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Effect of ethanol on the electrophysiological characteristics of pulmonary vein cardiomyocytes

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Abstract

Ethanol consumption has been considered to contribute to the occurrences of paroxysmal atrial fibrillation. Pulmonary veins are known to initiate atrial fibrillation. This study investigated whether ethanol may induce atrial fibrillation through increasing arrhythmogenic activity of pulmonary vein cardiomyocytes. Using the whole-cell clamp technique, the action potential and ionic currents were investigated in rabbit single pulmonary vein beating cardiomyocytes with and without (control) incubation of ethanol. Compared with control cardiomyocytes, pulmonary vein cardiomyocytes receiving 0.3 mg/ml or 1 mg/ml ethanol had shorter action potential duration, but had similar beating rates (2.6 ± 1.3, 2.7 ± 1.2, 2.7 ± 1.2 Hz) and incidences (45%, 41%, 32%) of delayed afterdepolarization. Pulmonary vein cardiomyocytes receiving ethanol had smaller L-type Ca^{2+} currents and larger transient outward currents, but had similar transient inward, delayed rectified outward, inward rectified and pacemaker currents. These results suggest that ethanol has no direct effect on the arrhythmogenic potential of pulmonary vein cardiomyocytes.

Keywords: Action potential; Atrial fibrillation; Ethanol; Pulmonary vein

1. Introduction

Atrial fibrillation is the most common sustained cardiac rhythm disturbance and is responsible for substantial morbidity and mortality in the general population. Previous studies have indicated that alcohol consumption may trigger the occurrence of paroxysmal atrial fibrillation (Ettinger et al., 1978; Cohen et al., 1988; Koskinen et al., 1990). On the other hand, several studies also showed that ethanol was not associated with occurrences of atrial fibrillation (Psaty et al., 1997; Kannel et al., 1998). Ethanol is known to have several cardiovascular effects (Regan, 1990). Previous studies have indicated that ethanol could shorten the action potential duration in cardiac Purkinje fibers (Williams et al., 1980) and enhance the spontaneous activity of sinoatrial cells (Carpentier and Gallardo, 1987). In contrast, other studies have shown that ethanol has little effect on the spontaneous activity of Purkinje fiber or sinoatrial cells (Williams et al., 1980; Jain and Carpentier, 1998). However, only a few studies have evaluated the effects of ethanol on membrane currents of cardiomyocytes. Ethanol decreased Ca^{2+} and Na^{+} currents in ventricular cardiomyocytes (Habuchi et al., 1995), and inhibited electrically induced Ca^{2+} transients in isolated cardiomyocytes (Thomas et al., 1989). Knowledge about the effects of ethanol on ionic currents of pacemaker cells was limited.

Pulmonary veins are important sources of ectopic beats for the initiation of paroxysmal atrial fibrillation and the foci

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Table 1
The action potential parameters of pulmonary vein cardiomyocytes with or without (Control) the administration of ethanol

	MDP (mV)	APA (mV)	APD_{20} (ms)	APD_{50} (ms)
Control ($n=20$)	-58 ± 2	84 ± 1	65 ± 6	105 ± 9
0.3 mg/ml ethanol ($n=12$)	-61 ± 2	84 ± 2	43 ± 5^a	77 ± 8^a
1 mg/ml ethanol ($n=13$)	-57 ± 3	81 ± 3	41 ± 5^b	75 ± 8^a

APA, amplitude of AP; MDP, maximal diastolic potential. Values are means ± S.E.

[a] $P<0.05$ versus the same parameter of the control group.

[b] $P<0.005$ versus the same parameter of the control group.

of ectopic atrial tachycardia and focal atrial fibrillation (Walsh et al., 1992; Haissaguerre et al., 1998; Chen et al., 1999). Previous studies have indicated that most paroxysmal atrial fibrillation was induced by the ectopic foci in pulmonary veins (Haissaguerre et al., 1998; Chen et al., 1999). They are also associated with the maintenance of atrial fibrillation (Pappone et al., 2000; Sueda et al., 2001). Pulmonary veins were known to contain cardiomyocytes with electrical activity and suggested to be subsidiary pacemakers or possibly induce atrial arrhythmias (Cheung, 1981; Blom et al., 1999; Chen et al., 2000; Saito et al., 2000). Studies of single cells showed that pulmonary veins had cardiomyocytes with distinct electrophysiological characteristics (Chen et al., 2001, 2002a,b). Furthermore, pulmonary veins were found to have arrhythmogenic activity from the enhancement of automaticity or induction of triggered activity in pulmonary veins cardiomyocytes with pacemaker activity (Chen et al., 2001, 2002a,b). Long-term atrial pacing, the important atrial fibrillation study model, can increase pulmonary vein arrhythmogenic activity to induce atrial fibrillation (Chen et al., 2001). Similarly, administration of thyroid hormone was shown to increase pulmonary vein arrhythmogenic activity, which may underlie the mechanisms of hyperthyrodism-induced atrial fibrillation (Chen et al., 2002a). All of these findings suggested that pulmonary veins play a critical role in the genesis of atrial fibrillation. Because pulmonary veins have particular arrhythmogenic properties, it is possible that ethanol could increase pulmonary vein arrhythmogenic activity and trigger ectopic beats from pulmonary veins to induce atrial fibrillation. The purpose of this study was to investigate whether ethanol would change the electrophysiological characteristics, arrhythmogenic activity and membrane currents of pulmonary vein cardiomyocytes.

2. Materials and methods

2.1. Isolation of pulmonary vein cardiomyocytes

The investigation conforms to the institutional *Guide for the Care and Use of Laboratory Animals.* As previously described (Chen et al., 2002a,b), 56 rabbits weighing 1–2 kg were anesthetized with intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed and the heart along with lung was quickly removed. The pulmonary veins were perfused in a retrograde manner via polyethylene tubing (outer diameter, 3.5 mm) cannulated through the aorta and left ventricle into the left atrium. The free end of the polyethylene tubing was connected to a Langendorff perfusion column for perfusion with oxygenated normal Tyrode solution (containing in mM: NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 10 and glucose 11; the pH was adjusted to 7.4 by titrating with 1 N NaOH) at 37 °C. The perfusate was replaced with oxygenated Ca^{2+}-free Tyrode solution containing 300 units/ml collagenase (Sigma, Type I) and 0.25 units/ml protease (Sigma, Type XIV) for 8–12 min, after which time the proximal pulmonary veins (8–12 mm) were cut away from the atrium and lung and placed in a dissection chamber containing Ca^{2+}-free oxygenated Tyrode solution. The piece of tissue was cut into fine pieces and gently shaken in 5–10 ml of Ca^{2+}-free oxygenated Tyrode solution until single cardiomyocytes were obtained. The solution was then

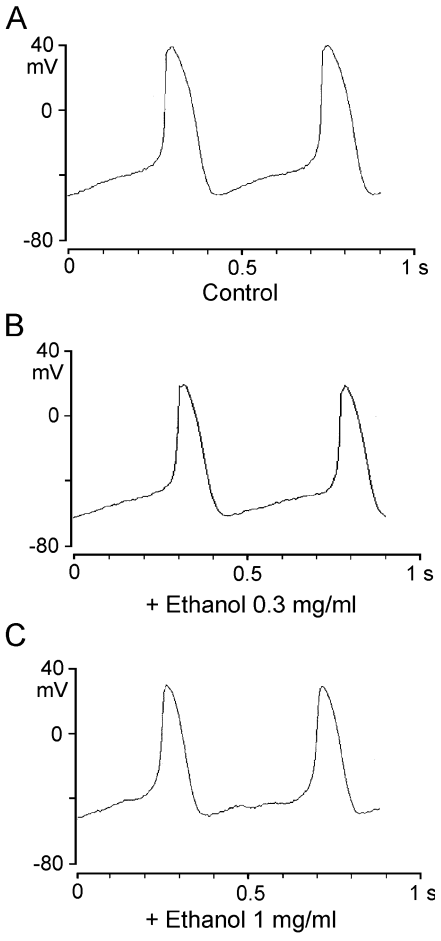

Fig. 1. Action potentials of pulmonary vein cardiomyocytes during spontaneous beating. Control pulmonary vein cardiomyocytes (panel A) had longer 20% or 50% of action potential duration than pulmonary vein cardiomyocytes receiving 0.3 mg/ml (panel B) and 1 mg/ml ethanol (panel C).

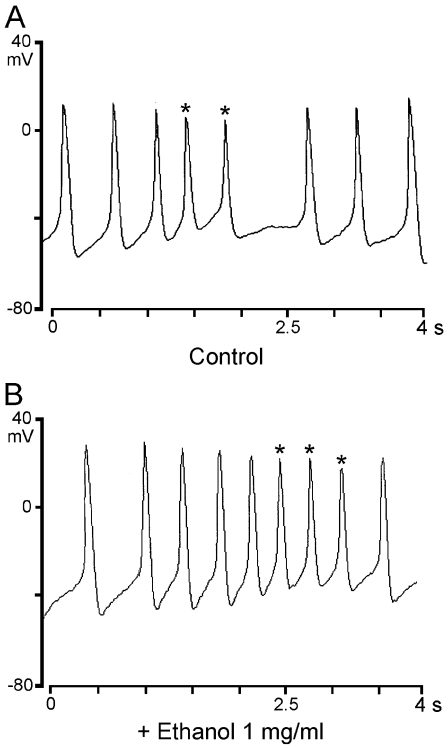

Fig. 2. Oscillatory potentials (asterisk) occurred in pulmonary vein cardiomyocytes without (panel A) or with (panel B) 1 mg/ml ethanol administration.

gradually changed to normal oxygenated Tyrode solution. Only cells with spontaneous activities and cross striations were used. The pulmonary vein cardiomyocytes with spontaneous activity were identified by the action potential configurations (less negative diastolic potential and spontaneous diastolic depolarization), constant spontaneous beating and smaller inward rectified K^+ current (I_{K1}). Experiments were carried out at 35 ± 1 °C, and the cells were allowed to stabilize in the bath for at least 30 min before experiments.

2.2. Electrophysiological and pharmacological study

Whole-cell patch-clamp was performed in pulmonary vein cardiomyocytes without (control) and with incubation of ethanol (0.3 or 1 mg/ml) over 30 min by means of an Axopatch 1D amplifier (Axon Instruments, Union, CA, USA). Borosilicate glass electrodes (o.d., 1.8 mm) were used, with tip resistances of 3–5 MΩ. Before formation of the membrane-pipette seal, tip potentials were zeroed in Tyrode solution. Junction potentials between the bath and pipette solution (9 mV) were corrected for action potential recording. Action potential and membrane currents were measured during superfusion with normal Tyrode solution with (control group) or without ethanol. The composition of pipette solution was as follows (in mM): KCl 20, K aspartate 110, $MgCl_2$ 1, Mg_2ATP 5, HEPES 10, EGTA 0.5, LiGTP 0.1, and Na_2 phosphocreatine 5, adjusted to pH 7.2 with 1 N KOH. To study the L-type calcium currents ($I_{Ca,L}$), micropipettes were filled with a solution containing (in mM) CsCl 130, $MgCl_2$ 1, Mg_2ATP 5, HEPES 10, EGTA 10, NaGTP 0.1, and Na_2 phosphocreatine 5 (at pH 7.2, adjusted with CsOH). NaCl and KCl in normal Tyrode solution were replaced by tetraethylammonium chloride and CsCl, respectively. The action potentials were recorded in current-clamp mode and ionic currents in voltage-clamp mode as described previously (Chen et al., 2001, 2002a,b). A small hyperpolarizing step from a holding potential of −50 mV to a testing potential of −55 mV for 80 ms was delivered at the beginning of each experiment. The area under the capacitative currents was divided by the applied voltage step to obtain the total cell capacitance. Normally 60–80% series resistance (R_s) was electronically compensated. After compensation, the aver-

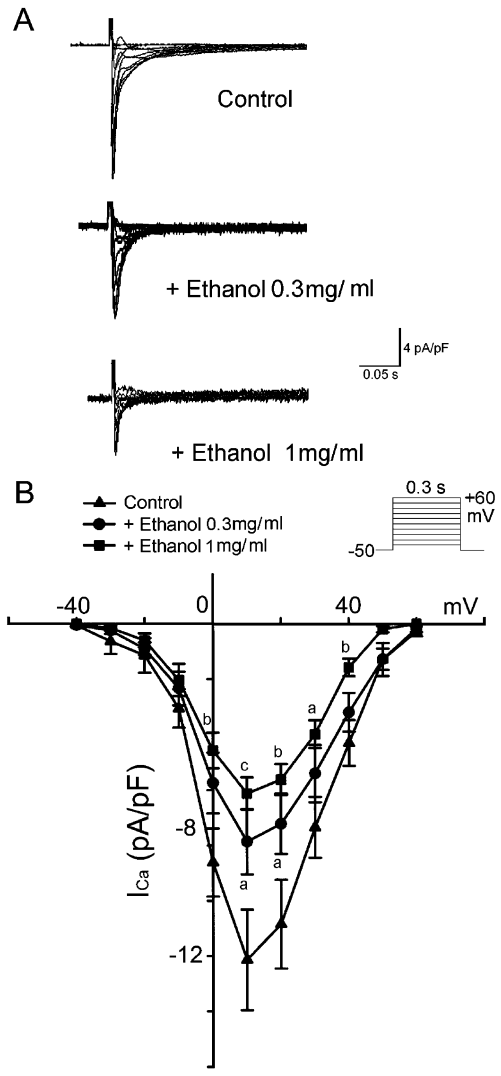

Fig. 3. Current traces (panel A) and I–V relationship (panel B) of $I_{Ca,L}$ in pulmonary vein cardiomyocytes with or without the administration of ethanol. Insets show the various clamp protocols. $^aP<0.05$, $^bP<0.01$, $^cP<0.005$, versus control PV cardiomyocytes.

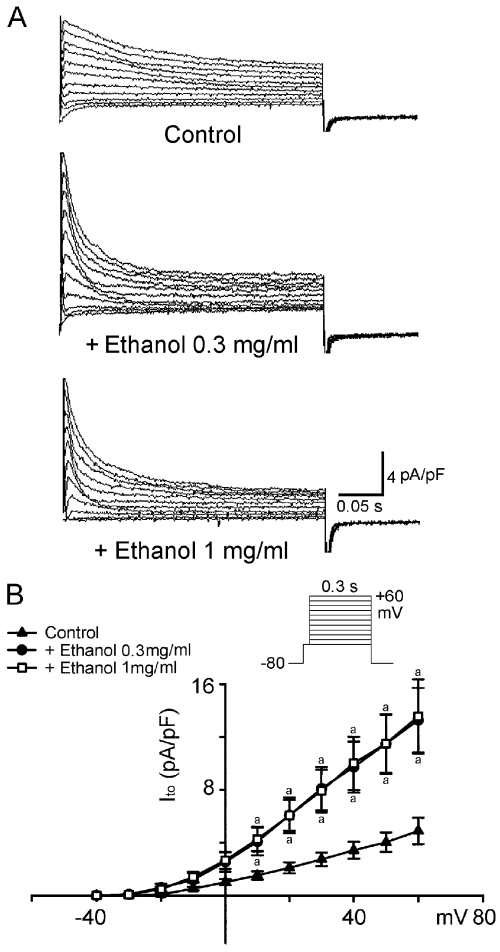

Fig. 4. Current traces (panel A) and I–V relationship (panel B) of I_{to} in pulmonary vein cardiomyocytes with or without the administration of ethanol. Insets of current traces show the various clamp protocols. [a]$P<0.05$ versus control pulmonary vein cardiomyocytes.

age time constant was 110 ± 8 μs (cell capacitance, 62 ± 2 pF, $n=249$), and average R_s was 1.7 ± 0.1 MΩ. Currents rarely exceeded 1.5 nA, and the maximal voltage error did not exceed 3 mV.

Voltage command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). Action potential measurements were begun 5 min after cell rupture. The steady-state action potential duration at 20% (APD_{20}) and 50% (APD_{50}) of full repolarization were only measured during spontaneous beating within the rates 2–3.5 Hz to minimize the effects of beating rate on action potential parameters. Recordings were low pass-filtered at half the sampling frequency. Data were sampled at rates varying from 2 to 25 kHz. Oscillatory potentials were defined as the action potentials spontaneously fluctuated at depolarized levels (Gonzalez and Vassalle, 1993).

$I_{Ca,L}$ were measured as inward currents during depolarization from −50 to +60 mV in 10 mV steps for 300 ms at a frequency of 0.1 Hz. In order to avoid the 'run-down' effects, $I_{Ca,L}$ were measured between 5 and 15 min after rupturing the membrane patch in each cardiomyocyte from control and ethanol groups. Transient outward currents (I_{to}) were studied with a double-pulse protocol. A 30 ms pre-pulse from −80 to −40 mV was used to inactivate sodium channel, followed by a 300-ms test pulse to +60 mV in 10 mV steps at a frequency of 0.1 Hz. $CdCl_2$ (200 μM) was added to the bath solution to inhibit $I_{Ca,L}$. 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_K) were measured as the peak outward current at the end of 1 s depolarization from −40 to +60 mV during the infusion of 200 μM $CdCl_2$ in the bath solution to inhibit $I_{Ca,L}$.

Transient inward current was induced by clamped potentials from −40 to +40 mV for a duration of 3 s and then repolarized to −40 mV. The amplitude of transient inward current was measured as difference between the peak of the transient current and the mean of current just before and after the transient current (Chen et al., 2001, 2002a).

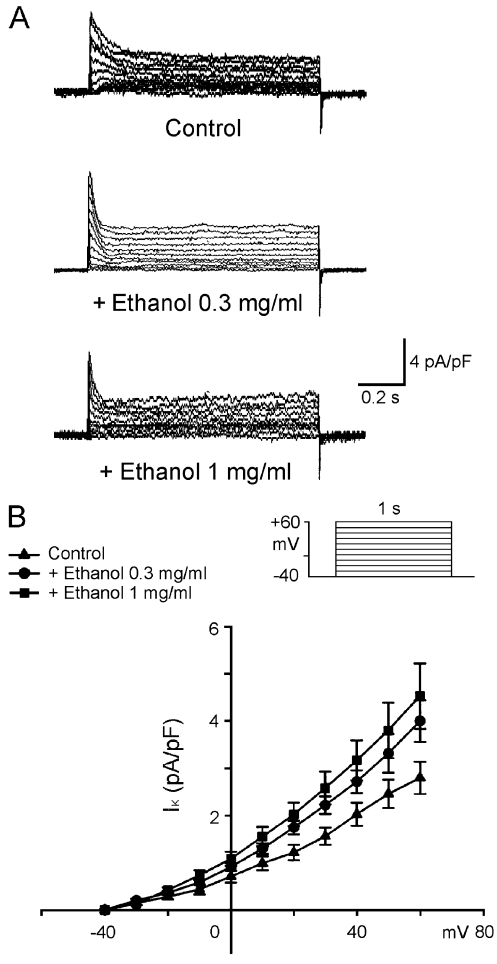

Fig. 5. Current traces (panel A) and I–V relationship (panel B) of I_K in pulmonary vein cardiomyocytes with or without the administration of ethanol. Insets show the various clamp protocols.

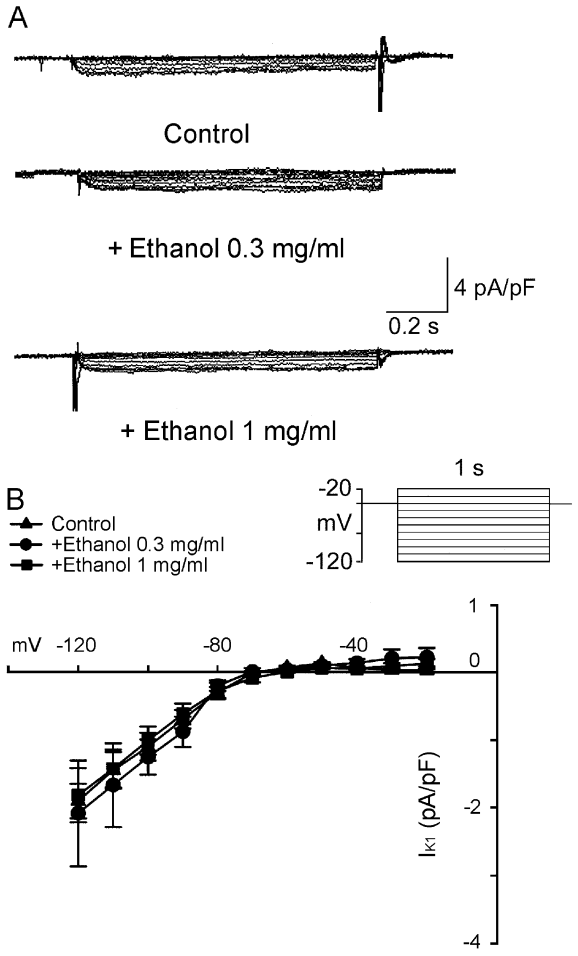

Fig. 6. Current traces (panel A) and I–V relationship (panel B) of I_{K1} in pulmonary vein cardiomyocytes with or without the administration of ethanol. I_{K1} were similar among control and pulmonary vein cardiomyocytes receiving 0.3 or 1 mg/ml ethanol. Insets show the various clamp protocols.

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 during the infusion of 200 μM $CdCl_2$ in the bath solution. The amplitudes of I_{K1} were measured as 1 mM barium sensitive currents. A progressive large inward current developed with slow voltage-dependent kinetics and was not inactivated during hyperpolarization from a holding potential of -40 mV to a test potential of -120 mV for 1 s. It was suppressed by 5 mM cesium and was measured as pacemaker current, I_f.

2.3. Statistics

Continuous variables are expressed as mean ± S.E.M. and the n is the number of experimental cells. The differences among the pulmonary vein cardiomyocytes with or without administration of ethanol were analyzed by one-way analysis of variance test. Multiple comparisons were analyzed with the Fisher's Least Significant Difference Test. Nominal variables were compared by Chi-square analysis with Yates correction or Fisher's exact test. A P value lower then 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of ethanol on action potentials of pulmonary vein cardiomyocytes

The beating rates were similar among the pulmonary vein cardiomyocytes receiving 0.3 mg/ml ethanol (2.6 ± 1.3 Hz, $n=44$) and 1 mg/ml ethanol (2.7 ± 1.2 Hz, $n=37$) and control (2.7 ± 1.2 Hz, $n=47$) ($P>0.05$). However, the cells receiving 0.3 mg/ml or 1 mg/ml ethanol had shorter APD_{20} and APD_{50} than the cells without the

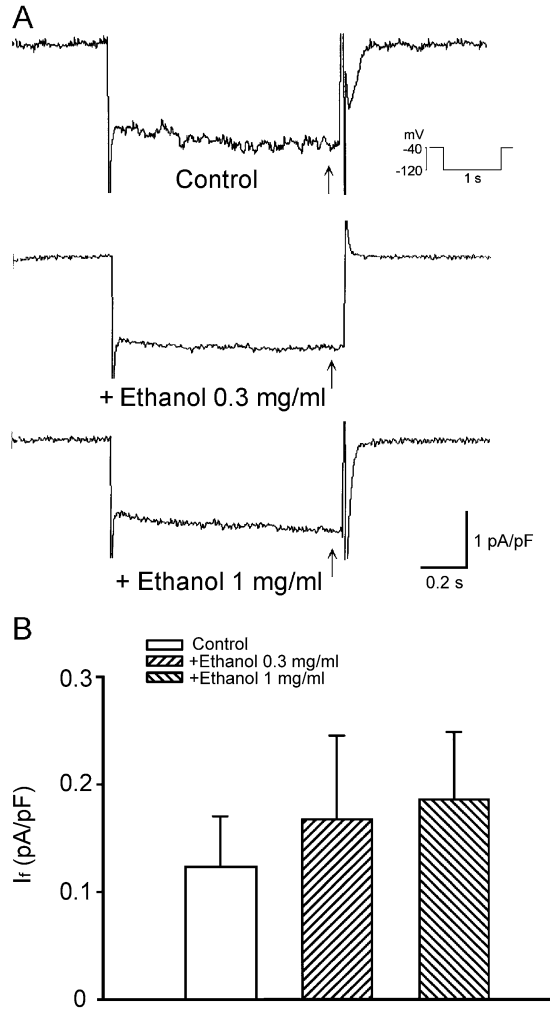

Fig. 7. Current traces (Panel A) and densities (Panel B) of I_f from pulmonary vein cardiomyocytes with and without administration of ethanol. There was similar I_f among pulmonary vein cardiomyocytes with or without administration of ethanol. Insets show the clamp protocols. I_f's are indicated by upward arrows.

administration of ethanol (Table 1). Fig. 1 shows the example of pulmonary vein cardiomyocytes with and without the administration of ethanol. The pulmonary vein cardiomyocytes with ethanol administration had shorter action potential duration than control during similar spontaneous beating rates. In addition, oscillatory potentials occurred in 10 (25%) control pulmonary vein cardiomyocytes, 11 (25%) pulmonary vein cardiomyocytes receiving 0.3 mg/ml ethanol and 11 (30%) pulmonary vein cardiomyocytes receiving 1 mg/ml ethanol ($P>0.05$). Fig. 2 shows the examples of oscillatory potentials generated before full repolarization in pulmonary vein cardiomyocytes with or without the administration of ethanol. Moreover, 15 of 47 (32%) control pulmonary vein cardiomyocytes, 20 of 44 (45%) pulmonary vein cardiomyocytes receiving 0.3 mg/ml ethanol and 15 of 37 (41%) pulmonary vein cardiomyocytes receiving 1 mg/ml ethanol had delayed afterdepolarization ($P>0.05$).

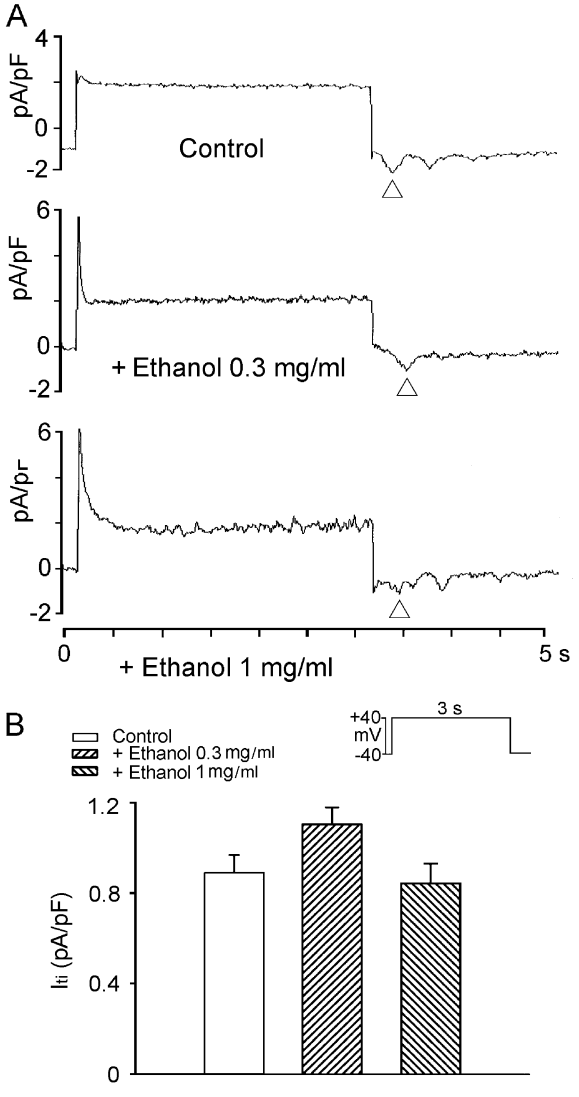

Fig. 8. Current traces (Panel A) and densities (Panel B) of I_{ti} from pulmonary vein cardiomyocytes with and without administration of ethanol. The arrows indicate the I_{ti} on repolarization to −40 mV after a depolarizing pulse from −40 to +40 mV for 3 s. Insets show the clamp protocols.

3.2. Effects of ethanol on membrane currents of pulmonary vein cardiomyocytes

3.2.1. Depolarization-induced currents

Fig. 3A shows the recordings of $I_{Ca,L}$ from the cells with or without the administrations of ethanol. Pulmonary vein cardiomyocytes receiving 0.3 mg/ml ($n=8$), and 1 mg/ml ethanol ($n=13$) have smaller current density of $I_{Ca,L}$ than control ($n=8$) pulmonary vein cardiomyocytes (Fig. 3B).

Fig. 4A shows the recordings of I_{to} from the cells with or without the administrations of ethanol. I_{to} were larger in cardiomyocytes receiving 0.3 mg/ml ($n=14$) or 1 mg/ml ($n=13$) ethanol than in control ($n=9$) pulmonary vein cardiomyocytes (Fig. 4B). However, the current densities of I_K were similar among control ($n=13$) and pulmonary vein cardiomyocytes receiving 0.3 mg/ml ($n=21$) or 1 mg/ml ($n=16$) ethanol (Fig. 5A). Fig. 5B shows the recordings of I_K from the cells with or without the administrations of ethanol.

3.2.2. Hyperpolarization-induced currents

Fig. 6A shows the recording of I_{K1} in the cells with or without the administration of ethanol. There were similar I_{K1} among control ($n=10$) and pulmonary vein cardiomyocytes receiving 0.3 mg/ml ($n=6$) or 1 mg/ml ($n=7$) ethanol (Fig. 6B). In addition, 36% of the 14 control cells, 43% of the 14 cells receiving 0.3 mg/ml ethanol and 50% of the 14 cells receiving 1 mg/ml ethanol had I_f ($P>0.05$). Fig. 7A shows the recordings of I_f in pulmonary vein cardiomyocytes with and without the administration of ethanol. The peak I_f density was similar among the cells with or without the administration of ethanol (Fig. 7B).

As the example shown in Fig. 8A, transient inward currents were present in 32 of 34 (94%) control pulmonary vein cardiomyocytes, 21 of 21 (100%) pulmonary vein cardiomyocytes receiving 0.3 mg/ml ethanol, and 33 of 37 (89%) pulmonary vein cardiomyocytes receiving 1 mg/ml ethanol ($P>0.05$). There were similar transient inward currents among these cells with or without the administration of ethanol (Fig. 8B).

4. Discussion

4.1. Major findings

This study demonstrated that ethanol shortened the action potential duration, but did not alter the automaticity or triggered activity of pulmonary vein cardiomyocytes. Ethanol decreased $I_{Ca,L}$ and increased I_{to}, but did not change transient inward currents, I_K, I_{K1}, and I_f.

4.2. Effect of ethanol on arrhythmogenic activity of pulmonary vein cardiomyocytes

Although previous studies have suggested that alcohol consumption may induce the occurrence of atrial fibrillation with the frequently used term "Holiday heart syndrome" (Ettinger et al., 1978; Cohen et al., 1988; Koskinen et al., 1990), the role of ethanol in the genesis of atrial fibrillation is still uncertain. Ethanol was shown to shorten the atrial action potential duration which would decrease atrial refractoriness and may facilitate the maintenance of atrial fibrillation (Regan, 1990). Increased circulating catecholamines, enhancement of sympathetic reaction, myocardial damage and altered myocardial metabolism have been suggested to be potential mechanisms underlying ethanol-induced atrial fibrillation (Mathews et al., 1981; Tiina et al., 1998). In contrast, ethanol was also found to have little effects on the occurrence of atrial fibrillation (Psaty et al., 1997; Kannel et al., 1998). In this study, as in our previous studies, we demonstrated the presence of spontaneous activity and triggered activitity in pulmonary vein cardiomyocytes (Chen et al., 2001, 2002a,b). The administration of ethanol shortened the action potential duration in pulmonary vein cardiomyocytes, which was similar to the known effects of ethanol on ventricular myocytes. However, unlike the findings in pulmonary veins after long-term atrial pacing or administration of thyroid hormone (Chen et al., 2001, 2002a), there were similar automaticity and triggered activity between pulmonary vein cardiomyocytes with or without the administration of ethanol. Additionally, ethanol has little effects on oscillatory potentials of pulmonary vein cardiomyocytes, it is possible that the spontaneous oscillatory potentials may prevent ethanol to exert an effect on this arrhythmogenic activity. It is known that the blood concentration of ethanol at which people received alcohol consumption is approximately 1–1.5 mg/ml and the concentration higher than 4 mg/ml could be lethal. Therefore, in order to correlate our study with clinical situations, we used 0.3 mg/ml and 1 mg/ml ethanol in this study. These results may suggest that ethanol has little arrhythmogenic effects on pulmonary vein cardiomyocytes, although, this in vitro experiment still does not completely exclude the possibility that ethanol may induce atrial fibrillation through its systemic effects or arising from the squeal of myocardial damage by ethanol (Mathews et al., 1981; Tiina et al., 1998).

4.3. Effect of ethanol on membrane currents of pulmonary vein cardiomyocytes

This study demonstrated that ethanol would suppress $I_{Ca,L}$ in pulmonary vein cardiomyocytes. Previous study also has shown that ethanol decreases $I_{Ca,L}$ in ventricular cardiomyocytes (Jain and Carpentier, 1998). However, little information was available about the effects of ethanol on potassium currents. This study showed that ethanol increased I_{to}, but not I_K or I_{K1} in pulmonary vein cardiomyocytes. These effects of ethanol on $I_{Ca,L}$ and I_{to} would contribute to the shortening of action potential duration in these cells.

I_f has been suggested to contribute to the automaticity of cardiomyocytes and may result in cardiac arrhythmia (DiFrancesco, 1986; Hoppe et al., 1998). Similarly, transient inward currents were also known to play a role in the genesis of delayed afterdepolarization and have been shown to induce arrhythmia (Tseng and Wit, 1987). It is not clear whether ethanol has a role in these currents. This study showed that there were similar I_f and transient inward currents between pulmonary vein cardiomyocytes with and without ethanol administration. Our previous studies also have shown that enhancement of I_f or transient inward currents play a role in the arrhythmogenic activity of pulmonary vein cardiomyocytes (Chen et al., 2001, 2002a). Therefore, the small effects of ethanol on I_f and transient inward currents may account for the similar automaticity and triggered activity between pulmonary vein cardiomyocytes with or without ethanol administration.

4.4. Conclusions

There was no effect of ethanol on the automaticity or triggered activity of pulmonary vein cardiomyocytes. These findings suggest that ethanol has no direct arrhythmogenic effects on pulmonary vein cardiomyocytes.

Acknowledgements

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