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Activation of β_3-Adrenoceptor Promotes Rapid Pacing-Induced Atrial Electrical Remodeling in Rabbits

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Key Words

β_3-adrenoceptor • Atrial electrical remodeling • L-type calcium current • Inward rectifier potassium current • Transient outward potassium current

Abstract

Cardiac electrophysiological function is under the regulatory control of the sympathetic nervous system. In addition to classical β-adrenoceptors (β-AR, including β_1- and β_2- subtypes), β_3-AR is also expressed in human heart and shows its distinctive functions. This study is aimed to elucidate the role of β_3-AR in the regulation of atrial fibrillation (AF), especially its role in rapid pacing-induced atrial electrical remodeling in rabbits. The rapid atrial pacing model was established by embedding electrodes in the right atrium pacing at a speed of 600 beats per minute. The protein level of β_3-AR in the atria was found significantly upregulated by western blot. The atrial effective refractory period (AERP) and its rate adaptation were decreased after pacing which were further shortened by BRL37344, a selective β_3-AR agonist, leading to the increase of AF inducibility and duration. Similarly, β_3-AR activation induced time-dependent shortening of action potential duration (APD), together with decrease of L-type calcium current ($I_{Ca,L}$) and increase of inward rectifier potassium current (I_{K1}) and transient outward potassium current (I_{to}) in rapid pacing atrial myocytes. Meanwhile, all the effects were abolished by specific β_3-AR antagonist, SR59230A. In summary, our study represents that activation of β_3-AR promotes the atrial electrical remodeling process by altering the balance of ion channels in atrial myocytes, which provides new insights into the pharmacological role of β_3-AR in heart diseases.

Introduction

Atrial fibrillation (AF) is highly prevalent and associated with pronounced morbidity, mortality and socioeconomic burden. This pathological entity is related to alteration of atrial electrophysiology that promotes its own occurrence and maintenance, often referred to as 'electrical remodeling'. Atrial electrophysiological remodeling caused by rapid atrial pacing was used to study the underlying mechanisms of AF [1]. Previous studies have shown that atrial tachycardia-induced electrical remodeling includes decrease of atrial effective refractory

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period (AERP) and loss of physiological rate adaptation [2-4]. In addition, standard microelectrode studies of human atrial preparations have proved that action potential duration (APD) changes are one of the important mechanisms for AF-induce ERP alterations [5]. Recently, several studies have demonstrated that shortening of AERP and APD during tachycardia-induced remodeling are the results of dysfunction of ion channels, including diminished L-type Ca^{2+} channel, increased activity of the inward rectifier K^+ channel (I_{K1}) and down-regulation of the transient outward K^+ channel (I_{to}) [6].

An imbalance of subnormal cardiac parasympathetic regulation coupled with elevated cardiac sympathetic activation is involved in many cardiovascular diseases including AF, while enhanced cardiac parasympathetic regulation is associated with restoration of β-AR balance and protection of AF induced by acute myocardial ischemia [7]. It has been recognized that at least three different adrenoceptor subtypes, β_1-, β_2- and β_3-adrenoceptors (β-ARs), participated in the regulation of cardiac function and electrical activity [8-10]. β_3-AR differs from β_1-and β_2-AR subtypes in its molecular structure and pharmacological functions [11]. Recently, the critical role of β_3-AR in regulation of ion channels has been paid more and more attention. For example, Bosch et al demonstrated that preferential β_3-AR agonist BRL37344 inhibited the slow rectifier potassium channel (I_{Ks}) leading to an increase of APD in guinea pig ventricular myocytes [12]. Daniel et al found that activation of β_3-AR with isoproterenol resulted in marked increase of Kir2.x potassium channels [13]. Moreover, Skeberdis et al proved that β_3-AR activation could stimulate the L-type Ca^{2+} current ($I_{Ca,L}$) and increase human atrial tissue contractility [14].

However, few studies have focused on the effects of β_3-AR on cardiac arrhythmogenesis *in vivo*. Recently, Zhou et al found that chronic subcutaneous administration with BRL37344 could significantly reduce the occurrence of ventricular tachycardia [15]. In contrast to its effect on ventricular myocardium, BRL37344 can produce positive inotropic effect in isolated right atrium [16]. Interestingly, previous studies suggested that the role of β_3-AR on human atrium was different from that in ventricles [14]. Thus, how β_3-AR affects atrial function during atrial arrhythmia remains unknown. Therefore, the present study was designed to evaluate the potential role of β_3-AR in early atrial electrical remodeling. Meanwhile, the effects of the selective β_3-AR agonist, BRL37344 on atrial electrical remodeling *in vivo* were examined. In addition, the effect of β_3-AR on the ion channels was assessed in rapid pacing atrial myocytes, which provided further insights into the mechanism of β_3-AR stimulation on atrial electrical properties. The current findings extended our knowledge regarding the β-adrenergic regulation of electrical properties in AF-induced remodeling and provided promising insight into the potential mechanisms involved in the atrial electrical remodeling.

Materials and Methods

Rabbit model of rapid atrial pacing

Male New Zealand white rabbits weighing 2.0~2.5 kg were housed individually in a room with a 12:12-hrs light-dark cycle and allowed unlimited food and water. The animals were randomly divided into three groups: sham (P0), rapid atrial pacing for 1 day (P1) and 7 days (P7) groups. All rabbits were anaesthetized with ketamine (Sigma Aldrich) and xylazine (Sigma Aldrich) mixture (7:1) at a dosage of 1.2 ml/3 kg (i.m.). After intubation and mechanical ventilation, medical thoracotomy was performed. One thin silicon plaque containing two pairs of electrodes (electrode diameter 1 mm; interelectrode distance 1 mm; distance between electrode pairs 10 mm) was sutured to the right atrium [17]. One pair was connected to a pacemaker (Fudan University, Shanghai, China). The pacemaker was implanted in a subcutaneous pocket on the back of the rabbit. The other was tunneled subcutaneously and exposed at the back of the rabbits used for electrophysiological measurements. After one week recovery, the rabbits in P1 group were paced at 600 beats per minute for 1 day, and the rabbits in P7 group were paced at 600 beats per minute for 7 days [18]. All animal procedures were approved by the Ethical Committee for Animal Experiments, Harbin Medical University, and confirmed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Experimental protocol

Each group was divided into three groups again: control, BRL37344 and SR59230A group respectively. In control group, rabbits were injected with saline and then performed electrophysiological measurement. In BRL37344 group, the rabbits were intervenous injected a preferential β_3-AR agonist, BRL37344 (Sigma-Aldrich, St Louis, MO, USA), sodium 4-{2-[2-hydroxy-2-(3-chlorophenyl)-ethylamino]propyl} phenoxyacetate esquihydrate, at a dose of 83 μg/kg [15, 19]. 20 minutes prior to treatment with BRL37344, the rabbits were injected with nadolol (Sigma-Aldrich, St Louis, MO, USA) at a dose of 1 mg/kg to exclude the stimulation effect of BRL37344 on β_1-and β_2-ARs [20]. Five minutes after treatment with BRL37344, electrophysiological measurement was performed. In SR59230A group, the rabbits were first pretreated with BRL37344 and nadolol the same as BRL37344 group, then injected with a selective β_3-AR antagonist, SR59230A 3-(2-ethylphenoxy)-1-[(1,S)-1,2,3,4-tetrahydronapth-1-ylamino]-2S-2-propanoloxalate (Sigma-Aldrich, St Louis, MO, USA), at a

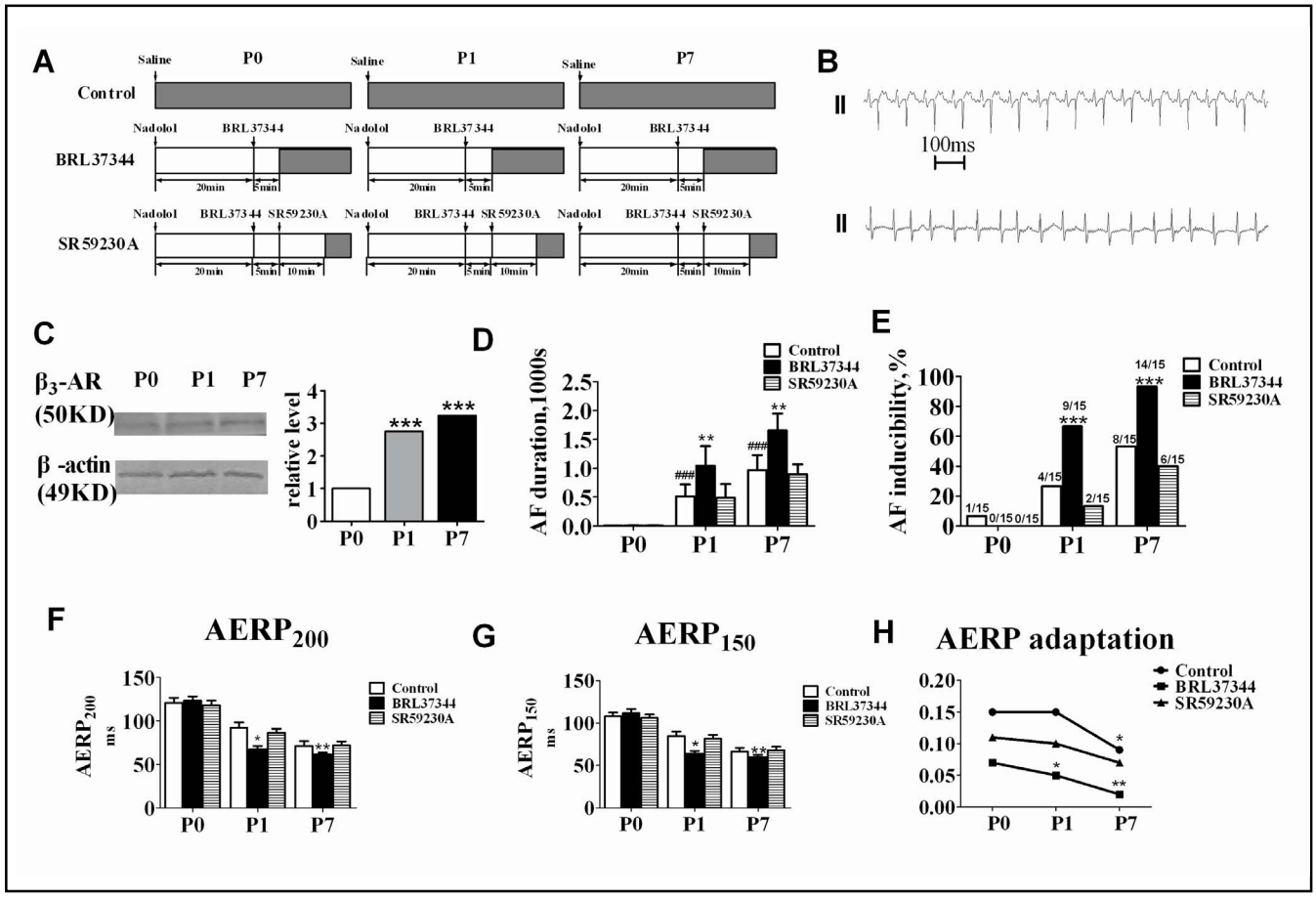

Fig. 1. Electrical measurement protocol and electrical property changes affected by rapid atrial pacing and β_3-adrenoceptor (β_3-AR) activation *in vivo*. (A) Diagram illustrating the experimental protocols for electrical measurement *in vivo*. The shadow part presented the process of electrical measurement. (B) Top panel: surface electrocardiographic electrogram (ECG) (lead II) in the rabbit during rapid atrial pacing with 600 beats/min. Bottom panel: atrial fibrillation (AF) was recorded in a surface ECG after burst atrial stimulus (paper speed 100 mm/s). (C) Effect of rapid atrial pacing on the protein expression of β_3-AR. Left, examples of western blot bands; right, quantified as mean ± SEM (n = 5). Levels of β_3-AR increased significantly after rapid atrial pacing for 1 day and 7 days. ***$p < 0.001$ vs. P0 group. (D) AF duration induced by rapid atrial pacing and β_3-AR stimulation. ###$p < 0.001$ vs. P0 group. **$p < 0.01$ vs. control group. (E) β_3-AR activation contributed to the increase of AF inducibility induced by burst atrial stimulus during different period of pacing time. (F) and (G) atrial effective refractory period (AERP) changes at basic cycle length of 200 ms (F) and 150 ms (G) during different pacing time. BRL37344 induced shortening of AERP progressively. *$p < 0.01$, **$p < 0.001$ vs. control group. (H) AERP rate adaptation affected by β_3-AR stimulation. The AERP rate adaptation became lower during rapid atrial pacing. BRL37344 made this maladaptation worse in both P1 and P7 groups, which was restored by SR59230A. *$p < 0.05$, **$p < 0.01$ vs. P0 group.

dose of 1 mg/kg [20]. 10 minutes later, electrophysiological measurement was repeated.

Electrophysiological measurements

AERPs were measured at two basic cycle lengths (BCLs, 150, 200 ms) [21]. A train of eight basic stimuli (S_1) were followed by a premature stimulus (S_2) of 150 ms initially. The coupling interval was shortened in steps by 5 ms until S_2 failed to produce an atrial response. S_1-S_2 intervals were then increased by 5 ms and decreased in 2 ms steps until S_2 capture failure. The AERP was defined as the longest S_1-S_2 interval failing to produce a response. The mean value of three AERP at each BCL was used for data analysis. The degree of AERP rate adaption was defined as difference of two AERPs divided by difference of corresponding BCLs. After AERP was measured, AF was induced with a train of 10 Hz, 2 ms stimuli to the right atrium at four times threshold current [22]. AF was defined as a rapid, irregular atrial rhythm with vary atrial electrogram morphology (Fig. 1B, bottom panel). AF was considered sustained if it persisted for more than 30 minutes, and was distinguished from atrial flutter on the basis of the irregularity of atrial electrogram morphology and frequency. AF was induced ten times if AF

duration was less than 10 minutes in order to estimate the average duration of AF. Two AF inductions were performed if episodes lasted between 10 and 30 minutes. If AF lasted for more than 30 minutes and no briefer episodes occurred, mean AF duration was based on this episode.

Western blot

The protein concentration was determined with Bio-Rad Protein Assay Kit (Bio-Rad, Mississauga, ON, Canada) using bovine serum albumin (BSA) as the standard. The membrane protein samples were extracted from rabbit atriums. Membrane protein sample (~150 μg) was fractionated by SDS-PAGE (7.5%-10% polyacrylamide gels) and transferred to PVDF membrane (Millipore, Bedford, MA). The sample was incubated overnight at 4 °C with the primary antibodies in 1:50~1:200. The expression of β_3-AR was examined with a polyclonal goat antibody raised against the rabbit β_3-AR (Santa-Cruz Biotechnology, Santa Cruz, CA).[23] Next day, the membrane was washed in TBS-T three times (10 min/each) and incubated for 2 hrs with the HRP-conjugated donkey anti-goat IgG (H+L) (1:600) in the blocking buffer. Western blot bands were normalized to β-actin (Santa-Cruz Biotechnology, Santa Cruz, CA) as an internal control.

Isolation of rabbit atrial myocytes

Atrial myocytes were isolated from the rabbits via enzymatic digestion with the procedures similar to previously described [24]. Hearts were rapidly excised after anaesthetization and mounted on the Langendorff apparatus and perfused retrogradely with the following solutions: 1 mM Ca^{2+} Tyrode, Ca^{2+}-free Tyrode, Ca^{2+}-free Tyrode containing collagenase (100 U/ml, type II, Worthington Biochemical) and 1% bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA). Then, the heart was removed and the atria and ventricles were separated. The atrial myocytes were minced in the storage solution and filtered. The freshly isolated myocytes were gently centrifuged and resuspended in the storage solution for subsequent patch-clamp experiments.

Whole-cell patch clamp recording

Patch-clamp techniques have been described in detail elsewhere [24, 25]. Individual currents were normalized to the membrane capacity to control for differences in cell size, being expressed as current density pA/pF. The amplitude of I_{to} was measured as the difference between the initial peak of I_{to} and the current level remaining at the end of the pulse. I_{K1} was measured as the magnitude of the current at the end of the pulse relative to zero reference. The amplitude of $I_{Ca,\ L}$ was measured as the difference between the peak inward currents and the currents remaining at the end of the pulse. In addition to the use of different solutions to optimize recordings of the currents of interest and to avoid unwanted currents, ion channel blockers were also used to prevent the contaminating currents. For studies on K^+ currents, I_{Na} was inactivated by holding the membrane at -40 mV and $I_{Ca,\ L}$ was blocked by $CdCl_2$ (0.3 mM) in the bathing solution. 4-aminopyridine (1 mM) was used to inhibit I_{to} for recording other currents and glyburide (10 μM) plus Mg-ATP (5 mM) in the pipette solution to prevent ATP-sensitive K^+ current. Dofetilide (1 μM) (Avanti Polar Lipid Inc., Alabaster, AL, USA) were used to block I_{Kr}, for I_{to} recordings.

Solutions

Tyrode's solution contained (mM): 126 NaCl, 2 $CaCl_2$, 5.4 KCl, 0.8 $MgCl_2$, 0.33 NaH_2PO_4, 10 dextrose, and 10 HEPES (pH adjusted to 7.4 with NaOH). The high-K^+ storage solution contained (mM): 20 KCl, 10 KH_2PO_4, 10 dextrose, 70 glutamic acid, 10 β–hydroxybutyric acid, 10 taurine, and 10 EGTA (pH adjusted to 7.4 with KOH). The pipette solution for K^+ current recordings contained (mM): 130 KCl, 1 $MgCl_2$, 5 Mg-ATP, 10 EGTA, and 10 HEPES (pH adjusted to 7.25 with KOH). The pipette solution for $I_{Ca,\ L}$ contained (mM): 20 CsCl, 110 Cesium aspartate, 1 $MgCl_2$, 5 Mg-ATP, 10 EGTA, and 10 HEPES (pH adjusted to 7.25 with CsOH). The internal pipette solution for AP recording contained the same components as for K^+ currents recording, except that EGTA was reduced to 0.05 mM. The extracellular solution for I_{to} and I_{K1} currents and AP recording was normal Tyrode's solution. The extracellular solution for $I_{ca,\ L}$ recordings contained (mM): 136 TEA-Cl, 5.4 CsCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES (pH adjusted to 7.25 with CsOH).

Data analysis

Group data are expressed as mean ± S.E. Statistical comparisons (two way-ANOVA followed by Dunnett's method) were carried out using graph prism 5.0. Chi-square analysis was used to compare the occurrence of AF in different groups. A two-tailed $p < 0.05$ was taken to indicate a statistically significant difference.

Results

Effect of rapid pacing on protein level of β_3-AR in rabbit atria

The effects of rapid atrial pacing on the expression of β_3-AR protein were illustrated in Fig. 1C. A significant increase of β_3-AR protein levels was first seen after one day of rapid atrial pacing ($p < 0.001$ vs. P0 group), and a further increase was observed after seven days ($p < 0.001$ vs. P0 group).

Effect of β_3-AR on AF inducibility and duration

AF duration was progressively increased after rapid atrial pacing, in addition, AF lasted much longer after BRL37344 stimulation in both P1 and P7 group (Fig. 1D). The numbers of rabbits with sustained AF were from none in P0 group to 8/15 (53%) in P7 group. Fig. 1E suggested that the inducibility of AF after stimulation with BRL37344 in the rapid atrial pacing rabbits were increased to 9/15 (60%) in P1 group, 14/15 (93%) in P7 group, respectively. After treatment with SR59230A, the inducibility of AF in these groups stayed no difference with the normal control level.

Effect of β_3-AR on AERP and AERP rate adaptation induced by rapid atrial pacing

The tachycardia-dependent reduction in AERP was dramatically affected by rapid atrial pacing. AERP at a cycle length of 200 ms ($AERP_{200}$) and 150 ms ($AERP_{150}$) were both time-dependently reduced in paced rabbits compared with those in P0 group (Fig. 1F, G). In P0 groups, there was no significant difference among these three groups. After pacing for 1 day, $AERP_{200}$ and $AERP_{150}$ were both shortened in BRL37344 group ($AERP_{200}$: 67.33 ± 4.00 ms vs. 92.22 ± 6.11 ms in control group, $p<0.01$; $AERP_{150}$: 64.22 ±3.32 ms vs. 84.67 ± 5.39 ms in control group, $p<0.01$). $AERP_{200}$ and $AERP_{150}$ were decreased more significantly by BRL37344 in P7 group compared with those in the control group ($AERP_{200}$: 61.33 ± 2.00 ms vs. 71.33 ± 5.39 ms in control group, $p < 0.001$; $AERP_{150}$: 60.00 ± 2.65 ms vs. 66.67 ± 4.24 ms in control group, $p < 0.001$). Meanwhile, the effects of BRL37344 on $AERP_{200}$ and $AERP_{150}$ in both P1 and P7 rabbits were inhibited by SR59230A (Fig. 1F, G). Notably, the equation of AERP rate adaptation = ($AERP_{200}$-$AERP_{150}$)/50 ms was employed. Fig. 1H showed that AERP adaptation was not markedly changed in P1 group compared with P0 group, whereas that in P7 group was significantly lower than that in P0 group (0.09±0.04 vs. 0.15±0.11, $p<0.05$). Meanwhile, the AERP rate adaptation after treated with BRL37344 was found significantly decreased both in P1 and P7 group compared with that in P0 group.

Effect of β_3-AR on APD after rapid atrial pacing

As illustrated in Fig. 2A, APD at 50% repolarization (APD_{50}) and 90% repolarization (APD_{90}) were remarkably shortened along with the course of rapid pacing. Then the effect of BRL37344 on APD of atrial myocytes was investigated in pacing rabbits. It was found that BRL37344 (10^{-7} M) slightly prolonged APD_{50} and dramatically prolonged APD_{90} (Fig. 2C-D). Interestingly, BRL37344 could significantly shorten APD_{50} and APD_{90} of atrial myocytes in P1 and P7 groups (Fig. 2E-H). Furthermore, all effects were completely abolished after administering with selective β_3-AR antagonist SR59230A (10^{-7} M) [26]. In addition, the resting membrane potential (RMP) was increased in P1 rabbits (-64.4 ± 4.8mV, n=9 cells, $p < 0.01$) and in P7 rabbits (-73.6 ± 5.5 mV, n=9 cells, $p < 0.001$) compared with that in P0 rabbits (-56.1 ± 5.6 mV) after rapid pacing. In P0 rabbits, RMP was reduced by BRL37344 (-46.6 ± 5.1 mV, n=9 cells, $p < 0.01$ vs. control group), but was increased slightly in P1 group (-70.0 ± 5.7mV, n=9 cells, $p < 0.05$ vs. control group) and in P7 group (-79.5 ± 3.2 mV, n=9 cells, $p < 0.05$ vs. control group).

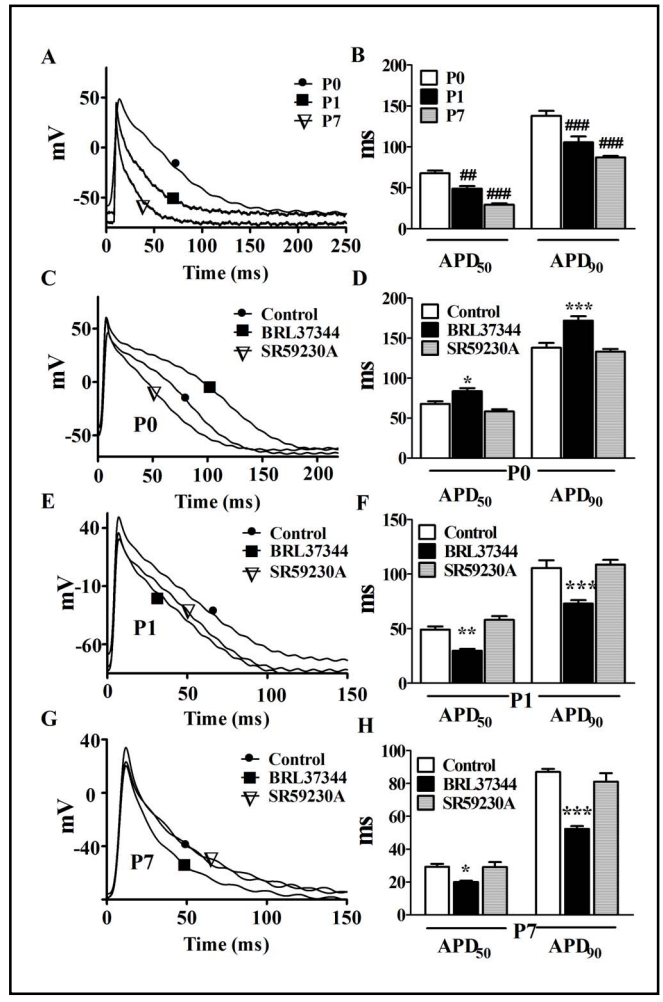

Fig. 2. Effects of pacing and BRL37344 on action potential duration (APD) of atrial myocytes. Action potentials were recorded in current-clamp mode. The action potentials were elicited by a train of 10-consecutive stimuli of 2-ms duration and twice threshold strengths at a frequency of 2 Hz. The recordings obtained from 10th pulses were taken as steady-state value and used for data analysis. Mean data are from control and atrial rapid pacing rabbits (n=9 cells). ##$p < 0.01$ vs. P0, ###$p < 0.001$ vs. P0, *$p < 0.05$ vs. control, **$p < 0.01$ vs. control, ***$p < 0.001$ vs. control.

Effect of β_3-adrenoceptor on $I_{Ca,L}$ after rapid atrial pacing

$I_{Ca,L}$ density was reduced progressively by rapid atrial pacing (Fig. 3). At +10mV, $I_{Ca,L}$ densities were -6.54 ± 0.34 pA/pF in P1 rabbits and -5.70 ± 0.51 pA/pF in P7 rabbits ($p < 0.001$ vs. P0 group), respectively compared with that in P0 rabbits (-13.45 ± 1.32 pA/pF). The

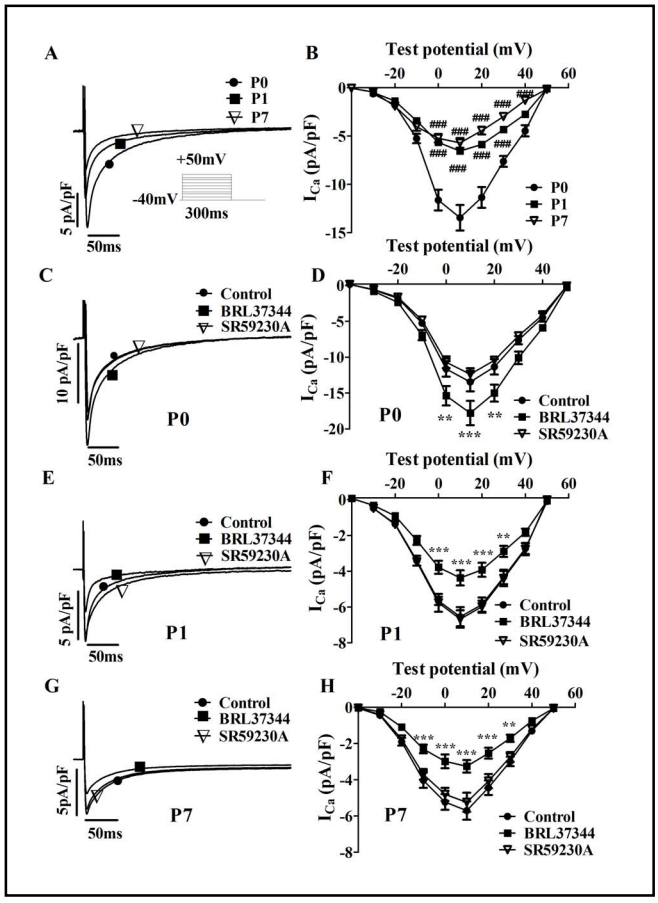

Fig. 3. Effects of pacing and BRL37344 on L-type calcium current ($I_{Ca,L}$) of atrial myocytes. Representative recording of $I_{Ca,L}$ (Fig. 3 A, C, E, G), averaged I-V relationships (Fig. 3 B, D, F, H) in different pacing time atrial myocytes. ###$p < 0.001$ vs. the P0 group. **$p < 0.01$ vs. the control group. ***$p < 0.001$ vs. the control group.

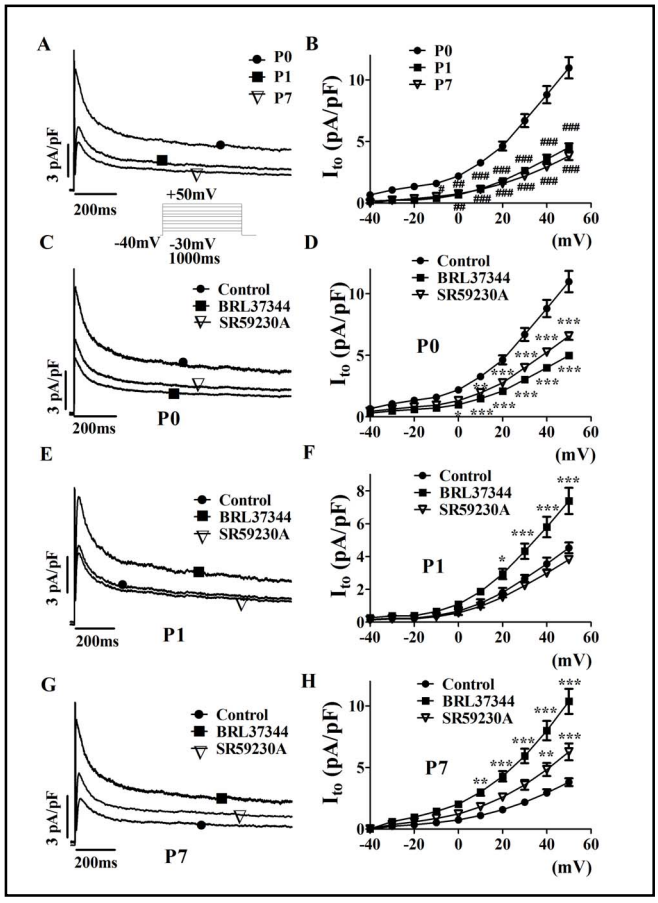

Fig. 4. Effects of pacing and BRL37344 on transient outward potassium current (I_{to}) of atrial myocytes. Left panel: representative recording of I_{to}, Right panel: average I-V relationships in atrial myocytes with different pacing time. ###$p < 0.001$ vs. the P_0 group. **$p < 0.01$ vs. the control group. ***$p < 0.001$ vs. the control group.

recordings of $I_{Ca\,L}$ at +10mV on atrial myocytes isolated from P0, P1 and P7 rabbits were showed in Fig. 3 C,E,G. As shown in Fig. 3D, BRL37344 increased $I_{Ca,L}$ in P0 rabbits at potentials ranging from 0 mV to +20 mV. After the myocytes were exposed to SR59230A, the effect of BRL37344 was inhibited. Interestingly, $I_{Ca,L}$ was decreased about 33% in P1 group (Fig. 3F) and about 43% in P7 group (Fig. 3H) by BRL37344. The peak $I_{Ca,\,L}$ was decreased about 28% in P1 group and 49% in P_7 group. The role of BRL37344 on $I_{Ca,\,L}$ was also abolished by SR59230A.

Effect of β_3-adrenoceptor on I_{to} after rapid atrial pacing

The densities of I_{to} were 4.53 ± 0.33 pA/pF in P1 rabbits and 3.81 ± 0.32 pA/pF in P7 rabbits respectively compared with 10.99 ± 0.87 pA/pF in P0 rabbits ($p < 0.001$, Fig. 4B). After exposure to BRL37344, the density of I_{to} current was significantly reduced in P0 rabbits (Fig. 4D). In contrast, the density of I_{to} current was increased after exposing to BRL37344 in P1 rabbits ($p < 0.001$ in Fig. 4F) and more significant in P7 rabbits ($p < 0.001$ in Fig. 4H). The role of BRL37344 was abolished after exposure to SR59230A.

Effect of β_3-adrenoceptor on I_{K1} after rapid atrial pacing

Investigations have observed that I_{K1} and I_{KACh} were increased in isolated human atrial cells of AF patients, which participated in the process of atrial electrical remodeling. In our present study, we mainly focus on voltage-gated channel, furthermore, we found that the

RMP of atrial myocytes were changed following prolongation of pacing and β_3-AR agonist treatment. Thus, the I_{K1} current was further measured. As shown in Fig. 5A, I_{K1} density was increased obviously after pacing 1day and 7 days. The peak densities of I_{K1} were -12.59 ± 1.35 pA/pF in P1 rabbits and -17.46 ± 1.30 pA/pF in P7 rabbits compared with -7.85 ± 0.40 pA/pF in P0 rabbits (Fig. 5B, $p < 0.01$). After exposure to BRL37344, the density of I_{K1} current was significantly reduced in P0 rabbits at test potentials between -120 mV and -100 mV (Fig. 5D). The I_{K1} current density was increased after BRL37344 treatment in P1 rabbits ($p < 0.05$ in Fig. 5F). The increase of I_{K1} inducing by BRL37344 was even more significant in P7 group. (Fig. 5H). Similar to $I_{Ca,L}$, the role of BRL37344 on I_{K1} current was eliminated by SR59230A.

Discussion

The main finding of the present study was that we have successfully revealed the association between β_3-AR and atrial electrical remodeling in a short-term rapid pacing rabbit model, which was supported by the following evidences: firstly, the level of β_3-AR protein was increased with the prolongation of rapid atrial pacing. Secondly, our study demonstrated that β_3-AR activation could promote the progression of atrial electrical remodeling by shortening AERP and AERP rate adaptation and thus increased AF inducibility and duration *in vivo*. Finally, we found that β_3-AR activation significantly decreased $I_{Ca,L}$ and increased I_{to} and I_{K1}, which might be the plausible mechanism for shortening of APD in atrial myocytes and AERP of atria (summarized in Fig. 6).

Previous studies have shown that AF produced a heterogeneous increase in atrial sympathetic stimulation [27]. Sympathetic hyperinnervation and nerve sprouting was demonstrated in a canine model of rapid atrial pacing [28]. These findings emphasized that prolonged activation of β-AR may result in harmful proliferative response and cell death. Moreover, β-ARs (mainly β_1- and β_2-AR) underwent functional desensitization after long-term exposure to catecholamine. Nevertheless, several studies suggested that β_3-adrenergic response could be preserved, however, β_1-and β_2-adrenergic responses were diminished during prolonged activation of the sympathetic nervous system [29]. Furthermore, studies *in vivo* and *in vitro* have shown that β_3-AR was activated only at a high concentration of catecholamine, which was close to pathophysiologically relevant state [20, 30]. In addition,

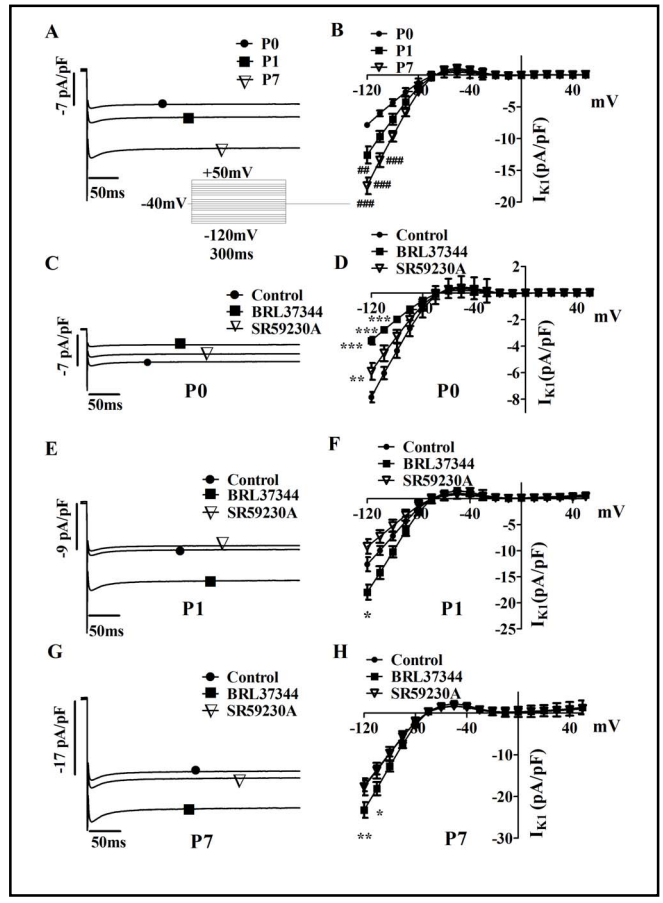

Fig. 5. Effects of pacing and BRL37344 on inward rectifier potassium current (I_{K1}) of atrial myocyes. Left panel: representative recording of I_{K1}, Right panel: averaged I-V relationships in different pacing time atrial myocytes. ##$p < 0.01$, ###$p < 0.001$ vs. the P0 group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. the control group.

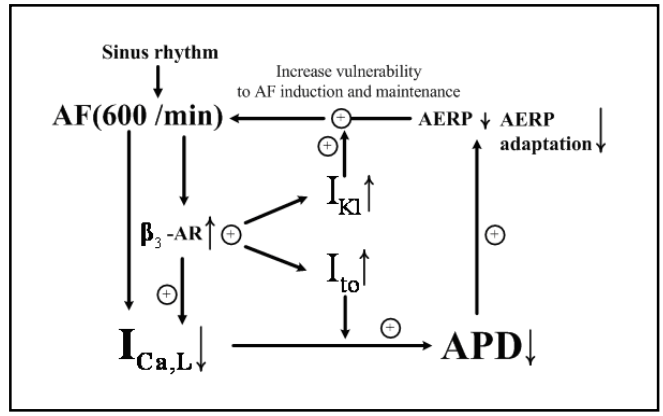

Fig. 6. Schematic illustrating the way in which AF and β_3-AR activation resulted in alternation of ion channels. Burst atrial pacing caused the upregulation of β_3-AR. After specific agonist BRL37344 stimulation, β_3-AR was activated and induced disturbance of several ion channels which contributed to shortening of APD and AERP, and in which way promoted AF initiation and perpetuation.

Chamberlain et al detected β_3-AR expression in human atrium [31] and Skeberdis et al found that β_3-AR activation increased human atrial tissue contractility and stimulated $I_{Ca,L}$ *in vitro* in physiological conditions [14]. Based on previous studies, we hypothesized that activation of β_3-AR due to exposure to the excess catecholamine might process this main adrenergic regulatory factor of AF. However, the exact role of β_3-AR in pathological conditions remained unclear. In our study, we found that BRL37344 stimulation, the number of sustained AF in rabbits induced by burst pacing was progressively increased and the increase continued following the prolongation of rapid atrial pacing, which was not observed in the sham rabbits. To explore the further underlying regulation of β_3-AR on electrical remodeling, we observed the characteristic features of tachycardia-induced atrial electrical remodeling [1, 32], and found that BRL37344 could remarkably decrease AERP and AERP adaptation in the rapid atrial pacing rabbits. Importantly, nadolol was used to eliminate the β_1-and β_2-AR effects, meanwhile the specific β_3-AR antagonist SR59230A was used to confirm of the crucial role of β_3-AR.

Evidences demonstrated that decrease of AERP caused by rapid atrial pacing was due to shortening of APD [33, 34]. The shortening of AERP and APD during atrial tachycardia-induced remodeling occurred within minutes after onset of AF, which might be due to the disturbance of ion channels (Fig. 3-5). The diminished amplitude of $I_{Ca,L}$ current contributed to APD shortening [34]. Rania Gaspo et al [21] found that β-AR number and affinity were not altered by chronic atrial tachycardia, suggesting that the reductions in $I_{Ca,L}$ caused by atrial tachycardia are not due to alterations in β_1 and β_2 -AR function. It was worth noting that in their study, only the classical β-AR, i.e. β_1 and β_2-AR function was investigated. However, whether β_3 -AR had participated in was unknown. In canine and rat ventricular myocytes of heart failure model, BRL37344 induced a negative inotropic effect, which was associated with an inhibition of $I_{Ca,L}$ and decreased amplitude of the intracellular calcium transients [16, 35]. Furthermore, BRL37344 led to a concentration-dependent APD shortening. The inhibition of BRL37344 in failure ventricular myocytes was greater than that in normal ventricular myocytes. However, Skeberdis demonstrated that β_3-AR activation by three β_3-AR agonists increased human atrial tissue contractility and stimulated the L-type Ca^{2+} channel [14]. Basing on previous studies, the role of β_3-AR on ventricle and atrium may be opposing. In our study, we found that β_3-AR stimulation by BRL37344 could significantly increase $I_{Ca,L}$ density of P0 rabbits, which was consistent with what Skeberdis had reported. Interestingly, β_3-AR stimulation remarkably decreased $I_{Ca,L}$ densities and shortened APD in atrial myocytes after rapid pacing in the present study. It was well known that in the model of rapid atrial pacing, the burst atrial tachycardia produced intracellular calcium overload that threatened cell viability. Initially calcium overload caused a markedly and functional reduction of the $I_{Ca,L}$ by a negative feedback mechanism. We found that BRL37344 slightly prolonged APD of no-pacing atrium, while shortened APD of rapid atrial paced myocytes, which was consistent with the changes of $I_{Ca,L}$ induced by BRL37344.

Recent investigations have shown that the modulation of APD by β_3-AR stimulation could also be achieved through the regulation on potassium channels [11, 12]. However, the effect β_3-AR on I_{to} has not been well studied. In our study, we observed that BRL37344 could increase the density of I_{to} in rapid paced atrial myocytes. Our findings is consistent with the previous report that rapid atrial pacing induced down-regulation of I_{to} [18]. However, the stimulation of β_3-AR conversely induced the increase of I_{to}, which is not paralleled with the change induced by atrial tachycardia model. But the increase of I_{to} induced by BRL37344 contributed to the shortening of APD, especially APD_{50}. It should be noted that we found activation of β_3-AR only increased the inward part of I_{K1}, while the outward part was not changed. These changes did not result in the shortening of APD but the increase of RMP. Previous studies showed that increase of I_{K1} in animal models of atrial tachypacing was associated with increased susceptibility to atrial arrhythmias [36]. Although the increase of I_{K1} induced by BRL37344 did not participate in the regulation of APD, it could aggravate the progress of atrial electrical remodeling by accelerating AF rotors.

Interestingly, the changes induced by β_3-AR activation in P0 rabbits were contradicting to the changes in rapid atrial pacing rabbits. It might be related to the level of β_3-AR expression. β_3-AR expression in atrium was lower than that in ventricle and this difference made β_3-AR activation play opposite roles between atrium and ventricle [8]. In a physiologic condition, β_3-AR activation induced the prolonged APD of atrial, which might play a protective role through negative feedback during intense adrenergic stimulation. In atrial tachycardiac condition, we found β_3-AR expression in atriums was up-regulated during the prolongation of rapid atrial pacing. Activation of β_3-AR in rapid atrial pacing rabbits might play a deleterious role with a persistent shortening of AERP and

APD, which would promote the development of atrial electrical remodeling.

It was worth noting that all these data were restricted to the β_3-AR stimulation by a preference β_3-AR agonist, BRL37344 on the rapid atrial pacing rabbits. Studies performed *in vivo* and *in vitro* described the complexity of β_3-AR pharmacology, suggesting a pronounced interspecies variability and the heterogeneous pharmacological profiles in different species [37]. Athough studies have proved that rabbit and human mRNA and protein of β_3-AR share respectively 89 and 92% homology in the sequenced part [23], the regulation of β_3-AR stimulation on human atium remains unclear. Skeberdis found that BRL37344 increased $I_{Ca,L}$ and contractility in human atrial myocytes, while recently, Christ found that BRL37344 mediated increases in $I_{Ca,L}$ in human atrial myocytes are restricted to low temperatures.[38]. So the possible regulation of β_3-AR stimulation in rabbit model in the present study should be prudently applied in AF patients in the future. Since different β_3-AR agonists shows their special affinity to β_1-, β_2-and β_3-ARs [39], further investigations were needed to perform other β_3-AR agonists in evaluation of their regulatory effects in AF model from other species.

Taken together, our present study revealed the role of β_3-AR in promotion of the electrical remodeling that activation of β_3-AR increased the inducibility and duration of AF by altering the dynamic balance of the ion channels. Better knowledge about regulatory role of β_3-AR in AF could lead to better control of AF and it may be novel therapeutic and drug targets.

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