Effects of allitridum on the transient outward potassium current in rats with heart failure

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Abstract

Objective To study the effect of allitridum on the transient outward potassium current (Ito) of ventricular myocytes in heart failure (HF).

Methods The dual enzymatic method was used to separate single ventricular myocytes from Sprague Dawley rats. Patch-clamping was used to record Ito and analyze the effect of allitridum on the current.

Results The Ito current had a significant decrease in the HF group, compared with the control group. The density of Ito in the HF group was increased after treatment of allitridum (30 μmol/L). The peak current densities of Ito were enhanced in the HF group from 6.01 ± 0.30 pA/pF to 8.41 ± 0.54 pA/pF (P < 0.01) at +50 mV after treatment with allitridum (30 μmol/L). We also determined the effect of allitridum on the gating mechanism of the Ito in the HF group.

Conclusions We found that allitridum increased the Ito by accelerating the activation of channels and shortened the time constants of inactivation, and allitridum decreased the remodeling of Ito in ventricular myocytes of rats with HF.

Keywords: Allitridum; Heart failure; Ion current; Ventricular myocyte

1 Introduction

Ventricular tissue damage and electrical remodeling are hallmarks of the failing heart. Transient outward K+ currents (Ito), which underlie phase 1 repolarization, contribute importantly to the early phase of an action potential and the normal propagation of activity in the ventricular myocardium. Ito is reported to significantly reduce heart failure (HF) by decreasing the pathologic remodeling of the heart.[9,10] Deng, et al.[12] found that allitridum could block Ito in human atrial myocytes. Our lab has previously shown that allitridum could effectively improve Ito currents that normally decrease in ventricular myocytes of spontaneously hypertensive rats. APD prolongation by decreasing Ito enhanced the risk of ventricular arrhythmia,[13] which could result in sudden death in HF patients. However, what effect allitridum may have on Ito in hearts undergoing HF has not been studied. Therefore, in the present study we investigated whether or not allitridum has a protective effect on HF through modulation of Ito.

2 Methods

2.1 Animals

All experimental procedures and protocols were carried out according to the Chinese Law on Animal Experimentation and approved by the Animal Experimental Committee of Chinese PLA General Hospital (China). The investigation conforms to 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).
Thirty Sprague-Dawley (SD) rats of 200–250 g body weight were purchased from animal center of Chinese PLA General Hospital. The animals were kept under secure, clean and controlled room temperature (25°C) with a 6:00 h to 18:00 h light cycle and were fed food and water ad libitum. The rats were randomly assigned to control (sham) or HF groups. The surgery was performed in male SD rats as described previously.\[14,15\] Briefly, animals in the HF group were anaesthetized with sodium pentobarbital (30 mg/kg body weight, i.p.). The abdominal aorta between the diaphragm and the renal artery was partially constricted with an eight gauge disposable needle, which produced approximately 50% stenosis of the aortic diameter. The same procedure was performed rats in the control sham group, except for the insertion of the 8G needle in the abdominal aorta. To characterize the model, echocardiographic measurements were obtained 16 weeks after surgery using a Vivid 7 Dimension cardiovascular ultrasound system (GE Healthcare, Fairfield, Connecticut, United States) as described previously.\[16\] The parameters measured are shown in Table 1.

### 2.2 Drugs and solutions

Allitridum reference standard was purchased from Solarbio Sci&Tech Co., Ltd., China. The Ca\(^{2+}\)-free Tyrode solution contained (in mmol/L): NaCl 137, KCl 5.4, MgCl\(_2\) 1.0, NaH\(_2\)PO\(_4\) 0.33, hydroxyethyl piperazine ethanesulfonic acid (HEPES) 10, and glucose 10 (pH 7.35, adjusted with NaOH). The Krebs buffer (KB) solution for cell storage contained (in mmol/L): KCl 40, KH\(_2\)PO\(_4\) 20, MgCl\(_2\) 3.0, KOH 70, L-glutamic acid 50, HEPES 10, taurine 20, glucose 10, and EGTA 0.5 (pH 7.35, adjusted with KOH).

For the \(I_{so}\) recordings, the internal pipette solution contained (in mmol/L): L-aspartic acid 140, MgATP 4, MgCl\(_2\) 1, EGTA 10, GTP 0.1, and HEPES 10 (pH 7.3, adjusted with KOH).

### 2.3 Cardiac ventricular myocyte isolation

Ventricular myocytes were isolated from the hearts of the rats as previously described with slight modifications.\[16,17\] Briefly, 5 min after the rats were heparinized (100 U/mL, 1mL/100 g, i.p.), the animals were anesthetized with 3% chloral hydrate (0.5 mL/100 g, i.p.). The heart was rapidly excised and mounted on the Langendorff apparatus and perfused via the aorta with oxygenated Ca\(^{2+}\)-free Tyrode solution for 5 min and then with Ca\(^{2+}\)-free Tyrode solution containing collagenase II (0.6 mg/mL, Worthington, USA), trypsin (0.24 mg/mL, Amresco, USA), and proteinase E (0.08 mg/mL, Amresco, USA) for 15–20 min at 37°C. Subsequently, the ventricular tissue was excised, cut into small pieces in a dish containing KB solution, and blown gently to obtain single ventricular myocytes. The cells were maintained at 4°C in KB solution until use. All of the solutions were continuously gassed with 95% O\(_2\) and 5% CO\(_2\) at 37°C. The single ventricular myocyte selected for electrophysiological measurements was rod-shaped, quiescent, Ca\(^{2+}\)-tolerant, and had clear cross-striations and a smooth and glossy surface.

### 2.4 Electrophysiological recordings

The whole-cell patch clamp technique was used to record the \(I_{so}\) using an Axopatch 700B amplifier with the pCLAMP 9.2 software (Axon Instruments, USA). Borosilicate glass patch pipettes (resistance = 3–5 M\(\Omega\)) were pulled using a vertical pipette puller (Narishige pp-830, Japan). The cells were maintained in external solution for 5 to 10 min after perfusion and the data were recorded after entering the cell for 5 min to stabilize the current. All of the recordings were performed at room temperature. Dofetilide was used to block delayed rectifier K\(^+\) current (\(I_{kr}\)). The \(I_{so}\) was recorded in the voltage-clamp mode.

### 2.5 Statistical analysis

Off-line leak correction was performed on all of the amplitude data. The pCLAMP 9.2 software (Axon Instruments, USA) and the Origin 6.1 software (Microcal Software, USA) were used for the data acquisition and analysis. The data are presented as the mean ± SE, where \(n\) represents the number of cells analyzed. The statistical comparisons between different groups were performed with ANOVA and Student’s \(t\)-test. Differences with a \(P\) value less than 0.05 were considered statistically significant.

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**Table 1. Assessment of cardiac function and remodeling in control and HF groups.**

| Heart index | Heart rate/min | LVEDd, mm | LVPWT, mm | IVSDT, mm | LVEF, % | Cm/pF |
|-------------|----------------|-----------|-----------|-----------|--------|-------|
| Ctrl        | 416 ± 17       | 3.22 ± 0.56 | 1.75 ± 0.12 | 1.82 ± 0.16 | 82.50 ± 7.22 | 73.44 ± 10.27 |
| HF          | 297 ± 63\*     | 5.85 ± 0.46 | 1.85 ± 0.30 | 1.95 ± 0.30 | 57.09 ± 9.78 | 161.75 ± 74.99 |

\(N\) = 15. Data are presented as mean ± SE. \(*p < 0.05\), Ctrl group vs. HF group. Cm/pF: the membrane capacitance/ picofarad; Ctrl: control group; LVEF: left ventricular ejection fraction; HF: heart failure; IVSDT: inter-ventricular septal thickness; LVEDd: left ventricular end-diastolic dimension; LVPWT: left ventricular posterior wall thickness.

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3 Results

3.1 Effects of allitridum on the $I_{to}$ in the HF group.

Parameters representing cardiac function and remodeling of control and HF group are shown in Table 1. Figure 1A shows $I_{to}$ was elicited by a 300 ms depolarizing pulse from a holding potential of $-80$ mV to a testing potential of $+70$ mV, and a conditioning test of $-40$ mV for 25 ms to eliminate the sodium current in a rat ventricular myocyte. The $I_{to}$ traces show that the amplitude of the $I_{to}$ was lower in the HF group compared with the control group. After treatment with 30 μmol/L allitridum, the $I_{to}$ was significantly increased in the HF group. According to the I-V curves, the current densities at +50 mV were 13.92 ± 0.40 pA/pF in the control group and 6.01 ± 0.30 pA/pF ($n$ = 10, $P < 0.05$) in the HF group, revealing a significant decrease in current density in response to HF. After treatment with allitridum (30 μmol/L), the mean current densities were markedly increased from 6.01 ± 0.30 pA/pF to 8.41 ± 0.54 pA/pF ($n$ = 10, $P < 0.01$) at +50 mV (Figure 2B). The concentration-

Figure 1. Effect of allitridum on $I_{to}$ in SHR ventricular myocytes. (A): The effects of 30 μmol/L allitridum on $I_{to}$ are shown. (B): The $I_{to}$ current densities increased in a test potential-dependent manner after exposure to 30 μmol/L allitridum. (C): Concentration-response relationship of the effects of allitridum. Ctrl: control group; HF: heart failure; EC50: 50% effective concentration; $I_{to}$: transient outward K$^+$ currents; SHR: spontaneously hypertensive rats.

Figure 2. Effects of allitridum on steady-state activity of $I_{to}$. $^a P < 0.05$ vs. HF group; $^b P < 0.05$ vs. HF group. (A): Steady-state activity curve of $I_{to}$ in HF group is shifted to more negative potentials by allitridum. (B): $K_{act}$ is no different between two groups. (C): $V_{1/2,act}$ of $I_{to}$ from HF is shifted to more negative potential compared with the Ctrl group. After exposure to allitridum, the decrease observed in $V_{1/2,act}$ in HF is partially restored. Ctrl: control group; HF: heart failure; $I_{to}$: transient outward K$^+$ currents, $K_{act}$: the slope factor the activation, $V_{1/2,act}$: the half-activation potentials.
response relationship of the effects of allitridum on $I_{to}$ currents (Figure 1C) showed that the 50% effective concentration ($EC_{50}$) of allitridum was 26.30 $\mu$mol/L, with a Hill coefficient of 0.94.

3.2 Effects of allitridum on steady-state activation kinetics of $I_{to}$ in the HF group

The steady-state activation curves of $I_{to}$ were determined using pulses from $-40$ mV to $+70$ mV at 10 mV increments for 300 ms, and were described assuming a Boltzmann function: $G/G_{\text{max}} = 1/[1 + \exp \{(V_m - V_{1/2,\text{act}})/k\}]$. The steady-state activation curve in the HF group shifted to the right significantly (Figure 2A). After administering allitridum (30 $\mu$mol/L), the right shift was partially reversed. The half-activation potentials ($V_{1/2,\text{act}}$, at which 50% of the channels are activated) in the control and the HF groups were 20.01 ± 2.50 mV and 6.01 ± 1.54 mV, respectively ($n$ = 10, $P$ < 0.05). Treatment with 30 $\mu$mol/L allitridum shifted the $V_{1/2,\text{act}}$ from 6.01 ± 1.54 mV to 10.94 ± 1.89 mV ($n$ = 10, $P$ < 0.05) in the HF group (Figure 2B). However, the slope factor ($k_{\text{act}}$) of the activated curve in the control group, the HF group, and the HF group after treatment with 30 $\mu$mol/L allitridum were not statistically different ($n$ = 10, $P$ > 0.05, Figure 2C). These results revealed that allitridum increased the $I_{to}$ current by accelerating activation of the channels.

3.3 Effects of allitridum on steady-state inactivation kinetics of $I_{to}$ in the HF group

The steady-state inactivation curves were described assuming a Boltzmann function: $I/I_{\text{max}} = 1/[1 + \exp \{(V_m - V_{1/2,\text{act}})/k\}]$. Compared with the control group, the steady-state inactivation curve in the HF group shifted to a more negative potential. In the presence of 30 $\mu$mol/L allitridum, the steady-state inactivation curve of the HF group showed no difference (Figure 3A). $V_{1/2,\text{inact}}$ (at which 50% of the channels are inactivated) in the control and the HF groups were $-75.5 \pm 5.3$ mV and $-81.1 \pm 4.07$ mV, respectively ($n$ = 10, $P$ > 0.01, Figure 3C). The half-inactivation potential in the HF group after treatment with 30 $\mu$mol/L allitridum was $-80.9 \pm 4.98$ mV ($n$ = 15, $P$ > 0.05) (Figure 3B). The slope factor ($k_{\text{inact}}$) of the inactivation curves in the control group, the HF group, and the HF group after treatment with 30 $\mu$mol/L allitridum were 5.01 ± 0.54 mV, 2.61 ± 0.35 mV, and 2.45 ± 1.78 mV, respectively. These values were not significantly different ($n$ = 15, $P$ > 0.05, Figure 3D).

Figure 3. Effects of allitridum on steady-state and closed-state inactivity of $I_{to}$. A: Steady-state inactivity curve of $I_{to}$ is shifted to more negative potentials in HF group; this change is not reversed by allitridum; B: Closed-state time constants of $I_{to}$ are shortened by allitridum; C: $V_{1/2,\text{inact}}$; and D: $K_{\text{inact}}$ of $I_{to}$ is not different between sham and HF groups. Ctrl: control group; HF: heart failure; $I_{to}$: transient outward $K^+$ currents; $K_{\text{inact}}$: the slope factor of the inactivation, $V_{1/2,\text{inact}}$: the half-inactivation potentials.
In the control group, 90% of $I_{to}$ channels were still open at 5000 ms, but only 50% of the channels were open in HF group at the same time point. After treatment with 30 μmol/L allitridum, however, the percentage of open $I_{to}$ channels in the HF group increased to 70% (Figure 3B). This result demonstrated that the closed-state time constants of $I_{to}$ were prolonged by allitridum in the HF group.

### 3.4 Effects of allitridum on the recovery from inactivation of $I_{to}$

Allitridum treatment had an effect on the trace of recovery curves from inactivation in the HF group (Figure 4A). The recovery curves from inactivation of the $I_{to}$ shifted left in the range of 0.01 ms to 10 ms after treatment of allitridum, though it had no effect on the range of 10 ms to 1000 ms in the HF group (Figure 4B).

The voltage dependence of the time course of inactivation was investigated in our study. Compared to the control, the time constants of inactivation were significantly prolonged throughout a range of potentials, especially at $-40$ mV ($P < 0.01$, $n = 17$, Figure 4C). After giving 30 μmol/L of allitridum to the HF group, this extension could be partially reversed ($P < 0.01$, $n = 17$).

### 4 Discussion

The primary active compound in garlic, allitridum, has displayed cardioprotective effects in several disease models. However, the mechanism(s) by which allitridum may alleviate HF remains unknown. The present study reveals several interesting findings in regards to the therapeutic potential of allitridum: (1) the current densities of $I_{to}$ in the HF group was decreased compared with the control group, and allitridum treatment partially rescued this defect in the HF group; (2) allitridum increased the $I_{to}$ by accelerating the activation process of the channels, and it prolonged the time constants of inactivation in HF group, while having no significant effect on the inactivation process. These major findings suggest that allitridum has a protective effect on HF.

Allitridum has been reported to have a protective effect in variety of heart diseases,[9,18] and it was shown that allitridum inhibits $I_{to}$ currents in human atrial myocytes (reference). A previous study in our lab revealed that $I_{to}$ decreased in spontaneously hypertensive rats, and allitridum could improve this decrease in a dose-dependent manner. In this study, we investigated the effects of allitridum on $I_{to}$ channels in rats with HF. Electrical remodeling in ventricular ion channels has been associated with enhanced risk of sudden death in patients with HF.[19] Functional down-regulation of K⁺ currents is a recurring theme in hypertrophied and failing ventricular myocardium.[20,21] The current study also found that $I_{to}$ was significantly decreased in response to HF; this result was consistent with our previous study. We also found that the current density of $I_{to}$ in HF group was lower than those in the control group. The acute application of allitridum increased $I_{to}$ in a concentration-dependent manner in the control group. The EC₅₀ was found to be 26.30 μmol/L. Allitridum significantly increased the current density of $I_{to}$ in the HF group.

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**Figure 4. Effects of allitridum on recovery from inactivation of $I_{to}$.** (A): The trace of recovery from inactivation of $I_{to}$ among groups. (B): Time constant of recovery from inactivation in HF is shortened by allitridum in the range of 10 ms, but not different from 10 ms to 1000 ms. (C): The time constants of inactivation were significantly shortened by allitridum treatment in the HF group. Ctrl: control group; HF: heart failure; $I_{to}$: transient outward K⁺ currents.
Furthermore, we determined the effect of allitridum on the gating mechanism of $I_{to}$ in the HF group. Compared with the control group, the steady-state activation curve of the HF group was shifted to a more positive potential. In the presence of 30 μmol/L allitridum, the steady-state activation curve was shifted to a more negative potential. This result suggests that the voltage-dependent steady-state activation of the $I_{to}$ channels was accelerated. The steady-state inactivation curves in each group were not significantly different. But allitridum increased the open channels of $I_{to}$ from 50% to 70% at 5000 ms in the HF group. Moreover, we found that time constants of closed state of $I_{to}$ prolonged significantly in the HF group, especially at ~40 ms. Treatment with allitridum significantly inhibited the modification of this particular parameter caused by HF disease. In addition, allitridum shifted the recovery curve from inactivation of $I_{to}$ to the left in the range of 0.01 ms to 10 ms, but there was no difference from 10 ms to 1000 ms in the HF group. These data suggest that allitridum increased the $I_{to}$ through facilitation of the steady-state activation and shortened the time constants of the closed state of $I_{to}$.

Huang, et al.[22] reported a reduction in $I_{to}$, accompanied with Ca$^{2+}$ overload, in hypertrophic ventricular myocytes. Our study only focused on the effect of allitridum on cardiac $I_{to}$ of rats with HF. Although we did not study Ca$^{2+}$ dynamics in the present study, our observations suggest that allitridum has the ability to alleviate HF-induced decreases in $I_{to}$.

In conclusion, we demonstrated that allitridum has a direct effect on the density and state of $I_{to}$ channels. These data suggest that allitridum may potentially serve as an effective treatment for patients with HF.

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