Allicin inhibits transient outward potassium currents in mouse ventricular myocytes

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Abstract. Allicin is the active constituent of garlic, a widely used spice and food. The remedial properties of garlic have also been extensively researched and it has been demonstrated that allicin is able to inhibit the transient outward potassium current (I_{to}) in atrial myocytes. However, the direct effect of allicin on I_{to} in ventricular myocytes has yet to be elucidated. In the present study, the effects of allicin on I_{to} in ventricular myocytes isolated from mice were investigated, using the whole-cell patch recording technique. The results revealed that I_{to} current was not significantly suppressed by allicin in the low-dose group (10 μmol/l; P>0.05). However, I_{to} was significantly inhibited by higher doses of allicin (30, 100 and 300 μmol/l; P<0.05 vs. control; n=6) in a concentration-dependent manner (IC_{50}=41.6 μmol/l). In addition, a high concentration of allicin (≥100 μmol/l) was able to accelerate the voltage-dependent inactivation of I_{to} in mouse ventricular myocytes. In conclusion, the present study revealed that allicin inhibited the I_{to} in mouse ventricular myocytes, which may be the mechanism through which allicin exerts its antiarrhythmic effect.

Introduction

Allicin is the active compound in garlic, a well-researched remedy that is widely used as a spice and food (1,2). It has been reported that garlic may reduce cholesterol levels, lower blood pressure, inhibit platelet aggregation, activate fibrinolysis and prevent atherosclerosis, while it also has antioxidant and anticancer effects (3-12). Garlic has also been reported to have an antiarrhythmic effect (13,14), which has been observed in ventricular and supraventricular arrhythmias (13). The incidence of ischemia/reperfusion-induced ventricular fibrillation in isolated perfused rat hearts was found to be reduced by garlic powder (15). Garlic significantly decreases the upper limit of vulnerability of ventricular fibrillation and improves defibrillation efficacy in a dose-dependent pattern (16,17). Martín *et al* (18) revealed that allicin inhibited the myocardial contraction and slowed the sinus rhythm. In a further study, Martín *et al* (19) demonstrated that garlic dialysate was able to prolong the effective refractory period and the sinus node recovery time of isolated rat atria, in addition to suppressing premature ventricular contractions and ventricular tachycardia in ouabain-intoxicated canines.

A study by Deng *et al* (20) revealed that allicin was able to inhibit transient outward potassium currents (I_{to}) in human atrial myocytes. However, the direct effect of allicin on I_{to} in ventricular myocytes has yet to be elucidated. Therefore, in the present study, the effects of allicin on I_{to} in ventricular myocytes isolated from mice were investigated, using the whole-cell patch clamp recording technique to test the effect of allicin on I_{to}, as detected via I_{to} amplitude and kinetics, including I_{to} activation, inactivation and recovery.

Materials and methods

Ethical approval. All animal procedures were approved by the Institutional Animal Care and Use Committee at Renmin Hospital of Wuhan University (Wuhan, China). The animals used in the present study were male C57 mice, aged 8-10 weeks.

Drugs and solution. Tyrode's solution was composed of the following: 130 mmol/l NaCl, 5.4 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 0.3 mmol/l Na_2HPO_4, 10 mmol/l HEPES and 10 mmol/l glucose. The pH of the solution was adjusted to pH 7.4 using NaOH. In addition, Ca^{2+}-free Tyrode's solution was used, without $CaCl_2$. The collagenase solution was composed of Ca^{2+}-free Tyrode's solution containing 0.6 mg/ml collagenase type II (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 0.1% bovine serum albumin, 20 mM taurine and 30 μM $CaCl_2$. Kraft-Brühe (KB) solution included 10 mmol/l taurine, 70 mmol/l glutamic acid, 25 mmol/l KCl, 10 mmol/l KH_2PO_4, 22 mmol/l glucose and 0.5 mmol/l ethylene glycol tetraacetic acid (EGTA). The pH of the KB solution was adjusted to pH 7.2 using KOH. Tyrode's solution was supplemented with 10, 30, 100 and 300 μmol/l allicin during allicin treatment. Furthermore, the pipette solution used in the study

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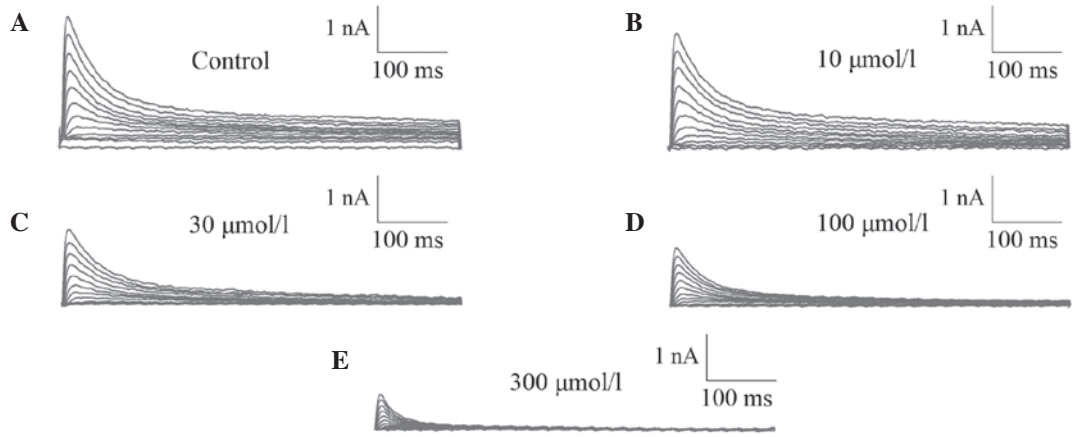

Figure 1. Effects of allicin on transient outward potassium currents (I_{to}) in mouse ventricular myocytes. The data reveal the representative voltage-dependent I_{to} (A) under control conditions, and (B) in the presence of 10 μmol/l allicin, (C) 30 μmol/l allicin, (D) 100 μmol/l allicin and (E) 300 μmol/l allicin. Data are presented as mean ± standard deviation.

consisted of 110 mmol/l K-aspartate, 20 mmol/l KCl, 8 mmol/l NaCl, 1 mmol/l $MgC1_2$, 1 mmol/l $CaC1_2$, 4 mmol/l MgATP, 0.1 mmol/l EGTA and 10 mmol/l HEPES, and was adjusted to pH 7.2 using KOH. Allicin was purchased from Xuzhou Ryen Pharm Co., Ltd (Xuzhou, China).

Isolation of ventricular cardiac myocytes. A total of 36 C57BL/6 mice, weighing 25.1±3.4 g, were heparinized [100 U; intraperitoneal injection (i.p.); Wangbang Co., Xuzhou, China] 15 min prior to sacrifice, anaesthetized by pentobarbital sodium (60 mg/kg; i.p.; Sigma-Aldrich, St. Louis, MO, USA) and sacrificed by cervical dislocation. Hearts were rapidly removed and retrogradely perfused at a temperature of 37˚C with the following solutions, according to Langendorff technique (21): i) Tyrode's solution (5 min); ii) Ca^{2+}-free Tyrode's solution (5 min); iii) collagenase solution (15 min); and iv) KB solution (5 min). Subsequent to the perfusion, the left ventricular free wall was dissected from the heart and placed in ice-cold KB solution. The tissue was then minced and titrated to free individual myocytes. Isolated cardiac myocytes were stored in KB solution at 4˚C until required.

Electrophysiology recording. Whole-cell patch clamp was performed on the myocytes using an EPC-9 amplifier (Heka Elektronik, Lambrecht, Germany), as previously described (21), and data was recorded and analyzed with a Pulse/Pulsefit software interface (version 8.31; Heka Elektronik). During the experiments, 1.5 ml myocytes were placed in the experimental chamber and mounted on the stage of an inverted microscope (IX70; Olympus Corporation, Tokyo, Japan) and perfused with Tyrode solution supplemented with 10, 30, 100 and 300 μmol/l allicin for 5 min at a rate of 2-3 ml/min at room temperature. In order to elucidate the effect of allicin on I_{to} in mouse ventricular myocytes, 6 cells were observed per solution influx, in triplicate. Pipettes had resistances of 2.5-3.5 MΩ when filled with pipette solution. Series resistance (Rs) was between 4-8 MΩ and was compensated by 80-90% to reduce the Rs. Current signals were filtered at 3 kHz by an 8-pole Bessel filter, digitized at a sampling rate of 1 kHz and recorded on a computer running Pulse/Pulsefit software, which was additionally used for the generation of voltage pulses and data analysis.

I_{to} recording. The total I_{to} was determined by 500 msec depolarizing pulses varying from -50 to +60 mV in 10 mV increments from a holding potential of -80 mV. In order to examine I_{to}, pre-pulse (100 msec, -40 mV) was used to inactivate I_{to} prior to activation steps with allicin, and I_{to} was measured by subtracting the currents before and after that pre-pulse. By dividing the measured current amplitude by the membrane capacitance (pA/pF), I_{to} values were reported as current densities.

The IC_{50} of allicin on I_{to} was fitted with Hill function using OriginPro version 8.0 software as follows: $E = E_{max}[1+(D/C)b]$, where E is the effect at concentration C, E_{max} is the maximum effect, D is the concentration for half-maximum action (IC_{50}) and b is the Hill coefficient.

Steady-state activation curve of I_{to}. Using the current-voltage (I-V) association for I_{to}, the voltage-dependent of steady-state activation curve for I_{to} was fitted to the Boltzmann equation as follows: $I/I_{max} = 1/[1+\exp((V_T - V_{1/2})/k)]$, where I_{max} is maximum current, V_T is the membrane potential, $V_{1/2}$ is the midpoint potential for activation and K is a slope factor (22).

Steady-state inactivation of I_{to}. The two-step voltage-clamp protocol was applied for steady-state inactivation of I_{to}, as previously described (21). The process involved an inactivating pre-pulse period that varied from -110 mV to +10 mV with a 1 sec pre-pulse, followed by a fixed 400 ms test pulse to +40 mV. The test current amplitude of I_{to} at each pulse potential was normalized to the maximal amplitude of this current (I/I_{max}). Data were fitted to the Boltzmann equation.

Recovery from inactivation of I_{to}. The time-dependence of reactivation was measured using an inactivating pulse (-40 mV, maintained for 500 msec). Following this, at variable time intervals (10-200 msec), a 500 msec test pulse at +40 mV was performed. The ratio of the current amplitude produced by the test pulse to the inactivating pulse (P2/P1) was plotted as a function of the time intervals. The time constant was calculated by data fitted to exponential functions.

Statistical analysis. All data are expressed as the mean ± standard deviation. Statistical analysis was performed using

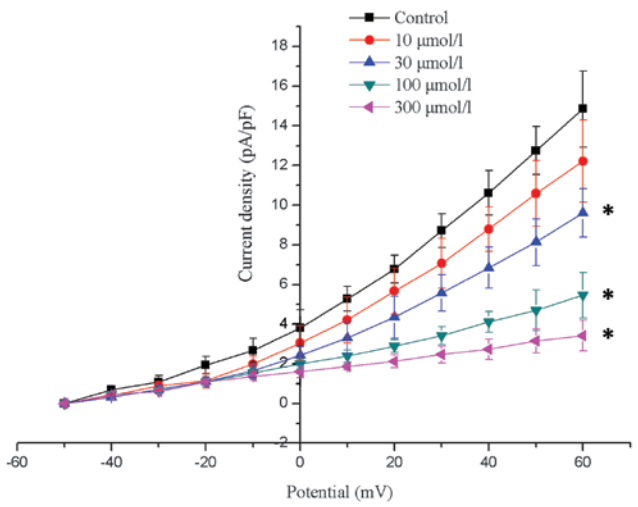

Figure 2. Current-voltage association for I_{to} in mouse ventricular myocytes following treatment with various allicin concentrations. Allicin inhibited I_{to} in a concentration-dependent manner. Data are presented as the mean ± standard deviation. *P<0.05 vs. control, n=6. I_{to}, transient outward potassium current.

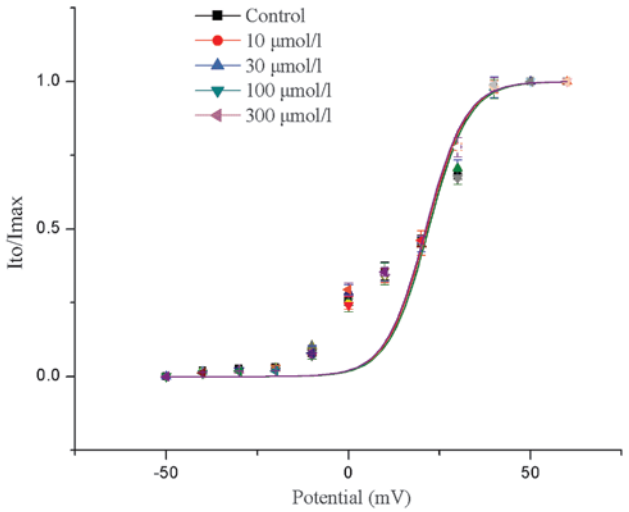

Figure 4. Voltage-dependent activation curve of I_{to} using the current-voltage association for I_{to}. Allicin had no significant effect on the voltage-dependence of the steady-state activation curve of I_{to} (P>0.05). Data are presented as the mean ± standard deviation. I_{to}, transient outward potassium current; I_{max}, maximum current.

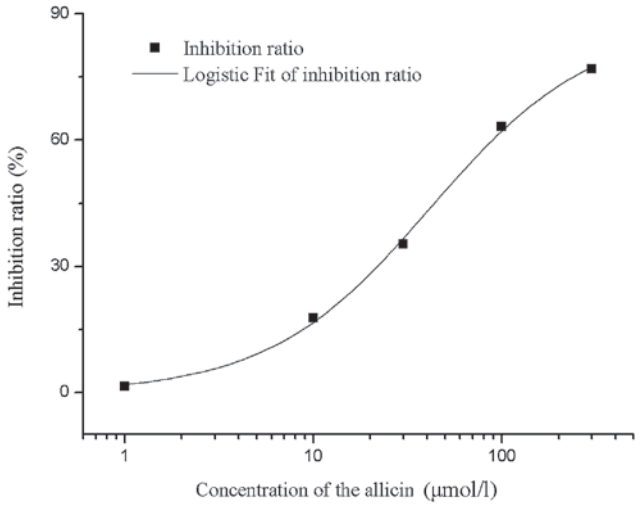

Figure 3. Dose-response association for inhibition of I_{to} by allicin. At a potential of +60 mV, treatment with 1, 10, 30, 100 and 300 μmol/l allicin decreased the peak I_{to} current by 1.5, 17.8, 35.3, 63.2 and 76.9%, respectively. The IC_{50} of allicin on I_{to} was fitted with Hill function and calculated to be 41.6 μmol/l, using OriginPro version 8.0 software (n=6). I_{to}, transient outward potassium current.

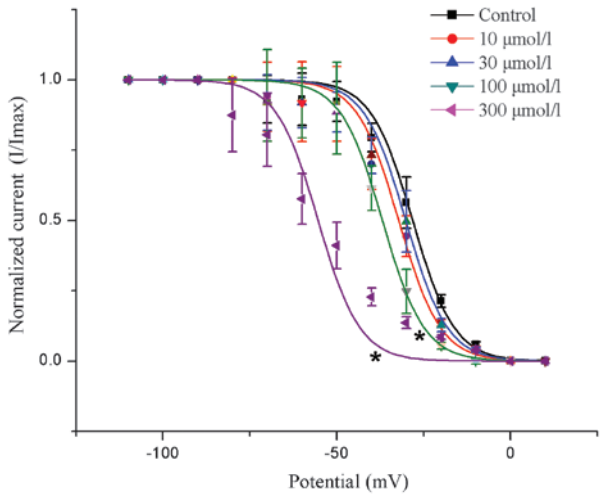

Figure 5. Voltage dependence of the inactivation (I/I_{max}) of I_{to} following treatment with various allicin concentrations. The voltage dependence of the inactivation of I_{to} was negatively shifted after treatment with high concentrations of allicin (100 and 300 μmol/l; *P<0.05 vs. control; n=6).Data are presented as the mean ± standard deviation. I/I_{max}, current / maximum current; I_{to}, transient outward potassium current.

a Student's t test and analysis of variance, performed on SPSS version 17.0 software (SPSS, Inc. Chicago, IL, USA). Patch-clamp data were analyzed using Origin version 8.0 (OriginLab Corporation, Northampton, MA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of allicin on voltage-dependent I_{to}. Allicin at 10, 30, 100 and 300 μmol/l was applied, respectively. I_{to} was blocked by allicin in a concentration-dependent manner. Currents were gradually decreased with the increase of allicin concentration. The representative current blocked by allicin at 10, 30, 100 and 300 μmol/l is shown in Fig. 1.

Fig. 2 displays the I-V association for I_{to} density prior to and following the application of 10, 30, 100 and 300 μmol/l allicin. The I_{to} was not significantly suppressed by allicin in the low dose (10 μmol/l; P>0.05); however, it was significantly suppressed by higher doses (30, 100 and 300 μmol/l; P<0.05; n=6) compared with the control.

In addition, Fig. 3 shows the dose-response association for the inhibition of I_{to} by allicin. At a potential of +60 mv, treatment with 1, 10, 30, 100 and 300 μmol/l allicin decreased the peak I_{to} by 1.5, 17.8, 35.3, 63.2 and 76.9%, respectively. The IC_{50} of allicin on I_{to} was fitted with Hill function and calculated

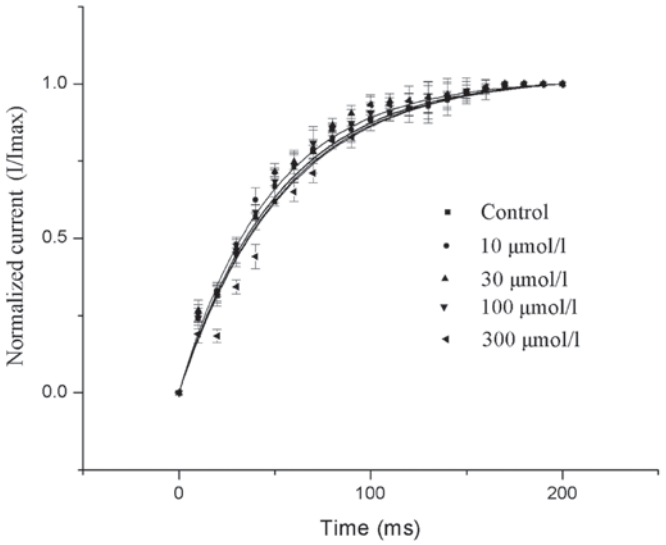

Figure 6. Effects of allicin on recovery from the inactivation of I_{to}. Allicin was not found to have a significant effect on the recovery from the inactivation of I_{to} (P>0.05 vs. control). I_{to}, transient outward potassium current; I/I_{max}, current / maximum current.

as 41.6 μmol/l (n=6 cells in each group) using OriginPro 8.0 software.

Effects of allicin on the steady-state activation curve of I_{to}. Allicin was not found to have a significant effect on the voltage-dependence of the steady-state activation curve of I_{to} (P>0.05; Fig. 4).

Effects of allicin on the steady-state inactivation of I_{to}. The results revealed that a low dose of allicin had no significant effect on the voltage-dependence of the inactivation (I/I_{max}) of I_{to} (control, $V_{1/2}$ = -28.2±4.7 mV; 10 μmol/l allicin, $V_{1/2}$ = -32.2±3.8 mV; 30 μmol/l allicin, $V_{1/2}$ = -30.1±3.6 mV; n=6; P>0.05, compared with the control). However, as shown in Fig. 5, high doses of allicin significantly shifted the voltage-dependence of the inactivation of I_{to} toward the negative potential (100 μmol/l allicin, $V_{1/2}$ = -36.9±4.1 mV; 300 μmol/l allicin, $V_{1/2}$ = -55.3±5.0 mV; n=6; P<0.05 compared with the control).

Effects of allicin on the recovery from inactivation of I_{to}. Allicin was not found to have a significant effect on the recovery from the inactivation of I_{to} following allicin treatment (P>0.05; Fig. 6).

Discussion

In the present study, allicin significantly inhibited I_{to} in mouse ventricular myocytes in a concentration-dependent manner. High-dose allicin (≥100 μmol/l) was able to significantly shift the voltage-dependence of the steady-state inactivation curve of I_{to} towards an increasingly negative potential. However, allicin did not have a significant effect on steady-state activation, or recovery from the inactivation of I_{to}.

Traditional Chinese medicine has been used for thousands of years for the treatment of cardiovascular diseases (23,24). In recent decades, garlic has been found to possess anti-arrhythmic effects (13,14). Several reports (25-27) have indicated that allicin is the predominant active component that is responsible for the majority of the biological activities of garlic, including attenuating ischemic injury, lowering blood pressure and antiarrhythmic effects (6,28,29). The chemical structure of allicin is CH_2=CH-CH_2-S(O)-S-CH_2-CH=CH_2, and it has been has been manufactured synthetically and produced worldwide (18). Although garlic has been discovered to be a significant antiarrhythmic agent, the exact mechanism has yet to be elucidated.

In the present study, allicin significantly inhibited I_{to} in mouse ventricular myocytes; however, it had no significant effect on steady-state activation, or recovery from inactivation of I_{to}, which is in agreement with previous findings (20). In the study by Deng *et al* (20), the research target was human atrial myocytes, and it was demonstrated that 30 μmol/l allicin was able to negatively shift the voltage-dependence of the steady-state inactivation curve of I_{to}. By contrast, in the present study, only high-dose allicin (≥100 μmol/l) was able to significantly shift the steady-state inactivation curve of I_{to} towards an increasingly negative potential. This may be due to allicin having different effects in different tissues and species. Allicin exerts its suppressive effect on I_{to} by changing the quantity and kinetic properties of I_{to}. In human atrial monocytes, I_{to} contributes to cardiac repolarization, whilst in the hearts of mice, I_{to} has a role in action potential repolarization (30,31). Notably, I_{to} is not uniformly distributed within the left ventricle in humans, mice and certain other mammals (32-35). In the left ventricular free wall, I_{to} is larger in epicardial compared with endocardial regions, which contributes to the regional variations of action potential (AP) profiles and results in a prominent AP notch in the epicardium, but not in the endocardium (36). It has been confirmed that a prominent I_{to} is important in physiological and pathophysiological process (37-41). The high incidence of phase 2 reentry and ventricular fibrillation during myocardial ischemia was partly due to the prominent I_{to}-mediated epicardial AP dome (42). In patients with coronary heart disease, the incidence of sudden mortality in men was significantly higher compared with that in women (43,44). This may be a result of a more prominent I_{to} in men compared with women (39). Thus, I_{to} block may be an effective therapy for arrhythmia (37).

In the present study, it was revealed that allicin was able to inhibit I_{to}, and may be the mechanism through which allicin exerts its antiarrhythmic effect. Antiarrhythmic therapeutics with low toxicity and low reverse use-dependence (RUD) effects are a focal point in antiarrhythmic drug research. Xing *et al* (23) confirmed that allicin has similar effects to amiodarone on the conduction system and cardiac electrophysiology. However, allicin possesses no RUD and this may contribute to multi-channel blockers. Furthermore, allicin appears to be safe for use in the majority of conditions (2) and is therefore likely to be a promising antiarrhythmic therapy.

In conclusion, the present study revealed that allicin inhibits I_{to} in mouse ventricular myocytes, which may be the mechanism through which allicin exerts its antiarrhythmic effect. Thus, allicin has demonstrated potential to be a promising antiarrhythmic therapy in the future; however, whether allicin exerts the same effect in other tissues or species requires further investigation.

Acknowledgements

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