Electrophysiology and Arrhythmogenic Activity of Single Cardiomyocytes From Canine Superior Vena Cava

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Background—The superior vena cava (SVC) has been proved to be a focal point in the initiation of paroxysmal atrial fibrillation. The autonomic nervous system plays an important role in the genesis of atrial fibrillation. However, the arrhythmogenic potentials of SVC and its responses to autonomic agents are not clear. The purpose of this study was to isolate single SVC cardiomyocytes and to investigate their electrophysiological characteristics, as well as the direct effects of autonomic agents.

Methods and Results—Canine SVC cardiomyocytes were isolated by perfusion with digestive enzymes. The action potentials and ionic currents were investigated in single SVC cardiomyocytes using the whole-cell clamp technique. Dissociation of the SVC yielded rod-shaped single cardiomyocytes with (n = 74, 51%) or without (n = 71, 49%) pacemaker activities. There were similar densities of inward Ca^2+, delayed rectifier K^+, transient inward, inward rectifier K^+, and pacemaker currents between SVC cardiomyocytes with and without pacemaker activity. SVC cardiomyocytes with pacemaker activity have, however, greater transient outward currents than those without pacemaker activity. In SVC cardiomyocytes, acetylcholine (5.5 μmol/L) abolished the spontaneous activities, but isoproterenol (10 nmol/L), atropine (10 μmol/L), and phenylephrine (10 μmol/L) accelerated the spontaneous activity and induced the occurrences of early or delayed afterdepolarizations.

Conclusions—These findings suggest that SVC cardiomyocytes have distinct action potentials and ionic current profiles that may be responsible for the arrhythmogenic activity of the SVC. (Circulation. 2002;105:2679-2685.)

Key Words: drugs ■ fibrillation ■ ion channels ■ veins

Thoracic veins are important foci in the initiation of paroxysmal atrial fibrillation.14 The superior vena cava (SVC) has been proved to be an arrhythmogenic vein.3 The proximal SVC in the adult mammal contains cardiac muscles connected to right atrium.5,8 Through multiple cellular electrophysiological studies, the SVC was found to have cardiomyocytes with fast response action potential6 or with phase 4 depolarization accompanied by the initiation of automatic activity.6 Another study also has reported abnormal automaticity and fibrillation induced by aconitine in the musculature of the SVC.9 The atrial muscle extension into the SVC was shown to be the source of the spontaneous ectopic activity recorded in the SVC.3 Our previous studies in multiple cells or single cells have shown that pulmonary veins (PVs) contain cardiomyocytes with or without spontaneous activities, which may underlie the high arrhythmogenic activities of PVs.10,11 Although clinical electrophysiological study has found that the SVC and PVs may have different electrophysiological characteristics,12 knowledge about the mechanisms underlying the arrhythmogenic activity of the SVC was limited. Because experiments utilizing intact tissue are complicated by the multiplicity of cell types and complex arrangement of cardiac cells within the tissue matrix, isolation of single SVC cardiomyocytes is important in evaluating the arrhythmogenic activity of SVC.

The autonomic nervous system is known to play an important role in the genesis of atrial fibrillation.13 Our previous studies10,11 in PVs have shown that autonomic agents have several effects on the electrical properties and arrhythmogenic activity of PVs. A clinical study12 showed that enhanced vagal reflex could inhibit SVC electrical activities. These findings suggest that it is important to investigate the effects of autonomic agents on SVC single cardiomyocytes. However, knowledge about the direct effects of autonomic agents on SVC is limited. The purposes of this study were to isolate single SVC cardiomyocytes and to investigate their electrophysiological characteristics and arrhythmogenic activity, as well as the responses of autonomic agents.

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Methods

Isolation of Single SVC Cardiomyocytes

Twenty-five healthy dogs were anesthetized with sodium pentobarbital (30 mg/kg IV), and the hearts were rapidly removed through a thoracotomy and dissected at room temperature. For the dissection of the SVC, the right atrium was opened by an incision from the right atrial appendage. The SVC was separated from the right atrium ~5 mm above the junction of the SVC and right atrium; thus, we avoided mixture of sinoatrial nodal cells in the SVC. After reversing the lumen, the SVC was perfused from the distal end through a polyethylene tube. The other end of the tubing was connected to a perfusion pump with a perfusion rate of 500 mL/h. The proximal end and side branches of the SVC were ligated with silk. The SVC was initially perfused with an oxygenated Tyrode solution at 37°C (NaCl 137, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, HEPES 10, and glucose 11 mmol/L; the pH was adjusted to 7.4 by titrating with 1 N NaOH), which was then replaced with Ca²⁺-free Tyrode solution containing 300 U/mL collagenase (Sigma, Type I) and 0.5 U/mL protease (Sigma, Type XIV). After the SVC softened, it was cut into five pieces and gently shaken until single cardiomyocytes were obtained. Only cells showing clear cross striations were used. To have stable recordings of action potentials (APs) or ionic currents, most experiments were carried out at room temperature (24°C to 26°C). Some cardiomyocytes also were experimented with under body temperature (37±1°C) to evaluate the arrhythmogenic activity of the SVC at the physiological state. The cells were allowed to stabilize in the bath for at least 30 minutes before experiments.

Electrophysiological and Pharmacological Study

The whole-cell patch-clamp technique was used by means of an Axopatch 1D amplifier (Axon Instruments). Borosilicate glass electrodes (outer diameter 1.8 mm) were used, with tip resistances of 3 to 5 MΩ. Before formation of the membrane-pipette seal, tip potentials were zeroed in Tyrode solution. Junction potentials (8 mV) were corrected for AP recordings. The pipette solution contained (in mmol/L) KCl 120, MgCl₂ 1, Na₂ATP 5, HEPES 10, EGTA 0.5, and CaCl₂ 0.01, adjusted to pH 7.2 with 1N KOH. In order to identify whether the cells had pacemaker activity, we did not add ionic currents blockers into the pipette solution. Normal Tyrode solution was used as a bath for AP and current (except L-type calcium current) recordings. The total cell capacitance was measured as described previously. APs were elicited by pulses of 2 ms and suprathreshold voltage (range 50 to 90 mV) at a frequency of 1 Hz. Voltage command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). AP measurements were begun 5 minutes after cell rupture, and the steady-state AP duration was measured at 50% (APD₅₀) and 90% (APD₉₀) of full repolarization. Data were sampled at rates varying from 2 to 25 kHz. Early afterdepolarization (EAD) was defined as the cells generating oscillatory potentials at depolarized levels.

Membrane currents were analyzed both from depolarization and hyperpolarization protocols. Depolarization-induced membrane currents were elicited at clamped potentials from −40 to 60 mV in 10-mV steps at a frequency of 0.1 Hz. L-type calcium currents (I_{Ca,L}) were measured from the peak inward current at the beginning of depolarization. A holding potential of −40 mV was used to inactivate the sodium channel. To minimize the contaminants from other ions, I_{Ca,L} were recorded during the administration of 10 μmol/L tetrodotoxin and 2 mmol/L 4-aminopyridine. Additionally, tetraethylammonium chloride and CsCl replaced NaCl and KCl, respectively, in the Tyrode solution. Transient outward currents (I_{to}) were studied with a double-pulse protocol. A 30-ms prepulse from −80 to −40 mV was used to inactivate the sodium channel, followed by a 300-ms test pulse to 60 mV in 20-mV steps at a frequency of 0.1 Hz. CdCl₂ (200 μmol/L) was added to the bath solution to inhibit I_{to}. I_{to} were measured as the difference between peak outward current and the outward current at the end of the test pulse. Delayed rectified outward potassium currents (I_{K1}) were measured as the difference between the holding current and the peak outward current at the end of 1 s depolarization from −40 to 60 mV in the presence of 200 μmol/L CdCl₂ in the bath Tyrode solution to inhibit I_{K1}.

Hyperpolarization-activated membrane currents were activated from −40 mV to test potentials ranging from −20 to −120 mV in 10-mV steps for 1 s at a frequency of 0.1 Hz. The amplitudes of inward rectified currents (I_{IR}) were measured as the peak instantaneous currents at the onset of hyperpolarization test pulses. A progressive large inward current developed with slow voltage-dependent kinetics and was not inactivated. It was suppressed by 5 mmol/L cesium and was measured as pacemaker current (I_{P}). The transient inward current (I_{trans}) was induced at clamped potentials from −40 to 40 mV for the duration of 3 s and then repolarized to −40 mV. The amplitude of I_{trans} was measured as difference between the peak of the transient current and the mean of the current just before and after the transient current.

Statistical Analysis

All quantitative data are expressed as mean±SEM. An unpaired t test was used to compare the differences between the SVC cardiomyocytes with and without pacemaker activity. The differences before and after drug administration were analyzed by a paired t test. We used χ² test with Yates’ correction or Fisher’s exact test for nonparametric data. A value of P<0.05 was considered statistically significant.

Results

Cell Morphologies and Action Potential Configurations

Dissociation of the canine SVC yielded single cardiomyocytes identified by the presence of striated myofibril bundles (Figure 1). The cardiomyocytes have rod-shaped morphologies with or without bifurcation. Fifty-one percent (n=74) of the cardiomyocytes beat spontaneously (0.7±0.4 Hz) or with spontaneous depolarization (20.7±2.8 mV/s; Figure 2A) and had the resting membrane potentials of −55±1 mV and AP amplitudes of 83±2 mV. The other cardiomyocytes (n=71, 49%) did not have pacemaker activity with a steady level of membrane potential and had no diastolic depolarization (Figure 2B). The cardiomyocytes without pacemaker activity had an APD₉₀ of 164±13 ms and APD₉₀ of 368±22 ms during 1-Hz electrical stimulation. The resting membrane potentials and AP amplitudes were −65±1 mV (P<0.001 versus the cells with pacemaker activity) and 85±3 mV (P>0.05 versus the cells with pacemaker activity). There were similar electrical capacitance (180±13 versus 190±12 pF, P>0.05), cell length (118±5 versus 116±4 μm,
P<0.05), and cell width (17±1 versus 17±1 μm, P<0.05) between the SVC cardiomyocytes with and without spontaneous activities.

As shown in Figure 3A, the SVC cardiomyocytes with pacemaker activity (n=18) had faster beating rates (3.1±0.3 Hz, P<0.001) at body temperature than at room temperature. At body temperature, 6 (33%) of these cells had delayed afterdepolarization (DAD), and 8 (44%) had EAD. Figure 3 (B and C) shows the examples of SVC cardiomyocytes with EAD or DAD at body temperature. Compared with those at body temperature, SVC cardiomyocytes with pacemaker activities had lower incidences of DAD (12%, P<0.05) or EAD (0%, P<0.001) at room temperature. In SVC cardiomyocytes without pacemaker activity (n=9), there was shorter APD90 (196±29 ms, P<0.001) and APD50 (57±11 ms, P<0.001) at body temperature than at room temperature.

Ionic Current Experiments
Depolarization-Induced Currents
The middle panels in Figure 2 show current tracings during depolarization in SVC cardiomyocytes. SVC cardiomyocytes both with and without pacemaker activity had a brief inward current on depolarization from a holding potential of −40 mV to −20 mV and above, which was consistent with the behavior of I_{Ca,L}. The I-V relationship of I_{Ca,L} was similar between the cardiomyocytes with (n=9) and without (n=8) pacemaker activities (Figure 4A). In addition, I_{to} with rapid activation kinetics, which increased progressively in amplitude with increasing depolarization step, was identified in SVC cardiomyocytes both with and without pacemaker activities. Figure 4B shows the current traces and I-V relationship of I_{to}. SVC cardiomyocytes without pacemaker activities (n=12) have smaller I_{to} as compared with the cells with pacemaker activities (n=9). Long depolarizing steps also induced a slowly activating, non-inactivating I_{K}. The I_{K} was similar between the cardiomyocytes with (n=12) and without (n=9) pacemaker activities (Figure 4C).

Hyperpolarization-Induced Currents
The right panels in Figure 2 show current tracings during depolarization in SVC cardiomyocytes. Hyperpolarizing steps from the −40 mV holding potential induced an instantaneous current with slow inactivation kinetics in SVC cardiomyocytes with and without pacemaker activities (Figure 2, right panels). The currents consistent with the properties of I_{K1} became pronounced progressively with increasing hyperpolarization steps and were suppressed by 0.5 mmol/L barium chloride. The densities of I_{K1} currents were similar between SVC cardiomyocytes with (n=58) and without (n=52) pacemaker activities (Figure 5A). In addition, I_{f} was identified in 26 (45%) of the 58 SVC cardiomyocytes with pacemaker activity and 25 (48%) of the 52 cardiomyocytes without pacemaker activity. Figure 5B shows examples of I_{f}.
in SVC cardiomyocytes with and without pacemaker activity. There was similar current density of \( I_f \) between SVC cardiomyocytes with \((0.069 \pm 0.019 \text{ pA/pF})\) and without \((0.062 \pm 0.016 \text{ pA/pF})\) pacemaker activity.

**Repolarization-Induced Currents**

Figure 5C shows examples of the \( I_{ti} \) from the SVC cardiomyocytes with and without pacemaker activity. \( I_{ti} \) was identified in 29 (48%) of 62 cardiomyocytes with and 16 (36%) of 45 cardiomyocytes without pacemaker activity \((P < 0.05)\). The density of \( I_{ti} \) was similar between the SVC cardiomyocytes with \((n=62, 0.0065 \pm 0.0011 \text{ pA/pF})\) and without \((n=45, 0.0041 \pm 0.0015 \text{ pA/pF})\) pacemaker activity.

**Effects of Autonomic Agents on the Arrhythmogenic Activity of SVC Cardiomyocytes**

Infusion of 5.5 \( \mu \text{mol/L} \) acetylcholine hyperpolarized the maximal diastolic potentials from \(-55 \pm 5\) to \(-64 \pm 6\) mV \((n=5, P < 0.005)\), and arrested the spontaneous activity in SVC cardiomyocytes. Figure 6A shows an example of the effect of acetylcholine on a SVC cardiomyocyte. The maximal diastolic potential was shifted from \(-42\) to \(-60\) mV, and the spontaneous activity was completely inhibited. In contrast, administration of atropine (10 \( \mu \text{mol/L} \)) increased the spontaneous activity from 0.4\( \pm 0.1\) to 1.3\( \pm 0.2\) Hz \((n=9, P < 0.005)\), depolarized the maximal diastolic potentials from...
Infusion of 10 nmol/L of isoproterenol increased the spontaneous activity from $0.2 \pm 0.1$ to $1.8 \pm 0.2$ Hz ($n=11, P<0.001$). In addition, isoproterenol increased the incidences of DAD from 19% to 73% ($P<0.05$) and EAD from 0% to 46% ($P<0.01$). Figure 6 (C and D) shows examples of isoproterenol-induced oscillatory afterpotentials (DAD and EAD) in a SVC cardiomyocyte. Isoproterenol increased $I_{\text{it}}$ from $0.007 \pm 0.003$ to $0.018 \pm 0.004$ pA/pF ($n=11, P<0.01$) and $I_{\text{f}}$ from $0.05 \pm 0.03$ to $0.13 \pm 0.06$ pA/pF ($n=11, P<0.05$) but did not change the $I-V$ relationship of $I_{\text{k1}}$. Infusion of 10 μmol/L phenylephrine also increased the spontaneous activity from $0.2 \pm 0.1$ to $1.2 \pm 0.2$ Hz ($n=10$), and induced the occurrences of DAD in 5 cells (50%) and EAD in 3 cells (30%). Figure 7 (A and B) shows examples of phenylephrine-induced DAD and EAD in SVC cardiomyocytes. Phenylephrine increased the $I_{\text{it}}$ ($0.003 \pm 0.002$ versus $0.014 \pm 0.006$ pA/pF, $P<0.05$) but did not change $I_{\text{f}}$ or $I_{\text{k1}}$. Figure 7C shows the current traces of $I_{\text{it}}$ before and after the administration of phenylephrine.

**Discussion**

**Isolation of SVC Cardiomyocytes**

Similar to the isolation of PV cardiomyocytes, we successfully isolated calcium-tolerated single SVC cardiomyocytes through the perfusion method. The SVC was known to have a myocardial extension in the outer layer and a vascular smooth muscle layer in the inner layer. In this experiment, after reversing the SVC lumen, the digestive enzymes would be in close contact with the myocardial sleeve during perfusion. The maintained electrophysiological characteristics suggest that this method is applicable for isolating cardiomyocytes from thoracic veins.

**AP Configurations and Ionic Currents of SVC Cardiomyocytes**

SVC cardiomyocytes were found to have cardiomyocytes with and without pacemaker activity, which was consistent with the previous in situ study in the SVC. The presence of cardiomyocytes with pacemaker activity suggests that automaticity may play a role in the arrhythmogenic activity of the SVC. The presence of DAD in SVC cardiomyocytes also suggests that triggered activity has a role in SVC electrical activity. The increase of beating rates and induction of triggered activity at body temperature further confirms that SVC has arrhythmogenic activity during physiological state. Therefore, the SVC cardiomyocytes with pacemaker activity may induce atrial arrhythmia through the enhancement of automaticity and induction of triggered activity. Because myocardial substrates play an important role in the genesis of arrhythmias, the myocardial sleeve of the SVC could provide abundant structural and electrical substrates for arrhythmogenesis. Moreover, cardiomyocytes with pacemaker activity are not known to be driven by electrical stimuli, which may result in slow electrical conduction or a
conduction block in the SVC. Previous studies have shown that there was conduction block in the SVC.\(^{3,6}\)

We identified multiple ionic currents in SVC cells, which was similar to the nature of cardiac cells or PV cardiomyocytes.\(^{11}\) Half of the SVC cardiomyocytes have \(I_f\) and thus have properties similar to those of PV or atrial cardiomyocytes.\(^{11,16}\) The similarity of \(I_f\) between SVC cardiomyocytes with and without pacemaker activity suggests that \(I_f\) does not play a critical role in the spontaneous activity of SVC cardiomyocytes. There were, however, some interesting differences in SVC cardiomyocytes. The small \(I_{K1}\) was similar in SVC cardiomyocytes with and without pacemaker activity, but was different from the findings in PV cardiomyocytes.\(^{11}\) In addition, the different \(I_{to}\) densities between the cells with and without pacemaker activity were also unique for the SVC. These findings suggest that SVC cardiomyocytes have distinct electrophysiological characteristics.

Effects of Autonomic Agents on SVC Cardiomyocytes

\(\beta\)-Adrenergic stimulation was known to play an important role in the genesis of paroxysmal atrial fibrillation. Previous in vivo and in vitro studies have shown that isoproterenol can accentuate electrical activity in PVs or the Marshall ligament.\(^{4,10}\) \(\beta\)-Adrenoceptor stimulation also increased the spontaneous activity of SVC cardiomyocytes. Moreover, similar to the findings in PVs,\(^{11}\) isoproterenol induced the occurrences of EAD and DAD in SVC cardiomyocytes. These findings confirm the previous results showing that triggered activity has a role in the arrhythmogenic activity in these vessels.\(^{2,10}\) The ionic currents study showed that isoproterenol increased \(I_f\) and \(I_{to}\); which was similar to results in the previous studies in other cardiomyocytes\(^{17,18}\) and which may account for the increase of spontaneous activity and DAD.
Previous studies have investigated the chronotropic action of phenylephrine with controversial results. The in vivo automaticity of the sinus node was depressed by \( \alpha_1 \)-adrenergic stimulation via the baroreceptor reflex because of an increase in arterial blood pressure. In vitro studies have shown that \( \alpha_1 \)-adrenergic stimulation would decrease automaticity in human atrium or canine Purkinje fibers. In contrast, phenylephrine has been shown to enhance automaticity in rabbit sinus node. The direct arrhythmic effect of phenylephrine, however, was not clear. Similar to the effects of isoproteenol on SVC cardiomyocytes, phenylephrine accelerated spontaneous activity and induced the occurrence of EAD and DAD. These findings suggest that \( \alpha_1 \)-adrenergic stimulation has direct arrhythmic effects. However, the increase of \( I_t \) (but not \( I_f \)) was different from the effects of isoproterenol.

The inhibitory effect of acetylcholine on the automaticity of SVC cardiomyocytes suggests that these cells have the nature of pacemaker cells. In addition, atropine has been shown for the first time to increase the arrhythmogenic activity of the SVC through an increase of spontaneous activity and an induction of triggered activity. This finding indicates that atropine not only is a muscarinic antagonist, but also has direct arrhythmogenic effects, and it suggests that vagal activity plays an important role in the electrophysiological properties of SVC.

Conclusions
This study demonstrates that the perfusion method can isolate a mixture of calcium-tolerant single cardiomyocytes from the SVC. The complicated electrophysiological characteristics and pharmacological responses of SVC cardiomyocytes may be responsible for their arrhythmogenic activity.

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