Neuronal Na\textsubscript{v}1.8 Channels as a Novel Therapeutic Target of Acute Atrial Fibrillation Prevention

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**Background**—Ganglionated plexus have been developed as additional ablation targets to improve the outcome of atrial fibrillation (AF) besides pulmonary vein isolation. Recent studies implicated an intimate relationship between neuronal sodium channel Na\textsubscript{v}1.8 (encoded by SCN10A) and AF. The underlying mechanism between Na\textsubscript{v}1.8 and AF remains unclear. This study aimed to determine the role of Na\textsubscript{v}1.8 in cardiac electrophysiology in an acute AF model and explore possible therapeutic targets.

**Methods and Results**—Immunohistochemical study was used on canine cardiac ganglionated plexus. Both Na\textsubscript{v}1.5 and Na\textsubscript{v}1.8 were expressed in ganglionated plexus with canonical neuronal markers. Sixteen canines were randomly administered either saline or the Na\textsubscript{v}1.8 blocker A-803467. Electrophysiological study was compared between the 2 groups before and after 6-hour rapid atrial pacing. Compared with the control group, administration of A-803467 decreased the incidence of AF (87.5% versus 25.0%, *P*<0.05), shortened AF duration, and prolonged AF cycle length. A-803467 also significantly suppressed the decrease in the effective refractory period and the increase in effective refractory period dispersion and cumulative window of vulnerability caused by rapid atrial pacing in all recording sites. Patch clamp study was performed under 100 nmol/L A-803467 in TSA201 cells cotransfected with SCN10A-WT, SCN5A-WT, and SCN3B-WT. *I*\textsubscript{Na,p} was reduced by 45.34% at −35 mV, and *I*\textsubscript{Na,L} by 68.57% at −20 mV. Evident fast inactivation, slow recovery, and use-dependent block were also discovered after applying the drug.

**Conclusions**—Our study demonstrates that Na\textsubscript{v}1.8 could exert its effect on electrophysiological characteristics through cardiac ganglionated plexus. It indicates that Na\textsubscript{v}1.8 is a novel target in understanding cardiac electrophysiology and SCN10A-related arrhythmias. ([J Am Heart Assoc. 2016;5:e004050 doi: 10.1161/JAHA.116.004050](https://doi.org/10.1161/JAHA.116.004050))

**Key Words:** atrial fibrillation • electrophysiology • ganglionated plexus • Na\textsubscript{v}1.8 • SCN10A

It has been recognized that cardiac autonomic nervous system activity is of great significance in the initiation and maintenance of atrial fibrillation (AF). Stimulation or inhibition of selective extracardiac neural structures may be a useful therapeutic option for cardiac arrhythmias, including AF, and other abnormalities. As the major part of the intrinsic cardiac autonomic nervous system, most cardiac ganglionated plexus (GPs) embedded in the fat pad around 4 pulmonary veins (PVs), play an important role in the early stage of AF. Increased GP activity can induce the increase of both parasympathetic and sympathetic activity, which promotes rapid focal firing in the PV myocardium. Clinical evidence also suggests that additional GP ablation to PV isolation could increase success rates in eliminating AF.

As a tetrodotoxin-resistant periphery nerve voltage-gated sodium channel, Na\textsubscript{v}1.8 (encoded by SCN10A) plays a significant part in the upstroke of action potential in neurons, and is responsible for repetitive firing. It is found primarily expressed in small- and medium-diameter nociceptive sensory neurons, which mediate pain perception. Genome-wide association study highlights the role of Na\textsubscript{v}1.8 in cardiac conduction and arrhythmic diseases. In addition to the canonical cardiac sodium channel Na\textsubscript{v}1.5/SCN5A, Na\textsubscript{v}1.8 is recently considered a “new cardiac sodium channel.” SCN10A is adjacent to SCN5A on the same chromosome, and there is 70.4% of similarity in the amino acid sequence between these 2 sodium channels. Our previous research shows Na\textsubscript{v}1.8 could physically interact with Na\textsubscript{v}1.5 by using co-immunoprecipitation when both are expressed in vitro.
Others prove that Nav1.8 could modulate the activity and expression of Na\textsubscript{v}1.5 at the transcriptional level, which may be intermediated by TBX3/5.\textsuperscript{10,11} Increasing evidence indicates that Na\textsubscript{v}1.8/SCN10A plays a critical role in AF. Both common and rare variants of SCN10A were associated with the risk of AF.\textsuperscript{12,13} Some rare SCN10A variants have been identified in patients with early-onset AF (rs141207048, rs202143516, rs202192818, rs139861061, et al), and the relevant mechanism implied by functional study in vitro might be through the modulation of peak sodium current (I\textsubscript{Na,p}) and late sodium current (I\textsubscript{Na,L}) of Na\textsubscript{v}1.8.\textsuperscript{14} Certain common variants, such as rs6795970, have been proved to be related to AF susceptibility.\textsuperscript{12,13} One recent study has suggested that blockade of Na\textsubscript{v}1.8 suppresses vagal-mediated AF most likely by inhibiting the neural activity of GP.\textsuperscript{15} However, the exact electrophysiological role of Na\textsubscript{v}1.8 in AF, especially at the early stage of acute AF, is uncertain, and the relevant mechanisms still need to be assessed. In this study, we aim to explore the expression of Na\textsubscript{v}1.8 and Na\textsubscript{v}1.5 in canine cardiac ganglia, and to evaluate the role of the Na\textsubscript{v}1.8 blocker A-803467 in cardiac GP in an acute AF canine model. We have further investigated the changes of the channel density and kinetic characteristics in the presence of A-803467 when the SCN5A and SCN10A coexpressed in vitro, which might provide the potential mechanism to explain its effect in a canine AF model.

Methods

Animal Preparation

Experiments were approved by the Animal Ethics Committee of Wuhan University under approval number 2015-0072 and followed the guidelines outlined by the Care and Use of Laboratory Animals of the National Institutes of Health. Sixteen mongrel dogs weighing from 20 to 25 kg were included in this study. Surgeries were performed under anesthesia with sodium pentobarbital with an initial dose of 50 mg/kg and an additional dose of 2 mg/kg per hour. A heating pad was used to maintain the core body temperature at 36.5 ± 1.5°C. All dogs were ventilated with room air by a positive pressure respirator. Bilateral thoracotomy was conducted at the fourth intercostal space, as previously described\textsuperscript{16,17} (Figure 1A and 1B). In brief, multielectrode catheters were sutured to obtain recordings at the surface of the atrium and PVs and to pace at the left atrial appendage (LAA). All recordings were displayed on a computerized Bard Electrophysiology System (CR Bard Inc, Billerica, MA). High-frequency stimulation (20 Hz, 0.1 ms duration, square waves) was applied at the fat pad to identify GPs by a bipolar electrode stimulator (Grass-S88; Astro-Med, West Warwick, RI). Anterior right ganglionated plexi (ARGP) was located at the RSPV-atrial junction and superior left ganglionated plexi (SLGP) at the left superior PV (LSPV)-atrial junction. A successful GP stimulation was marked at the 50% sinus rate slowing or second- or third-degree atrioventricular block developing.

Study Protocol

Two groups were set in this study based on injecting saline (0.5 mL per GP) or the selective Na\textsubscript{v}1.8 blocker A-803467 (1 μmol/0.5 mL per GP) into both ARGP and SLGP. After drug administrating, RAP was delivered at the LAA (20 Hz, 10 diastolic threshold) for 6 hours. At the end of RAP, targeted parameters-effective refractory period (ERP) and window of vulnerability (WOV) were evaluated as previously described.\textsuperscript{16,18} Briefly, we measured ERP by using S1-S1 programmed stimulating at 330 ms interval and S1-S2 interval from 150 ms with decrementing at 10 and 1 ms when approaching the refractory period (S1:S2=8:1, 10 TH). To delineate AF inducibility, WOV was calculated as the difference between the longest and the shortest S1-S2 interval. ERP dispersion was assessed as the coefficient of variation of ERP at all 8 recording sites. AF was defined as irregular atrial rates related with irregular atrioventricular conduction at more than 500 bpm lasting at least 5 seconds. AF cycle length was measured as the averaged first 10 fibrillation waves at the onset of AF. The number of AF episodes and averaged AF duration were also recorded at each group. Representative traces in the electrophysiological study before and after administrating A-803467 at GPs are shown in Figure 1C and Figure 1D.

Tissue Preparation and Immunofluorescence

Cardiac ganglia were quickly obtained from 3 canine heart fat pads located at the atria posterior wall. After that, they were placed into normal Krebs solution in ice, dissected under a stereomicroscope, and put into liquid nitrogen for flash freezing. Cryosections from embedded ganglia tissue were fixed in 4% paraformaldehyde for 10 minutes, permeabilized in 0.3% Triton X-100 in PBS for 15 minutes, and all blocked in 5% donkey serum for 30 minutes at room temperature. Tissue sections were incubated at 4°C overnight with primary antibody specific for rabbit polyclonal anti-Nav1.8 (Alomone Labs, Jerusalem, Israel), rabbit polyclonal anti-Nav1.5 (Alomone Labs, Jerusalem, Israel), goat polyclonal anti-Nav1.5 (Abcam, Cambridge, MA), goat polyclonal anti-choline acetyltransferase (ChAT, CHEMICON International, Temecula, Canada), and mouse monoclonal anti-tyrosine hydroxylase (TH, Alpha Diagnostic International, San Antonio, TX). Alexa 488-conjugated secondary antibody was then added according to a different source of primary antibody and incubated for 90 minutes at room temperature. Stained samples were
Coexpression of NaV1.5 and NaV1.8 and Patch Clamp Study

TSA-201 cells transfected with SCN5A, SCN10A, and SCN3B plasmids were used for patch clamp study. A plasmid encoding EGFP was used to identify transfected cells. Briefly, transient transfection using FuGENE 6 (Roche Diagnostics, Indianapolis, Indiana) was carried out with SCN10A, SCN5A, and SCN3B with a molar ratio of 5:5:1 (for a total of 2.25 μg of DNA). The cells were grown in GIBCO DMEM medium (No. 10566, Gibco With FBS [No. 16000] and antibiotics [No. 15140], Life Technologies) on polylysine-coated 35 mm culture dishes (Cell+, Sarstedt, Newton, NC). Cells were placed in a 5% CO₂ incubator at 37°C for 24 to 48 hours prior to patch clamp study.

Membrane currents were measured using whole-cell patch clamp techniques. All recordings were obtained at room temperature (20–22°C) using an Axopatch 200B amplifier equipped with a CV-201A head stage (Axon Instruments Inc./Molecular Devices, Union City, CA). Currents were filtered with a 4-pole Bessel filter at 5 kHz and digitized at 50 kHz.

Series resistance was compensated at around 80% to assure that the command potential was reached within microseconds with a voltage error <2 mV. Cells were allowed to stabilize for 10 minutes after establishment of the whole-cell configuration before current was measured. Macroscopic whole-cell Na⁺ current was recorded by using bath solution perfusion containing (in mmol/L) 140 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 Dextrose (pH 7.35 with NaOH). Osmolarity was adjusted to 310 mmol/kg with sucrose. Patch pipettes were fabricated from 1.5 mm OD borosilicate glass capillaries.
(Fisher Scientific Inc, Hampton, NH). They were pulled using a gravity puller (Model PP-830, Narishige International USA, Inc, East Meadow, NY) to obtain resistances between 0.8 and 2.2 MΩ when filled with a pipette solution containing (in mmol/L) 10 NaF, 105 CsF, 20 CsCl, 2 EGTA, and 10 HEPES with a pH of 7.35 adjusted with CsOH and an osmolality of 300 mmol/kg with sucrose.

Specific voltage-clamp protocols assessing channel activation and fast inactivation were used, as depicted in the figure insets. Cardiac sodium channel current ($I_{Na}$) was elicited by depolarizing pulses ranging from −90 to +40 mV in 5 mV increments with a holding potential of −120 mV. Peak currents ($I_{Na,p}$) were measured and $I_{Na}$ densities (pA/pF) were attained by dividing the obtained cell capacitance. Activation properties were determined from $I/V$ relationships by normalizing $I_{Na,p}$ to driving force and maximal $I_{Na}$, and plotting normalized conductance versus $V_m$. Voltage dependence of steady-state inactivation was obtained by plotting the normalized $I_{Na,P}$ (40-ms test pulse to −0 mV after a 1000-ms conditioning pulse from −140 to −10 mV with the holding potential of −120 mV) versus $V_m$. The steady-state channel availability and inactivation curves were fitted to the Boltzmann equation, $I/I_{max}=1/(1+exp((V-V_{1/2})/\kappa))$, to determine the membrane potential for half-maximal activation/inactivation ($V_{1/2}$) and the slope factor ($\kappa$). Pulses for recovery from inactivation were of 100 ms duration for P1 and 50 ms for P2. The peak current elicited during the second pulse was normalized to the value obtained during the initial test pulse. It was analyzed by fitting data to a double exponential function: $I(t)/I_{max}=Af\times(1−exp(−t/\tau_f))+As\times(1−exp(−t/\tau_s))$, where Af and As are the fractions of fast and slow inactivating components, respectively, and $\tau_f$ and $\tau_s$ are their time constants.

All data acquisition and analysis were performed using pCLAMP version 9.2 (Molecular Devices, Sunnyvale, CA), Excel (Microsoft, Redmond, WA), and Origin 7.5 (MicroCal Software, GE Healthcare, Pittsburgh, PA).

Statistics
All continuous data are presented as mean±standard error (SE). Student $t$ test was used to analyze those continuous data. For nonparametric data, Fisher exact test was used to compare 2 groups. Statistical significance was selected at a 2-sided $P$ value of 0.05.

Results
**Na$_v$1.8 and Na$_v$1.5 Expression in Canine Cardiac Ganglia**
As shown in Figure 2, adult canine cardiac ganglia were partially dissected and labeled by neuron marker PGP9.5, TH,

![Figure 2. Immunochemical study of Nav1.8, Nav1.5, PGP9.5, anti-choline acetyltransferase (ChAT), and anti-tyrosine hydroxylase (TH) in partial dissected intracardiac ganglia. Protein expression of Nav1.8 (A and B), Nav1.5 (C and D), PGP9.5 (E and F), ChAT (G and H), and TH (I and J) present in intracardiac ganglia. Images on the left side show fluorescein isothiocyanate green fluorescence. Images on the right side show nonstained components of the ganglia. Calibration bars in (A through D) are 20 μm and in (E through J) are 50 μm.](image-url)
and ChAT. Both adrenergic and cholinergic neurons can be found in those ganglia (Figure 2G through 2J). Staining of Na\textsubscript{v}1.8 and Na\textsubscript{v}1.5 separately proved their expression on canine GP (Figure 2A through 2D). The 2 sodium channels can be observed in both membrane and cytoplasm under high magnification and no significant differences were found in regard to the channel distribution in adrenergic and cholinergic neurons (data not shown).

Effect of A-803467 on ERP and AF Inducibility in GPs

Representative traces in the electrophysiological study before and after administrating A-803467 at GPs are displayed in Figure 1C and Figure 1D. Mean ERP at baseline between the control and A-803467 groups was similar at all 8 recording sites (97.98±6.86 vs 97.95±7.30 ms, P>0.05, 10x threshold). After administrating saline or A-803467, respectively, to the 2 groups at ARGP and SLGP, rapid pacing was implemented at LAA. The ERPs were reevaluated at the end of 6-hour RAP by the same protocol. Compared with the control group, the shortening of ERP at all PV and atrial sites was suppressed in the A-803467 group (Figure 3, P<0.05).

ERP dispersion and cumulative WOV (ΣWOV) were calculated and AF inducibility was compared between the 2 groups before and after RAP. No baseline differences at ERP dispersion or ΣWOV were found between the 2 groups before RAP (ERP dispersion: 0.15±0.15 vs 0.14±0.13 ms; ΣWOV: 19.30±3.51 vs 18.13±2.34 ms; P>0.05 [Figure 4D and 4E]). After 6-hour RAP, both ERP dispersion and ΣWOV were significantly increased in the control group. However, injecting A-803467 at GPs attenuated the increase in ERP dispersion and ΣWOV apparently at all sites, underlying the decrease in AF inducibility (Figure 4D and 4E, ERP dispersion: 0.29±0.15 vs 0.15±0.13, ΣWOV: 180.70±20.47 vs 21.64±5.84 ms; P<0.01).

After 6-hour RAP, spontaneous AF occurred in 7 dogs in the control group (N=8), whereas it occurred in only 2 dogs in the A-803467 group (N=8), with an incidence of 87.50% versus 25.0% (Figure 4C). In addition, administrating A-803467 can also shorten AF duration and prolong AF cycle length in contrast to that in the control group (AF duration: 47.05±10.45 seconds vs 18.18±6.59 seconds, P<0.05; AF cycle length: 79.21±1.24 versus 129.77±17.41 ms, P<0.05 [Figure 4A and 4B]).

Characterization of A-803467 at 100 nmol/L on SCN5A-SCN10A-SCN3B Cotransfected TSA201 Cells

Previous reports have shown the abundant expression of β3 subunit (SCN3B) in dorsal root ganglion neurons and have emphasized that the β3 subunit can promote the trafficking of Na\textsubscript{v}1.8 and help its expression on membrane in comparison to other β subunits without changing gating properties.\textsuperscript{19,20} Based on that, SCN5A-SCN10A-SCN3B-WT was cotransfected into TSA201 cells in the present study to depict the electrophysiological characteristics of the sodium channel composed of SCN5A-SCN10A under 100 nmol/L A-803467. A significant reduction was shown with 100 nmol/L A-803467 on the current-voltage relationship, whereas no difference was found at the same concentration with SCN5A-SCN3B-WT (Figure 5A). Furthermore, electrophysiological characteristic properties were implemented on the cells transfected with SCN5A-SCN10A-SCN3B-WT. Other and our previous results have reported the extremely low current density when SCN10A was transfected into TSA201 cells.
with or without sodium β subunit.9,21 Hence, the blocking effect of A-803467 was not performed on SCN10A-SCN3B-WT transfected TSA201 cells. A-803467 reduced maximum I\textsubscript{Na,P} by 45% (I\textsubscript{Na,P}, from \(\bar{I}_{0}\) 758.77 pA/pF, \(n=8\); \(P<0.01\)) with similar I-V curve (INa,P amplitudes at \(-35\) mV) between the control and A-803467 groups (Figure 5B and 5C). In addition, INa,L was measured under 100 nmol/L A-803467 between 295 and 300 ms by the test pulse at \(-20\) mV from HP of \(-120\) mV at the rate of 0.2 Hz (Figure 5D). Relative INa,L (percentage of INa,P) decreased from 0.52% to 0.20%. The inhibitions of INa,P and INa,L under the same protocol were also compared. Both the INa,P and INa,L in control conditions were regarded as 100%. As presented in Figure 5E, after applying A-803467, a more pronounced inhibition with INa,L than INa,P was shown: INa,P was decreased 42.02\%\pm3.88\% and INa,L was decreased 68.57\%\pm5.86\% (\(n=8\) for each group, \(P=0.02\)).

Figure 6A shows a hyperpolarized shift of steady-state inactivation by the influence of 100 nmol/L A-803467 under a 100 ms prepulse to various potentials in 10 mV step followed by a 20 ms test pulse to 0 mV. A-803467 caused a prominent negative shift of the \(V_{1/2}\) of inactivation from \(-83.76\pm2.38\) to \(-95.77\pm3.20\) mV (\(n=12\), \(P<0.05\)), predicting less availability of channels open at physiological resting potential or sustained depolarization with the drug. As shown in Figure 6B and the Table, there was no significant difference in steady-state activation before and after applying the drug (\(V_{1/2}: -49.52\pm2.74\) and \(-51.95\pm1.38\) mV, \(n=12\) [\(P=0.44\)]; \(\kappa: -5.21\pm0.48\) and \(-5.78\pm0.24\), \(n=12\) [\(P=0.30\)])

Recovery from inactivation was also assessed before and after drug administration by using a standard 2-pulse protocol: first test \(-10\) mV for 100 ms then recovering at \(-120\) mV for a various duration before a second pulse at \(-10\) mV for 50 ms. As displayed in the representative traces (Figure 6C), A-803467 inhibited the process of recovery from inactivation. A 2-exponential equation was used for fitting, and time constants were compared. Both the fast (\(\tau_f\)) and slow (\(\tau_s\)) time constants were much slower in the presence of 100 nmol/L A-803467 (\(\tau_f: 2.87\pm0.38\) versus \(6.46\pm0.58\), \(n=14\) [\(P<0.01\)]; \(\tau_s: 20.51\pm0.61\) versus 64.37\pm15.47, \(n=14\) [\(P=0.01\), Figure 6D, Table]).

For further study, use-dependent block (UDB) was investigated in the absence and presence of A-803467 via a series of 40 pulses to \(-20\) mV from \(-120\) mV holding potential at the rate of 1, 2, and 10 Hz. The proportion of the 40th and first current amplitudes was compared between control and drug conditions under these 3 different frequencies. For the control group, the proportions of INa,P were 97.38%, 95.80%, and 93.89%, and for the drug were 95.95%, 89.81%, and 83.96%, at the rate of 1, 2, and 10 Hz, respectively (Figure 7B). The inhibition effect of A-803467 was more evident at faster rates (reduction at 1/2/10 Hz was 3.68%/6.06%/12.78%, \(n=9\) [\(P<0.01\))]. Figure 7A shows the exemplified traces at 10 Hz. Both traces of the 2 conditions were

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positioned at the same scale so that the noticeable reduction under drug influence was more obvious in comparison. Together with the inhibition of recovery from inactivation, A-803467 may interact with the inactivated state of these sodium channels. In addition, a frequency-dependent reduction was also observed in \(I_{\text{Na,L}}\). Figure 7D shows the relative current proportion of \(I_{\text{Na,L}}\) at -20 mV in the rate of 2 Hz. In the control condition, there was more inhibition of \(I_{\text{Na,L}}\) than that in \(I_{\text{Na,P}}\), with 68.99±3.67% versus 95.80±1.75% remaining after the 40th pulse. When the drug was applied, the value of \(I_{\text{Na,L}}\) and \(I_{\text{Na,P}}\) decreased to 60.64±3.98% and 89.43±2.06% (reduced rate of \(I_{\text{Na,L}}\) versus \(I_{\text{Na,P}}\); 12±0.04% versus 6±2%, N=8 for each group \([P=0.04]\)). The result indicates preferential UDB of A-803467 on \(I_{\text{Na,L}}\) than \(I_{\text{Na,P}}\).
Discussion

In this study, we demonstrate that, in a canine acute AF model, pharmacological inhibition of Nav1.8 on GP can prevent electrical remodeling at PV and atrial myocardium and further reduce the incidence of paroxysmal AF. Sodium channel properties were obviously affected by the Na\textsubscript{v1.8} blocker when SCN5A-SCN10A-SCN3B-WT coexpressed in vitro, which indicates a possible underlying mechanism of our animal model results. The transfected sodium channel complex with 100 nmol/L A-803467 displayed decreased INa, and faster inactivation, slower recovery, and stronger UDB on both INa,\textsubscript{P} and INa,\textsubscript{L} in contrast to the characteristics before the drug.

Table. Effects of A-803467 on the Kinetics Gating Parameters in SCN10A-SCN5A-SCN3B Coexpression TSA201 Cells

|                      | Inactivation | Activation | Recovery |
|----------------------|--------------|------------|----------|
|                      | V\textsubscript{1/2}, mV | \kappa, mV | No. | V\textsubscript{1/2}, mV | \kappa, mV | No. | \tau\textsubscript{r}, ms | \tau\textsubscript{s}, ms | No. |
| Control              | –83.76±2.38 | 7.70±0.39  | 12  | –49.52±2.74  | 5.21±0.48  | 12  | 2.87±0.38  | 20.51±5.61  | 14  |
| A-803467, 100 nmol/L | 95.77±3.20* | 8.94±0.48* | 12  | –51.95±1.38  | 5.78±0.24  | 12  | 6.46±0.58* | 64.37±15.47* | 14  |

Parameters of inactivation and activation were calculated from the Boltzmann function. V\textsubscript{1/2} is the voltage for half-maximal availability or activation and \kappa is the slope factor. Parameters of recovery were fitted to a double exponential function.

*P<0.05 vs control group. Data are from Figure 6 and are reported as mean±SEM.
Focal firing and vulnerable substrate are requisite for AF initiation and maintenance. One study by Nattel and colleagues verified that GPs played a vital role in the experimental AF related to atrial tachycardia remodeling. Compared with small effect on AF vulnerability by PV isolation, GP ablation could reduce AF vulnerability and attenuate AF maintenance by prolonging ERP, reducing AF duration and dominant frequency of fibrillatory activity. In addition, GP stimulation increases vagal and sympathetic tone, which induces early afterrepolarization formation, enhances Ca\(^{2+}\) transient facilitating trigger activity, and shortens action potential duration, especially at PV myocardium, increasing heterogeneous repolarization and prompting substrate and re-entry within atria.\(^3\,24,25\) RAP can shorten ERP and increase ERP dispersion and ΣWOV, which were all reversed after GP ablation. Implementing GP ablation or autonomic blockers (atropine or propranolol) followed by RAP can prevent induced spontaneous AF and atrial electrical remodeling.\(^26,27\) In our study, administering A-803467 can effectively attenuate ERP shortening and suppress increased ERP dispersion and ΣWOV, indicating a pharmacological denervation effect by this selective Na\(_{\text{1.8}}\) blocker. Voltage-gated sodium channels are responsible for generating and propagating action potentials and are critical in neuronal firing and excitability. Therefore, our results suggest that blocking Na\(_{\text{1.8}}\) can decrease GP firing, inhibit its activity, and prevent the initiation and maintenance of RAP-induced AF. Similar findings were observed by Qi and colleagues.\(^15\) They applied a different AF-induced model by vagus nerve stimulation (VNS) to evaluate cardiac conduction and AF inducibility after injecting A-803467 on ARGP and inferior right ganglionated plexi at the same concentration. They concluded that blockade of Na\(_{\text{1.8}}\) could suppress the effect of VNS on sinus rate, PR interval, and ventricular rate when AF occurred during VNS. The values of ERP in the control group after GP injection were lower than ours probably because of VNS. Previous study demonstrated that ARGP and SLGP in the intrinsic cardiac autonomic nerve system collected the nerve from both vагosympathetic trunk as integration center, and either ARGP or SLGP ablation could eliminated ERP shortening during vagosympathetic trunk stimulation. Ablating ARGP could also eliminate AF inducibility during high-frequency atrial pacing. It identified that ARGP and SLGP are the main connections in the neural pathway to control intrinsic

Figure 7. Use-dependent block (UDB) in the control and A-803467 100 nmol/L groups. A and B, Current recordings between the 2 groups. Pulse duration was 20 ms, and the holding potential was −120 mV and given at a rate of 0.1 seconds (10 Hz). C, UDB of \(I_{\text{Na,P}}\) in the control and A-803467 groups at 1, 2, and 10 Hz. D, UDB of \(I_{\text{Na,L}}\) in the control and A-803467 groups at 2 Hz.
autonomic activity in AF. Therefore, ablation (drug denervation) of ARGP and SLGP could be sufficient to show the effect of AF prevention. In the present study, we chosen ARGP and SLGP for injection and applied 6-hour RAP after injection to assess the effect of blocking Na<sub>1.8</sub> on acute AF directly. Decreased ERPs were found in all atrium and pulmonary recording sites, which were suppressed when A-803467 were administrated in advance. This could suggest that blocking Na<sub>1.8</sub> could avoid functional substrate forming in PV myocardium, probably as a result of inhibiting GP function and atrial electrical remodeling. Although we could not deny the fact that sodium pentobarbital has been reported vagolytic and may increase the ERP value when applying, the effect of A-803467 on ERP in the present study is still established, since both groups were treated equally with the anesthetic to eliminate bias between groups, and the minimum dosage was used to reduce the possibility of unnecessary influence, as in our previously published studies. Scherlag et al. injected lidocaine into GPs, leading to the loss of AF inducibility in 6 of 7 dogs. Our results displayed an apparent inhibition with 25.0% AF incidence when applying A-803467, compared with 87.5% in the control group (odds ratio=21.00). As a class I antiarrhythmic drug and local anesthetic, lidocaine proved to have a pronounced effect in inhibiting Na<sub>1.8</sub> current, enhancing UDB, and regulating gating properties. A-803467, as a selective Na<sub>1.8</sub> blocker, is used in alleviating Na<sub>1.8</sub>-involved neuropathic pain. In recombinant HEK293 cells, A-803467 was blocked human Na<sub>1.8</sub> (IC<sub>50</sub>=8 nmol/L), and was >100-fold selective versus human Nav1.2, Nav1.3, Nav1.5, and Nav1.7 (IC<sub>50</sub> >1 µmol/L). In the present study, TSA201 cells were transfected with SCN5A-SCN10A-SCN3B, A-803467 at 100 nmol/L potently inhibited I<sub>Na,<sup>P</sup></sub> by 42.02%, and it suppressed more in I<sub>Na,<sup>L</sup></sub> by 68.57%. Gating properties were also changed under A-803467 with hyperpolarized steady-state inactivation and delayed recovery from inactivation. Interestingly, the left-shifted steady-state inactivation was quite consistent with the effect of A-803467 in isolated mouse intracardiac neurons delineated by Verkerk et al. Like other sodium channel blockers, our results support that A-803467 might block sodium channels in an inactivated state and the number of trapped inactivated sodium channels might be increased during repeated stimulation. Obvious delay of recovery was displayed as 2 times slower in τ<sub>r</sub> and more than 3 times in τ<sub>s</sub>. As expected, UDB was also observed under A-803467. Inhibition in I<sub>Na,<sup>P</sup></sub> could reduce neuronal excitability, which might explain the pharmacological denervation effect. The changes in gating properties, together with UDB, indicate reduced availability of the sodium channel under sustained depolarization or repetitive stimulation, especially in a RAP-induced AF model.

In the present study, we utilized the SCN5A-SCN10A-SCN3B cotransfected model to analyze the effect of A-803467 on sodium current and gating properties to provide a possible explanation for the results we found in animal experiments. Compared with SCN5A-SCN3B transfected TSA-201 cells, the current density was significantly increased and was sensitive to A-803467 when SCN10A were added together with SCN5A-SCN3B. Since others’ and our studies have reported the extremely low current density in SCN10A-SCN3B transfected cell lines, it is reasonable to deduce that the increased current is not an arithmetic addition of current component produced by SCN10A. It implies that SCN10A might interact with SCN5A as a new sodium complex (Figure 8), and A-803467 could have an effect on SCN10A-SCN5A complex, as indicated in our patch clamp study. Although the complicated protein network of this new complex needs to be further explored, this model cells might be help us to understand the electrophysiological role of Na<sub>1.8</sub> in arrhythmias.

Many association studies have discovered the intensive relationship between SCN10A variants and AF phenotype or incidence. In addition to the significant effect on GP, Na<sub>1.8</sub> might directly influence the electrophysiological characteristics of cardiac tissue with suppressed trigger activity and reduced substrate during AF (Figure 8). The decreased current in I<sub>Na,<sup>P</sup></sub> by A-803467 might prolong ERP in atrium by virtue of postrepolarization refractoriness and reduce excitability at the fast rate due to UDB. The prolonged ERP might also prevent the formation of functional substrate during acute AF. Decreased I<sub>Na,<sup>L</sup></sub> might also cause conduction block to break the reentrant circuit. The significantly reduced I<sub>Na,<sup>L</sup></sub> seen in our study might suppress the incidence of early afterdepolarization during AF. Yang et al. also showed that 30 nmol/L A-803467 can remarkably reduce I<sub>Na,<sup>L</sup></sub> in mouse and rabbit myocytes with less effect on I<sub>Na,<sup>P</sup></sub>. In addition, the inhibition of sodium channel by A-803467 can reduce Na<sup>+</sup> in myocytes during repolarization, which may prevent the Ca<sup>2+</sup> loading to inhibit delay after repolarization. The suppressed trigger activity and reduced substrate could also be potential mechanisms for inhibiting AF initiation and maintenance.

Genome-wide association studies have opened up a new horizon in understanding cardiac electrophysiology. Several independent loci of SCN10A were indicated to increase PR and/or QRS intervals, which were regarded as intermediate phenotypes, suggesting the risk of conduction disease and arrhythmia susceptibility. There is an increased number of studies that focus on the relationship of SCN10A/Na<sub>1.8</sub> with cardiac diseases. In spite of several disputes such as the expression of Na<sub>1.8</sub> in the heart, Na<sub>1.8</sub> is critical in cardiac electrophysiology. Our previous study first reported 17 putative pathogenic SCN10A variants in 25 of 150 Brugada syndrome probands with a positive proband yield of 16.7%,
approaching our historical yield of 20.1% for SCN5A. Subsequent studies from others also emphasize the importance of SCN10A in both rare and common variants in Brugada syndrome.9 One recent study concluded that rs6795970 might be associated with cardiac conduction abnormalities in patients with hypertrophic cardiomyopathy.40 Therefore, it has come a long way as the “new cardiac sodium channel,” and the exact roles of Na,1.8 in arrhythmia still have a long way to go. It can be considered as a novel target in understanding cardiac electrophysiology, and its selective blocker or other relative drug might be promising in SCN10A-related arrhythmias.

Study Limitations
We did not record GP activity directly in our RAP-induced canine AF model in the absence or presence of A-803467. However, previous studies have demonstrated that blocking GP activity can inhibit RAP-induced atrial electrical remodeling and AF inducibility. After we administrated A-803467 in GPs, the shortening of the ERP in all recording sites and the increase in ERP dispersion and ΣWOV were all attenuated, potently indicating the suppressed GP activity under the effect of A-803467. Since neurotransmission is not static and circumscribed, the Na,1.8 blockade within the GPs on AF can have effects throughout the cardiac neuraxis. Due to the plasticity in the nervous system, based on present data, we cannot deduce that the observed effects may be limited only to the GPs. In fact, it may not be primarily mediated by GP blockade. Meanwhile, our previous study showed the colocalization of Na,1.5 and Na,1.8 when we transfected their cDNA separately into TSA201 cells. It would be more desirable if we could demonstrate this relationship in canine GP directly. However, our patch clamp study displayed that SCN5A could evidently increase SCN10A current in TSA201 cells, which can be blocked by A-803467. This phenomenon could indirectly explain the interaction between Na,1.5 and Na,1.8 to some extent.

Conclusions
In the present study, we found that the Na,1.8 selective inhibitor could decrease the incidence of acute AF with suppressed electrical remodeling of atrial and PV myocardium in a RAP-induced AF model. Na,1.8 could be a promising target in early AF, as GP activity were more important in early electrical
remodeling process of “AF begets AF” compared with progressed stages with prominent fibrosis and structural remodeling. In addition, we displayed that Na\textsubscript{v}1.8 could probably interact with Na\textsubscript{v}1.5, which may form a novel functional complex affecting cardiomyocyte and (or) cardiac GP properties, as shown in Figure 8. And it could be supported by our electrophysiological study with noticeable changes in INa\textsubscript{S} and gating kinetics under A-803467. This novel sodium complex could be involved in AF and other SCN10A-related arrhythmias.

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Disclosures
None.

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