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Full paper

Atrial-selective block of sodium channels by acehytisine in rabbit myocardium☆

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ABSTRACT

Acehytisine, a multi-ion channel blocker, can markedly inhibit I_{Na}, I_{Ca}, I_{Kur}, I_f at various concentrations and effectively terminate and prevent atrial fibrillation (AF) in patients and animal models, but the molecular mechanism underlying its blockage remains elusive. In this study, we investigated the effects of acehytisine on action potentials and sodium channels of atrial and ventricular myocytes isolated from rabbit, using whole-cell recording system. We found that acehytisine exerted stronger blocking effects on sodium channels in atria than in ventricles, especially at depolarization (IC_{50}: 48.48 ± 7.75 μmol/L in atria vs. 560.17 ± 63.98 μmol/L in ventricles). It also significantly shifted steady state inactivation curves toward negative potentials in atrial myocytes, without affecting the recovery kinetics from inactivation of sodium channels in the same cells. In addition, acehytisine inhibited I_{Na} in a use-dependent manner and regulated slow inactivation kinetics by different gating configurations. These findings indicate that acehytisine selectively blocks atrial sodium channels and possesses affinity to sodium channel in certain states, which provides additional evidence for the anti-AF of acehytisine.

1. Introduction

Atrial fibrillation (AF), the most prevalent sustained clinical arrhythmia, increases morbidity and mortality. Although invasive electrophysiological procedures have gained significant progress in treating AF, antiarrhythmic drugs (AADs) remain the mainstay for the management of AF patients. However, the efficacy and/or safety of AADs are far from ideal. An important limitation of currently available agents is the risk of inducing severe ventricular arrhythmias and/or organ toxicity. Therefore, it is imperative to develop more effective and safer anti-AF agents. Currently, administration of atrial-selective sodium channel blockers has been considered a novel and effective strategy for the management of AF (1–3). Several sodium channel blockers (ranolazine, AZD1305, dronedarone, Wenxin Keli) have been experimentally demonstrated to produce atrial-selective electrophysiological effects that allow them to effectively suppress AF with minimal side effects in the ventricle (4–9). However, clinical evidence of their effectiveness in treating AF remains scarce.

Acehytisine (previously named Guanfu base A), a novel diterpene alkaloid, is isolated from the root of *aconitum coreanum*

Abbreviations: AAD, antiarrhythmic drug; AF, atrial fibrillation; AP, action potential; APD_{90}, action potential duration at 90%; CAST, Cardiac Arrhythmias Suppression Trail; CL, cycle length; HCN, hyperpolarization-activated and cyclic nucleotide-gate; HERG, human ether-a-go-go related gene; I–V, current–voltage; RMP, membrane potential; TdP, Torsade de Pointes; V_{max}, maximum upstroke velocity resting.

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(Levl.) raipaics, a herbal medication in China for centuries (10). In both cellular and global electrophysiological studies, acehytisine has been verified as a multi-ion channel blocker (sodium channel, calcium channel, potassium channel, HCN channel (11–17)) which prevents arrhythmias with low toxicity in animal models (18,19). Acehytisine, after passing phases II and III clinical studies, has been approved for the treatment of paroxysmal supraventricular tachycardia in China in 2005 (20). Currently, it is undergoing phase IV clinical study. Acehytisine has also been shown to effectively terminate AF in mongrel dog models induced by vagal stimulation and topical application of acetylcholine (21,22), but the mechanism of its action has not been elucidated completely. Since acehytisine can effectively terminate superaventricular arrhythmia but does not induce ventricular arrhythmia concomitantly (20), we postulate that acehytisine could selectively block sodium channels in atrium, which may involve suppression and prevention of AF. Therefore, we set out to investigate the electrophysiological characteristics of acehytisine in rabbit atrial myocytes in this study with the hope to provide novel insights into its anti-AF mechanisms.

2. Materials and methods

2.1. Isolation of cardiomyocytes

Single cardiomyocytes were enzymatically isolated with procedure as described in the online supplementary methods.

2.2. Electrophysiological recording

The I_{Na} and action potential recordings were conducted under voltage and current clamp configuration with the techniques as described in the online supplementary methods.

2.3. Drugs

All reagents used in this study were described in the online supplementary methods.

2.4. Data analysis

All results in text, tables and figures were presented as means ± SD. from 'n' experiments. The data acquisition and analysis were described in the online supplementary methods.

3. Results

3.1. Electrophysiological distinctions between atrial and ventricular myocytes

The transmembrane action potentials (APs) and ion currents were recorded by patch-clamp technique in atrial and ventricular myocytes respectively. The electrophysiological distinctions were showed in Table 1, Figs. 1 and 2. On the one hand, the resting membrane potential (RMP) was much more depolarized in cells from atria than that from ventricle ($P < 0.01$, n = 7 to 7). On the other, the maximum upstroke velocity (V_{max}) was greater and AP duration at 90% (APD_{90}) of repolarization was longer in ventricular myocytes than those in atrial ones ($P < 0.05$, n = 7 to 7). In addition, a smaller membrane capacitance and a greater current density in atrial were demonstrated than those in ventricular cells while a greater current density was obtained under hyperpolarization (−90 mV vs. −120 mV) both in atria and ventricle.

In I–V relationship, current density peaked at −38.54 ± 2.43 mV in atrial and at −31.42 ± 4.75 mV in ventricular cells, whereas the reversal potentials were not different. The half-inactivation voltage ($V_{1/2}$) in atrial myocytes was more negative (−8.76 mV) than that recorded in ventricular myocytes, whereas the activation curves remained similar between atria and ventricles. In terms of differences of recovery curve, the recovery kinetics from inactivation state were obtained faster in ventricular than atrial myocytes. These results indicate that a greater percentage of sodium channels in atria than in ventricles was inactivated at a specified resting or take-off potential.

3.2. Effects of acehytisine on AP in myocardium

The effects of acehytisine on the AP configuration were shown in Table 1 and Fig. 1. When a train of relatively slow frequency of stimulation was applied (cycle length, CL = 800 ms), 10 μmol/L

Table 1
Electrophysiological distinctions between atrial and ventricular myocytes and the effects of acehytisine on APs and sodium currents.

		Control		Achytisine	
		Atrium	ventricle	Atrium	ventricle
RMP (mV)		−77.71 ± 2.62	−88.14 ± 1.77**	−75.57 ± 2.07	−87.57 ± 2.14
V_{max} (V/s)	CL = 800ms	138.57 ± 6.80	176.91 ± 19.04*	98.75 ± 4.08‡	172.67 ± 19.60
	CL = 300ms	131.18 ± 5.18	183.32 ± 33.76*	69.67 ± 7.77‡	175.30 ± 21.41
APD_{90} (ms)	CL = 800ms	216.76 ± 15.83	258.12 ± 40.30*	224.16 ± 29.56	253.57 ± 22.84
	CL = 300ms	159.30 ± 12.01	204.99 ± 33.76**	201.30 ± 18.45‡	212.67 ± 19.06
Membrane capacitance	(pF)	67.69 ± 7.21	112.34 ± 15.87**		
Current density	V_h = −120 mV	−73.75 ± 14.23	−36.25 ± 7.74**		
(pA/pF)	V_h = −90 mV	−52.68 ± 11.37	−19.66 ± 4.54**		
I–V relationship	MAX (mV)	−38.57 ± 2.43	−31.42 ± 4.75*	−39.12 ± 2.51	−32.93 ± 3.47
	REV (mV)	25.71 ± 3.45	24.28 ± 1.88	24.91 ± 2.46	24.49 ± 2.14
Activation curve	$V_{1/2}$ (mV)	−45.68 ± 0.49	−44.64 ± 0.54	−40.83 ± 0.55†	−44.85 ± 0.63
	k	6.37 ± 0.46	6.28 ± 0.52	6.53 ± 0.49	6.75 ± 0.41
Inactivation curve	$V_{1.2}$ (mV)	−84.64 ± 0.93	−75.88 ± 0.69**	−95.92 ± 0.49‡	−74.68 ± 0.60
	k	5.04 ± 0.53	5.81 ± 0.46	5.94 ± 0.38	5.37 ± 0.63
Recovery curve	τ (ms)	25.18 ± 0.77	13.97 ± 0.30**	27.01 ± 0.96	16.29 ± 0.88

Abbreviations: RMP, resting membrane potential; V_{max}, maximum upstroke velocity; APD_{90}, action potential duration at 90% of total repolarization (ms); CL, cycle length; V_h, holding potential; MAX, membrane voltage at maximum current density; REV, reversal potential; $V_{1/2}$, half-activation or half-inactivation potential; k, slope factor; τ, time constant. Value are represented as mean ± SD. *$P \leq 0.05$ and **$P \leq 0.01$ vs. Atrium; †$P \leq 0.05$ and ‡$P \leq 0.01$ vs. Control.

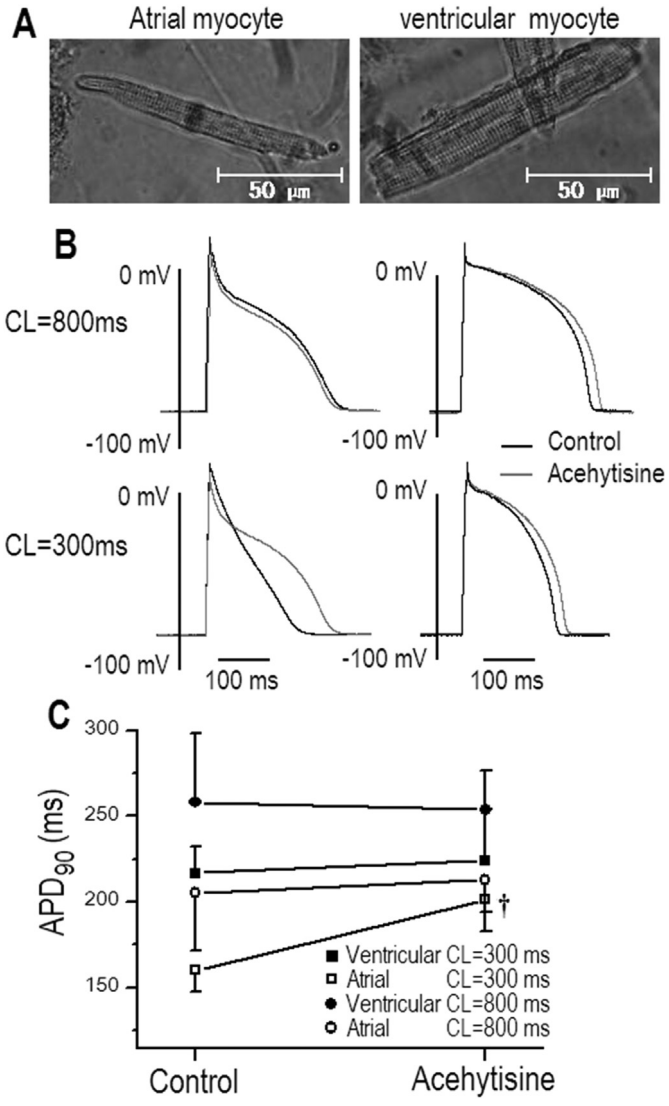

Fig. 1. Myocytes and effects of acehytisine on action potentials in atrial and ventricular myocytes. (A) Typical morphology of atrial and ventricular myocyte isolated from rabbit heart. (B) Representative recordings of action potentials were superimposed before (control), during superfusion of 10 μmol/L acehytisine in atrial and ventricular myocytes, respectively. (C) The summary data of APD_{90} in absence and presence of 10 μmol/L acehytisine. †$P < 0.01$ vs. control.

acehytisine caused a significant decrease in the V_{max} ($P < 0.01$, n = 7) with slight depolarization of RMP ($P > 0.05$, n = 7) in atrial myocytes without changing APD_{90} in both atria and ventricle ($P > 0.05$, n = 7 to 7). When a faster frequency of stimulation (CL = 300 ms) was applied, acehytisine significantly prolonged APD_{90} in atrial myocytes ($P < 0.01$, n = 7) without changing the V_{max} and APD_{90} (CL = 300 ms) in ventricular myocytes ($P > 0.05$, n = 7). Meanwhile, with further shortening in the interval of pacing, the values of V_{max} were more obviously reduced in atrial myocytes ($P < 0.01$, n = 7). These results suggest that the atrioventricular difference of AP might result from atrial-selective effects of acehytisine on various ion channels in myocardium.

3.3. Atrial-selective blocking effects of sodium channels

The concentration-dependent blockages of sodium channels by acehytisine were compared between atrial and ventricular myocytes (Fig. 3A and B). I_{Na} was activated at −40 mV from a holding potential of −120 mV. Firstly, the time-course of the effects of acehytisine on sodium channels were assessed for a suitable time point to study drug effects. After control was recorded, the atrial or ventricular myocytes were superfused with 50 μmol/L acehytisine or vehicle (negative control) and the voltage steps were applied again every 2 s. Markedly, an early onset of inhibition by acehytisine in atria than in ventricles, more rapid and stronger, was observed. The blockage approached a stable state in 1–4 min after drug application. Current amplitudes were partially recovered but did not return to the predrug control level when blank bath solution was washed (n = 4, Fig. 3C). Thus, all data were collected at 5 min in presence of the drug. Acehytisine inhibited I_{Na} expressed in atrial and ventricular myocytes in a concentration-dependent manner with the average IC_{50} values of 193.47 ± 15.70 μmol/L and 845.17 ± 60.96 μmol/L ($P < 0.01$, n = 7 to 7), and the Hill coefficients of 0.64 ± 0.16 and 0.67 ± 0.22 ($P > 0.05$, n = 7 to 7), respectively, suggesting that acehytisine inhibited I_{Na} with a potency that was 4.3-fold greater in atria than in ventricle. However, membrane potentials of cardiac myocytes, in normal conditions, hardly shift to hyperpolarization state (−120 mV), under which almost all sodium channels are in pre-open state. We then investigated the concentration-dependent blockages of I_{Na} in atrial and ventricular myocytes under the holding potential of −90 mV, near the resting potential. We found that IC_{50} calculated from the dose–response curves of inhibitory action was lower in atrial than in ventricular myocytes (48.48 ± 7.75 μmol/L vs. 560.17 ± 63.98 μmol/L, $P < 0.01$, n = 7 to 7), but Hill coefficients were not distinct in myocardium (0.66 ± 0.24 vs. 0.68 ± 0.19, $P > 0.05$, n = 7 to 7). These results indicate that acehytisine exerted atrial-dominant blocking effects on sodium channel, especially under depolarized condition.

3.4. Effects of acehytisine on I–V relationship, activation and inactivation kinetics of I_{Na}

The effects of I–V relationship by acehytisine were illustrated in Fig. 2G. Compared with control condition, the I–V relationship (including maximum activated potential and reversal potential) underwent no major changes. Meanwhile, suppression was similar at each potential tested in the presence of 50 μmol/L acehytisine ($P > 0.05$, n = 7 to 6). The effects of acehytisine on activation and inactivation kinetics of I_{Na} in atrial and ventricular myocytes were shown in Figs. 4 and 5 and Table 1. After applying 50 μmol/L acehytisine, half-activation voltage of I_{Na} was slightly shifted to left (<5 mV) in atrial cells ($P < 0.05$, n = 7). In contrast, in ventricular myocytes, the activation characteristics of I_{Na} were not clearly modified by acehytisine ($P > 0.05$, n = 7). Similarly, this drug shifted markedly the inactivation curve by −11.28 mV ($P < 0.01$, n = 6) in atrial cells, whereas it was not altered by acehytisine in ventricular cells ($P > 0.05$, n = 7).

3.5. Effects of acehytisine on recovery kinetics of I_{Na}

To determine the rate of recovery of I_{Na} from the drug-induced blockage, a train of double-pulse protocols were employed as shown in Figs. 4 and 5. In both atrial and ventricular myocytes, the time courses of I_{Na} recovery, expressed by a single exponential function with a time constant, were not altered in the presence of 50 μmol/L acehytisine (data in Table 1), suggesting that acehytisine had very rapid unbinding kinetics (rate of dissociation of drug from the sodium channel).

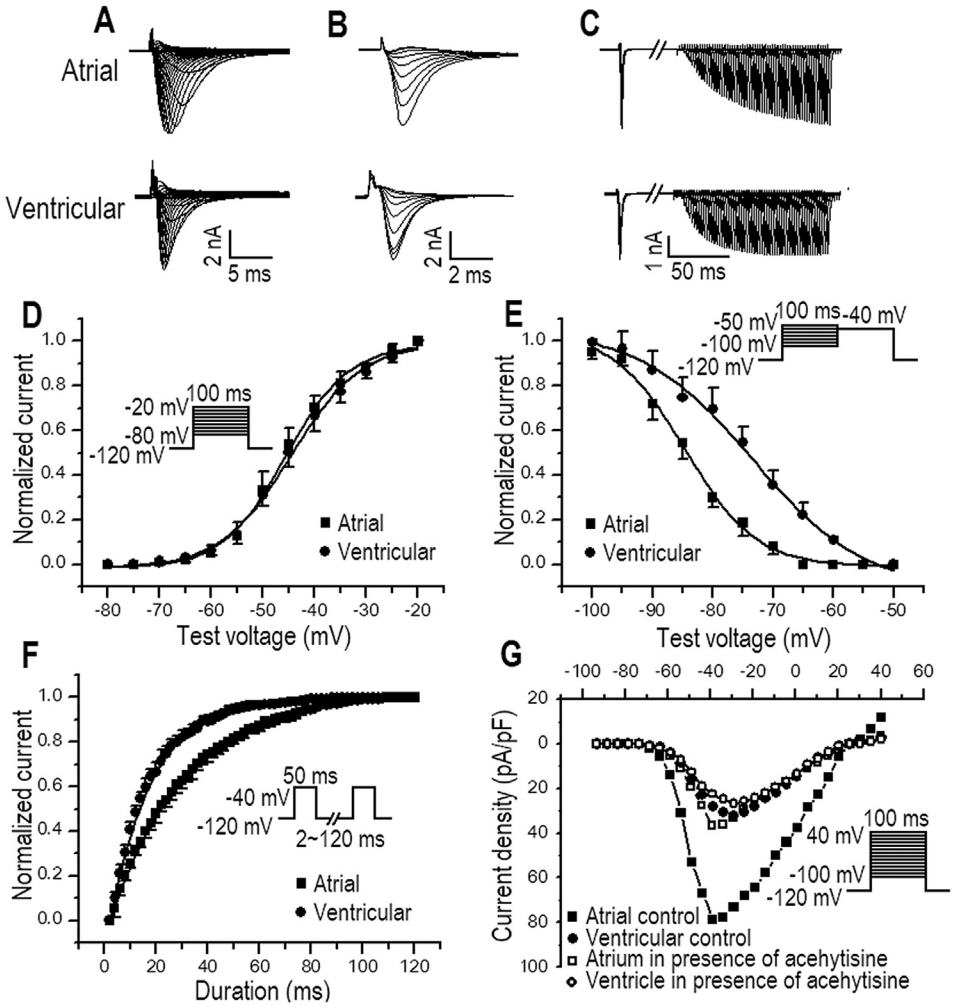

Fig. 2. Distinctions of kinetics of sodium channels between atrial and ventricular myocytes. Tracings of activation (A), inactivation (B) and recovery(C) currents in atrial and ventricular myocytes. The normalized steady-state activation (D), inactivation (E) and recovery (F) curves of sodium currents were plotted in atrial and ventricular myocytes. (G) The current–voltage relationships of sodium channels in absence and presence of acehytisine.

3.6. Positive rate-dependent blockage of acehytisine on I_{Na}

The rate-dependent blockage of I_{Na} by acehytisine in atrial myocytes was presented in Fig. 6. In control, the peak amplitude of I_{Na} barely had any changes after 20 repetitive depolarizing pulses at 0.5–4 Hz (n = 5). On the contrary, 10 μmol/L acehytisine ($P > 0.05$, n = 6) produced a progressive decline of the I_{Na} amplitude by a 1–4 Hz depolarization when the holding potential was clamped to −90 mV ($P < 0.05$–0.01, n = 6). The suppression of I_{Na} by acehytisine was enhanced along with an increase in the frequency of stimuli, showing a positive rate-dependent manner. However, when the holding potential was shifted to hyperpolarization (−120 mV), inhibition of I_{Na} was found only at the fastest frequency of stimuli (4 Hz, $P < 0.05$, n = 6). In addition, we found that the amplitudes of sodium currents at the first pulse did not decrease since no marked blocking effect occurred during the diffusion of 10 μmol/L acehytisine at various frequencies. The aforementioned results suggest that acehytisine inhibited I_{Na} without tonic blocking effects since it did not bind to sodium channels in the pre-open (resting) state.

3.7. Effects of acehytisine on slow inactivation of I_{Na}

To assess the blockage of I_{Na} during depolarization, a function of various durations of depolarization protocol, which can be clarified to the time course of blocking development by acehytisine, was applied as shown in Fig. 7. In comparison to control in which current amplitude gradually diminished over time and nearly achieved a plateau after 1 s, I_{Na} was speedily decreased by 47% at 1000 ms, and remained persistently reduced after 1000 ms in the presence of 10 μmol/L acehytisine. This phenomenon was more prominent in the presence of 50 μmol/L acehytisine than in its low concentration. Meanwhile, the time course of inactivation was fitted to a single exponential equation with τ values of 146.88 ± 55.55 ms (n = 6) while the time course of inactivation can be simulated finely by a double exponential equation both in the presence of 10 and 50 μmol/L acehytisine with two τ values (τ_{fast} and τ_{slow}) representing the effects of acehytisine on fast and slow inactivation course of I_{Na} respectively. The values of τ_{fast} and τ_{slow} were 48.50 ± 4.60 ms (vs. control, $P < 0.01$, n = 6) and 1333.06 ± 35.4 ms after 10 μmol/L acehytisine administration, and 22.11 ± 1.37 ms (vs.

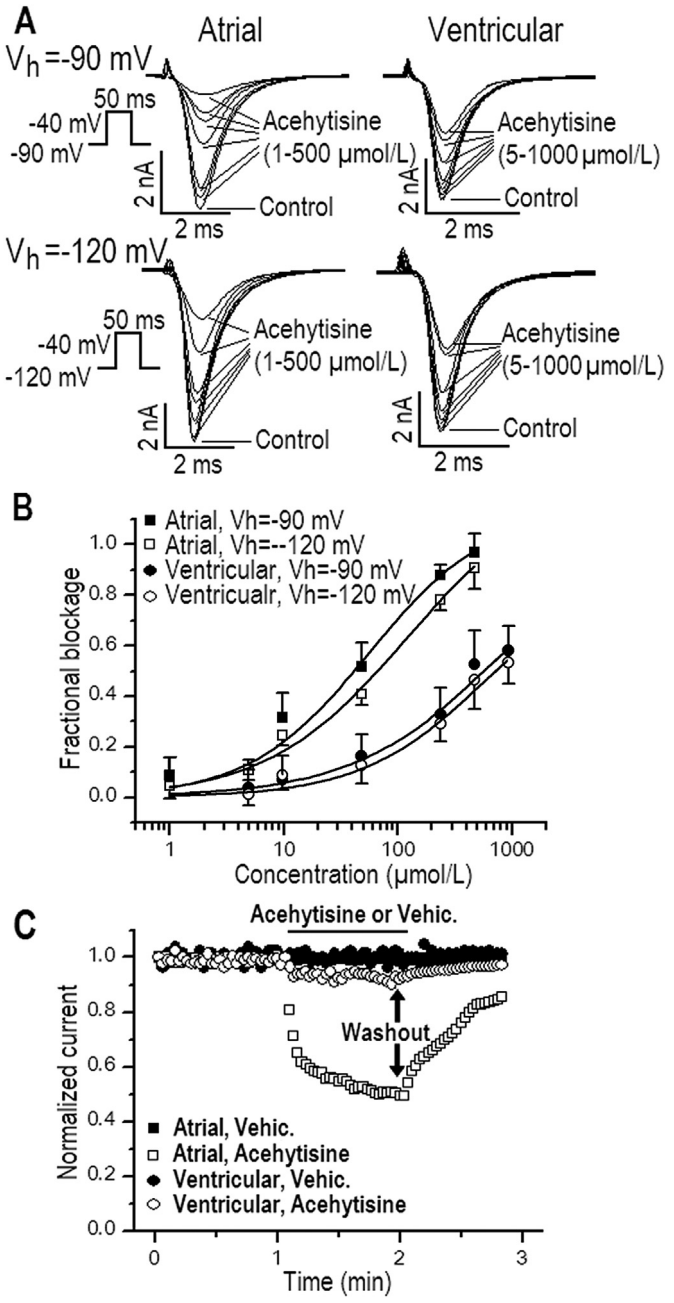

Fig. 3. Concentration-dependent blockage of acehytisine in atrial and ventricular myocytes. (A) Original current tracings of sodium channel were superimposed before (control) and after superfusion of acehytisine in atrial and ventricular myocytes at holding potentials of −90 mV or −120 mV (B)The concentration–response curve is plotted based on data from panel A and B, and fitted by the Hill equation. (C) Typical time course of sodium channels for a vehicle control and for 100 μmol/L acehytisine washed in and out in atrial and ventricular cells. The current amplitudes were not decayed significantly in the control condition. Arrows represented washout.

10 μmol/L acehytisine, $P < 0.01$, n = 6) and 1375 ± 10.61 ms (vs. 10 μmol/L acehytisine, $P > 0.05$, n = 6) after 50 μmol/L acehytisine administration. These results indicate that acehytisine is an I_{Na} blocker with affinity to multi-state sodium channels and that it might bind to sodium channels in both open and inactivated states.

4. Discussion

Recently, acehytisine has been classified mainly as a type I AAD with multi-ion channel blocking effects (11–17). Using concentrations comparable to those in clinical trials, we found that 1–500 μmol/L acehytisine significantly inhibited atrial I_{Na}. Huang et al. (10) and Yang et al. (23) have determined the plasma concentrations of acehytisine (49.98 μg/ml and 14.49 μg/ml) in human. In this study, we postulate that acehytisine can block sodium channels in vivo and possesses anti-arrhythmic effects under the therapeutic concentration. Our work demonstrates that inhibition of I_{Na} by acehytisine was significantly larger in atrial myocytes than in ventricular ones. Meanwhile we also show that atrial sodium channel had a higher current density than its ventricular counterpart, which was consistent with previous evidences in canine (5). The values of IC_{50} between atria and ventricles clearly differed by approximately one order of magnitude, especially in depolarized condition. Similarly, Suzuki et al. have also shown that amiodarone has stronger inhibitory effects on sodium channels in atria than in ventricle (IC_{50}: 1.4 ± 0.3 μmol/L in atria vs. 40.4 ± 11.9 μmol/L in ventricles) (24). It seems that amiodarone is more atrial-selective than acehytisine. However, simple comparison of our study with that of Suzuki et al. is not readily justified because of differences in experimental conditions, methods, and animals.

To explain the different effects of acehytisine on atrioventricular sodium channels, we studied the dynamic characteristics of sodium channels in the presence of acehytisine. The greater depolarization of RMP in atria than in ventricle often lead to larger fractions of sodium channels in atria under the state of inactivation than those in ventricle (2,25). Thus, we investigated the difference of blockage by acehytisine under various holding potentials. We found that acehytisine (at low concentration) did not affect sodium channels from hyperpolarization of membrane potential while it induced the significant suppression of I_{Na} in rabbit myocardium when cell membrane was shifting to positive potentials. This suggests that acehytisine, like amiodarone (26,27), has low affinity on the resting state of channel, but can exert state-dependent blockage on sodium channel. By using a series of protocols to further evaluate the channel state of drug binding, we found that acehytisine not only significantly shifted steady state inactivation curves toward negative potentials, but also slightly shifted steady state activation curves toward positive potentials in atrial myocytes. While the former suggests that the rate of sodium channel inactivation was accelerated, the latter indicates that the course of channel opening was delayed. The changes in activation and inactivation kinetics can reduce the I_{Na} "window current" or "late I_{Na}", which is an important origin of arrhythmia, systolic dysfunction and cell injury (28). Like ranolazine, acehytisine also possesses the strong blocking activity for late I_{Na} (29). In addition, I_{Na} at the first pulse was not reduced in presence of acehytisine, since no marked blocking effect appeared during the diffusion drug at various frequencies, suggesting that acehytisine might interact with the inactivation state or open state of sodium channel but not the resting state.

To further validate this hypothesis, we also investigated the effects of acehytisine on slow inactivation of I_{Na}. We found that acehytisine could change the kinetics of both fast and slow inactivation of sodium channels. It speedily decreased the amplitude of I_{Na} at early depolarization and persistently reduced it after late depolarization, while it affected the control differently, with sodium channels being quickly inactivated at early depolarization, but not continuously blocked while the time courses of depolarization being prolonged. As we know, sodium currents include two components that possess different gating configurations. In presence of acehytisine, sodium channels at different states have several specific-affinities to drugs, suggesting that the kinetics of inactivation differed from that in control. Based on these results, we conclude that acehytisine can preferentially block open and inactivated sodium channels with the detailed sites binding to sodium channels being validated by several electropharmological

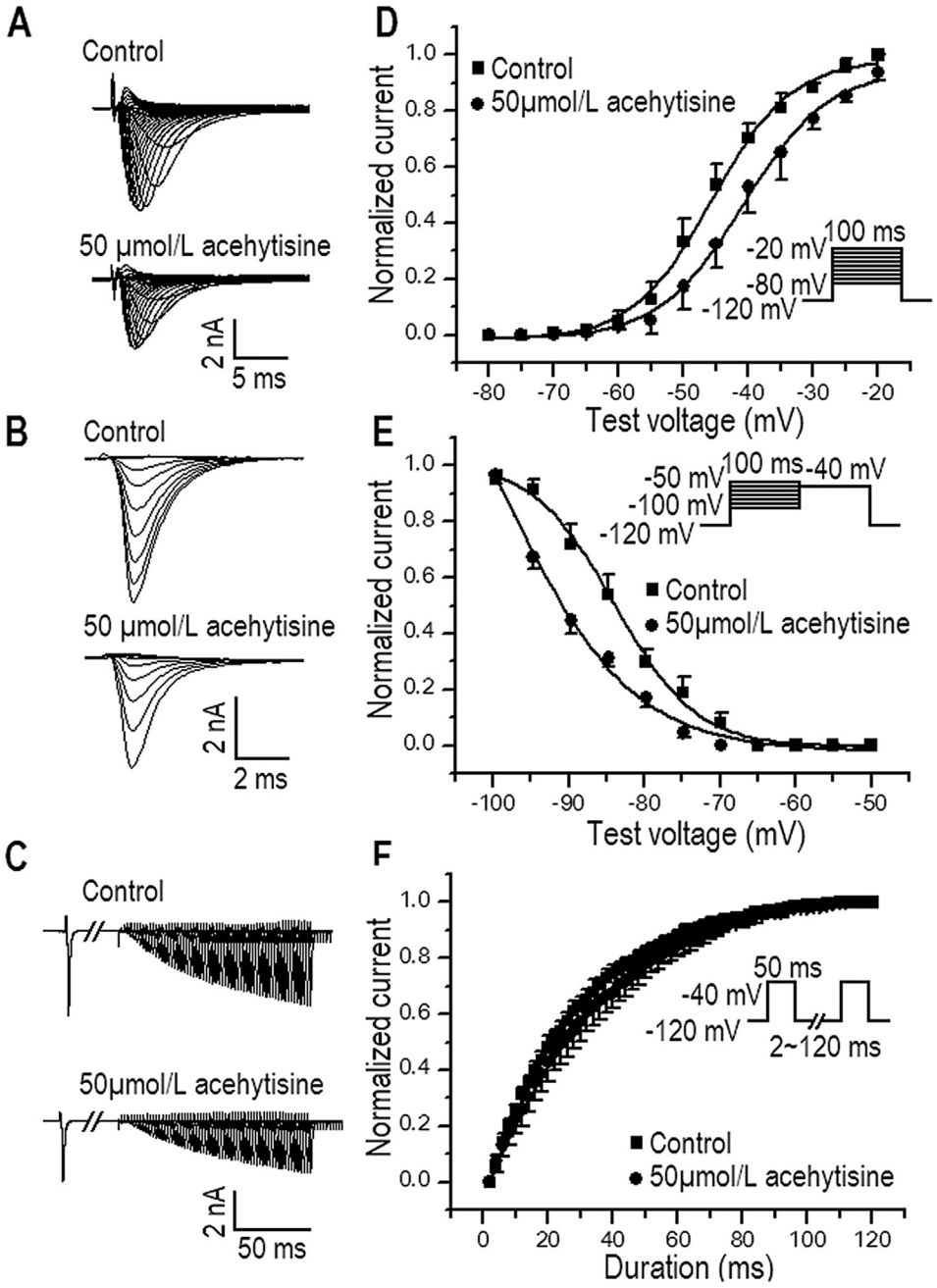

Fig. 4. Effects of acehytisine on steady-state activation, inactivation and recovery from inactication of I_{Na} in atrial myocytes. (A–C) Activation, inactivation and recovery from inactication of I_{Na} of atrial myocytes before and after superfusion of 50 μmol/L acehytisine, respectively. (D–F) The effects of 50 μmol/L acehytisine on steady-state activation, inactivation and recovery curves of I_{Na} in atrial myocytes, respectively.

experiments on model cells heterologously expressing mutational channel proteins.

We also found that acehytisine inhibited I_{Na} in a positive rate-dependent manner, consistent with the results from our recording AP. The faster the frequency of stimulation, the more frequently the channels become activated and inactivated in unit time and the more frequently the channels opened and closed. Thus, high frequency stimulation increased the probability of drug binding to the receptor sites of the sodium channel, thereby reducing sodium channels shifting from the resting state to the open state. As sodium channels accumulate in the inactivation state, only a small proportion of sodium channels are left to be activated. That is, the amount of channel opening time was reduced in unit time. The more frequently channels open (the faster the heart rate is), the stronger the blocking effect of acehytisine becomes, a mechanism resembling the effects of ranolazine, a preferred drug for atrial tachyarrhythmia treatment. In addition, acehytisine did not modify the recovery kinetics from inactivation, indicating that it rapidly dissociated from the sodium channel under repolarization, a property recently suggested to contribute to atrial-selectivity of sodium channel blocker (2,3,25).

In addition, the results from APs showed that acehytisine produced the larger descent of V_{max}, whose value depends on the activity of sodium channels, in atrial than in ventricular myocytes, consistent with outcomes from our ion channel recording.

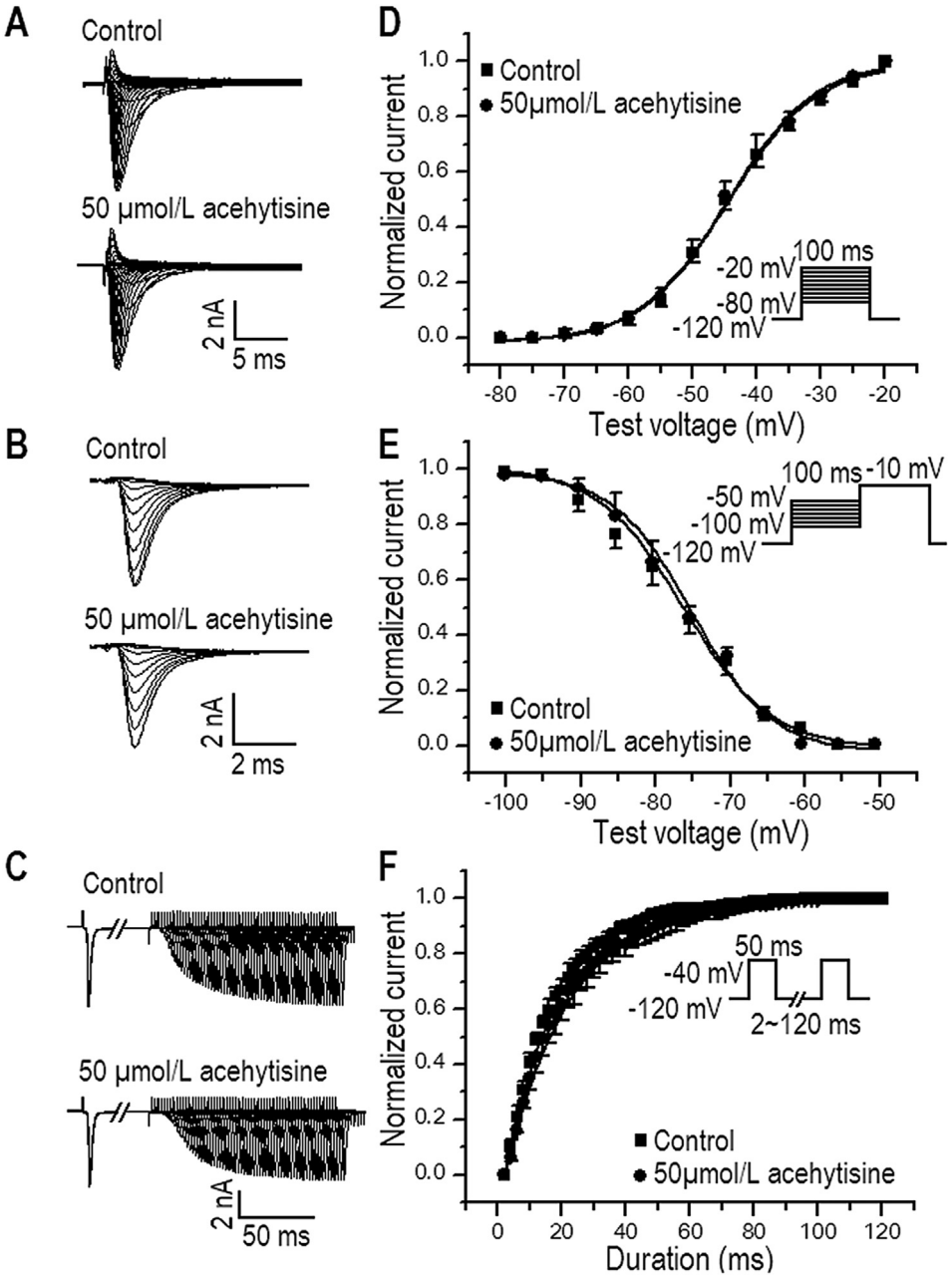

Fig. 5. Effects of acehytisine on steady-state activation, inactivation and recovery from inactication of I_{Na} in ventricular myocytes. (A–C) Activation, inactivation and recovery from inactication of I_{Na} of ventricular myocytes before and after superfusion of 50 μmol/L acehytisine, respectively. (D–F) The effects of 50 μmol/L acehytisine on steady-state activation, inactivation and recovery curves of I_{Na} in ventricular myocytes, respectively.

Meanwhile, it did not prolong the duration of AP in ventricular myocytes, a fact attributable to its little effect on HERG channel with the exception of the concentration exceeding 1000 μmol/L (15,17), indicating that acehytisine is a safe AAD with little the side-effect on QT interval prolongation and TdP. Together with the inhibition of other ion channels, I_{Kur}, HCN currents by acehytisine, atrial APD_{90} were significantly prolonged at fast pacing, which could enhance blocking effects of sodium channels by prolongation of the depolarizing time. Comparing to sinus rhythm, the pharmacological action can be enhanced during fast atrial rate and long APD under state of AF. Furthermore, it has been reported that AF, originated from pulmonary vein myocardium (a special type of atrial myocardium) could be effectively terminated by sodium channel blocker such as pilsicainide in experiments and clinical trials (30–32). In all, seeking and developing atrial–selective sodium channel blockers with high efficiency and low toxicity has been considered as a novel strategy for the anti-AF (1–3).

In summary, the present work suggests that acehytisine exerts atrial-selective blockage on sodium channels and may have high affinity to certain states of channels. Like other selective blockers, acehytisine has rapid kinetics on sodium channels which may contribute to the substance's anti-AF efficacy and its reduced proarrhythmic effects at the ventricular level. Therefore, our findings are likely to shed important light on the clinical applications of acehytisine in the treatment of AF.

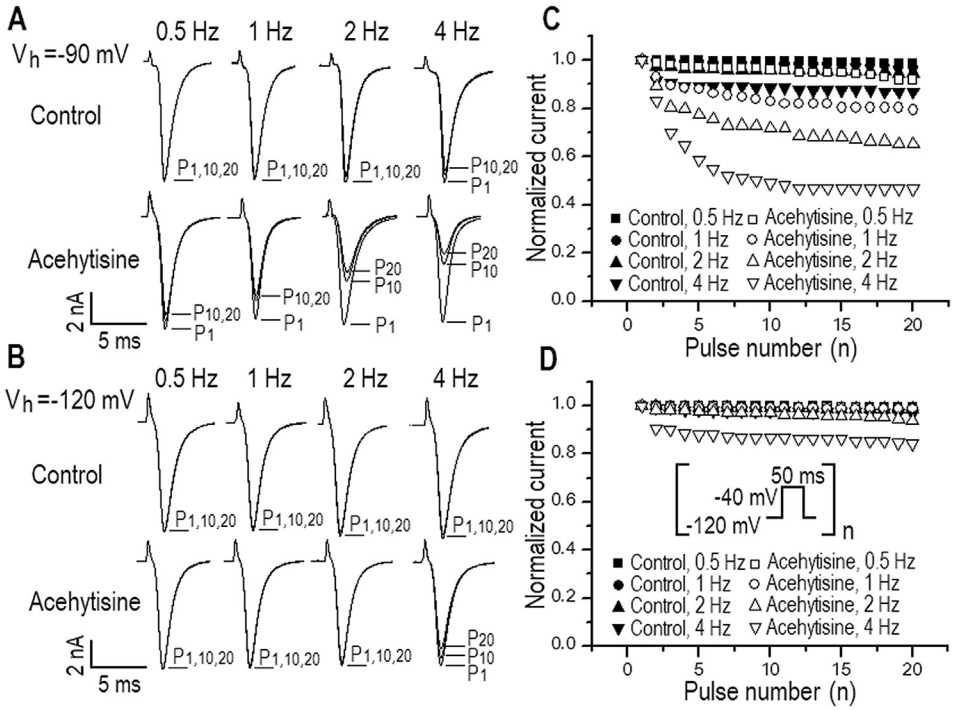

Fig. 6. Rate-dependent blockage of I_{Na} by acehytisine in atrial myocytes. Superimposed tracings of I_{Na} obtained from different experiments: in control at 0.5–4 Hz, and in the presence of 50 μmol/L acehytisine at 0.5–4 Hz, when holding potentials of −90 mV (A) or −120 mV (B) were applied. (C) and (D) The normalized currents of sodium channels were plotted against each pulse number based on data from panel A and B, respectively.

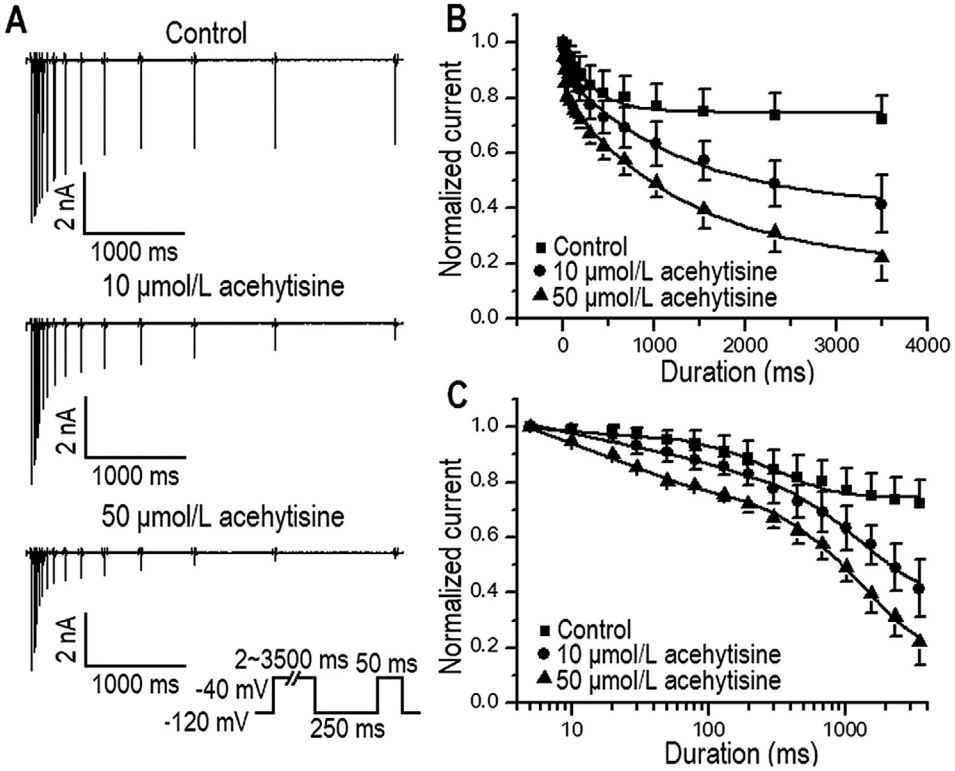

Fig. 7. Effects of acehytisine on slow inactivation of I_{Na} in atrial myocytes. (A) Tracings of I_{Na} in atrial myocytes before and after superfusion of 10 or 50 μmol/L acehytisine. The normalized currents of sodium channels were plotted against various durations of depolarization and fitted by the single or double exponential equation, X axis was expressed by arithmetic (B) or logarithm (C).

Conflict of interest

The authors indicated no potential conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

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