

Research Article

Serum Positive for the Autoantibody against the β_1 -Adrenoceptor from Chinese Patients with Congestive Heart Failure Decreases I_{ss} in Mouse Cardiac Myocytes

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Autoantibodies targeting the β_1 -adrenergic receptor (AAB- β_1) display agonist-like effects, which may have a pathogenic role in the progression of heart failure. Here, we used the electrophysiological recordings to explore the effects of AAB- β_1 -positive serum from Chinese patients with heart failure on the activity of the peak transient outward potassium current (I_{to}) and the end 50 ms steady-state potassium current (I_{ss}) in mouse cardiac myocytes. We found that the AAB- β_1 -positive serum had no effect on the activity of I_{to} , but it produced a decrease in the currents of I_{ss} . A low concentration of positive serum (1/100) had a small inhibitory effect on I_{ss} . However, positive serum at 1 : 10, 1 : 20, and 1 : 50 significantly decreased I_{ss} . The concentration-dependence analysis showed that the EC_{50} of AAB- β_1 -positive serum was 1/60.24 and its nH was 2.86. It indicated that the AAB- β_1 could inhibit I_{ss} in mouse cardiomyocyte in a concentration-dependent manner.

1. Introduction

It has become increasingly clear that autoimmune disorders are a feature of congestive heart failure (CHF) of various etiologies [1, 2]. Over the past few decades, several autoantibodies have been detected in the serum of patients with CHF, including autoantibodies against α -adrenoceptor [3, 4], β_1 -adrenoceptor [5–9], and M_2 -adrenoceptor [10–12]. Autoantibodies targeting the second extracellular loop of the β_1 -adrenergic receptor (AAB- β_1) are specifically associated with the effects of β -blocker therapy and correct prediction of ventricular tachycardia and sudden death in patients with idiopathic dilated cardiomyopathy [8, 13]. *In vivo*, AAB- β_1 can induce β_1 -adrenergic receptor uncoupling, which causes cardiomyocyte apoptosis and sustained calcium influx that results in cardiac electrical instability [14]. These results

suggest that AAB- β_1 displays agonist-like effects that may have a pathogenic role in the progression of heart failure.

Additional evidence has revealed the electrophysiological effects of AAB- β_1 . AAB- β_1 and the IgG fraction containing this antibody significantly enhanced I_{ca} amplitude of adult rat ventricular myocytes [15]. The previous study also showed that purified autoantibodies enhanced cell shortening, prolonged action potential duration, and increased calcium current amplitude of rat ventricular myocytes; these positive effects of AAB- β_1 were indeed mediated via the β_1 -adrenoceptor [14, 16]. However, the effects of AAB- β_1 on voltage-gated potassium channels in mouse ventricular myocytes remained unclear.

In cardiac myocytes, voltage-gated K^+ currents are responsible for the repolarization of the membrane potential and, therefore, influence action potential duration (APD).

Previous studies have described a steady state outward K^+ current aside from the transient outward K^+ current in rat ventricular cells [17–19]. This steady state current displayed a weak voltage-dependent inactivation and was negatively regulated by the β -adrenergic agonist isoprenaline. Thus, this steady state current might play an important role in determining APD during neurohormonal regulation. Therefore, we explored the effects of AAB- β_1 -positive serum from Chinese patients with CHF on the activity of the peak transient outward potassium current (I_{to}) and the end 50 ms steady state potassium current (I_{ss}) in mouse cardiac myocytes.

2. Materials and Methods

2.1. Patients. Fourteen patients admitted to the Department of Cardiology of QiLu Hospital of Shandong University with stable CHF enrolled and submitted to serological tests, coronary angiography, and electro- and echocardiography to discard those with the following pathological conditions: Chagas' disease, hypertrophic cardiomyopathy, acute coronary syndrome, severe hypertension, valvular heart disease, alcohol or drug abuse, insulin-dependent diabetes mellitus, and severe infection. All selected patients had left ventricular ejection fractions (LVEF) $\leq 45\%$ determined by echocardiography (*M* mode). They were receiving standard therapy, including angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, diuretics, and digitalis glycosides during the study. None of them were being treated with β -blockers at enrollment. Five control sera were obtained from voluntary healthy blood donors. The blood was collected and fractioned, and the serum was stored at -20°C until immunological and/or electrophysiological assays were performed. This study was performed in compliance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of QiLu Hospital. All patients gave informed consent for participation.

2.2. Autoantibodies. The target peptide was a fusion protein corresponding to the putative sequence of the second extracellular loop of the human β_1 -adrenergic receptor (amino acids 197 to 222: H-W-W-R-A-E-S-D-E-A-R-R-C-Y-N-D-P-K-C-C-D-F-V-T-N-R), which was commercially synthesized. Peptide purity was ascertained by mass spectroscopy analysis. The presence of autoantibodies was determined by ELISA. ELISA was carried out as previously described [12, 20] with the following modifications: the wells of microtiter plates were coated with this peptide ($10\ \mu\text{g}/\text{mL}$) and incubated for 2 hours. After washing the plate 3 times, $100\ \mu\text{L}$ of 3% skim milk was added to each well for 2 hours. Then $100\ \mu\text{L}$ of patient serum (at dilutions starting from 1:20) was added to the coated wells of the microtiter plate. After washing the plate 3 times, an affinity-purified antihuman immunoglobulin G peroxidase-conjugated antibody (diluted 1:5000) was added to each well for 1 hour. After washing the plates 4 more times, bound peroxidase-conjugated antibody were detected by incubation with the chromogenic substrate for peroxidase. The reaction was stopped with $50\ \mu\text{L}$ of

sulfuric acid, and the optical density was determined at 450 nm. A positive reaction was defined as ≥ 2.5 times the background level. Autoantibodies directed against the β_1 -adrenergic receptor were detected in 6 patients (43%) by ELISA.

2.3. Cell Isolation. Ventricular myocytes were dissociated from the hearts of mice according to previously published protocol [14]. Briefly, 8-week-old male Kunming mice (30–40 g) were anaesthetized with pentobarbitone sodium (30–40 mg/kg), which were injected intravenously together with heparin (100 IU/kg). The heart was removed, washed in a cold calcium-free Joklik MEM (Sigma) solution, and perfused for 5 min on a Langendorff apparatus with the same calcium-free Joklik MEM (containing 11.0 g/L Joklik MEM and 10 mmol/L HEPES, the pH was adjusted to 7.3 with NaOH) warmed to 37°C . The heart was then perfused with collagenase-containing solution (collagenase, 1 mg/mL, Worthington, and BSA 1 mg/mL). After approximately 15 min, the ventricles were removed, placed in fresh solution, cut into $1\ \text{mm}^3$ sections, and gently agitated to dissociate the myocytes. Single ventricular myocytes were collected in KB solution (composition in mM: 30 KCl, 35 KOH, 3 MgSO_4 , 50 L-glutamic acid, 0.5 EGTA, 20 taurine, 10 glucose, and 10 HEPES; pH adjusted to 7.2 with KOH). Cells were stored at $22\text{--}24^\circ\text{C}$.

2.4. Electrophysiological Recordings. The cardiac myocytes were transferred to a recording chamber mounted on an inverted microscope (NIKON TE2000-U) at least 10 min before patch clamping. Micropipettes were made from borosilicate glass capillary with an outside diameter of 1.5 mm. After being fire-polished and filled with pipette solution (composition in mM: 115 K-aspartate, 5 KCl, 4 Na_2ATP , 7 MgCl_2 , 5 EGTA, and 10 HEPES; pH was adjusted with NaOH to 7.2), the resistance was 2–4 M Ω . The junction potential between the patch pipettes and bath solution was nullified immediately before G Ω seal formation. Cell capacitances were read from the potentiometer to set transient capacitances to zero. After the pipette and cell transient capacitance were compensated, the membrane was ruptured with gentle suction to obtain the whole cell voltage-clamp configuration using PCS-5200 micro-operation (Burleigh, USA). Signals were amplified with HEKA EPC-10 patch clamp amplifier and controlled with the Pulse software (HEKA, Lambrecht, Germany). Signals were sampled at 3 kHz and filtered at 1 kHz. The voltage protocol was a 1-s depolarizing step from -50 to $+50\ \text{mV}$ in $10\ \text{mV}$ increments from a holding potential of $-60\ \text{mV}$. The peak of the current was the transient outward potassium channel current (I_{to}), and the end 50 ms of plateau potential current was I_{ss} . All experiments were performed at room temperature ($22\text{--}25^\circ\text{C}$). The ventricular myocytes were perfused with normal bath solution (BS, composition in mM: 135 ChCl, 5.4 KCl, 1.2 MgCl_2 , 0.5 CdCl, 10 glucose, and 10 HEPES; pH was adjusted to 7.4 with NaOH) for 10 min to stabilize the currents. For analysis of autoantibody

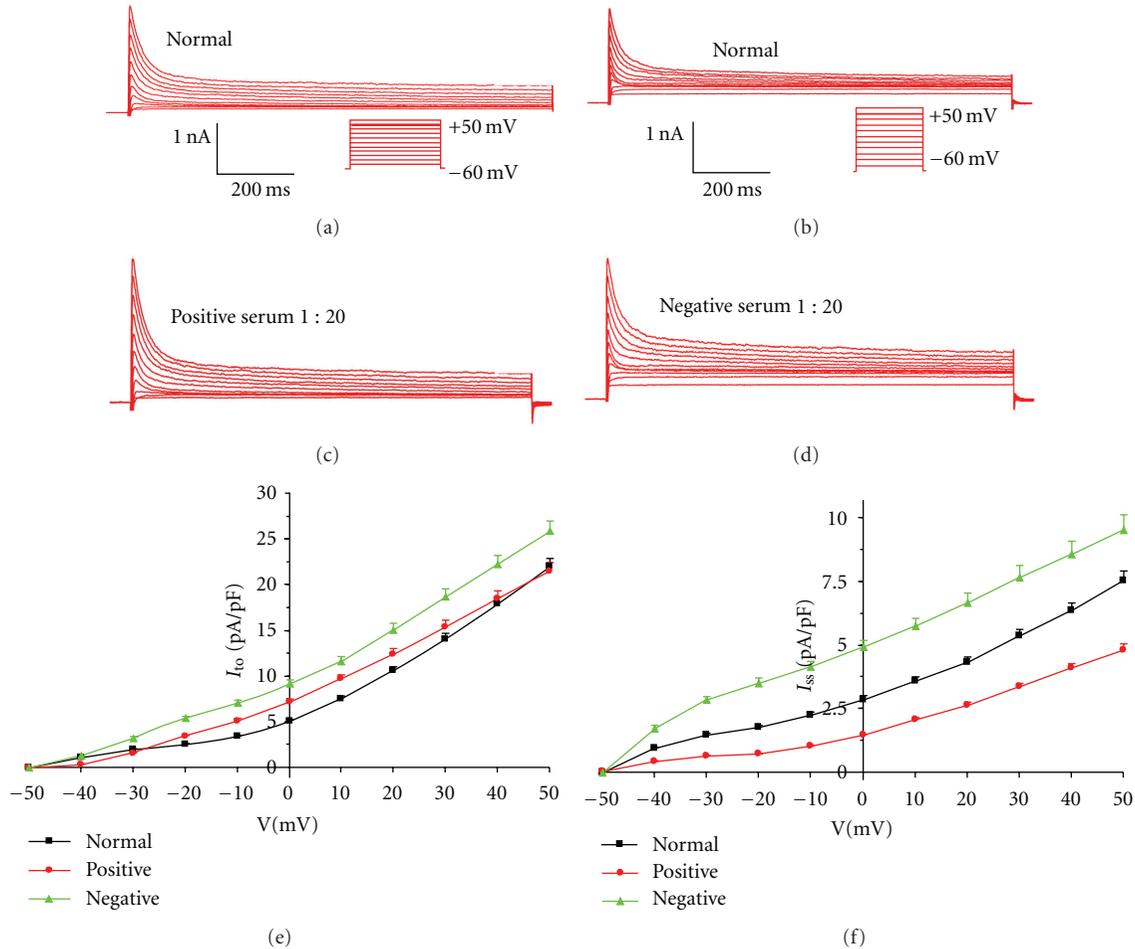


FIGURE 1: Effect of AAB- β_1 -negative and -positive serum of CHF patients on outward K^+ currents of mouse cardiomyocytes: (a), (b), (c), and (d), current traces obtained with 1-s depolarizing step from -50 to $+50$ mV in 10 mV increments from a holding potential of -60 mV. Voltage protocols are shown below the current traces. Under the present experimental conditions, the peak of the current was I_{to} , and the end 50 ms of plateau potential current was I_{ss} . (e), (f), corresponding current-voltage relationships during the application of AAB- β_1 -negative or -positive serum of CHF patients at the dilution of 1:20. AAB- β_1 negative serum had no effect on the activity of I_{to} , I_{ss} and the corresponding current-voltage curves (b, d, e, f). The AAB- β_1 -positive serum had no effect on activity of I_{to} , but it produced a decrease in the currents of I_{ss} (a, c, e, f).

effects, cells were separately perfused with BS including AAB- β_1 -negative serum and AAB- β_1 -positive serum for 5 min. For the concentration-dependence analysis of autoantibody effects, cells were perfused with the following bath solutions: serum dilution ranging from 1/100, 1/50, 1/20 to 1/10.

The recordings were analyzed using IGOR and the Origin software. The value of current was expressed with the density of current (pA/pF) to eliminate the capacitance error. Current amplitude was determined as the difference between peak inward current and current at the end of the depolarising step.

2.5. Statistics. All of the data were presented as the means \pm S.E. One-way ANOVA with repeated measures and analysis of variance were used for statistical analysis where appropriate. Statistical analysis was performed using the SPSS12.0 software, and $P < .05$ was considered statistically significant.

AAB- β_1 -positive serum dilution-response curves were fitted using the equation: $I = a/(1 + (EC_{50}/\text{dilution})^{nH})$, where a was the amplitude of the I_{ss} current, the EC_{50} was the dilution where a half-maximal response was induced, and nH was the Hill coefficient.

3. Results

Under these experimental conditions (in the presence of ChCl and CdCl to block the Na^+ currents and Ca^{2+} currents, resp.), outward K^+ currents were recorded in mouse myocytes. These readings were composed of rapidly activating and inactivating currents (I_{to}) and slowly activating but noninactivating current (I_{ss}) (Figure 1(a), 1(b), 1(c), 1(d)).

The AAB- β_1 -negative serum of CHF patients had no effect on the activity of I_{to} and I_{ss} in mouse ventricular myocytes (Figures 1(b) and 1(d)). The negative serum also

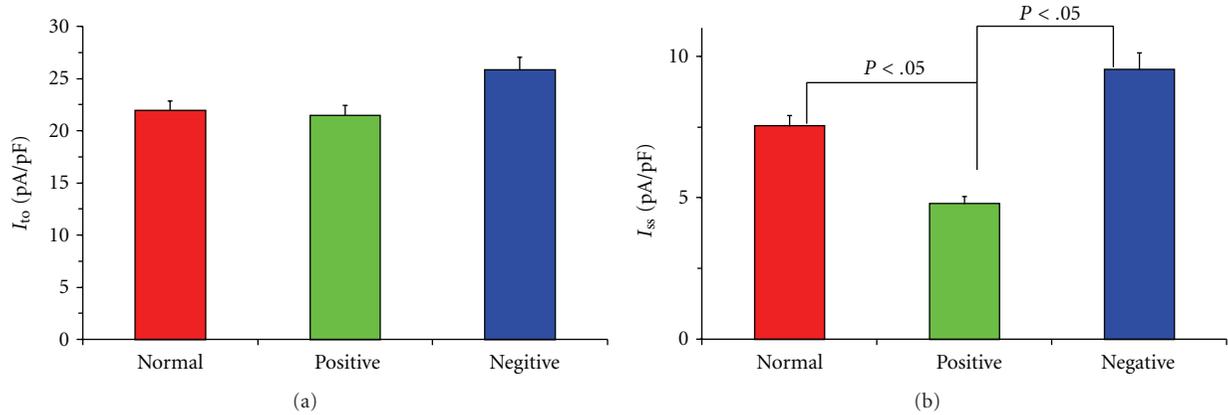


FIGURE 2: Comparison of AAB- β_1 -negative serum and -positive serum from patients with heart failure (dilution at 1:20) on the mean current of outward K^+ currents at a voltage of 50 mV. Compared to currents at normal bath solution and AAB- β_1 -negative serum ($n = 5$ cells, dilution at 1:20), AAB- β_1 -positive serum ($n = 7$ cells, dilution at 1:20) had no effect on I_{to} ($P > .05$). I_{ss} on the AAB- β_1 -positive serum decreased significantly compared to the normal bath solution ($P < .05$) and had also significant decrease compared to the AAB- β_1 -negative serum ($P < .05$).

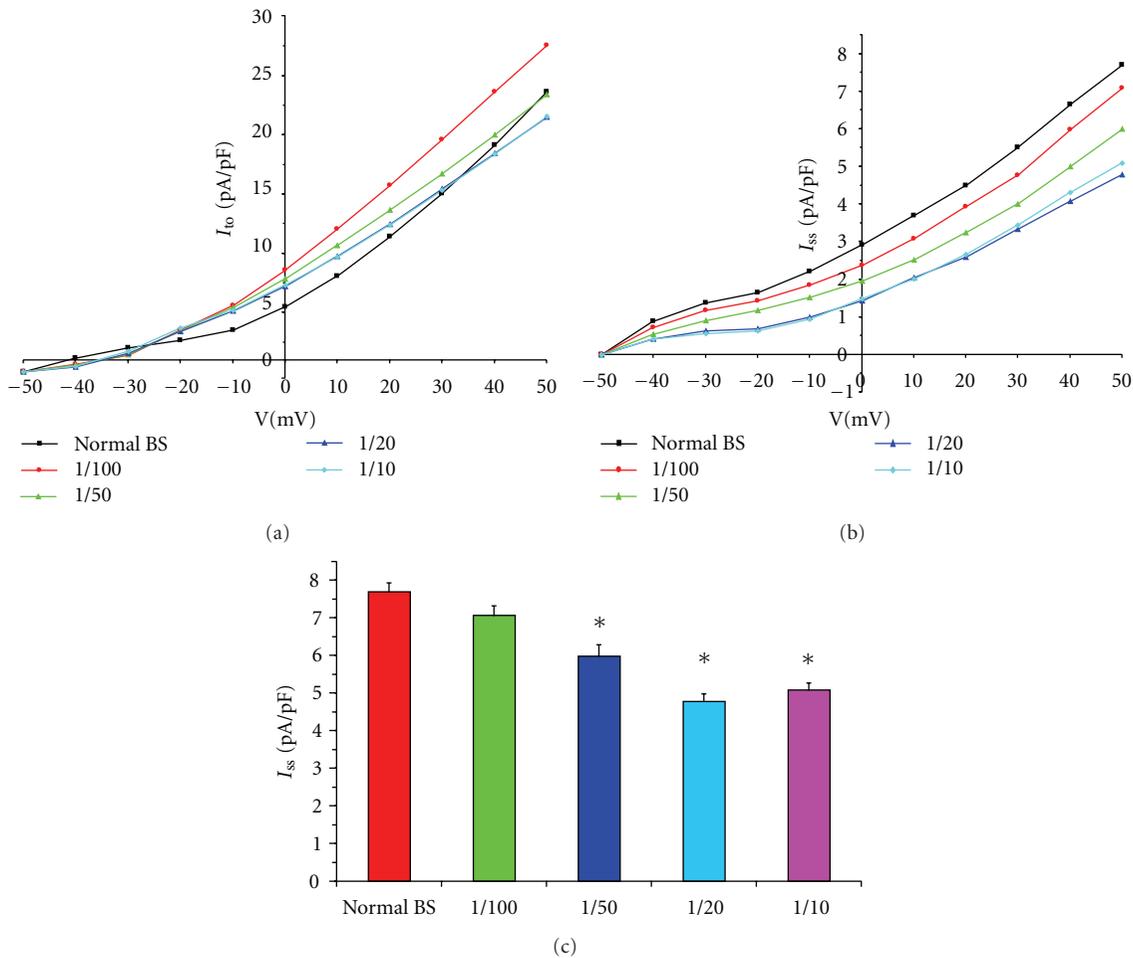


FIGURE 3: Different concentrations of AAB- β_1 -positive serum (dilution ranging from 1:100, 1:50, 1:20 to 1:10) on the outward K^+ currents at a voltage of 50 mV: I_{to} showed no changes at different concentrations of AAB- β_1 -positive serum (a). A low concentration of AAB- β_1 -positive serum (1:100) had a small inhibitory effect on I_{ss} (b, c). However, AAB- β_1 -positive serum at 1:10, 1:20, and 1:50 significantly decreased I_{ss} ($P < .05$, b, c). Additionally, there were no significant differences in currents at 1/10 and 1/20 AAB- β_1 -positive serum treatment (b, c). AAB- β_1 -positive serum had no effect on the I - V relationship at any concentration (a, b). * $P < .05$.

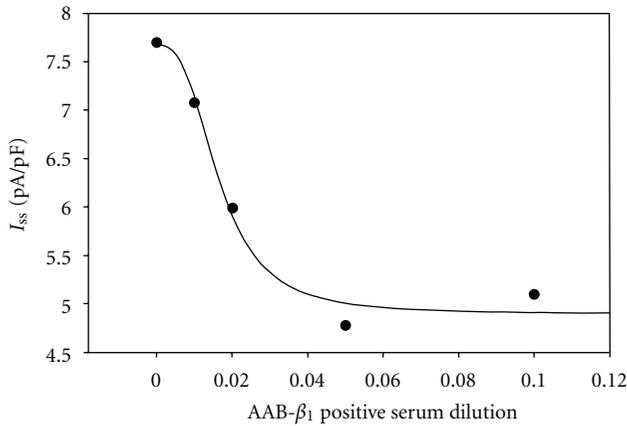


FIGURE 4: Concentration-dependent inhibition of AAB- β_1 -positive serum on I_{ss} activated at a potential of 50 mV: EC₅₀ (dilution of AAB- β_1 positive serum that produces the half-maximum blockade) of AAB- β_1 -positive serum was 1 : 60.24 and nH was 2.86.

showed no effect on the current-voltage curves of I_{to} and I_{ss} (Figures 1(e) and 1(f)). The AAB- β_1 -positive serum had no effect on activity of I_{to} , but it produced a decrease in the currents of I_{ss} (Figure 1(a), 1(c), 1(e), 1(f)). Compared to the currents at normal bath solution and AAB- β_1 -negative serum (dilution at 1 : 20), AAB- β_1 -positive serum (dilution at 1 : 20) had no effect on I_{to} but caused a significant decrease in I_{ss} myocyte currents ($P < .05$, Figure 2).

Compared with the normal bath solution, the current density of I_{to} showed no change at different concentrations of AAB- β_1 -positive serum (Figure 3(a)). A low concentration of AAB- β_1 -positive serum (1/100) had a small inhibitory effect on I_{ss} (Figures 3(b) and 3(c)). However, AAB- β_1 -positive serum at 1 : 10, 1 : 20 and 1 : 50 significantly decreased I_{ss} ($P < .05$, Figures 3(b) and 3(c)). Additionally, there were no significant differences in the I_{ss} currents between 1/10 and 1/20 AAB- β_1 -positive serum (Figures 3(b) and 3(c)) treatments. Similarly, AAB- β_1 -positive serum had no effect on the I - V relationship at any concentration (Figures 3(a) and 3(b)). The concentration-dependence analysis showed that the EC₅₀ of AAB- β_1 -positive serum was 1/60.24, and its nH was 2.86 (Figure 4).

4. Discussion

Increasing evidence demonstrates that the contribution of AAB- β_1 to the pathogenesis of chronic heart failure is not just a correlation. In the present study, we found for the first time that serum positive for autoantibodies against the β_1 -adrenoceptor decreases the current density of I_{ss} in mouse ventricular myocytes in a concentration-dependent manner, with no effect on I_{to} . AAB- β_1 -positive serum at the dilution of 1 : 10, 1 : 20, and 1 : 50 significantly decreased I_{ss} . Concentration-dependence analysis showed that the EC₅₀ was 1/60.24 and nH was 2.86.

The autoantibodies for the β_1 -adrenergic receptor have been found in sera not only from patients with idiopathic dilated cardiomyopathy [5], but also from patients with CHF

of various etiologies [21, 22]. Previous studies have conclusively demonstrated that autoantibodies targeting the second extracellular loop of the β_1 -adrenergic receptor showed agonist-like effects: inducing receptor uncoupling, causing cardiomyocyte apoptosis, and permitting sustained calcium influx [14, 23]. In the present study, serum positive for autoantibodies against the β_1 -adrenoceptor decreased the current density of I_{ss} without any effect on I_{to} , which is similar to the inhibitory effect of the β_1 -adrenergic agonist isoprenaline [17]. From this close resemblance of macroscopic I_{ss} after stimulation with AAB- β_1 and isoprenaline, we suggest that both activators mediate their effects via similar signal transduction pathways.

Autoantibodies are thought to induce activation of the receptor that leads to intracellular signaling involving the classical PKA pathway [24–27]. Other groups have reported effects of purified autoantibodies or AAB- β_1 -positive serum on calcium channels. Christ et al. found that immunoglobulin G derived from patients positive for the β_1 -adrenoceptor autoantibodies increased Ca^{2+} current to a similar extent, but prolonged the plateau of duration of action potentials to a lesser extent compared to isoprenaline [14]. However, Del Corso et al. found that serum from patients with IDC induced a significant decrease in isoproterenol-stimulated L-type Ca^{2+} currents in rabbit ventricular myocytes. This activation is known to involve the PKA pathway [12]. Furthermore, Christ et al. concluded that AAB- β_1 may not only enhance I_{Ca} via stimulation of the β_1 -adrenoceptors, but may also inhibit this β_1 -adrenoceptor-mediated increase upon stimulation with catecholamines [16]. In our study, AAB- β_1 -positive serum inhibited I_{ss} in a concentration-dependent manner with no effect on the current-voltage curves. Therefore, the regulatory effect of AAB- β_1 on ion channel currents may all involve the classical PKA pathway in the different studies.

Furthermore, AAB- β_1 -positive serum only decreased I_{ss} with no effect on I_{to} , which was similar to the results of β_1 -adrenergic agonist isoprenaline treatment [17]. Several hypotheses can be proposed to account for such a difference in threshold dose and potency. The channels may be more easily accessible to phosphorylation in I_{ss} than I_{to} , which is a possible effect according to the theory of cAMP compartmentalization. Another possibility is that the channels may be more sensitive to phosphorylation in I_{ss} than I_{to} ; for example, phosphorylation at one site on I_{ss} may be sufficient to induce an effect while I_{to} requires phosphorylation of several sites. The above suggested mechanisms may also lead to the dose-dependence of I_{ss} .

5. Conclusions

Autoantibodies against β_1 -adrenoceptor from Chinese patients with congestive heart failure can inhibit I_{ss} in mouse cardiomyocytes in a concentration-dependent manner. Because I_{ss} plays an important role in the repolarization of action potentials, AAB- β_1 may influence action potential duration via this current.

5.1. Study Limitations and Clinical Implications. In the present study, we did not investigate the mechanism behind the inhibitory effect of AAB- β_1 on I_{ss} . It would require much more work to establish whether AAB- β_1 inhibits I_{ss} directly or indirectly. Recent studies reported that AAB- β_1 may influence the effects of β -blocker therapy and that specific removal of AAB- β_1 by immunoadsorption can improve cardiac function in patients with DCM. These results suggest that anti- β_1 -adrenergic receptor autoantibodies have a pathogenic role in the onset and progression of heart failure. Because I_{ss} has biophysical properties of being slowly activated and noninactivated (steady state), AAB- β_1 may prolong repolarization and action potential duration by inhibiting I_{ss} ; this would subsequently result in cardiac electrical instability.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgments

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