The Effect of Amiodarone on K⁺ Channel Current in the Normal and Hypertrophied Rat Heart

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The basis for the unique effectiveness of amiodarone treatment on cardiac arrhythmias is incompletely understood. We investigated and compared the effects of amiodarone on K⁺ channels of hypertrophied ventricular myocytes with normal ventricular myocytes in rats. The pressure overload hypertrophy models of rats were established by partial ligation of ascending aorta for 4 weeks. Ventricular myocytes were exposed to 1, 10 and 50 µmol/l amiodarone, and whole cell patch-clamp technique was used to study the effects of amiodarone on outward currents, such as delayed rectifier outward K⁺ current (I_K), slowly activating delayed rectifier outward K⁺ current (I_{Ks}), transient outward K⁺ current (I_{to}) and rectifier K⁺ current (I_{K1}). Compared with the control group, the current density of I_K, I_{K1} and I_{to} were all decreased in hypertrophied myocytes group. Hyper-concentration of amiodarone (10 and 50 µmol/l) inhibited I_{to} of the control group (37.5% ± 5.8% and 54.3% ± 5.7%) and the hypertrophied myocytes group (9.3% ± 2.3% and 22.8% ± 3.0%), but had effect on I_{K1} neither the control nor hypertrophied myocytes group. 10 µmol/l amiodarone inhibited I_{Ks} 23.3% ± 6.2% in the control group and 50.1% ± 9.8% in the hypertrophied myocytes group was higher than that in control, whereas I_{to} was lower in hypertrophy group. We concluded amiodarone do inhibit I_{to} and I_K in rat normal and hypertrophied cardiomyocytes. Amiodarone application should be treated difference between hypertrophied heart and normal heart.

Key words — amiodarone, K⁺ current, hypertrophied myocytes, patch clamp

INTRODUCTION

Amiodarone is the most effective and strangest antiarrhythmic drug ever developed. Amiodarone works through many different mechanisms. It fits into two separate categories of antiarrhythmic drugs such as Class I and Class III. It has been referred to as a Class III antiarrhythmic agent.¹⁾ However, it appeares electrophysiologic effects of Class I and IV agents and minor Class II characteristics.²⁾ Especially, it has been considered that the effects of amiodarone could depend on its direct effect on cell membrane channels.³⁾ The pharmacological actions of this compound are very complex. In the past we always used normal myocytes as research objects, but little know there was any difference of K^+ channels between normal and hypertrophy myocytes. It is well known that transient outward K^+ current (I_{to}), delayed rectifier outward K^+ current (I_K), and rectifier K^+ current (I_{K1}), are critical potassium channels for the movement of K^+ across the cell membrane. We compared the effects of amiodarone on these currents between normal and hypertrophied myocytes, and to comprehend the rational usage of amiodarone on hypertrophied myocytes in this study.

MATERIALS AND METHODS

Model of Hypertrophy — A total of 40 healthy Sprague–Dawley rats (9–11 weeks old, either sex, weight 210 to 300 g) were used in the study. All the rats used in the following experiments were subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations from the Declaration of Tongji University. The

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rats were randomly divided into two groups (control group and hypertrophy group). Cardiac hypertrophy was induced by pressure overload produced by partial ligation of the abdominal aorta by using the method described by Anderson PG.^{4, 5)} The control group was the sham-operated group; the aorta was dissected without application of the ligation. After operation, both groups were fed up with normal fodder and tap water in different cages for one month.

Venticular Myocytes Isolation — Ventricular myocytes were isolated from the hearts of rats using previous protocols.⁶⁾ Briefly, hearts were rapidly excised and cyclo-perfused with low calcium Tyrode's solution containing 0.08% collagenase, 0.006% protease, and then get single ventricular myocyte. The single ventricular myocyte selected for study is rod-shape, had clear striations and smooth and glossy surface.

Patch Clamp Studies — The whole-cell configuration of the patch clamp technique was used in single ventricular myocyte. We recorded K⁺ currents in a bath solution and a pipette solution contained (mmol/l): I_K recording: the external solution choline-Cl 145, MgCl₂ 2, Ethylene glycol-bis-(2-aminoethyl)-tetraacetic acid (EGTA) 1, 4-(2-Hydroxyethyl)piperazine-1-ethanesulonic acid (HEPES) 5, glucose 5.5; pH = 7.4 (LiOH). The pipette solution: KCl 140, MgCl₂ 1, K₂ATP 5, HEPES 5, EGTA 10; pH = 7.3 (KOH); I_{K1} recording: the external solution: choline-Cl 145, KCl 5, MgCl₂ 1, EGTA 5, HEPES 10, glucose 10; pH = 7.4 (KOH). The pipette solution: KCl 140, CaCl₂ 1, EGTA 10, HEPES 5, K₂ATP 5; pH = 7.3 (KOH); Ito recording: the external solution: NaCL 140, KCL 4, CaCL₂1.5, MgCL₂1, CdCL₂0.5, HEPES 5, glucose 10; pH = 7.4 (NaOH). The pipette solution: KCL 140, MgCL₂ 1, K₂ATP 5, EGTA 5, HEPES 10; pH = 7.3 (KOH).

I_K was elicited from the holding potential of -50 mV to +60 mV and by 450 ms pulses with 10 mV increments. I_{K1} was elicited from the holding potential of -120 mV to +60 mV and by 200 ms pulses with 10 mV increments. I_{to} was elicited from the holding potential of -80 mV and by 300 ms pulses with 10 mV increments from a potential -40 mV to +70 mV. We perfuse cell with amiodarone hydrochloride (SANOFI Co., Hangzhou, China; molecular weight 681.78) of 1, 10 and 50 µmol/l, and every cell was perfused 2–3 concentration steps. Then the change of currents were recorded and concentration-dependent inhibition were analysed. The rapidly activating delayed

rectifier outward K^+ current (I_{Kr}) blocker dofetilide (UK 68798; molecular weight 441.57) was used to record slowly activating delayed rectifier outward K^+ current (I_{Ks}). All recordings are at room temperature. The external solution was filled with $95\%O_2$ and 5%CO₂. The tip resistance of the pipette was 2– 4 mV when filled with internal solution. Compensated series resistance $1.57 \pm 0.16 \text{ M}\Omega$. Cell capacitance averaged $25.8 \pm 5.5 \text{ pF}$ (*n* = 10 per group). To normalize for differences in total membrane area, current densities (in pA/pF) were calculated by dividing the total current by the membrane capacitance of the cell. Data were sampled at 10 kHz and filtered at 2 kHz by using an Axopatch 200A amplifier (Axon Instruments, Union City, CA, U.S.A.). Statistical Analysis ---- pCLAMP 9.0 software was used for data acquisition and analysis values are presented as means \pm S.D. Statistical comparisons between the different amiodarone concentrations groups were obtained by analysis of variance (ANOVA). Comparisons between control and hypertrophied myocytes group means were performed with Student's t test. Differences with p < 0.05were considered significant, completed by SPSS 11.5 Statistical package.

RESULTS

Heart Characteristics

The rat hearts were significantly larger in hypertrophy group ($850 \pm 13 \text{ mg}$, n = 20) than in control group ($702 \pm 43 \text{ mg}$, n = 15). However, there was no difference in body weight (BW) between the two groups. The mean of left ventricular weight (LVW) were $508 \pm 38 \text{ mg}$ in control group and $649 \pm 45 \text{ mg}$ in hypertrophy group. Heart weight (HW) index (heart weight/body weight, HW/BW) and left ventricular weight index (left ventricular weight/body weight, LVW/BW) in the hypertrophy group were 21.2% and 26.3% greater than those in the control group. They were statistical significant (Table 1).

Effects of Amiodarone on I_{to}, I_{K1} and I_K

The outward currents in normal myocytes were differed from hypertrophied myocytes. The current density of I_K , I_{to} and I_{K1} were all decreased in the hypertrophied group compared to the control group (Table 2). Hypo-concentration amiodarone had no effect on I_{to} on normal myocytes, whereas for hyper-concentration amiodarone (10 and 50 µmol/l amiodarone) decreased

 Table 1. Measurement Results of Body Weight, Heart Weight, Left Ventricular Weight and Their Ratios (mean ± S.D.)

	BW	HW	LVW	HW/BW	LVW/BW
	(g)	(mg)	(mg)	(mg/g)	(mg/g)
Control	243 ± 15	702 ± 43	508 ± 38	2.83 ± 0.17	2.18 ± 0.13
Hypertrophy	237 ± 18	$850 \pm 13^*$	$649\pm45^*$	$3.55\pm0.19^*$	$2.90\pm0.17^*$

BW, LVW and HW indicate body weight, left ventricular weight and heart weight. p < 0.05 compared to control group.

Table 2. The Comparison of the Current Density between the Normal and Hypertrophied Myocytes (pA/pF)

	I _K	I_{Ks}	I _{to}	I_{K1}
Control $(n = 6)$	7.24 ± 0.85	5.73 ± 0.53	34.60 ± 2.73	10.26 ± 0.59
Hypertrophy $(n = 6)$	$4.18 \pm 0.33^{**}$	$3.05 \pm 0.79^{**}$	$23.24 \pm 1.18^{**}$	$7.93 \pm 1.28^*$

*p < 0.05, **p < 0.01, compared to control group.



Fig. 1. The Effects of 10 and 50 μ mol/l Amiodarone on Current-voltage Curve of I_{to} in Normal Myocytes



Fig. 2. The Effects of 10 and 50 μ mol/l Amiodarone on Current-voltage Curve of I_{to} in Hypertrophied Myocytes



Fig. 3. The Effect of 1 and $10 \,\mu$ mol/l Amiodarone on Current-voltage Curve of I_{Ks} in Normal Myocytes



Fig. 4. The Effect of 1 and $10 \mu mol/l$ Amiodarone on Currentvoltage Curve of I_{Ks} in Hypertrophied Myocytes

 $37.5\% \pm 5.8\%$ and $54.3\% \pm 5.7\%$ summit current density of I_{to} on normal myocytes. On hypertrophied myocytes, 10 and $50\,\mu$ mol/l amiodarone decreased $9.3\% \pm 2.3\%$ and $22.8\% \pm 3.0\%$ summit current density of I_{to}. So for I_{to}, the inhibition action of amidarone on hypertrophied myocytes was lower (Figs. 1, 2). For I_{K1}, 10 and 50 µmol/l amiodarone had no effects on I_{K1} of normal and hypertrophied myocytes.

 I_{K} is the major outward current responsible for ventricular repolarization. Amiodarone inhibited I_{K} not only on normal myocytes but also on hypertrophied myocytes. 1, 10 and 50 µmol/l amiodarone decreased current density of I_K by $22.78\% \pm 3.77\%$, $34.08\% \pm 7.1\%$ and $63.32\% \pm 7.87\%$. On hypertrophied myocytes, there were $17.31\% \pm 6.13\%$, $26.44\% \pm 8.62\%$ and $64.35\% \pm 7.28\%$. I_K comprises two distinct current components IKs and IKr. Dofetilide, a Class III antiarrhythmic agent and a selective blocker of IKr made the recorded delayed rectifier current represent IKs. The inhibition potent of amiodarone on I_{Ks} of hypertrophied myocytes was higher than that of control (Figs. 3, 4); 10 µmol/l amiodarone decreased $23.3\% \pm 6.2\%$ current density of I_{Ks} on normal myocytes and $50.1\% \pm 9.8\%$ on hypertrophied myocytes.

DISCUSSION

Heart disease often involves both hypertrophic and failure cellular phenotypes. Cardiac hypertrophy is associated with a significantly increased risk of cardiovascular morbidity and mortality, much of which stems from electrical remodeling and arrhythmogenesis. At the same time the antiarrhythmic research was most based on normal myocytes, whether they have same action on pathosis myocytes was unknown. As a result, means of treating hypertrophy-associated arrhythmias remain disappointingly ineffective. So our research tries to discuss the ionic mechanism by using amiodarone and further to observe the medicine action not only on normal myocytes but also on hypertrophied myocytes. In the past, much research focused on L type Ca current (I_{Ca-L}) and the Na⁺/Ca²⁺ exchanger (I_{NCX}). Only reduced density of I_{to} is the most consistent electrophysiologic change observed in hypertrophied heart.^{7,8)} For other outward currents studies were still controversial. Outward currents repolarization are numerous and complex on ventricular myocytes, and there is substantial interspecies variation in the profile of repolarizing currents, such as in small animal I_{to} is the prominent repolarization current, while in large animal I_K is the prominent repolarization current.⁹⁾ We choice the rat as the study model, because there are advanced technical and easy to make the model.^{4, 5)}

Amiodarone's significant anti-muscarinic activity is one aspect of its adverse effect profile. Amiodarone's blockade of muscarinic receptors in both rat heart and brain was first reported in 1984.¹⁰⁾ As previously mentioned, amiodarone induced a prolongation of ventricular repolarization that can be explained by the decrease in I_{to} and I_{Ks} current densities. In the rat, I_{Ks} is also decreased by long-term amiodarone,¹¹⁾ probably because of the decreased expression in Kv1.5 Potassium voltage-gated channel subfamily A member 5 (KCNA5).¹²⁾ We tried utilizing acute amiodarone application in rat normal and hypertrophied myocytes and reveal the effect on I_{to} and I_{Ks}. That would be useful for the antiarrhythmias in the clinic.

 I_K was the major outward current responsible for ventricular repolarization and composed with two distinct current components I_{Ks} and I_{Kr}. Amiodarone has inhibition effects on K⁺ channels.¹³⁾ IC 50 of amiodarone was 19.1 µmol/l for ATP-sensitive potassium channels of adult rat heart.¹⁴⁾ We had chosen 1, 10 and 50 µmol/l as acute amiodarone application. 1, 10 and 50 µmol/l decreased current density of I_K by 22.78% \pm 3.77%, 34.08% \pm 7.1% and $63.32\% \pm 7.87\%$ on normal myocytes. On hypertrophied myocytes, there were $17.31\% \pm 6.13\%$, $26.44\% \pm 8.62\%$ and $64.35\% \pm 7.28\%$. In some previous studies, amiodarone induced a prolongation of ventricular repolarization due to the decrease in Ito and IKs current densities in different models.^{9, 15, 16}) In our research, 10 and 50 µmol/l amiodarone had no effects on I_{K1} and can inhibit I_{to} and I_K, but the inhibition on hypertrophied myocytes was lower than control. We also found that acute amiodarone application had inhibition effects on I_{Ks} , and the inhibition effect on hypertrophied myocytes was higher than control group. 10 µmol/l amiodarone decreased $23.3\% \pm 6.2\%$ current density of I_{Ks} on normal myocytes and $50.1\%\pm9.8\%$ on hypertrophied myocytes. In other words, I_{Ks} of hypertrophied myocytes had more sensitivity for amiodarone. Because Electrophysiologic chatacteristics of ventricular wall are heterogeneous across the ventricular wall. IKr is expressed equally in subendocardium, M cell, and epicardium. The repolarization of M cell more rely on IKr because IKs

is expressed little in M cell. But In pressure overload hypertrophy models, because of the swelling volume of hypertrophied myocytes, I_{Ks} was expressed unequally across the ventricular wall, the current density of IKs on subepicardial myocytes was higher than on the M cells. Although the inhibition of I_{Ks} also make the QT interval longer, the dispersion of refractoriness was little.¹⁷⁾ Therefore, electro-reconstruction of hypertrophied myocytes mainly displayed the abnormality of repolarization currents. That could explain hypertrophied heart show the prolonged QT interval and longer action potential, which is the electrophysiological basis of the arrhythmia.^{18, 19)} So amiodarone with a multifaced pharmacological profile mostly inhibited IKs and partly blocked Ito that could prevent or inhibit ventricular arrhythmia in hypertrophied heart.

Our results contribute to understanding of the unique profile of amiodarone in the treatment mechanism of hypertrophied and normal heart. Amiodarone do inhibit outward K^+ current (I_{to} and I_K) in rat cardiomyocytes. To compare between the hypertrophied group and the control group, I_{to} and I_{Ks} appeared difference effects of amiodarone. That indicate we should treat difference between hypertrophied heart and normal heart, when we use amiodarone in the clinic. However, their clinical relevance remains unclear for at least two reasons: the choice of species dictated by the availability of extensive genomic information; The absence of primary cardiac pathology with or without arrhythmias. It is therefore essential to investigate whether remodeling is also observed in humans and how it interferes with the remodeling resulting from cardiac pathologies.

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