



Effects of sodium ferulate on the ultrarapid delayed rectifier K^+ current in human atrial myocytes[☆]

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Abstract

Objective: To study the effects of sodium ferulate on the ultrarapid delayed rectifier K^+ current (I_{Kur}) in human atrial myocytes. **Methods:** Human atrial myocytes were isolated by enzyme dispersion method. I_{Kur} in human atrial myocytes were recorded by using the whole cell patch clamp. The changes of I_{Kur} were compared in the absence and the presence of sodium ferulate. **Results:** There was no effect of 0.4 g/L sodium ferulate on I-V relation of I_{Kur} . However, 0.4 g/L sodium ferulate inhibited I_{Kur} to some degrees at each test pulse. The current densities of I_{Kur} at +60 mV decreased from 4.997 ± 0.35 PA/PF to 3.331 ± 0.26 PA/PF ($n = 6$, $P < 0.05$). The inhibitory effect was concentration-dependent. IC_{50} was (0.41 ± 0.03) g/L and the Hill coefficient was 0.95 ± 0.05 . **Conclusion:** Sodium ferulate as a potassium channel blocker can inhibit I_{Kur} in human atrial myocytes effectively.

Key words: sodium ferulate; ultrarapid delayed rectifier K^+ current; patch clamp

INTRODUCTION

Sodium ferulate is a deviation from ferulate acid extracted from two Chinese medicines, angelica and szechwan lovage rhizome. To date, it is known that sodium ferulate has characteristics such as; blood vessel dilation, anti-platelet activation, and anti-oxidation and free radical elimination^[1-3]. Some investigations show that it may play a role in the blockade of potassium channel, and may have an anti-arrhythmic effect^[4]. The ultrarapid delayed rectifier (potassium current) is the main component as the repolarization of the atrial myocytes action potential. The abnormality of I_{Kur} takes an important responsibility for the occurrence of atrial tachy-arrhythmias such as atrial fibrillation (AF) and

atrial flutter^[5-8]. And interestingly, gene encoding for this channel only expresses in human atrial and not in respective ventricle myocytes. Therefore, $Kv1.5$ potassium channel, as the main molecular basis of I_{Kur} , may be a novel and ideal target for the development of the drugs to treat atrial fibrillation (AF)^[9-14]. So this experiment aims to observe the effect of sodium ferulate on the I_{Kur} of human atrial myocytes by patch clamp.

MATERIALS AND METHODS

Objective

Atrial myocytes were isolated from specimens of human right atrial appendage obtained from patients [(40.69 \pm 15.27) years old; male 11, female 9] undergoing cardiac surgery. The procedure for obtaining the tissue was approved by the Ethics Committee of the University of Huazhong science and technology. All atrial specimens were grossly normal at the time of cardiac surgery, and all patients were free of supraventricular

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tricular tachyarrhythmias and symptomatic congestive heart failure.

Chemicals and solution preparation

Calcium-free cardioplegic solution for transport of specimens contained (in mmol/L): KH_2PO_4 50, MgSO_4 8.0, adenosine 5.0, HEPES 10, glucose 140, mannitol 100, and taurine 10, with pH adjusted to 7.3 with KOH. The standard Tyrode's solution contained (in mmol/L): NaCl 140, KCl 5.0, MgCl_2 1.0, CaCl_2 1.0, NaH_2PO_4 0.33, HEPES 5.0, glucose 10, with pH adjusted to 7.4 with NaOH. For the atrial tissue wash, Calcium chloride was omitted. High-potassium storage medium contained (in mmol/L): KCl 10, K-glutamate 120, KH_2PO_4 10, MgSO_4 1.8, taurine 10, HEPES 10, EGTA 0.5, glucose 20, and mannitol 10, with pH adjusted to 7.3 with KOH. The pipette solution contained (in mmol/L): KCl 20, K-aspartate 110, MgCl_2 1.0, HEPES 10, EGTA 5.0, and GTP 0.1, Na_2 -phosphocreatine 5.0, Mg_2 -ATP 5.0, with pH adjusted to 7.2 with KOH. For I_{Kur} determination, BaCl_2 (200 mmol/L) and CdCl_2 (200 mmol/L) were added to the superfusion to block I_{K_1} and I_{Ca} . Atropine (1.0 mmol/L) was used to minimize possible acetylcholine-activated K current ($I_{\text{K}_{\text{Ach}}}$) contamination during the current recording. Sodium Ferulate was provided by GUOTAI medicine limited company (Sichuan, China). Collagenase II was purchased from Worthington company (USA), protease type XXII from Sigma (USA), all other chemicals were from Sigma (USA).

The isolation of atrial myocytes

After excision, the samples were quickly immersed in oxygenated, nominally Calcium-free cardioplegic solution for transport to the laboratory. Atrial myocytes were enzymatically dissociated with a modified procedure as following described^[15]. Briefly, the myocardial tissue was minced with a sharp blade and then placed in a 15 ml tube containing 10 ml of the Calcium-free Tyrode solution (36.1 °C), gently agitated by continuous bubbling with 100% O_2 . After 15 min (5 min at a time in fresh solutions), the chunks were incubated for 50 min in a similar solution containing 150~200 U/ml collagenase, 1.2 U/ml protease, and 1mg/ml bovine serum albumin (Sigma aldrich, USA). Subsequently, the supernatant was discarded and the chunks were re-incubated in a fresh enzyme solution with the same composition, but without protease. Microscope examination of the medium was performed every 5~10 min to determine the number and the quality of the isolated cells. When the yield appeared to be maximal, the chunks were suspended in a high potassium medium and gently blown with a pipette. The isolated myocytes were kept at room temperature in the medium for at least 1 h before study.

The recording of the patch clamp

The whole-cell and/or perforated patch-clamp techniques were used as described previously. Briefly, borosilicate glass electrodes (1.2 mm *A*) were pulled with a Brown-Flaming puller (model P-97, Sutter Instrument Co., Novato, CA, USA) and had tip resistances of 2~3MO when filled with pipette solution. A 3M KCl-agar salt bridge was used as reference electrode. Liquid junction potentials were compensated before the pipette touched the cell. After a gigaseal was obtained, the cell membrane was ruptured by gentle suction to establish the whole-cell configuration to record I_{Kur} . The patch clamp amplifier (Axopatch 200-B, Axon company, USA) connected with AD/DA converter (Digidata 1322A, Axon company, USA) and computer (HP company, USA) was used to release.

Statistical analysis

The values are presented as mean \pm s. Nonlinear curve fitting was performed using clampfit (Axon company, USA) and/or Sigmaplot (SPSS Science, Chicago, IL, USA). Paired Student's *t* tests were used to evaluate the statistical significance of differences between two group means. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Representative voltage dependent I_{Kur} (capacitance compensated) was recorded at 0.2 Hz in a typical experiment with a 100 ms prepulse from the holding potential +50 mV to +40 mV to inactivate I_{to_1} , followed by stepping in 10 mV 150 ms test pulses from -40 mV and +60 mV after a 10 ms interval, then to -30 mV. The relationship of I_{Kur} voltage-current was observed before and after the presence of 0.4 g/L sodium ferulate. 0.4 g/L sodium ferulate did not change the correspondence relationship of voltage-current of I_{Kur} . The maximal current density is at the potential of +60 mV. But 0.4 g/L sodium ferulate inhibited the current density of I_{Kur} at every voltage level respectively. The current density of I_{Kur} decreased from 4.99 ± 0.35 PA/PF to 3.33 ± 0.26 PA/PF ($n = 6$, $P < 0.05$; Fig 1).

At the condition with a 100 ms prepulse to +40 mV to inactivate I_{to_1} , followed by 150 ms test pulses from -50 to between -40 and +50 mV after a 10 ms interval, then to -30 mV, the different dose between 0.05~0.40 g/L was used to observed the inhibitory effect of sodium ferulate on I_{Kur} . The inhibitory effect of sodium ferulate on I_{Kur} is concentration dependent. The medium effective concentration is (0.41 ± 0.03) g/L by fitting according to the hill equation. The Hill coefficient is (0.95 ± 0.05) (Fig 2).

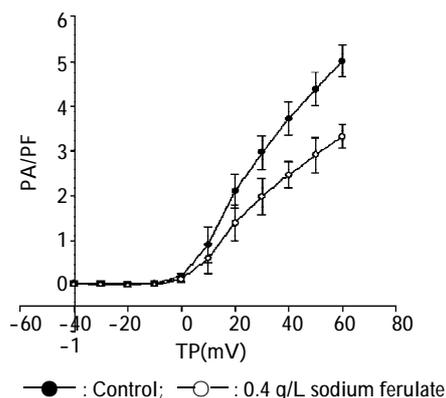


Fig 1 The effect of sodium ferulate on I_{Kur} , the I-V relationship of I_{Kur} during control, in the presence of 0.4 g/L sodium ferulate

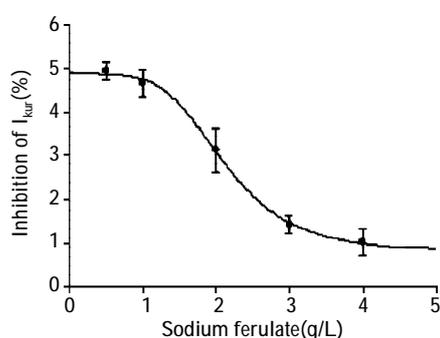


Fig 2 The concentration dependent effect of sodium ferulate on I_{Kur} of human atrial myocytes

DISCUSSION

Atrial fibrillation (AF), one of the most frequent tachyarrhythmias, can induce angina pectoris and congestive heart failure and even cause the genesis of the blood vessel embolism. Atrial myocytes have electrophysiological remodeling when atria fibrillation occurs, which may play an important role in the occurrence, persistence and recrudescence of AF^[16-17]. The main characteristics of AF are the reduction of the action potential duration and effective refractory period and re-entry wavelength of the atria myocytes^[18-19]. Anti-AF drugs, such as class III anti-arrhythmic drugs including Amiodarone, Dofetilide and Sotalol administered usually in clinic, have an effect of blocking the potassium channels^[20]. These drugs can block the repolarization potassium current of the atria myocytes, prolong APD and ERP and then lead to the prolongation of the re-entry wavelength. I_{Kur} Similar to other outer repolarization potassium currents, I_{Kur} is also highly responsible for repolarization in the human heart. 50% inhibition of this current could cause up to 66% prolongation of the human atrial myocyte action potential^[14]. Moreover, the genes that encode this potassium channel only express in human atrial, and not ventricle myocytes. So the blockade

of this potassium current may avoid the occurrence of the atria arrhythmia. Therefore the blockade of the ultrarapid delayed rectifier potassium is a new and ideal target for the treatment of AF.

Sodium ferulate, extracted from Dangui and Chuanxiong, is widely administered in the treatment of coronary artery disease, cerebral infarction and thromboangiitis obliterans due to its extensive pharmacological action with low toxicity, non-inducing malformation and non-mutagenesis. During the past few years, there have been some investigations showing that sodium ferulate may have an anti-arrhythmic effect^[21-23]. Wang and Li successively reported the effect of sodium ferulate on APD of the guinea pig columnar papillary muscle and monophasic action potential of rabbit ventricular myocardium^[4,24]. It can obviously prolong the APD₅₀, APD₉₀ and MNAP₅₀, MNAP₉₀. With the effect of significant prolongation of APD and ERP, it is presumed that this effect may be related to the blockade action of sodium ferulate on the delayed rectifier potassium channels. As an important member of potassium channels in human atrial myocyte, I_{Kur} may be the target of sodium ferulate. Our results showed that sodium ferulate can dose-dependently inhibit the I_{Kur} in human atria myocytes effectively as a potassium channel blocker and the value of IC_{50} and Hill coefficient were (0.41 ± 0.03) and (0.95 ± 0.05) , respectively. However, further in vivo experiments still need to be done to further identify its pharmacological effect on treating AF.

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