tachyarrhythmia that would not terminate without cardioversion. The data of the weekly changes in ERP are therefore not available. It is possible that the differences in remodeling between group 1 and group 2 were simply caused by different ERPs induced by BTX injection, which lengthened the atrial ERP, rendering reduced atrial capture rates in the group 1 animals and thereby less remodeling. However, the following lines of evidence do not support this possibility. First, Oh et al demonstrated that the BTX effects on GPs dissipated within 3 weeks.[14] Had all that we observed been caused by different ERPs and thereby different atrial capture rates, AF should have begun to occur in group 1 animals in the eighth week, lagging 3 weeks behind the group 2 animals. Figure 2, however, showed an almost absence of AF in group 1 animals for the entire 12-week period. Second, the ERP of the RA appendage, where rapid pacing was delivered, was not lengthened by BTX injection (Figure 1A). Therefore, group 1 and 2 animals should have had similar atrial capture rates to start with.

Conclusions

Via temporary suppression of the cardiac ANS by injecting BTX into the major atrial GPs, AF simulated by RAP failed to promote a favorable substrate to sustain AF. These findings indicate that early intervention to suppress autonomic remodeling may break the vicious cycle of "AF begets AF" and prevent AF from progressing to more advanced stages.

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Disclosures

None.

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