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The effect of allicin on the late sodium current associated with LQT3 syndromes caused by SCN5A mutation

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Abstract

Objective We aimed to study the effect of the Allicin (All) on late sodium currents ($I_{Na,\ late}$) induced with several SCN5A genetic mutations, which were involved in long QT3 syndrome (LQT3). **Methods** WT and SCN5A mutation plasmids were transfected into human embryonic kidney (HEK293) cells. Patch-clamp technique was used in order to record Nav1.5 current and analyze the current affected by All. **Results** Compared with WT, significant increase of $I_{Na,\ late}$ was observed from F1473S-SCN5A, T535I-SCN5A and E1784K-SCN5A. At -20 mV of test potential, the densities of $I_{Na,late}$ were 3.12 ± 0.32 pA/pF of F1473S, 5.33 ± 0.56 pA/pF of T535I and 2.86 ± 0.23 pA/pF of E1784K, respectively. After exposure to 30 μM All, $I_{Na,late}$ densities of SCN5A mutations were respectively reduced to 1.11 ± 0.05 pA/pF of F1473S, 0.9 ± 0.5 pA/pF of T535I and 1.0 ± 0.3 pA/pF of E1784K, close to the level of WT(0.3 ± 0.03 pA/pF). Furthermore, deactivation kinetic process of three SCN5A mutations was delayed with slow time constants lengthening of deactivation. **Conclusions** All decreases $I_{Na,\ late}$ densities of SCN5A mutations with LQT3.

Keywords: Allicin; Long QT3 syndrome; Late sodium currents; Patch clamp; SCN5A gene mutation

1 Introduction

Long-QT syndrome type 3 (LQT3) is a rare type of long-QT syndrome caused by gain-of-function mutations of the SCN5A gene, which codes for the alpha-subunit of the Nav1.5 channel protein.[1] LQT3 is characterized by prolonged QTc interval on electrocardiogram and manifest with syncope, seizures and sudden cardiac death during rest or sleep.[2,3]

Nav1.5 is encoded by SCN5A is the main sodium channel in the heart. The pore-forming type alpha subunit Nav1.5 channel protein was encoded by the gene SCN5A.[4,5] The gene of SCN5A has four transmembrane domains with six transmembrane sections per domain and locates on human chromosome 3p22, which is a large (> 6000 bp), highly conserved gene sequencethat is in various species.[6] SCN5A mutations have become progressively clear that they may combine different electrophysiological properties and induce complex phenotypes. More than 80 SCN5A mutations have been identified in LQT3 patients. SCN5A mutations from LQT3 family members were involved in an increase in late sodium current ($I_{Na,\ late}$). Under physiological conditions, the amplitude of $I_{Na,\ late}$ is less than 1% of peak sodium current ($I_{Na,\ peak}$). However, $I_{Na,late}$ is increased as a result of delayed inactivation procedure of current due to the genetic mutation of sodium channel.[7] This effect will lead to an abnormal repolarization of the ventricle and atrium.[8] Being a new target of anti-arrhythmic agents, the blockade of $I_{Na,\ late}$ can be beneficial to suppress arrhythmias such as LQT3 syndrome.

Allicin (All), the main active component of garlic, was reported to have cardiovascular protective effects.[9,10] The chemical formula of allitridum is showed in Figure 1. It was also reported that All possessed of antiarrhythmic effects.[11] In this study, we focused on examining the effect of All on $I_{Na,\ late}$ of three SCN5A mutations related to LQT3, including F1473S-SCN5A, T353I-SCN5A and E1784K-SCN5A.

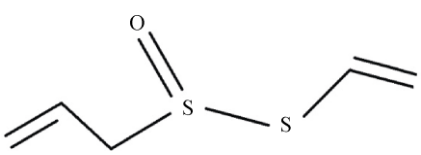

Figure 1. The chemical formula of Allicin. Allicin, a trithiocarbonate allyl ethers compounds, is extracted from the bulb of Allium sativum, Allium, Liliaceae.

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2 Materials and Methods

2.1 Drugs and solutions

All was purchased from Solarbio Sci & Tech Co., Ltd., China. To record the $I_{Na,\ late}$, human embryonic kidney (HEK293) cells were bathed in a solution containing (in mM) NaCl 130, $CaCl_2$ 2, CsCl 5, $MgCl_2$ 1.2, HEPES 10, and glucose 5, at pH 7.4, adjusted with CsOH. The glass pipettes were filled with a solution of (in mM) CsCl 60, cesium aspartate 80, EGTA 11, HEPES 10, Na_2ATP 5, at pH 7.2, adjusted with CsOH.

2.2 Transfection into HEK293 cells

HEK 293 cells (ATTC, Manassas, VA, USA) were maintained under 5% CO_2 in humidified air at 37 °C as indicated for biochemical analysis. Transient transfection of SCN5A channel mutation and WT cDNA plasmids pcDNA3.1 2.0 μg into the cultured cells was performed by using Lipofectamine (Life Technologies, Gaithersrburg, MD, USA) following the manufacturer's instructions. GFP cDNA was co-transfected as a reporter gene (EBo-pCD vector, American Type Culture Collection). After 6 h, the transfection medium was replaced with the regular HEK293 medium. The day before the experiment, the Petri dish was prepared, and GFP-positive cells identified using confocal imaging were patch-clamped 48–72 h after transfection.

2.3 Patch clamp experiments

The current was recorded using whole cell patch-clamp technique with a MultiClamp 700B amplifier (Axon Instruments). Data were sampled at 10 kHz and subsequently filtered at 5 kHz for analysis (Digidata 1322A, Axon Instruments). Patch pipettes were pulled from borosilicate glass on a P-97 horizontal puller (Sutter Instruments). Low-resistance electrodes (< 2 MΩ) were used to record the I_{Na}, and 2 MΩ – 5.5 MΩ resistance electrodes were used for Kv4.3. A routine series resistance compensation was performed for values > 80% to minimize voltage clamp errors. Thus, the uncompensated R_{series} was < 2 MΩ. The membrane capacitance, compensated by approximately 80% – 90% of their initial value, was measured on each cell.

2.4 Parameters designed for recording the current

Recordings were performed at room temperature. The $I_{Na,\ late}$ was measured 500 ms after depolarization to –20 mV and determined by subtracting the background currents measured in the presence of tetrodotoxin (50 μM, Sigma) from tetrodotoxin-free recordings.

2.5 Data analysis

Off-line leak correction was performed on all of the amplitude data. The data were presented as the mean value ± SD, with *n* representing the number of cells analyzed. Origin (Microcal Software) and pCLAMP version10.4 (Axon Instruments) were used for data analysis. The concentration of drugs to yield a 50% blockade of the hERG current (IC_{50}) was obtained by fitting the data to a Hill equation: $I/I_0 = 1/ [1 + ([C]/IC_{50})^{nH}]$, where I_0 and I are the current amplitudes measured in the absence and presence of drugs respectively, [C] is the concentration of drugs in the external solution, and nH is the Hill coefficient. The time course of inactivation was fitted using a two-exponential function: $I\ (t)\ /I_{max} = A_1 \times \exp\ (-t/\text{Tau1}) + A_2 \times \exp\ (-t/\text{Tau2})$, where the values for A refer to the amplitudes, and Tau1 or Tau2 refers to the fast and slow time constants, respectively. I refers to the current, and t refers to time. All data were fitted using a nonlinear least-squares minimization method.

2.6 Statistical analysis

The SPSS computer program (version 17.0) was used for statistical analyses. Statistical analyses between the experimental groups were performed using Student's t-test. One way analysis of variance (ANOVA) was used in comparing multiple groups. Significance between any two groups was evaluated by ANOVA followed by a Student-Newman-Keuls post hoc test. *P*-value < 0.05 was considered statistically significant.

3 Results

3.1 Effect of All on $I_{Na,late}$ of SCN5A

To study the contribution of $I_{Na,\ late}$ at steady state, we measured the current using a 500-ms depolarization pulse before and after treatment with 30 μM All. Representative current traces of F1473S-SCN5A, T535I-SCN5A, E1784K-SCN5A and WT of SCN5A are shown in Figure 2. At a test potential of -20 mV, the $I_{Na,late}$ densities of three mutations were increased from 0.91 ± 0.02 pA/pF of the WT to 4.12 ± 0.32 pA/pF of F1473S, 5.33 ± 0.56 pA/pF of T535I and 3.86 ± 0.23 pA/pF of E1784K, respectively ($P < 0.01$, $n = 10$). After exposure to 30 μM All, the $I_{Na,late}$ densities of SCN5A mutations were respectively reduced to 1.11 ± 0.05 pA/pF of F1473S,1.32 ± 0.5 pA/pF of T535I and 0.97 ± 0.3 pA/pF of E1784K ($P < 0.01$, $n = 10$), close to the level of WT (0.3 ± 0.03 pA/pF).

In another experiment, the WT and T353I currents were recorded before and after direct perfusion with 30 μM All for 5 min.[12] Interestingly, neither the WT current nor the

T353I current was affected by direct All perfusion.

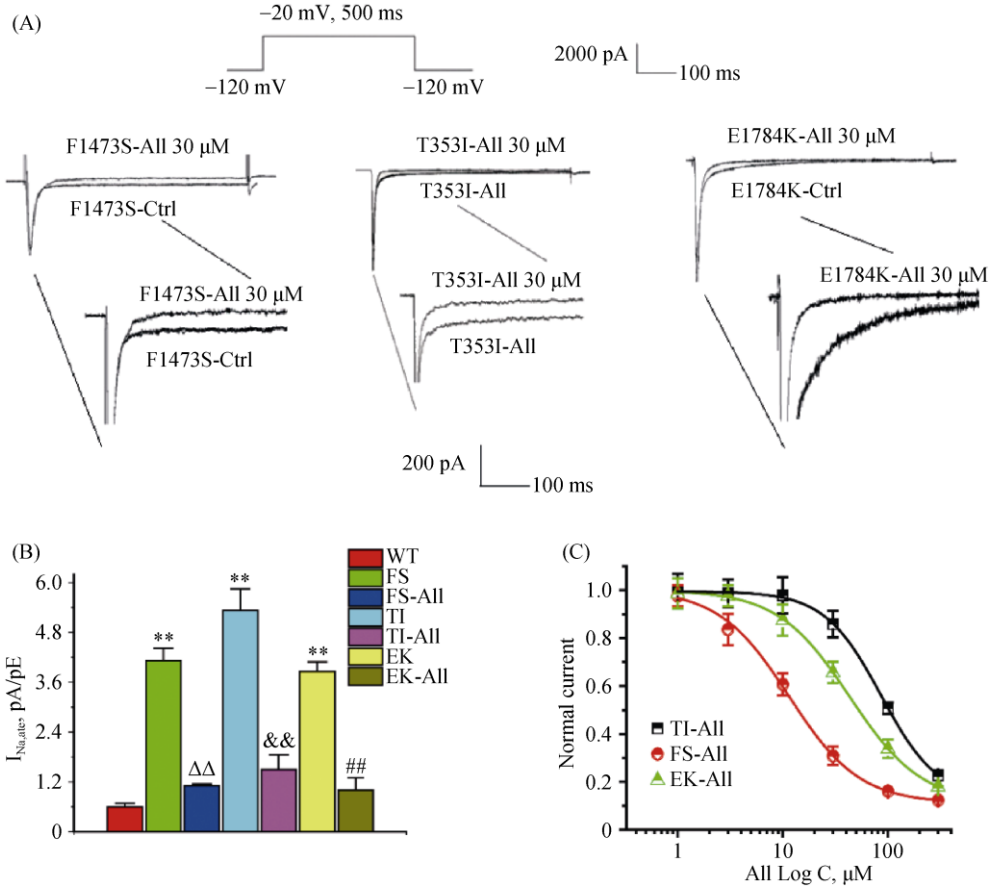

Figure 2. Effect of All on $I_{Na,late}$. (A): Representative $I_{Na,late}$ current traces recorded from WT, F1473S, T353I and E1784K before and after exposure to 30μM All; (B): at the test potential of −20 mV, $I_{Na,late}$ current densities of F1473S, T353I and E1784K are significantly increased compared with WT. After co-incubation with 30 μM All, $I_{Na,late}$ current densities are significantly decreased; (C) effects of ALL on $I_{Na,late}$. $I_{Na,late}$, which is inhibited by All in a concentration-dependent way, in different concentration. IC_{50} are 15.2 ± 2.2 μM for F1473S, 41.8 ± 3.6 μM for T535I and 18.1 ± 3.2 μM for E1784K. **: $P < 0.01$, compared with WT. ΔΔ: $P < 0.01$, compared with FS. &&: $P < 0.01$, compared with TI. ##: $P < 0.01$, compared with EK. All: Allicin; $I_{Na,late}$: late sodium currents.

3.2 Concentration-dependent effect of All on $I_{Na,late}$ of SCN5A

We measured the effects of different concentrations of All on $I_{Na,late}$ of SCN5A mutations. The cells were incubated for 24 h, and then the medium was replaced by bath solution containing different concentrations of ALL: 1, 3, 10, 30, 100 and 300 μM, respectively. It was found that $I_{Na,late}$ were inhibited by All in a concentration-dependent way. Plots of the half-maximum inhibition concentration value (IC_{50}) were 15.2 ± 2.2 μM for F1473S, 41.8 ± 3.6 μM for T535I and 18.1 ± 3.2 μM for E1784K respectively. The Hill coefficients were 0.87 for F1473S, 1.29 for T535I and 0.98 for E1784K respectively (Figure 2).

3.3 Effect of All on inactivation kinetic characteristics of $I_{Na,late}$ of SCN5A

As $I_{Na,late}$ is involved in change of inactivation kinetics of Nav1.5 channel. We analyzed the effect of All on the fast inactivation kinetics of three SCN5A mutants. Depolarizing steps from 2 to 50 ms were tested to ensure full current decay. Three mutation channels demonstrated a significantly longer time constant of the slow component (Tau2) compared with the WT channel over the entire voltage range from −70 mV to + 20 mV. The slower decay (Tau2) indicated a decelerated inactivation of open channels. The fast time constant (Tau1) proportion of the mutant under each test potential did not change compared with WT. Furthermore, Tau 2 of three mutations appeared shorter after exposure to 30 μM All, but Tau 1 was not different to that of control (Figure 3).

3.4 Effect of All on $I_{Na,peak}$ and ratios of $I_{Na,late}/I_{Na,peak}$ of SCN5A

$I_{Na,peak}$ was elicited using depolarizing steps from −70 mV to + 40 mV for 500 ms from a holding potential of −120 mV. $I_{Na,peak}$ representative current traces of three channels and WT with or without co-incubation with All for 24 h are shown in Figure 4. The peak current densities of F1473S, T353I and E1784K were −103.5 ± 6.6 pA/pF, −138.4 ± 10.2 pA/pF and −93.1 ± 8.7 pA/pF. After 30 μM All treatment, they were −265.1 ± 12.7 pA/pF, −284.2 ± 16.9 pA/pF and −241.3 ± 15.5 pA/pF respectively ($P < 0.01$, $n = 10$, Figure 4).The ratios of the late current/peak current ($I_{Na,late}/I_{Na,peak}$) of F1473S, T353I and E1784K were reduced from 3.98%, 3.78% and 4.15% to 0.41%, 0.52% and 0.43% by 30 μM All treatment ($P < 0.01$, $n = 10$), which were close to 0.21% of WT.

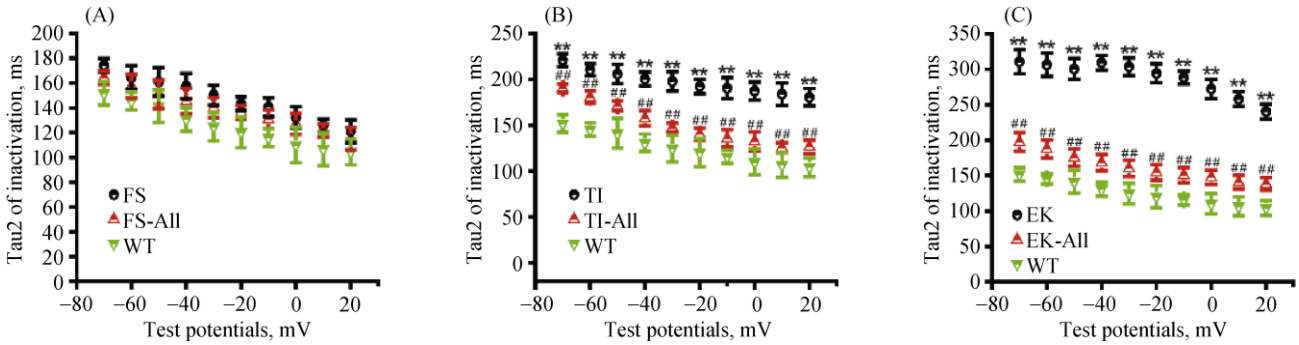

Figure 3. Effect of All on time constants of inactivation of SCN5A current. (A): slow time constants of F1473S are not significantly different among WT, F1473 and F1473-All cells; (B) the prolonged slow time constants of inactivation of T353I are shortened by 30 μM All treatment; (C) compared with WT, slow time constants of inactivation of E1784K are significantly lengthened and the prolonged slow time constants of inactivation of E1784K are relieved by 30 μM All and close to those of WT. **: $P < 0.01$, compared with WT. All: Allicin.

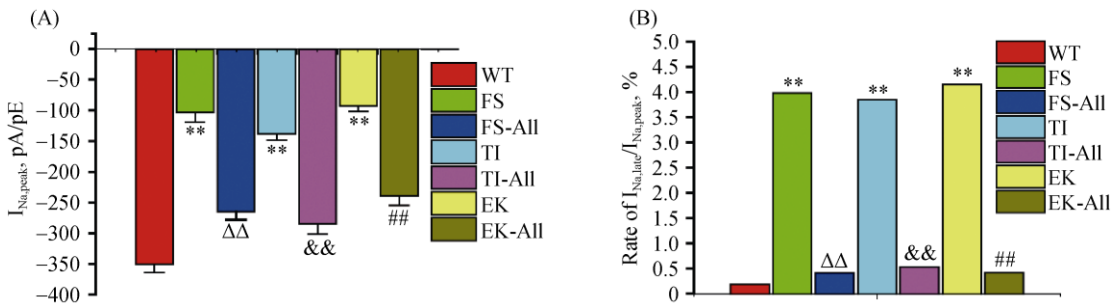

Figure 4. Effect of All on $I_{Na,peak}$ and ratios of $I_{Na,late}/I_{Na,peak}$. (A) The peak current densities of WT, F1473S, T353I and E1784K before and after incubated with 30 μM All. (B) The effect of All on the ratios of $I_{Na,late}/I_{Na,peak}$. Ratios of $I_{Na,late}/I_{Na,peak}$ of three mutations are significantly reduced, which is close to that of WT. **: $P < 0.01$, compared with WT. ΔΔ: $P < 0.01$, compared with FS. &&: P < 0.01, compared with TI. ##: $P < 0.01$, compared with EK. All: Allicin; $I_{Na,late}$: late sodium currents.

4 Discussion

LQT3 is caused by gain-of-function mutations of the Nav1.5 channel, which is a rare disease but causes high susceptibility to potentially lethal ventricular tachyarrhythmias.[8] Not very effective treatments are available for this syndrome except for invasive implantation of defibrillators (ICD) or pacemakers in some cases.[13] In our study, it was found that All can inhibit $I_{Na,late}$ of three mutations of SCN5A, F1473S-SCN5A, T353I-SCN5A and E1784K-SCN5A. This indicates that All can reduce LQT3 events.

$I_{Na,late}$ from normal myocytes is generally small, but it may be enhanced by more than several folds in various pathological conditions such as ischemia/reperfusion, heart failure and Nav1.5 sodium channel gene mutations. A sustained Na^+ current has been recognized to contribute to the plateau phase of the action potential of cardiomyocytes. In clinical terms, patients often exhibit QT interval prolongation at lower heart rates, which have an increased risk for cardiac events.[14] Patients with LQT3 who received pharmacological treatment show different efficacy. Sodium channel blockers such as mexiletine and flecainide have been a potential treatment option for patients with LQT3. Mexiletine appeared to significantly shorten QT interval among LQT3 patients.[15] Ruan, *et al*.[16] hold the opinion that treatment effect of mexiletine on LQT3 patients has

mutations in specific, which suggest examining, whether mexiletine can be effective for a specific patient. Similar to mexiletine, flecainide is also shown to shorten the QT interval in LQT3 patients by preferentially blocking increased $I_{Na, late}$ of SCN5A mutants.[17] In this study, we find that All play roles in three kinds of mutations, and this effect showed concentration-dependent characteristic. IC_{50} of F1473S and E1784K were lower than that of T353I. It suggests All may reduce LQT events by inhibiting $I_{Na, late}$ in spite of the different sensitivity of drugs to different mutations.

Furthermore, we find that the main mechanism of inhibition of All on $I_{Na, late}$ current is its effect on the deactivation dynamics of mutation Nav1.5 channels, especially the slow time constant of deactivation. All can significantly shorten the slow time constant of deactivation of T353I and E1784K, while having no effect on F1473S. Among them, $I_{Na, late}$ of T353I and E1784K are rarely induced by channel reopening during sustained depolarization in two different modes, including burst openings and scattered late openings.[8] Slowly as the burst opening mode undergoes, it can complete voltage-dependent inactivation and deactivates upon repolarization quickly. But scattered late openings are inactivated very slowly and may include a truly late current. $I_{Na, late}$ increase of F1473S may be explained by other mechanisms, such as the expression and location change of cell membrane channel proteins, which need further investigation.

Another interesting finding is that All inhibits the $I_{Na, late}$ and increases the $I_{Na, peak}$ meanwhile. The ratios of $I_{Na, late}/I_{Na, peak}$ of three mutations are remarkably decreased to the level close to that of WT. In normal condition, the amplitude of $I_{Na, late}$ is less than 1% of $I_{Na, peak}$. It can be increased due to impaired inactivation of the sodium channel with a variety of mutations.[18] The increased ratio of $I_{Na, late}/I_{Na, peak}$ is likely to contribute to the sodium overload observed in LQT3, which may play a role in the abnormalities of ventricular repolarization. This drug thereby appeared well-suited for balancing consequences of $I_{Na, late}$ and $I_{Na, peak}$. On the other hand, Brugada syndromes may be related to the reduction of $I_{Na, peak}$ caused by Nav1.5 channel mutation.[19] This indicates that All might have a potential inhibition effect on both LQT3 and Brugada syndromes, especially the overlap phenotype of SCN5A mutations.

Due to the unavailability of gene knockout mouse model with different SCN5A mutations, we did not test the effects of drugs on action potential from myocardial cells and QTc of electrocardiogram from animals with SCN5A mutations. This is the limitations of our study. Further research will be needed in the future.

Acknowledgments

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