The role of pulmonary veins vs. autonomic ganglia in different experimental substrates of canine atrial fibrillation

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Aims

Pulmonary vein (PV)-encircling ablation, which is effective in suppressing atrial fibrillation (AF), damages autonomic ganglia near the PV ostia. This study examined the effects of PV isolation (PVI) vs. peri-PV ganglionic plexus ablation (GPA) in two discrete canine AF models: ventricular tachypacing (240 bpm, 2 weeks)-induced congestive heart failure (CHF), and atrial tachypacing (400 bpm, 1 week)-induced atrial tachycardia remodeling (ATR).

Methods and results

All PVs were isolated with an epicardial radiofrequency clamp in nine CHF and eight ATR dogs. Peri-PV ganglionic plexi (identified by bradycardic responses to high-frequency stimulation) were ablated in six CHF and five ATR dogs with an epicardial radiofrequency-ablation pen. Electrophysiologic measurements, including 240-electrode AF mapping, were obtained and dominant frequencies (DFs) determined. Atrial growth associated protein-43 (GAP-43) and neurofilament-M (NF-M) expression were determined immunohistologically. In CHF, neither PVI nor GPA affected AF duration, DF or the already low AF vulnerability. In ATR, PVI reduced AF vulnerability (75 ± 6% to 55 ± 11%, P < 0.05) but did not alter AF duration or DF. In contrast, GPA prolonged atrial refractory period and decreased AF vulnerability (75 ± 8% to 30 ± 10%, P < 0.05), AF duration (617 ± 246 to 39 ± 23 s, **P < 0.01), and DF (11.4 ± 0.6 to 8.6 ± 0.3** Hz, left atrium) in ATR dogs. Both GAP-43 and NF-M expression were decreased in CHF (by 63.1** and 60.0%**) and increased in ATR (by 65.5** and 92.1%, P < 0.001) compared with control.

Conclusions

PVs play a minor role in experimental AF due to CHF or ATR, but autonomic ganglia are important in AF related to ATR. Differential neural remodelling may contribute to varying effects of GPA in discrete AF substrates.

Keywords

Autonomic nervous system • Pulmonary vein • Ablation • Atrial fibrillation • Animal model

1. Introduction

Atrial fibrillation (AF), the most common sustained arrhythmia, is associated with increased cardiovascular morbidity and mortality, but the underlying mechanisms remain poorly understood. Pulmonary vein (PV)-encircling ablation is frequently effective in suppressing AF; however, PV ablation also affects autonomic ganglia located near the PV ostia. Autonomic denervation may contribute to the efficacy of the procedure.

PV ablation suppresses AF in many patients. Some animal studies also suggest a role of PVs in experimental AF, but their precise contribution remains controversial. AF in dogs with congestive heart failure (CHF) induced by ventricular tachypacing (VTP) presents features suggesting PV focal reentry and triggered activity. Atrial fibrosis and conduction abnormalities may also produce a widespread reentry substrate in CHF. AF related to atrial tachycardia remodeling (ATR) induced by atrial tachypacing can present rapid focal PV activations, but resection of all PVs does not suppress AF in coronary-perfused left atrial (LA)-PV preparations from ATR dogs. Autonomic denervation is common following PV ablation and correlates with reduced AF recurrence. Selective autonomic denervation alone, without PV ablation, can prevent AF...
We previously reported that intact PVs are not needed to maintain experimental cholinergic AF, whereas ablation of peri-PV ganglionic plexi prevents vagal AF. The contribution of PVs and autonomic nerves in other AF substrates remains to be elucidated. The present study examined the role of PV isolation (PVI) vs. peri-PV ganglion-plexus ablation (GPA) in two different canine AF models: 1. VTP-induced CHF; 2. atrial tachypacing-induced ATR.

2. Methods

2.1 Animal models and experimental groups

Animal-handling procedures were approved by the Montreal Heart Institute animal-research ethics committee and conform with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Publication No. 85-23, revised 1996). Fifty mongrel dogs (weight, 20.7–38.2 kg) were studied in the following series: (1) effects of PVI (n = 9) vs. peri-PV GPA (n = 6) on AF in dogs with CHF (CHF dogs) induced by 14-day VTP (240 bpm), (2) effects of PVI (n = 8) vs. GPA (n = 5) on AF in dogs with ATR (ATR dogs) induced by 7-day atrial tachypacing (400 bpm), (3) effects of PVI (n = 5) vs. GPA (n = 5) in control dogs, and (4) another set of CHF, ATR, and control dogs (n = 4 each) for cardiac-tissue sampling for confocal imaging of nerve-sprouting markers, growth associated protein-43 (GAP-43) and neurofilament-M (NF-M).

Dogs were subjected to VTP and atrial tachypacing as previously described. Dogs were anaesthetized with ketamine (5.3 mg/kg, iv), diazepam (0.25 mg/kg, iv), and halothane (1.5%). In CHF dogs, a bipolar pacing lead was inserted into the right-ventricular apex under fluoroscopic guidance, and connected to a subcutaneous pacemaker (Vitatron, Minneapolis, MN). After 24-h post-operative recovery, 14-day VTP at 240 bpm was initiated. The final open-chest electrophysiologic study (EPS) was performed on day 14. In ATR-dogs, bipolar pacing leads were inserted into the right-ventricular apex and right-atrial (RA) appendage (RAA) and connected to pacemakers. Atrophicentric block was created by radiofrequency-catheter ablation. The right-ventricular demand-pacemaker was programmed to 80 bpm. After 24-h post-operative recovery, 7-day atrial tachypacing at 400 bpm was initiated. The final open-chest EPS was performed on day 7.

2.2 Study protocols

At the terminal open-chest study, EPS was performed during sinus rhythm and repeated before and after each ablation. Atrial and ventricular tachypacemakers were deactivated. All animals were in sinus rhythm when tachypacemakers were deactivated: spontaneous AF was not observed. Dogs were anaesthetized with morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, iv, followed by 29.25 mg/kg/h), and ventilated mechanically. Body temperature was maintained at 37°C. A femoral artery and both femoral veins were cannulated for pressure monitoring and drug administration. A median sternotomy was performed, and bipolar electrodes were hooked into the RAA and LA appendage (LAA). Five silicon sheets containing 240 bipolar electrodes were sutured onto the atrial surfaces as previously described for electrophysiologic mapping. A programmable stimulator was used to deliver 2× threshold current, 2-ms duration pulses. Atrial effective refractory period (ERP) was measured with 10 basic stimuli (S1) followed by premature extrastimuli (S2) with 5-ms decrements. The longest S1–S2 interval failing to capture defined the ERP. The mean of three ERP determinations at each basic cycle length (BCL) was used. ERPs were measured at BCLs of 150, 200, 250, 300, and 360 ms in the RAA and LAA, and at a BCL of 300 ms at six additional sites. AF vulnerability was determined as the percentage of atrial sites at which AF (>1 s) was induced by single extrastimuli. To estimate mean AF duration in each dog, AF was induced with 1–10 s burst pacing (10-Hz, 4× threshold current). AF was induced 10 times for AF duration <20 min and 5 times for AF durations of 20–30 min. Prolonged AF (>30 min) was terminated by direct-current electrical cardioversion. A 20-min rest period was allowed before continuing measurements. If prolonged AF was induced twice, no further AF induction was performed.

2.3 Conduction velocity and phase-delay analysis

Conduction velocity was determined from regressions of electrode distances on activation times. To evaluate conduction properties, phase-delay analysis was performed as previously reported. P5 (5th percentile of the phase-delay histogram) reflects the shortest phase delays, P95 (95th percentile) the longest phase delays, P50 (median phase-delay value) overall conduction, and P95−P50 (difference between 95th and 95th percentile normalized to median phase-delay value) is a conduction-heterogeneity index independent of overall conduction-velocity changes.

2.4 Dominant-frequency analysis

The frequency content of fibrillatory activity was analysed by fast Fourier transformation of filtered (fifth-order Butterworth) rectified epicardial bipolar potentials over 40-s periods during AF. Dominant-frequency (DF) values for each of the 240 electrodes was determined based on the peak in the power spectrum between 5 and 20 Hz. Mean LA and RA DFs were calculated by averaging all values in each atrium.

2.5 Ganglion-sparing pulmonary-vein isolation

All PVs were isolated with a bipolar epicardial radiofrequency clamp device as previously described. Before PVI, autonomic ganglia were localized by vagal response to epicardial high-frequency subthreshold stimulation (20-Hz, 0.4-ms pulse width, current–intensity half atrial capture threshold), and the clamp was positioned distally to avoid damage to ganglia. Sinus-rate slowing (sinus cycle-length prolongation >50% over baseline), atrophicentric block, and asystole were considered vagal responses. Vagal responses to high-frequency subthreshold stimulation were re-checked after PVI to ensure that the autonomic ganglia had been functionally spared. Radiofrequency energy was delivered at 32.5 W. A dramatic rise in impedance indicating lesion completion required 10–30 s. If signs of vagal response were observed during radiofrequency energy application, energy delivery was suspended and the clamp repositioned distally. Each PV was mapped epicardially on six segments (anterior and posterior cranial, anterior and posterior caudal, ventral, dorsal) with a bipolar electrode probe for the presence of PV potentials. PVI was defined as a loss of PV response to LA stimulation and of LA response to PV stimulation at all the PV sites distal to the ablation line. Persistent PV isolation was verified in the same way at the end of each experiment.

2.6 Autonomic ganglion plexus localization and ablation

Peri-PV ostial autonomic ganglionic plexi were located up to 1 cm from right PV ostia and 2 cm from left ostia. RF-power energy was delivered to ganglionic plexi via a bipolar epicardial radiofrequency pen device (35 W through a 100-Ω resistance). Successful GPA was defined by abolition of vagal responses to high-frequency subthreshold stimulation at the same regions that elicited responses before ablation. Transmural lesions were avoided, and there was no evidence of conduction block after GPA. PV potentials were recorded before and after each GPA to verify intact PV conduction.

2.7 Immunofluorescence and confocal imaging of autonomic innervation

LA-tissue samples were harvested, cryosectioned (14-μm thickness), and immunostained with rabbit anti-GAP-43 or rabbit anti-NF-M primary antibodies (Millipore) and Alexa Fluor-conjugated donkey anti-rabbit...
secondary antibody (647 nm, Invitrogen). GAP-43 was used to label nerve sprouting. 21 NF-M, which is found in more mature nerves, was used to quantify functional mature fibres. Slides were examined with a laser-scanning confocal LSM7 microscope (Carl Zeiss). Tile Z-stack acquisitions of 3 × 3 images were performed with a 25×/0.8 plan apochromat objective (oil) zoom-out 0.6 to maximize the surface studied to 1.7 × 1.7 mm. Images were obtained every 1 μm on the Z-axis to cover complete tissue thickness. Excitation was performed at 633 nm with a He/Ne laser and emission was collected at 670 nm. Images were further deconvolved using the maximum likelihood estimation algorithm and maximum intensity full projections were then performed. Projections were analysed with Image Pro Plus 6.2 (Media Cybernetics). The surface occupied by GAP-43 or NF-M positive nerves was normalized to the total surface of the imaged tissue.

2.8 Data analysis

Continuous variables are expressed as mean ± SEM. For comparisons involving single repeated measures only, paired t-tests were used. Multiple-group comparisons were obtained with one-way ANOVA or repeated measures ANOVA as appropriate, with Bonferroni-adjusted t-tests to compare individual mean differences if ANOVA was significant. All data satisfied statistical criteria for normal distribution except for AF duration, which was normalized by logarithmic transformation. Two-tailed P < 0.05 indicated statistical significance.

3. Results

Overall group characteristics and haemodynamic data at final open-chest study are presented in Table 1. Body weight increased after 14-day VTP in CHF dogs. CHF dogs had higher left-ventricular end-diastolic (11.8 ± 0.6 mm Hg) and left (10.2 ± 0.7 mm Hg) and right (8.3 ± 0.4 mm Hg) atrial pressures than control (2.1 ± 0.6, 3.9 ± 0.6, 3.4 ± 0.4 mm Hg, respectively) and ATR (3.8 ± 0.6, 5.0 ± 0.4, and 4.0 ± 0.3 mmHg, respectively) dogs.

Table 1 Group characteristics and haemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>CTL-PVI (n = 5)</th>
<th>CTL-GPA (n = 9)</th>
<th>CHF-PVI (n = 9)</th>
<th>CHF-GPA (n = 6)</th>
<th>ATR-PVI (n = 8)</th>
<th>ATR-GPA (n = 5)</th>
</tr>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>29.4 ± 2.0</td>
<td>28.9 ± 0.9</td>
<td>27.7 ± 1.9</td>
<td>31.5 ± 0.8</td>
<td>27.4 ± 2.6</td>
<td>31.6 ± 1.7</td>
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<tr>
<td>Pressures (mmHg)</td>
<td></td>
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<tr>
<td>Systolic BP</td>
<td>116 ± 8</td>
<td>113 ± 4</td>
<td>77 ± 7</td>
<td>78 ± 13</td>
<td>118 ± 12</td>
<td>116 ± 9</td>
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<td>Diastolic BP</td>
<td>72 ± 4</td>
<td>72 ± 4</td>
<td>44 ± 4</td>
<td>51 ± 9</td>
<td>60 ± 8</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>LVEDP</td>
<td>2.0 ± 0.9</td>
<td>2.1 ± 0.8</td>
<td>12.0 ± 0.6</td>
<td>11.4 ± 1.1</td>
<td>5.0 ± 0.4</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>LAP</td>
<td>3.4 ± 0.7</td>
<td>4.2 ± 0.8</td>
<td>9.7 ± 0.9</td>
<td>10.8 ± 1.2</td>
<td>5.2 ± 0.7</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>RAP</td>
<td>2.8 ± 0.6</td>
<td>3.8 ± 0.6</td>
<td>7.9 ± 0.6</td>
<td>9.0 ± 0.6</td>
<td>4.3 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

P † vs. CTL-PVI, P ‡ vs. CTL-GPA, P § vs. ATR-PVI, and P ¶ vs. ATR-GPA.

AF dogs had shorter ERPs compared with control dogs. DF in ATR dogs was faster in LA, but not in RA, than control dogs. Supplementary material online, Figure S1 shows baseline electrophysiologic data for all groups according to ablation-group assignment: results were not different between PVI and GPA groups for each model.

A representative example of PVI is provided in Supplementary material online, Figure S2. Discrete PV-potentials recorded pre-PVI were abolished in all the PVs after PVI. All PIs were successfully isolated in all PVI animals. A representative example of GPA is shown in Supplementary material online, Figures S3 and S4. In Supplementary material online, Figure S3, the vagal response induced by ganglion-plexus stimulation before GPA was abolished after GPA. However, higher output stimulation still captured atrial myocardium, indicating still-excitable underlying atrial tissue. In Supplementary material online, Figure S4, left-sided ganglion-plexus stimulation induced sinus slowing and AV block. Right-sided GPA abolished left-sided ganglion-plexus stimulation-induced sinus slowing but not AV block. Left-sided GPA then abolished all effects. Full abolition of all ganglion-plexus responses was achieved by GPA in all dogs.

3.1 Effects of PVI and GPA on atrial fibrillation in CHF-associated structural remodelling

Figure 2 shows electrophysiologic data before and after PVI (A−F) and GPA (G−L) in CHF dogs. PVI and GPA did not affect AF duration, DF, or atrial ERPs in CHF dogs. AF vulnerability was low in CHF and was not affected by PVI or GPA. Atrial conduction velocity and the conduction-heterogeneity index are shown in Supplementary material online, Figure S5: neither PVI (A−C) nor GPA (D−F) affected atrial conduction in CHF dogs.

3.2 Effects of PVI and GPA on atrial fibrillation in atrial tachycardia-induced remodelling

Figures 3A−F show electrophysiologic data before and after PVI in ATR dogs. PVI decreased AF vulnerability (from 75 ± 6 to 55 ± 11%, P < 0.05), but did not affect mean AF duration, DF, or atrial ERPs significantly.
Figure 1  Electrophysiologic variables and indices of AF promotion at baseline (pre-ablation) in each model. (A) Mean AF duration, (B) AF vulnerability, (C) mean DF in RA and LA and (D–F) atrial ERPs. *p < 0.05, **p < 0.01, ***p < 0.001 vs. control. CTL, control; CHF, congestive heart failure; ATR, atrial tachycardia remodelling; RAA, RA appendage; RAPW, RA posterior wall; RAIW, RA inferior wall; RABB, RA Bachmann’s bundle; LABB, LA Bachmann’s bundle; LAIW, LA inferior wall; LAPW, LA posterior wall; LAA, LA appendage.

Figure 2  Effects of PVI (A–F) and GPA (G–L) on electrophysiologic variables and indices of AF promotion in CHF dogs. Open bars and circles indicate baseline, closed bars and circles post-ablation results.
Ganglion-plexus stimulation induced sinus-rate slowing before GPA, but not after GPA. AF induced by burst pacing was sustained for more than 30 min before GPA; however, it lasted a maximum of 9 s after GPA. Overall electrophysiological data before and after GPA in ATR dogs are provided in Figures 3G–L. GPA prolonged ERPs in both atria, reduced AF vulnerability (from 75 ± 8 to 30 ± 10%, P < 0.05), decreased DF in both LA (from 11.4 ± 0.6 Hz to 8.6 ± 0.3 Hz, P < 0.05) and RA (from 9.9 ± 0.6 Hz to 8.3 ± 0.1 Hz, P < 0.01), and abbreviated mean AF duration (from 617 ± 246 to 39 ± 23 s, P < 0.01) in ATR dogs. Overall, GPA decreased AF vulnerability by 45 ± 13%, compared with the 20 ± 6% reduction produced by PVI (P = 0.08).

The differences in effect on DF are illustrated for one representative dog in each group in Figure 5. In CHF dogs (Figure 5A–D), there were only small regional DF differences and limited effects of ablation on DF values, without regional selectivity of ablation-induced changes. DF values were significantly greater in LA than in RA before ablation in ATR dogs (LA 11.4 ± 0.9 Hz vs. RA 9.9 ± 0.3 Hz, P < 0.05 in PVI group, Figures 3C and 5E; LA 11.4 ± 0.6 Hz vs. RA 9.9 ± 0.6 Hz, 9 s, P < 0.01).
3.3 Effects of PVI and GPA on atrial ERP in control dogs

The effects of PVI and GPA in control dogs are illustrated in Supplementary material online, Figure 5. PVI did not affect atrial ERPs significantly in control dogs. In contrast, GPA prolonged atrial ERPs significantly in all atrial regions (e.g., from 107 ± 6 to 120 ± 4 ms, P < 0.05, LAA; from 109 ± 4 to 128 ± 4 ms, P < 0.001, RAA; BCL 300 ms). GPA-induced ERP prolongation was similar for ATR compared with control dogs in the RAA (by 15 ± 5 ms, 21 ± 8% in ATR vs. 18 ± 3 ms, 17 ± 4% in control; BCL 300 ms); however, for the LAA, GPA increased ERP more prominently in ATR (by 28 ± 7 ms, 56 ± 18%; BCL 300 ms) than control dogs (by 13 ± 5 ms, 14 ± 6%; BCL 300 ms, P = 0.12 and P < 0.05, respectively vs. ATR). The larger effect of GPA in ATR LAAs suggested possible neural remodelling, which could account for the efficacy of GPA in ATR dogs. The lack of GPA effect on atrial ERPs in CHF dogs (Figure 2J and K), in contrast to its clear effect in control dogs, is compatible with decreased atrial autonomic innervation in CHF.

3.4 Effects of CHF and ATR on autonomic innervation

Autonomic innervation was assessed in LAA of control, CHF and ATR dogs. As illustrated in Figure 6A, GAP-43 staining was significantly decreased in CHF compared with control. ATR demonstrated the opposite effect, increasing GAP-43 staining vs. control. NF-M immunostaining (Figure 6B) showed similar changes. Quantitative analysis confirmed these observations (Figures 6C and D). Both GAP-43 and NF-M positive nerve densities decreased in CHF (by 63.1 and 60.0%, respectively, P < 0.01 for each) and increased in ATR (by 65.5 and 92.1%, P < 0.01, P < 0.001, respectively vs. control).

4. Discussion

Here, we assessed the roles of PVs and ganglionic plexi in the AF substrate associated with ATR and CHF in dogs. Neither PVI nor peri-PV GPA suppressed AF in VTP-induced canine CHF. Peri-PV GPA effectively suppressed AF related to ATR, while PVI had a quantitatively smaller effect on AF vulnerability and none on AF maintenance in ATR dogs. Intact PVs electrically connected to the LA are thus not essential for AF maintenance in either model, whereas intact autonomic ganglia play an important role in AF maintenance in the presence of ATR.

4.1 Autonomic denervation in human atrial fibrillation

Autonomic denervation increases the success rate of PV ablations in human AF. Abolition of vagal reflexes evoked by radiofrequency energy application during standard circumferential PV ablation correlated with reduced AF recurrence. Adding GPA to PV antrum isolation reduced AF recurrence (3 of 33 patients) compared with PVI alone (8 of 27 patients) in another study. Combined GPA and PVI prevented AF recurrence in 17 of 18 patients with vagally mediated paroxysmal AF. Stand-alone autonomic denervation also suppresses AF in some patients without PV ablation: selective autonomic denervation alone prevented the recurrence of vagally mediated paroxysmal AF in two of seven patients. In another study, GPA alone suppressed the recurrence of paroxysmal AF in 5 of 19 patients; however, the efficacy was lower than that of circumferential PV ablation alone (12 of 19 patients). Surgical dissection of the anterior fat pad reduced AF incidence after open heart surgery in one study; however, other findings contradict this result. In the present study, GPA was effective only in ATR, but not in CHF-related AF models. This result suggests that GPA effects may depend on the specific AF substrate, consistent with the variable clinical results. PVI was without effect in either CHF- or ATR-related AF. We intentionally isolated PVs distally to the sites of vagal responses to avoid damage to...
ganglia. In contrast, the standard lesion sets used in clinical PV ablation frequently encompass autonomic ganglia and eliminate vagal responses, even without specifically targeting such sites.\textsuperscript{4,15} Interference with cardiac autonomic nerve activity may contribute to the efficacy of PV ablation in suppressing AF, at least in some patients.

4.2 Previous studies of autonomic denervation in animal AF models

Various ablation procedures have been reported to suppress experimental vagal AF, including multiple linear radiofrequency-energy ablations in both atria,\textsuperscript{25} transvascular catheter ablation of the third fat pad,\textsuperscript{26} and epicardial radiofrequency-ablation of cardiac autonomic ganglionic plexi.\textsuperscript{19,27} Cryoablation of the left and right stellate ganglia and the cardiac branch of the left vagal nerve delayed the development of sustained AF and eliminated spontaneous paroxysmal AF episodes in a long-term atrial tachypacing canine AF model.\textsuperscript{28} However, the intrinsic autonomic ganglia can manifest spontaneous neural activity independent of extrinsic control,\textsuperscript{29} and the function/role of local cardiac ganglionic plexi may differ from those of extrinsic autonomic nerves.\textsuperscript{29,30} To our knowledge, the present study is the first to demonstrate the efficacy of local cardiac GPA in non-vagal animal AF models.

4.3 Role of vagal and sympathetic factors in atrial fibrillation

Both vagal and sympathetic activation shorten atrial ERP and may promote AF induction and maintenance by multiple circuit re-entry. Vagal influences are particularly effective in promoting AF maintenance.\textsuperscript{31,32} Vagal stimulation increases heterogeneity in atrial repolarization and stabilizes atrial microre-entrant circuits.\textsuperscript{31} However, complex interactions between vagal and sympathetic nerves are involved in the genesis of AF.\textsuperscript{28,33–35} Simultaneous sympathovagal discharges were observed preceding onsets of paroxysmal AF in an atrial tachypacing-induced canine AF model, in which left stellate ganglion nerve and left vagal nerve activities were continuously recorded.\textsuperscript{28} α-Adrenoceptor blockade suppressed atrial tachyarrhythmias induced by mediastinal nerve stimulation without affecting bradycardic responses.\textsuperscript{35} Adrenergic and cholinergic nerves are closely juxtaposed in autonomic ganglia and it is difficult to selectively ablate either alone.\textsuperscript{13,36} Ganglionic plexi were localized by vagal response to high-frequency subthreshold stimulation in the present study; however, both cholinergic and adrenergic withdrawal may have contributed to the efficacy of GPA. Although adrenergic and cholinergic effects are generally considered to oppose each other, they likely act in concert to promote AF.\textsuperscript{34}
4.4 Autonomic remodelling in the AF substrate related to atrial tachycardia remodelling

ATR-induced remodelling of cardiac ionic currents is a major contributor to AF promotion.\textsuperscript{37} Important changes include downregulation of L-type Ca\textsuperscript{2+} current, upregulation of background inward-rectifier current (I\textsubscript{K1}) and increased constitutive acetylcholine-dependent current (I\textsubscript{KACH}).\textsuperscript{37} Constitutive I\textsubscript{KACH} plays a significant role in action potential duration abbreviation and AF promotion.\textsuperscript{38,39} \(\beta_1\)-adrenocceptor stimulation enhances constitutive I\textsubscript{KACH}, whereas \(\alpha_1\)-adrenergic stimulation inhibits it.\textsuperscript{40,41} Withdrawal of \(\beta_1\)-adrenergic activity may have contributed to the efficacy of GPA in ATR-related AF via reduced constitutive I\textsubscript{KACH}. On the other hand, reduced vagal tone may also have contributed by decreasing agonist-activated I\textsubscript{KACH}.

Previous studies have shown increased atrial sympathetic innervation after atrial tachypacing in dogs\textsuperscript{28,42,43} and goats.\textsuperscript{44} Increased sympathetic innervation is also found in patients with persistent AF.\textsuperscript{45} We noted increased staining with GAP-43 (marker of immature sympathetic innervation is also found in patients with persistent AF).\textsuperscript{45} Increased staining with GAP-43 and Nurr1 (marker for immature sympathetic innervation in AF) in the only study from which data are available, long-term atrial tachypacing tended to increase atrial cholinergic innervation in dogs.\textsuperscript{28}

4.5 Possible limitations

Clinical and experimental studies suggest eventual recovery of autonomic function after radiofrequency ablation-induced autonomic denervation.\textsuperscript{15,27,41} The long-term effects of GPA on ATR promotion of AF may differ from acute effects. We studied specific durations of ATR and VTP. Different durations of tachypacing may have produced different results.

In this study, we used dog models of AF substrates, manifesting much more persistent AF than control dogs following AF induction. These models are likely relevant to patients with persistent AF substrates, but do not mimic the strongly enhanced triggers observed in many clinical AF paradigms, particularly paroxysmal AF. The absence of a role of the PVs in AF maintenance in our models does not bear on the clearly established participation of the PVs, particularly as sources of focal triggers and tachyarrhythmic activity, in many AF patients.

4.6 Conclusions

The results of this study argue against a significant role of PVs in the AF promotion resulting from ATR or CHF in dogs. On the other hand, the autonomic ganglia are important in ATR-induced AF promotion, possibly because of LA hyperinnervation. The dependence of ganglion-ablation effects on the specific AF substrate may relate to the large variability that has been reported for the effects of ablation-induced autonomic denervation among different patients and series.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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