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Calmodulin kinase II inhibition prevents arrhythmic activity induced by alpha and beta adrenergic agonists in rabbit pulmonary veins

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Abstract

The autonomic nervous system and calcium regulation play important roles in the pathophysiology of atrial fibrillation. Calmodulin regulates the calcium homeostasis and may mediate the proarrhythmic effects of autonomic nervous agents. The purpose of this study was to compare the effects of β- and α-adrenoceptor agonists on the pulmonary vein electrical activity and evaluate whether calmodulin kinase II inhibitors may change the effects of the adrenoceptor agonists on the pulmonary vein arrhythmogenesis. Conventional microelectrodes were used to record the action potentials in isolated rabbit pulmonary vein tissue specimens before and after the administration of isoproterenol, phenylephrine and KN-93 (a calmodulin kinase II inhibitor). In the tissue preparation, isoproterenol (0, 0.1, 3 μM) increased the beating rates (1.5±0.2, 1.6±0.2, 2.3±0.3 Hz, $n=10$, $P<0.001$) with the genesis of early afterdepolarizations (EADs, 0%, 40%, 50%, $P<0.05$) and increased the amplitude of the delayed afterdepolarizations (DADs, 0.6±0.3, 1.7±0.4, 3.9±1.0 mV, $P<0.05$). Phenylephrine (0, 1, 10 μM) also increased the beating rates (1.4±0.2, 1.6±0.2, 1.9±0.2 Hz, $n=12$, $P<0.001$), incidence of EADs (0%, 8%, 50%, $P<0.05$) and amplitude of the DADs (0.4±0.2, 1.2±0.4, 2.6±0.8 mV, $P<0.05$). KN-93 did not change the pulmonary vein beating rates or action potential duration. However, in the presence of KN-93 (1 μM), isoproterenol (3 μM) and phenylephrine (10 μM) did not induce any EADs or DADs in the pulmonary veins. In conclusion, calmodulin kinase II inhibition may prevent adrenergic induced pulmonary vein arrhythmogenesis.

Keywords: Adrenoceptor agonist; Atrial fibrillation; Calmodulin kinase II inhibitor; Pulmonary vein; Triggered activity

1. Introduction

Atrial fibrillation is the most common sustained arrhythmia in clinical practice and induces cardiac dysfunction and strokes. Pulmonary veins are an important focus for the generation of atrial fibrillation (Chen et al., 1999; Haissaguerre et al., 1998). The pulmonary veins are known to contain cardiomyocytes with and without pacemaker activity and are suggested to be subsidiary pacemakers which can induce atrial arrhythmias (Chen et al., 2001, 2002b). Abnormal calcium handling has been reported to induce atrial fibrillation and also plays a role in the pathophysiology of pulmonary vein arrhythmogenecity. The activation of the L-type calcium currents, Na^+/Ca^{2+} exchange (NCX) and transient inward currents may induce triggered activity with the genesis of early and delayed afterdepolarizations (EADs/DADs), which may contribute to the pulmonary vein arrhythmogenic activity (Chen et al., 2002a,b, 2001). It is known that calmodulin (CaM) and Ca^{2+}–CaM-dependent kinase II (CaMKII) are pivotal in modulating the Ca^{2+} influx, sarcoplasmic reticulum Ca^{2+} release, and sarcoplasmic reticulum Ca^{2+} uptake during the excitation–contraction coupling (Anderson et al., 1994; Hohenegger and Suko, 1993; Li et al., 1997; Lokuta et al., 1995; Takasago et al., 1991; Witcher et al., 1991). Increased CaMKII activity has been linked to EADs and

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also can facilitate the transient inward currents, stimulate the L-type Ca^{2+} channels and increase the NCX currents (Anderson et al., 1998; Wu et al., 1999). It is possible that CaMKII may play an important role in the pulmonary vein arrhythmogenesis.

The autonomic nervous system plays a critical role in the pathophysiology of atrial fibrillation. Previous studies reported that the sympathetic tone influenced the occurrence of atrial fibrillation through an increase in the automaticity and triggered activity (Coumel, 1996; Liu and Nattel, 1997). Electrical stimulation of the autonomic ganglia at the atrium-pulmonary vein junction can transform the pulmonary vein focal electrical activity into atrial fibrillation (Scherlag et al., 2005). In addition, isoproterenol may increase the automaticity and induce the triggered activity in pulmonary veins (Chen et al., 2002b). However, it remains unclear whether there are different effects between β- and α-adrenoceptors on the pulmonary veins. The β adrenergic activation may increase the intracellular and sarcoplasmic reticulum Ca^{2+} load through the activation of protein kinase A (Talosi et al., 1993). The α adrenergic activation also increases the Ca^{2+} influx due to its effects on the prolongation of the action potential duration (Liu and Kennedy, 1998; O-Uchi et al., 2005). It is known that an increase in the sarcoplasmic reticulum Ca^{2+} content may induce a diastolic Ca^{2+} release with the genesis of DADs and shorten the diastolic depolarization period with the acceleration of the pacemaker rates (Maltsev et al., 2004). Therefore, the increase in the intracellular calcium concentration caused by both β and α adrenergic stimulation may induce pulmonary vein arrhythmogenic activity. The known effect that CaMKII inhibitor prevents structural remodeling due to excessive β adrenergic stimulation further suggests its anti-adrenergic potential (Zhang et al., 2005). The purposes of this study were to compare the effects of β-and α-adrenoceptor agonists on the pulmonary vein electrical activity and evaluate whether CaMKII inhibitors (KN-93, 2-[N-(2-hydroxyethyl)]-N-(4-methoxybenzenesulfonyl)]-amino-N-(4-chlorocinnamyl)-N-methylbenzylamine) may reduce the arrhythmogenic effects of adrenoceptor agonists on the pulmonary veins.

2. Materials and methods

2.1. Rabbit pulmonary vein tissue preparations

This investigation conformed to the institutional *Guide for the Care and Use of Laboratory Animals*. The rabbits (weight, 1–2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed and the heart with the lungs was removed. For dissection of the pulmonary veins, the left atrium was opened by an incision along the mitral valve annulus extending from the coronary sinus to the septum in Tyrode's solution with a composition (in mM) of 137 NaCl, 4 KCl, 15 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 2.7 $CaCl_2$, and 11 dextrose. The pulmonary veins were separated from the atrium at the left atrium-pulmonary vein junction and separated from the lungs at the ending of the pulmonary vein myocardial sleeves. The preparation size was about 10 mm in length and 5 mm in width. One end of the preparation, consisting of the pulmonary veins, atrium-pulmonary vein junction, and atrial tissue (within 1 mm in length), was pinned with needles to the bottom of a tissue bath. The other end (distal pulmonary vein) was connected to a Grass FT03C force transducer with a silk thread. The adventitia or epicardial side of the preparations faced upwards. The tissue was superfused at a constant rate (3 ml/min) with Tyrode′s solution which was saturated with a 97% O_2–3% CO_2 gas mixture. The temperature was maintained constant at 37 °C and the preparations were allowed to equilibrate for 1 h before the electrophysiological study.

2.2. Electrophysiological and pharmacological studies

The transmembrane action potential of the pulmonary veins was recorded by means of machine-pulled glass capillary microelectrodes filled with 3 M of KCl and the pulmonary vein preparation was connected to a WPI model FD223 electrometer under a tension with 150 mg. Thus, the proarrhythmia seen at baseline is stretch induced. The electrical and mechanical events were displayed simultaneously on a Gould 4072 oscilloscope and Gould TA11 recorder. The signals were recorded with DC coupling and a 10-KHz low-pass filter cutoff frequency using a data acquisition system. Signals were recorded digitally with a 16-bit accuracy at a rate of 125 KHz. An electrical stimulus with a 10-ms duration and suprathreshold strength (30% above the threshold) were provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Isoproterenol (0, 0.1, 3 μM), phenylephrine (0, 1, 10 μM), and KN-93 (0, 0.1, 1 μM) were superfused more than 15 min to test the pharmacological responses. The 90% action potential duration (APD_{90}) and contractile force were measured (more than 10 min after the superfusion) during a 2 Hz electrical stimulus before and after the drug administration in the pulmonary veins without spontaneous activity. EADs were defined as the interruption of the smooth contour of phase two or three of the action potentials (Damiano and Rosen, 1984). DADs were defined as the presence of a spontaneous depolarization of the impulse after full repolarization had occurred (Cranefield and Aronson, 1988). The EADs and DADs were selected from consistent deflections without abrupt changes of resting membrane potential and action potential morphology. The spontaneous activity was defined as a constant occurrence of spontaneous action potentials without using any electrical stimuli. Only the beating rates, EADs, DADs and incidences of non-sustained accelerated pulmonary vein spontaneous activity were measured (more than 10 min after the superfusion) in the pulmonary veins with spontaneous activity.

2.3. Statistical methods

All continuous variables are expressed as the mean ± S.E.M. One way ANOVA was used to compare the baseline characteristics among different groups. A paired *t*-test or repeated ANOVA was used to compare the differences before and after the drug administration. An unpaired *t*-test was used to compare those with and without the presence of KN-93. Nominal variables were compared by a Chi-square test with a Yates correction or Fisher's exact test. A statistically significant difference was defined as having a $P<0.05$.

3. Results

3.1. Effects of isoproterenol, phenylephrine and KN-93 on the pulmonary vein electrical activity

Spontaneous activity was demonstrated in 34 (49%) of the 69 tissue specimens during the experiment by selected load. The tissue with spontaneous activity had a less negative resting membrane potential as compared to that without spontaneous activity (−76±1 vs. −79±1 mV, $P<0.05$). The baseline resting membrane potential, APD_{90} and beating rate were similar among different experiment groups (Table 1).

Fig. 1A shows an example of the effects of isoproterenol on the pulmonary veins with spontaneous activity. Isoproterenol (0, 0.1, 3 μM) concentration-dependently increased the beating rates in the pulmonary veins ($n=10$) with spontaneous activity (Fig. 1B). In addition, isoproterenol induced the occurrence of non-sustained accelerated pulmonary vein spontaneous activity in 4 of 10 pulmonary veins at a concentration of 0.1 μM and in 7 of 10 pulmonary veins at a concentration of 3 μM. Fig. 1C shows an example of isoproterenol-induced accelerated pulmonary vein spontaneous activity. In the pulmonary veins without spontaneous activity, isoproterenol (3 μM) shortened the APD_{90} from 81±6 to 65±8 ms ($P<0.05$) in 6 (50%) of the 12 pulmonary veins, but prolonged the APD_{90} from 79±5 to 87±6 ms ($P<0.05$) in the other 6 pulmonary veins (Fig. 1D). The baseline resting membrane potentials (−79±1 vs. −80±2 mV, $P>0.05$) and APD_{90} (81±6 vs. 79±5 ms, $P>0.05$) were similar among these pulmonary veins. However, isoproterenol (0, 0.1, 3 μM) consistently increased the contractile force (Fig. 1E), incidence and amplitude of EADs, and amplitude of the DADs (Fig. 1F, Table 2). The EADs were occasionally accompanied by a pulmonary vein aftercontraction (Fig. 1F).

Fig. 2A shows an example of the effects of phenylephrine on the pulmonary veins with spontaneous activity. Phenylephrine (0, 1, 10 μM) concentration-dependently increased the beating rates ($n=12$) in the pulmonary veins with spontaneous activity (Fig. 2B). Phenylephrine induced the occurrence of non-sustained accelerated pulmonary vein spontaneous activity in 2 of 12 pulmonary veins at a concentration of 1 μM and in 4 of 12 pulmonary veins at a concentration of 10 μM. Fig. 2C shows an example of accelerated pulmonary vein spontaneous activity induced by phenylephrine. In the pulmonary veins without spontaneous activity ($n=11$), phenylephrine (0, 1, 10 μM) lengthened the APD_{90} and increased the contractile force (Fig. 2D). As the example shows in Fig. 2E, phenylephrine increased the incidence and amplitude of EADs or DADs in the pulmonary veins (Table 2). Moreover, the cycle lengths before EADs were longer than those before DADs (673±122 vs. 529±83 ms, $P<0.01$) in the pulmonary veins ($n=14$) with both EADs and DADs occurrences after superfusing isoproterenol or phenylephrine.

KN-93 (0, 0.1, 1 μM) did not change the beating rates (1.5±0.2, 1.4±0.2, 1.4±0.2 Hz, $n=12$, $P>0.05$), APD_{90} (83±3, 86±3, 84±3 ms, $n=12$, $P>0.05$) or contractile force (100%, 101±5%, 102±3%, $P>0.05$) in the pulmonary veins (Fig. 3A and B). Nevertheless, in the four pulmonary veins with DADs before the drug administration, KN-93 (0.1 μM) had no effect on the DADs (0.8±0.4 vs. 0.8±0.3 mV, $P>0.05$), but KN-93 (1 μM) reduced the amplitude of the DADs from 0.8±0.4 to 0 mV (Fig. 3C).

3.2. Effects of KN-93 on the isoproterenol-induced arrhythmogenicity

In the presence of KN-93 (1 μM), isoproterenol (3 μM) increased the beating rates in the pulmonary veins ($n=7$) with spontaneous activity (Fig. 4A, B). However, compared with those without KN-93, isoproterenol (0.1, 3 μM) accelerated the pulmonary vein beating rates to a less extent (9% vs. 12%; and 18% vs. 56%, respectively, $P<0.05$) and only induced one episode of non-sustained accelerated pulmonary vein spontaneous activity at a concentration of 3 μM. In the presence of KN-93 (1 μM), the incidence (14%) of isoproterenol (3 μM) induced non-sustained accelerated pulmonary vein spontaneous activity was lower than that (70%) of pulmonary veins without KN93 ($P=0.05$). Moreover, isoproterenol did not induce the occurrence of any EADs or DADs in the presence of KN-93 (Table 2). In the pulmonary veins ($n=12$) without spontaneous activity, isoproterenol (3 μM) shortened the APD_{90} from 85±4 to 73±3 ms ($P<0.05$) in 7 (58%) of the 12 pulmonary veins, and prolonged the APD_{90} from 79±3 to 88±1 ms ($P<0.05$) in the other 5 pulmonary veins (Fig. 4C). In the presence of KN-93 (1 μM), the baseline resting membrane potentials (−81±2 vs. −79±1 mV, $P>0.05$) and APD_{90} (85±6 vs. 81±8 ms, $P>0.05$) were similar

Table 1
Baseline electrophysiological characteristics in the pulmonary veins receiving isoproterenol, phenylephrine, and combinations with KN-93

	Pulmonary veins without spontaneous activity			Pulmonary veins with spontaneous activity	
	RMP[a] (−mV)	APD_{90}[b] (ms)	n	Beating rate (Hz)	n
Receiving isoproterenol	80±1	80±3	12	1.5±0.2	10
Receiving phenylephrine	79±1	81±7	11	1.4±0.2	12
Receiving KN-93	80±1	83±3	12	1.5±0.2	12
Receiving isoproterenol+KN-93 pre-treatment	80±1	81±4	12	1.3±0.1	7
Receiving phenylephrine+KN-93 pre-treatment	80±2	82±3	6	1.4±0.2	6

There were no statistical significances from comparisons among groups by using one way ANOVA. Values represent the mean±S.E.M.
[a] Denotes the resting membrane potential.
[b] Denotes the 90% action potential duration.

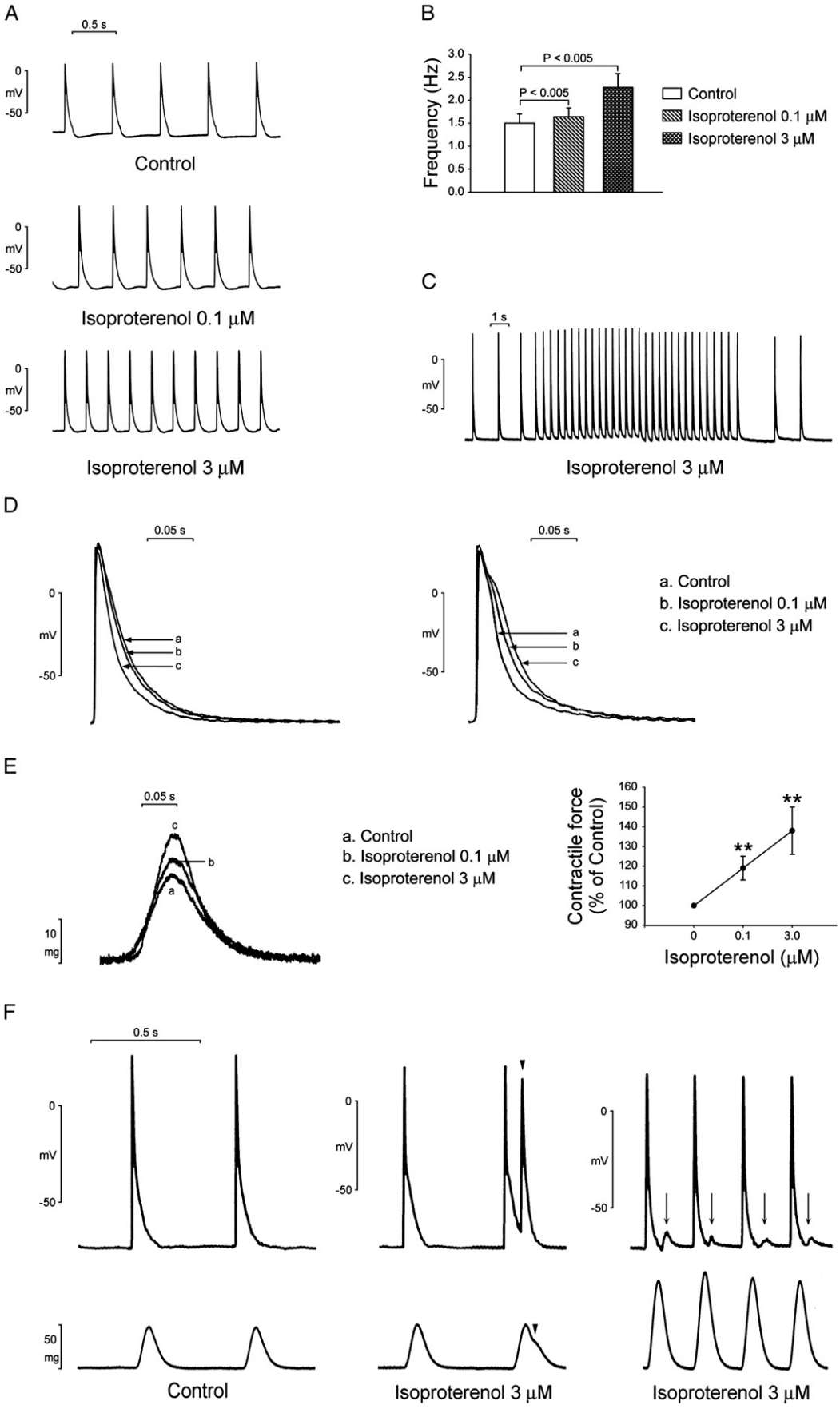
A
0.5 s
0
mV
-50
Control
Isoproterenol 0.1 μM
Isoproterenol 3 μM
B
Frequency (Hz)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P < 0.005
P < 0.005
Control
Isoproterenol 0.1 μM
Isoproterenol 3 μM
C
1 s
Isoproterenol 3 μM
D
0.05 s
a
b
c
a. Control
b. Isoproterenol 0.1 μM
c. Isoproterenol 3 μM
E
0.05 s
10
mg
a. Control
b. Isoproterenol 0.1 μM
c. Isoproterenol 3 μM
Contractile force
(% of Control)
160
150
140
130
120
110
100
90
**
**
0
0.1
3.0
Isoproterenol (μM)
F
0.5 s
50
mg
Control
Isoproterenol 3 μM
Isoproterenol 3 μM

Table 2
Effects of isoproterenol and phenylephrine in the absence or presence of KN-93 on the pulmonary vein arrhythmogenesis in spontaneous active tissue

Concentration (μM)	Early afterdepolarizations				Delayed afterdepolarizations			
	Without KN-93		With KN-93 (1 μM)		Without KN-93		With KN-93 (1 μM)	
	Incidence (%)	Amplitude (mV)	Incidence (%)	Amplitude (mV)	Incidence (%)	Amplitude (mV)	Incidence (%)	Amplitude (mV)
Isoproterenol	$n=10$		$n=7$		$n=10$		$n=7$	
0	0	0	0	0	40	0.6±0.3	0	0
0.1	40	3.4±1.6	0	0	70	1.7±0.4	0[a]	0[a]
3	50	7.6±3.7	0[a]	0[a]	80	3.9±1.0	0[a]	0[a]
P value	<0.05	<0.05	NS	NS	NS	<0.05	NS	NS
Phenylephrine	$n=12$		$n=6$		$n=12$		$n=6$	
0	0	0	0	0	25	0.4±0.2	0	0
1	8	0.2±0.2	0	0	58	1.2±0.4	0[a]	0[a]
10	50	2.3±0.9	0[a]	0[a]	83	2.6±0.8	0[a]	0[a]
P value	<0.05	<0.05	NS	NS	<0.05	<0.05	NS	NS

The *P* value was derived from the Chi-square test with a Yates correction or repeated measures of ANOVA testing the dose-dependency of isoproterenol and phenylephrine. Values represent the mean±S.E.M.

[a] $P<0.05$ denotes the differences between KN-93 treated and untreated tissues within the same isoproterenol or phenylephrine concentrations.

among these pulmonary veins. However, isoproterenol (0, 0.1, 3 μM) consistently increased the contractile force (Fig. 4D). The extent of the increase in the contractile force by isoproterenol (3 μM) was less than that without KN-93 (12% vs. 38%, $P<0.05$).

As the examples show in Fig. 5A through D, after the administration of isoproterenol (3 μM), the KN-93 (1 μM) did not change the pulmonary vein beating rates, EADs, DADs, APD_{90}, or contractile force in the pulmonary veins ($n=8$).

3.3. Effects of KN-93 on the phenylephrine-induced arrhythmogenicity

In the presence of KN-93 (1 μM), phenylephrine increased the beating rates in the pulmonary veins ($n=6$) with spontaneous activity (Fig. 6A and B). However, the percentage of the pulmonary vein beating rate acceleration caused by phenylephrine (10 μM) was less than that without KN-93 (28% vs. 39%, $P<0.05$). Phenylephrine only induced non-sustained accelerated pulmonary vein spontaneous activity in 1 (17%) of 6 pulmonary veins at concentrations of both 1 μM and 10 μM, which was less than that without the presence of KN-93 (17% vs. 33%, $P<0.05$). Moreover, phenylephrine did not induce any occurrence of EADs or DADs in the presence of KN-93 (1 μM, Table 2). In the pulmonary veins ($n=6$) without any spontaneous activity, phenylephrine (1, 10 μM) lengthened the APD_{90} and increased the contractile force in the presence of KN-93 (1 μM, Fig. 6C). However, the extent of the increase in the APD_{90} (14% vs. 31%, $P<0.05$) and contractile force (28% vs. 47%, $P<0.05$) with the phenylephrine (10 μM) administration was less in the presence of KM-93 than in that without KN-93.

As the examples show in Fig. 7A through D, after the administration of phenylephrine (10 μM), the KN-93 (1 μM) did not change the PV beating rates, EADs, DADs, APD_{90} or contractile force in the pulmonary veins ($n=7$).

4. Discussion and conclusions

4.1. Effects of β- and α-adrenoceptor agonists on the pulmonary vein electrical activity

In this study, similar to the results in the previous studies (Chen et al., 1999, 2002b; Priori and Corr, 1990), isoproterenol was demonstrated to increase the pulmonary vein spontaneous activity and triggered activity. These results suggest the importance of β adrenergic stimulation in the pulmonary vein arrhythmogenic activity. Moreover, isoproterenol may either prolong or shorten the action potential duration in the pulmonary veins. The inconsistent effects of isoproterenol on the action potential duration may arise from the consequence of the unequal responses of various ionic currents and suggest the presence of multiple populations of cardiomyocytes in the pulmonary veins (Chen et al., 2002b). Previous studies also reported that isoproterenol may prolong or shorten action potential duration on the ventricular myocytes (Charpentier and Rosen, 1994; Priori and Corr, 1990; Reuter, 1974). These results may aggravate the dispersion of the refractoriness and further facilitate the genesis of microreentry, which has been proposed to be one of the mechanisms of pulmonary vein arrhythmogenesis (Misier et al., 1992).

A previous study has shown that phenylephrine plays a role in the pathophysiology of atrial fibrillation (Leitch et al.,

Fig. 1. Effect of isoproterenol on the pulmonary vein electrical activity. A. Tracings showing the effects of isoproterenol on the pulmonary vein beating rates from the same recording site. B. The average data of the effects of the different concentrations of isoproterenol on the pulmonary vein beating rates. C. An example of non-sustained accelerated pulmonary vein spontaneous activity after superfusing with isoproterenol. D. Superimposed tracings of the effects of different concentrations of isoproterenol on the action potential configurations. The left panel shows the progressive shortening of the action potential duration caused by isoproterenol and the right panel shows the progressive lengthening of the action potential duration caused by isoproterenol. The action potentials were elicited by electrical stimuli at 2 Hz. E. Superimposed tracings and the concentration-response curve of the effects of the different concentrations of isoproterenol on the contractile forces. $^{**}P<0.01$ versus the control. F. Tracings of isoproterenol induced an EAD with an aftercontractions (arrows heads) and DADs (arrows) in the pulmonary veins from the same recording site.

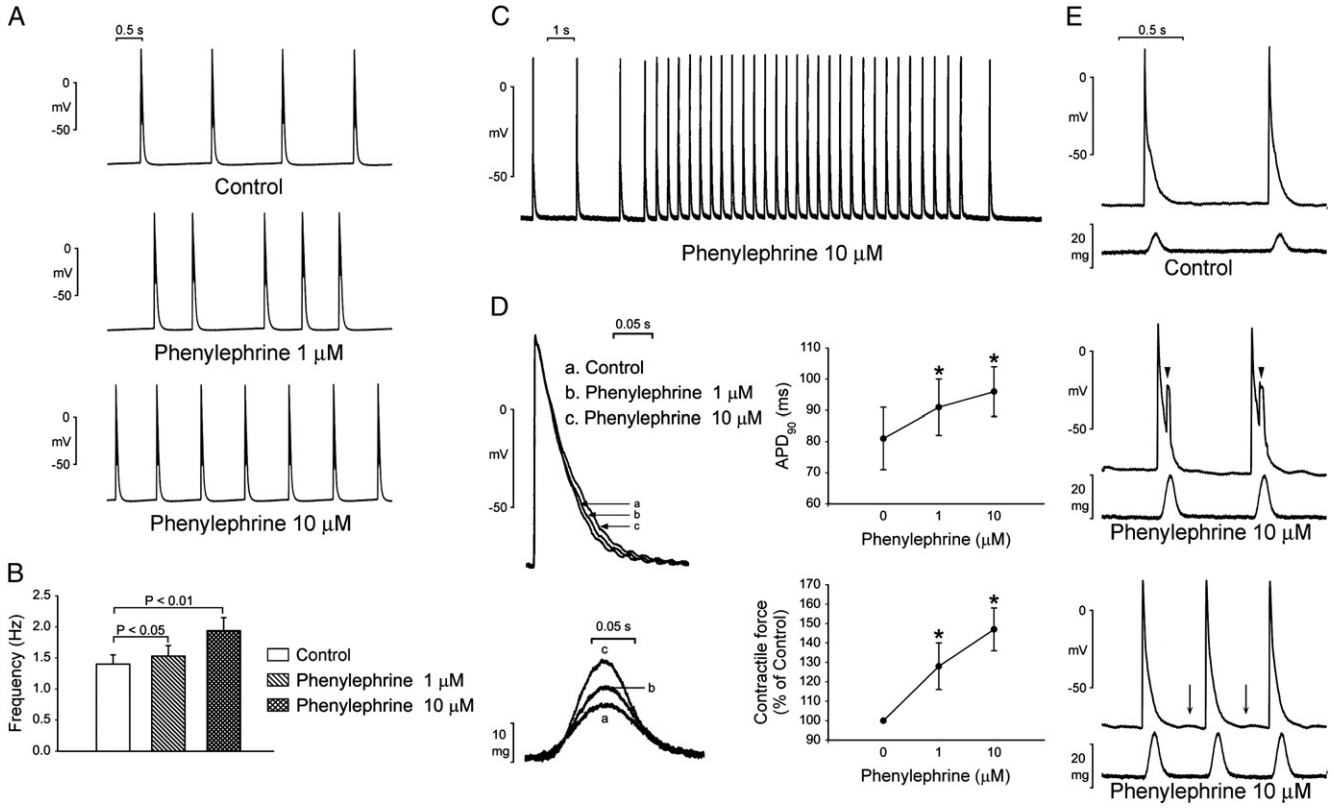

Fig. 2. Effects of phenylephrine on the pulmonary vein electrical activity. A. Tracings showing the effects of phenylephrine on the beating rates in the pulmonary veins with spontaneous activity from the same recording site. B. The average data of the effects of the different concentrations of phenylephrine on the pulmonary vein beating rates. C. An example of non-sustained accelerated pulmonary vein spontaneous activity after superfusing with phenylephrine. D. Superimposed tracings and the concentration-response curves of the effects of the different concentrations of phenylephrine on the action potential configurations and contractile forces. The action potentials were elicited by electrical stimuli at 2 Hz. *$P<0.05$ versus the control. E. Tracings showing that phenylephrine induced EADs (arrow heads) and DADs (arrows) in the pulmonary veins from the same recording site.

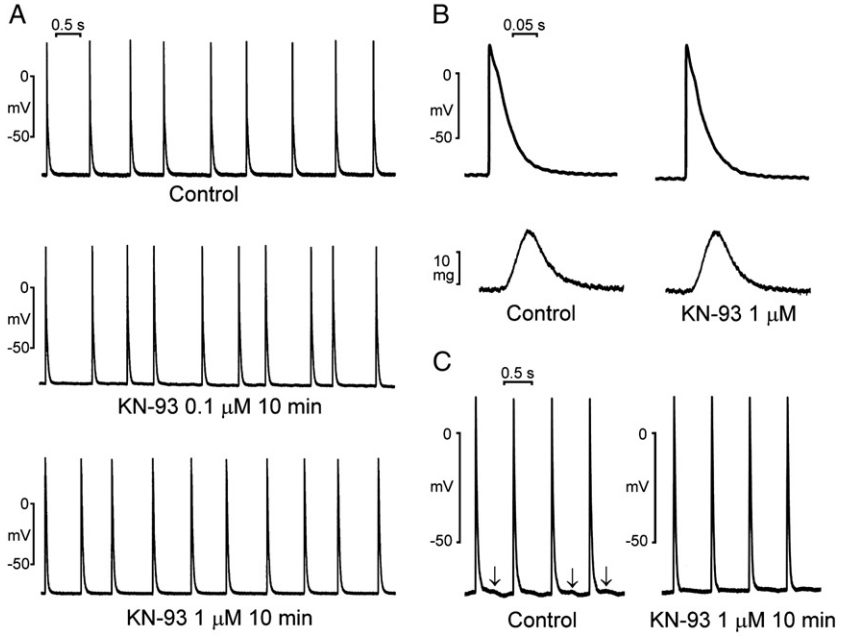

Fig. 3. Effect of KN-93 on the pulmonary vein electrical activity. A. Tracings showing the effects of KN-93 on the pulmonary vein beating rates from the same recording site. B. Tracings showing the effects of KN-93 on the action potential configurations and contractile forces. The action potentials were elicited by electrical stimuli at 2 Hz. C. Tracings showing that KN-93 suppressed the baseline DADs (arrows) in the pulmonary veins from the same recording site.

1997). The α adrenergic stimulation has been shown to suppress the pulmonary vein firings due to a baroreceptor reflex from an increase in the blood pressure (Tai et al., 2000). The in vitro study has shown that phenylephrine enhances spontaneous activity in pacemaker cells (Berthelot et al., 1982). However, the electrophysiologic and arrhythmogenic effects of phenylephrine on the isolated pulmonary vein specimens remain obscure. Although phenylephrine might have a weak β adrenergic activity, the effect is shown to be more prominent in a concentration of more than 20 μM (Gusovsky et al., 1987). In this study, phenylephrine (up to 10 μM) was found to increase the pulmonary vein beating rates and triggered activity. These results were similar to the known effects of phenylephrine on the cardiomyocytes from the superior vena cava (Chen et al., 2002c). Phenylephrine was demonstrated to increase the intracellular calcium and NCX (Liu and Kennedy, 1998; O-Uchi et al., 2005; Reinecke et al., 1997). Those effects may induce pulmonary vein arrhythmogenesis because modulation of the NCX has been found to contribute to the pulmonary vein electrical activity (Chen et al., 2006; Patterson et al., 2005; Wongcharoen et al., 2006). However, the prolongation of the action potential duration by phenylephrine may reduce the genesis of microreentry and at least in part decrease the pulmonary vein arrhythmogenesis.

4.2. Effect of KN-93 on the pulmonary vein electrical activity and adrenoceptor agonists

It has been demonstrated that CaMKII inhibition reduces the sarcoplasmic reticulum Ca^{2+} leak through a reduction in the phosphorylation of the ryanodine receptors, which may induce

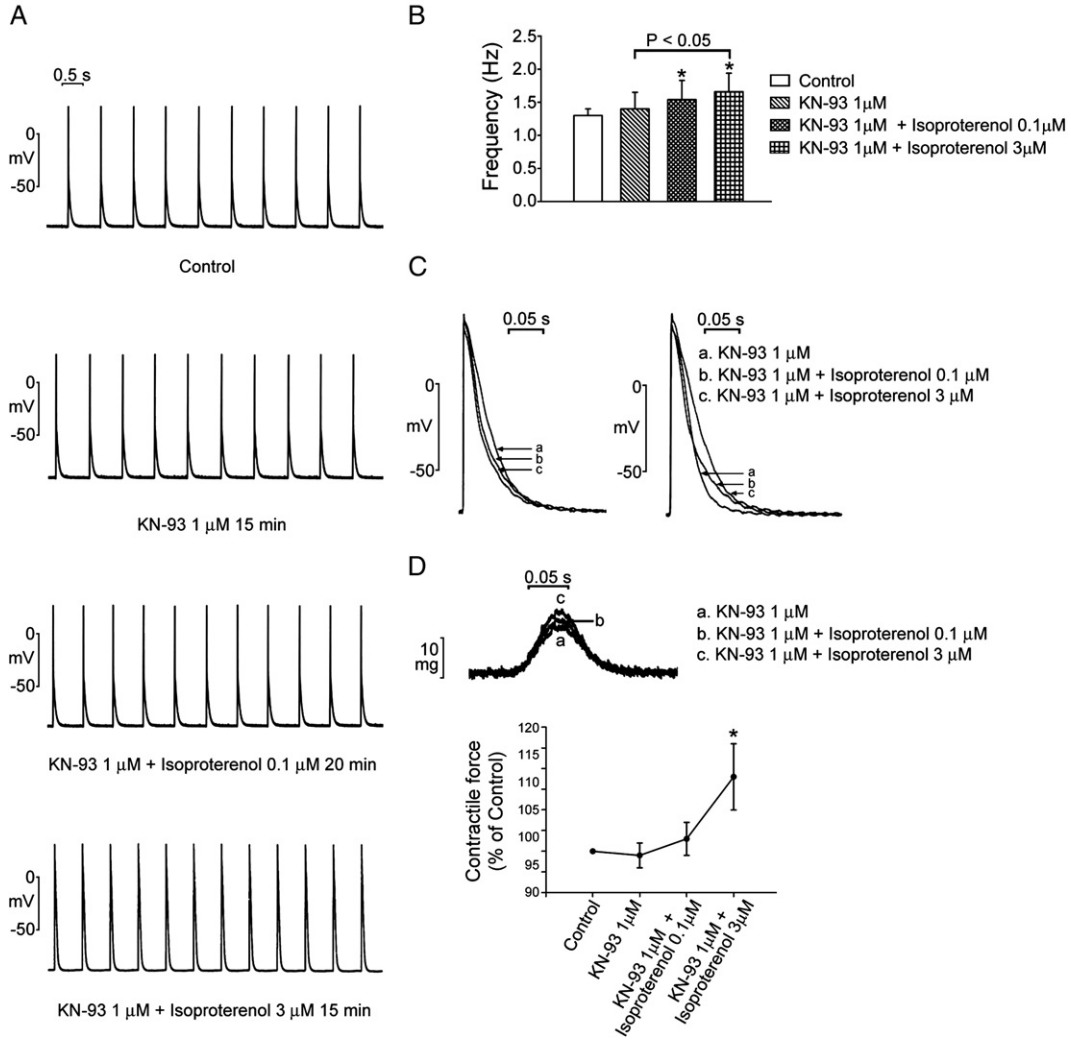

Fig. 4. Prevention effects of KN-93 on the isoproterenol induced pulmonary vein electrical activity. A. Tracings showing the effects of isoproterenol on the pulmonary vein beating rates from the same recording site in the presence of KN-93 (1 μM). B. The concentration-responses of isoproterenol on the pulmonary vein beating rates in the presence of KN-93 (1 μM). *$P<0.05$ versus those in the absence of KN-93 at the same isoproterenol concentration. C. Superimposed tracings of the effects of the different concentrations of isoproterenol on the action potential configurations in the presence of KN-93 (1 μM). The left panel shows the progressive shortening of the action potential duration caused by the isoproterenol and the right panel shows the progressive lengthening of the action potential duration caused by the isoproterenol. The action potentials were elicited by electrical stimuli at 2 Hz. D. In the presence of KN-93 (1 μM), the superimposed tracings and concentration-responses of the effects of the different concentrations of isoproterenol on the contractile forces of the pulmonary veins. *$P<0.05$ versus before the isoproterenol administration.

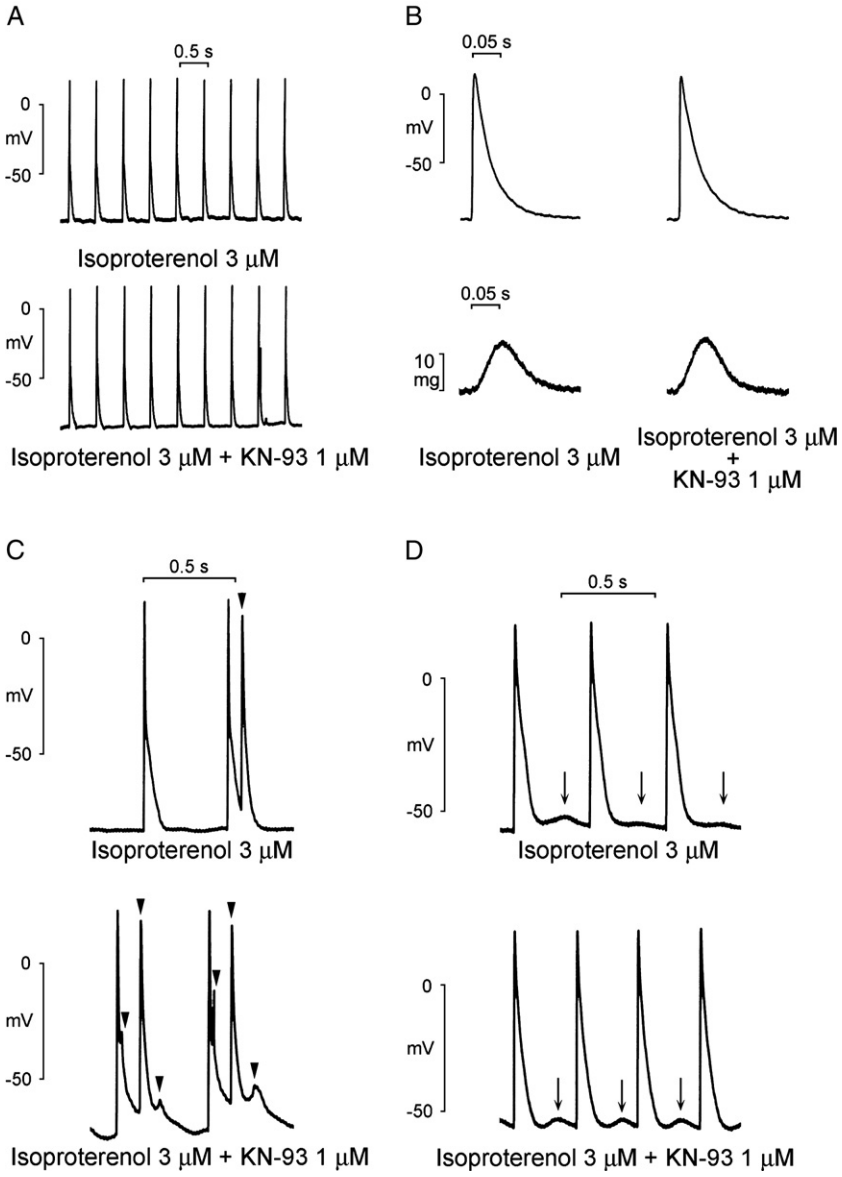

Fig. 5. Effect of KN-93 on the isoproterenol-induced pulmonary vein arrhythmogenicity. A. Tracings showing the effects of the KN-93 on the pulmonary vein beating rates from the same recording site in the presence of isoproterenol (3 μM). B. Tracings showing the effects of KN-93 on the action potential configurations and contractile forces in the presence of isoproterenol. The action potentials were elicited by electrical stimuli at 2 Hz. C. Tracings showing the effects of KN-93 on the isoproterenol-induced EADs (arrow heads) from the same recording site. D. Tracings showing the effects of KN-93 on the isoproterenol-induced DADs (arrows) from the same recording site.

the genesis of triggered activity (Li et al., 1997). Ai et al. (2005) reported that the inhibition of CaMKII may decrease the sarcoplasmic reticulum Ca^{2+} leak due to hyperphosphorylated ryanodine receptors in a rabbit heart failure model. Honjo et al. (2003) have found that abnormal ryanodine receptors may contribute to the arrhythmogenic potential of the pulmonary veins. The decrease in the DADs caused by KN-93 in the pulmonary veins at baseline raises the possibility that a sarcoplasmic reticulum Ca^{2+} leak may have a role in the pulmonary vein arrhythmogenesis.

In this study, KN-93 was found to prevent isoproterenol-induced triggered activity, which indicated the role of CaMKII in the arrhythmogenesis of β-adrenoceptor stimulation. It is possible that KN-93 may block the effects of β-adrenoceptor activation-induced cyclic AMP generation and protein kinase A phosphorylation by binding to CaMKII and further prevent an intracellular calcium overload through a reduction in the phosphorylation of ryanodine. Although a previous study has shown that KN-93 (1 μM) may inhibit the L-type calcium currents in ventricular myocytes (Anderson et al., 1998), the insignificant effects of KN-93 (1 μM) on the pulmonary vein beating rates at baseline suggests that CaMKII inhibition is the major antiarrhythmic mechanism. Previous studies (Chen et al., 2004; Wongcharoen et al., 2006) also have shown that the regulation of the calcium homeostasis in the pulmonary veins without interfering with the L-type calcium currents can modulate the pulmonary vein electrical activity. KN-93 also has been found to inhibit voltage-gated potassium (Kv) channels independent of the CaMKII

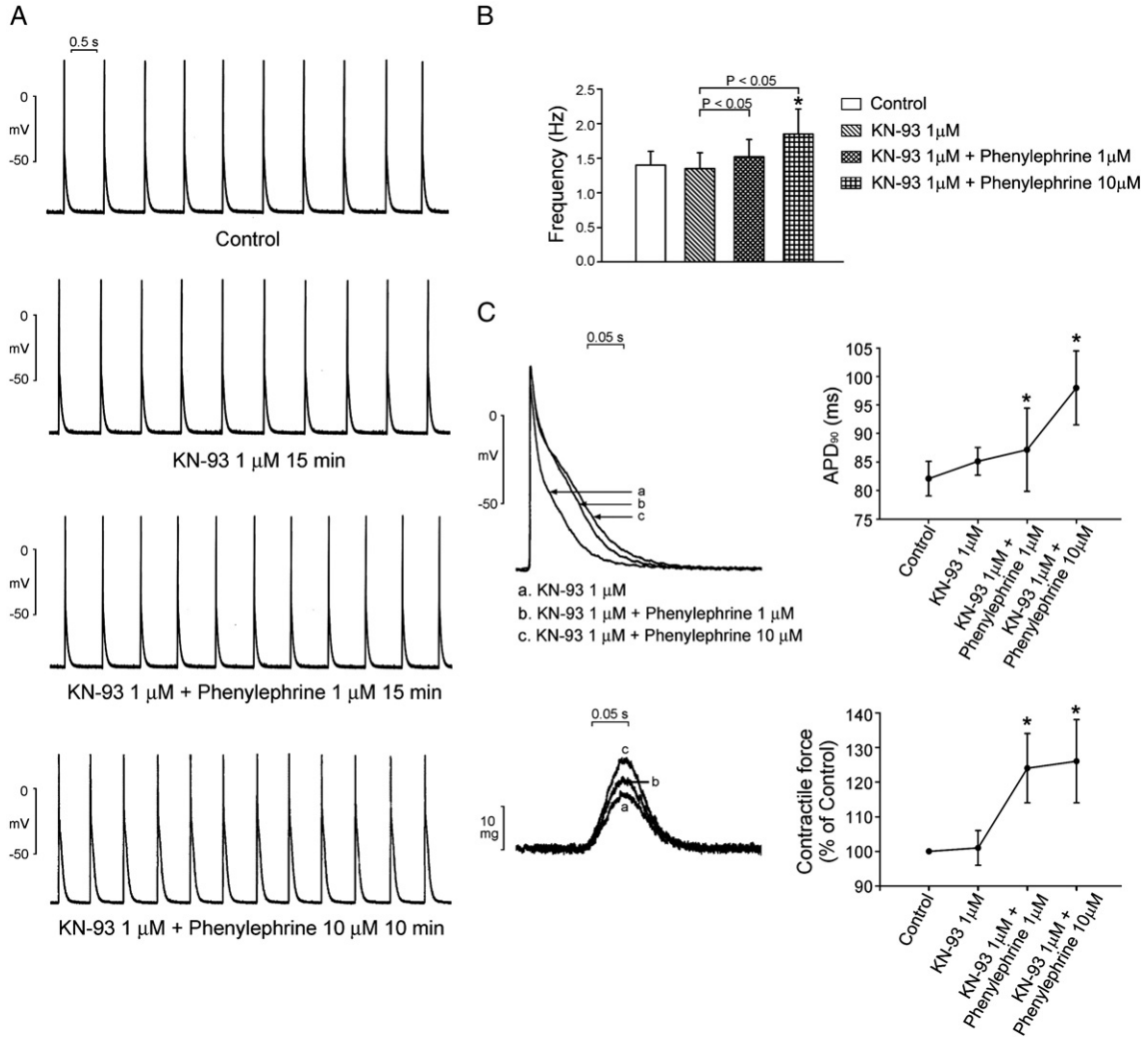

Fig. 6. Prevention effects of KN-93 on phenylephrine induced pulmonary vein electrical activity. A. Tracings showing the effects of phenylephrine on the pulmonary vein beating rates from the same recording site in the presence of KN-93 (1 μM). B. The concentration-responses of phenylephrine on the pulmonary vein beating rates in the presence of KN-93. *$P<0.05$ versus those in the absence of KN-93 at the same concentration of phenylephrine. C. Superimposed tracings and concentration-responses of the effects of the different concentrations of phenylephrine on the action potential configurations and contractile forces in the presence of KN-93. The action potentials were elicited by electrical stimuli at 2 Hz. *$P<0.05$ versus those before the phenylephrine administration.

inhibition (Anderson et al., 1998; Rezazadeh et al., 2006). However, the inhibition of the Kv channels is expected to increase the incidence of triggered activity and prolong the action potential duration, which was not found in this experiment. Therefore, the main effect of the KN-93 in the suppression of the triggered activity was suggested to arise from the inhibition of the CaMKII.

The α-adrenoceptor stimulation induced hypertrophic responses have been demonstrated to be prevented by CaMKII inhibition (Ramirez et al., 1997). In this study, KN-93 could effectively prevent the phenylephrine-induced pulmonary vein arrhythmogenic activity, which also suggested the important link between the adrenoceptor stimulation and CaMKII. The inhibition of CaMKII by KN-93 would prevent the phenylephrine-induced pulmonary vein spontaneous activity and triggered activity caused by an increase in the intracellular calcium concentration. Moreover, this study showed that KN-93 was ineffective in terminating the isoproterenol or phenylephrine induced pulmonary vein arrhythmogenesis. This could be caused by the fact that KN-93 did not directly counteract the action of the isoproterenol or phenylephrine. Once an increase in the intracellular calcium concentration caused by the sympathomimetic agents has sufficiently progressed, KN-93 may not be able to decrease the Ca^{2+} overload of the sarcoplasmic reticulum.

In this study, pretreatment with KN-93 was found to prevent the α-and β-adrenoceptor agonist induced triggered activity of the rabbit pulmonary veins, which suggests KN-93 may be a potential agent in treating atrial fibrillation. However, KN-93 did not abolish the α-and β-adrenoceptor agonist induced triggered activity on the rabbit pulmonary veins. These findings suggest that KN-93 may be useful in the prevention but not the intervention of atrial fibrillation. Moreover, the data in this experiment should be interpreted with caution due to the limitations of the study. Without intracellular calcium recordings, we did not directly evaluate the effects of sympathomimetic agents and KN-93 on calcium handling in the pulmonary

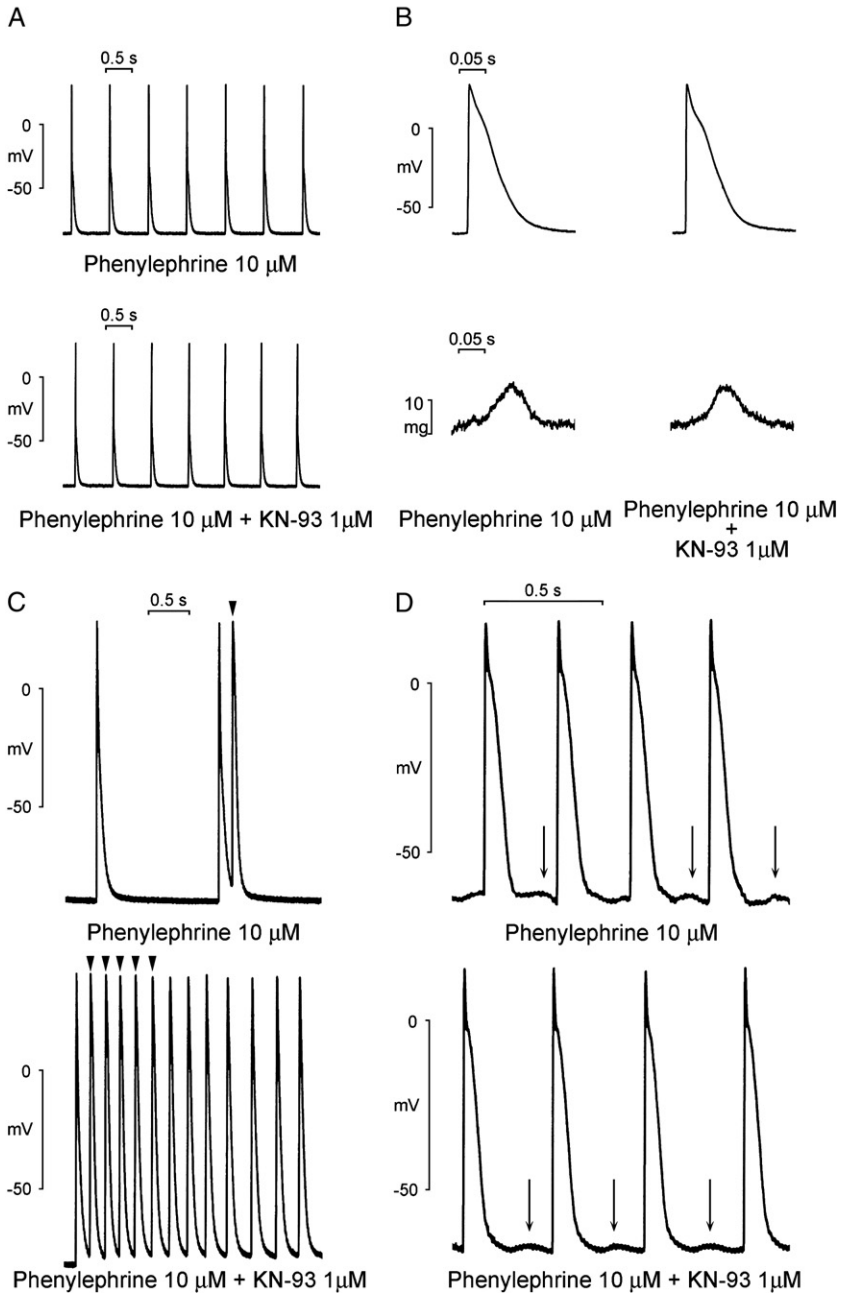

Fig. 7. Effect of KN-93 on the phenylephrine-induced pulmonary vein arrhythmogenicity. A. Tracings showing the effects of KN-93 on the pulmonary vein beating rates from the same recording site in the presence of phenylephrine (10 μM). B. Tracings showing the effects of KN-93 on the action potential configurations and contractile forces in the presence of phenylephrine. The action potentials were elicited by electrical stimuli at 2 Hz. C. Tracings showing the effects of KN-93 on the phenylephrine (10 μM) with EADs (arrow heads) from the same recording site. D. Tracings showing the effects of KN-93 on the phenylephrine-induced DADs (arrows) from the same recording site.

veins. However, this study has demonstrated that KN-93 may attenuate the arrhythmogenic effects (automaticity and triggered activity) of isoproterenol and phenylephrine. These findings strongly suggest that CaM and CaMKII may regulate the calcium homeostasis to alter the pulmonary vein electrical activity.

In conclusion, CaMKII plays a pivotal role in the α and β adrenergic-induced pulmonary vein arrhythmogenesis. The preventive effects of KN-93 on the isoproterenol and phenylephrine-induced pulmonary vein arrhythmogenesis suggest that KN-93 may be a novel anti-arrhythmic agent and has a potential role in treating atrial fibrillation.

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