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Carvedilol blocks the cloned cardiac Kv1.5 channels in a β-adrenergic receptor-independent manner

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ABSTRACT

Carvedilol, a non-selective β-adrenergic blocker, is widely used for the treatment of angina pectoris and hypertension. We examined the action of carvedilol on cloned Kv1.5 expressed in CHO cells, using the whole-cell patch clamp technique. Carvedilol reduced the peak amplitude of Kv1.5 and accelerated the inactivation rate in a concentration-dependent manner with an IC_{50} of 2.56 μM. Using a first-order kinetics analysis, we calculated $k_{+1} = 19.68\ \mu M^{-1}\ s^{-1}$ for the association rate constant, and $k_{-1} = 44.89\ s^{-1}$ for the dissociation rate constant. The apparent K_D (k_{-1}/k_{+1}) was 2.28 μM, which is similar to the IC_{50} value. Other β-adrenergic blockers (alprenolol, oxprenolol and carteolol) had little or no effect on Kv1.5 currents. Carvedilol slowed the deactivation time course, resulting in a tail crossover phenomenon. Carvedilol-induced block was voltage-dependent in the voltage range for channel activation, but voltage-independent in the voltage range for full activation. The voltage dependences for both steady-state activation and inactivation were unchanged by carvedilol. Carvedilol affected Kv1.5 in a use-dependent manner. When stimulation frequencies were increased to quantify a use-dependent block, however, the block by carvedilol was slightly increased with IC_{50} values of 2.56 μM at 0.1 Hz, 2.38 μM at 1 Hz and 2.03 μM at 2 Hz. Carvedilol also slowed the time course of recovery from inactivation of Kv1.5. These results indicate that carvedilol blocks Kv1.5 in a reversible, concentration-, voltage-, time-, and use-dependent manner, but only at concentrations slightly higher than therapeutic plasma concentrations in humans. These effects are probably relevant to an understanding of the ionic mechanism underlying the antiarrhythmic property of carvedilol.

1. Introduction

Catecholamines play an important role in cardiac electrophysiological function, but they exert complex effects on cardiac action potential profiles. Norepinephrine modulates the duration of the action potential biphasically in guinea-pig cardiac ventricular muscle: low concentrations prolong the action potential duration, while high concentrations shorten it [1]. On the other hand, sympathetic nerve stimulation abbreviates atrial refractoriness in canine atrial myocytes [2]. Adrenergic stimulation modulates most cardiac ion channels, which are important determinants of action potential shapes. For example, isoproterenol and norepinephrine increase the delayed rectifying K^+ current in guinea-pig heart cells, which explains the shortening of action potential duration observed at high concentrations of catecholamines [3].

Carvedilol is a non-selective β-adrenergic blocker that is widely used for the treatment of angina pectoris and hypertension [4]. Carvedilol reportedly exerts antiarrhythmic activity [5,6]. The pharmacological effects of this drug on cardiac arrhythmia can be largely attributed to β-adrenergic receptor blocking [6]. Anti-adrenergic therapies with β-adrenergic blockers are also reported to reduce cardiovascular mortality in long-QT syndrome [7]. However, carvedilol has reportedly inhibited many different types of cardiac ion channels. For instance, carvedilol blocked cardiac repolarizing K^+ currents, including the slowly and rapidly activating delayed rectifier, the ultra-rapid activating delayed rectifier, the transient outward K^+ currents, ATP-sensitive K^+ currents, and HERG in various cell types [5,8–10]. Carvedilol also inhibited Nav1.5 currents, and L- and T-type Ca^{2+} currents in expressed cell lines and in rabbit ventricular myocytes [8,11,12]. Because antiarrhythmic effects may be a reflection of changes in action potential duration, these results raise the possibility that some of the effects of carvedilol on cardiac arrhythmias are attributable to inhibitory effects on various cardiac repolarizing K^+

Abbreviations: Kv, voltage-gated K^+ channel; CHO, Chinese hamster ovary; HERG, human ether-a-go-go related gene.

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currents. Indeed, carvedilol caused action potential duration prolongation in rabbit heart pacemaker cells and ventricular myocytes [8,13], which confers both antiarrhythmic and proarrhythmic potential [14]. However, carvedilol treatment has reduced the incidence of ventricular fibrillation in feline myocardium and the frequency of ventricular tachycardia in heart failure, thus producing significant improvement in mortality [15–17]. The presence of Kv1.5 in the human atrium has been reported, and it plays important roles in the early phase of atrial repolarization in humans [18,19]. Kv1.5 is, therefore, a primary therapeutic target for the pharmacological management of arrhythmias [20]. Because there is no report that examines the detailed mechanism of action of carvedilol on Kv1.5 channels, we investigated the effect of carvedilol on Kv1.5 currents and compared its potency with that of other β-adrenergic blockers.

2. Materials and methods

2.1. Cell preparation

Rat Kv1.5 cDNA was stably transfected into CHO cells (American Type Culture Collection, Manassas, VA, USA) using the Lipofectamine reagent (Invitrogen, Grand Island, NY, USA), as described previously [21,22]. The cell lines were maintained in a humidified incubator at 37 °C with 5% CO_2. The cells were cultured in 50-ml polystyrene culture flasks in Iscove's modified Dulbecco's medium (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM glutamine, 0.1 mM hypoxanthine, 0.01 mM thymidine, and 0.3 mg/ml geneticin (Invitrogen). The cells were passaged every 2–3 days. They were detached from the culture flask using brief tryptin-EDTA (Invitrogen) treatments and were seeded onto glass coverslips (12-mm diameter; Fisher Scientific, Pittsburgh, PA, USA) in a 35-mm petri dish 12–24 h prior to use. The coverslips with adherent cells were placed in a continuously perfused recording chamber for electrophysiological recording (RC-13; Warner Instruments, Hamden, CT, USA).

2.2. Electrophysiological recording

The whole-cell configuration of a patch-clamp technique was used to record Kv1.5 currents at room temperature (22–24 °C). Currents were acquired with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Patch pipettes were pulled from borosilicate glass capillaries (PG10165-4; World Precision Instruments, Sarasota, FL, USA) using a programmable horizontal microelectrode puller (P-97; Sutter Instrument Co., Novato, CA, USA). Voltage errors were minimized using 70–80% series resistance compensation if the Kv1.5 currents exceeded 1 nA. Command voltage generation, data acquisition, and data analysis were performed on an IBM-compatible Pentium computer running pClamp 10.0 software (Molecular Devices) using a Digidata 1322A interface (Molecular Devices).

2.3. Solutions and drugs

Cells were perfused with an extracellular bath solution containing (in mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, adjusted to pH 7.3 with NaOH. The intracellular pipette solution contained (in mM) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 EGTA, adjusted to pH 7.3 with KOH. The measured osmolarity of the extracellular bath solution was 300–340 mOsm. Carvedilol, alprenolol (Tocris, Ellisville, MO, USA), oxprenolol and carteolol (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) as stock solutions, and the concentrations used in the current study were then diluted with the extracellular solution to obtain the desired concentration. The concentration of DMSO in the final dilution was less than 0.1%, and this concentration had no effect on Kv1.5 currents [22,23].

2.4. Data analysis

Curve fitting and data analysis were done using Clampfit 10.0 (Molecular devices) and Origin 8.0 software (Origin Lab Corp., Northampton, MA, USA). The concentration–response relationship for the current block by carvedilol was fitted to the Hill equation:

$$f = \frac{1}{1 + [\mathrm{D}]/(IC_{50})^{n^{\mathrm{H}}}} \tag{1}$$

where f is the fractional block, [D] is the carvedilol concentration, IC_{50} is the carvedilol concentration that causes 50% block, and n^{H} is the Hill coefficient. The first-order blocking scheme was used to describe the drug–channel interaction. The apparent rate constants of association (k_{+1}) and dissociation (k_{-1}) were obtained from the following equations [24]:

$$\tau_{\mathrm{D}} = \frac{1}{k_{+1}[\mathrm{D}] + k_{-1}} \tag{2a}$$

$$K_{\mathrm{D}} = \frac{k_{-1}}{k_{+1}} \tag{2b}$$

in which τ_{D} is the time constant of the fast initial drug-induced current decay. The voltage dependence of steady-state activation was derived by normalizing the tail currents at −40 mV after stepping the depolarizing voltage from −60 to +50 mV. The activation curves were fitted with the Boltzmann equation:

$$y = \frac{1}{1 + \exp[-(V - V_{1/2})/k]} \tag{3}$$

where $V_{1/2}$ is the potential when the conductance is half-maximal, V is the test potential, and k represents the slope factor for the activation curve. The voltage dependence of steady-state inactivation was calculated from a two-pulse protocol. The currents were produced by a 250-ms depolarizing pulse of +50 mV with potentials between −60 and +20 mV by an accretion of 10 mV with 20 s of preconditioning pulses. The curves were fitted with the following equation:

$$\frac{I - I_{\mathrm{c}}}{\mathrm{I}_{\max} - I_{\mathrm{c}}} = \frac{1}{1 + \exp[(V - V_{1/2})/k]} \tag{4}$$

in which $I_{\max}$ represents the maximum current from the absence of inactivation, and I_{c} represents a non-inactivating current at the most depolarized 20 s preconditioning pulse. V, $V_{1/2}$ and k are the test potential, the point at which channels are half-inactivated, and the slope factor, respectively. The non-inactivating residual current was removed by subtracting it from the actual value. The dominant time constant of activation was determined by fitting a single exponential function to the latter 50% of activation [25]. The voltage dependence of the block was determined as follows: the fractional block was measured at individual test potentials and the voltage dependence of the fractional block was fitted with a Woodhull equation:

$$f = \frac{[\mathrm{D}]}{[\mathrm{D}] + K_{\mathrm{d}}(0) \times \exp(-z\delta FV/RT)} \tag{5}$$

where $K_{\mathrm{d}}(0)$ is the apparent affinity at 0 mV (the reference voltage), z is the charge valence of the drug, δ is the fractional electrical distance (i.e., the fraction of the transmembrane electric field sensed by a single charge at the receptor site), F is Faraday's constant, R is the gas constant, and T is the absolute temperature. In the present study, 25.4 mV was used as the value of RT/F at 22 °C.

2.5. Statistical analysis

All data are expressed as the mean ± S.E. A Student's *t*-test and one-way analysis of variance, followed by the Bonferroni test for comparisons of multiple groups, were used for statistical analysis. *P* values were considered significant at $P < 0.05$.

3. Results

3.1. Concentration- and time-dependent block of Kv1.5

Fig. 1A shows the whole-cell Kv1.5 current recordings evoked every 10 s by 250-ms test pulses to +50 mV from a holding potential of −80 mV, before and after application of various concentrations of carvedilol (0.3–10 μM). Under control conditions, the Kv1.5 currents were rapidly activated and then slowly inactivated during 250-ms depolarizing pulses. External application of carvedilol inhibited the peak amplitude of Kv1.5 and caused a dramatic change in the time course of the current, increasing the apparent rate of current decay in a concentration-dependent manner. Thus, the steady-state currents recorded at the end of the 250-ms depolarizing pulse were much lower than the peak currents, and were used as an index of block (Fig. 1B). A nonlinear least-square fit of the Hill equation yielded an IC_{50} value of 2.56 ± 0.05 μM with a Hill coefficient of 1.68 ± 0.10 (n = 9). Under control conditions, the dominant activation time constant was 1.16 ± 0.21 ms (n = 9) with a 250-ms depolarizing pulse of +50 mV. In the presence of 3 μM carvedilol, the activation time constant was 1.09 ± 0.19 ms (n = 9), which indicates that the activation kinetics were not altered by carvedilol. Under control conditions, Kv1.5 inactivation fit well to a curve describing a single exponential function (208.38 ± 12.35 ms, n = 9). However, the inactivation of Kv1.5 in the presence of carvedilol was better described by a biexponential function. The acceleration in the inactivation rate of Kv1.5 caused by carvedilol was concentration-dependent (Fig. 1C). At 0.3, 1, 3 and 10 μM, carvedilol decreased the fast time constants (τ_D) to 23.93 ± 2.68, 15.88 ± 2.53, 8.43 ± 0.59, and 4.20 ± 0.16 ms, respectively, but did not affect the slow time constants (205.78 ± 12.15, 208.26 ± 9.66, 206.06 ± 6.92, and 201.71 ± 6.67 ms, respectively). Plotting τ_D at +50 mV against each concentration and fitting it to a linear equation yielded the apparent association rate (k_{+1}) and dissociation rate (k_{-1}) constants of 19.68 ± 1.63 $\mu M^{-1}\ s^{-1}$ and 44.89 ± 8.55 s^{-1}, respectively (n = 9). The theoretical K_D value was derived from these values by k_{-1}/k_{+1}. The calculated K_D was 2.28 ± 0.04 μM, which was reasonably close to the IC_{50} obtained from the concentration–response curve shown in Fig. 1B. Thus, the time constant of carvedilol-induced current decay represents a good approximation of the drug-channel interaction kinetics. The Kv1.5 currents were completely deactivated during a 250-ms repolarizing pulse of −40 mV with a time constant of 18.36 ± 1.86 ms (n = 12) (Fig. 2). In the presence of 3 μM of carvedilol, the initial amplitude of the tail current was decreased and its subsequent decline was considerably slowed with a deactivation time constant of 45.26 ± 2.83 ms (n = 12, $P < 0.01$), which caused a tail crossover phenomenon.

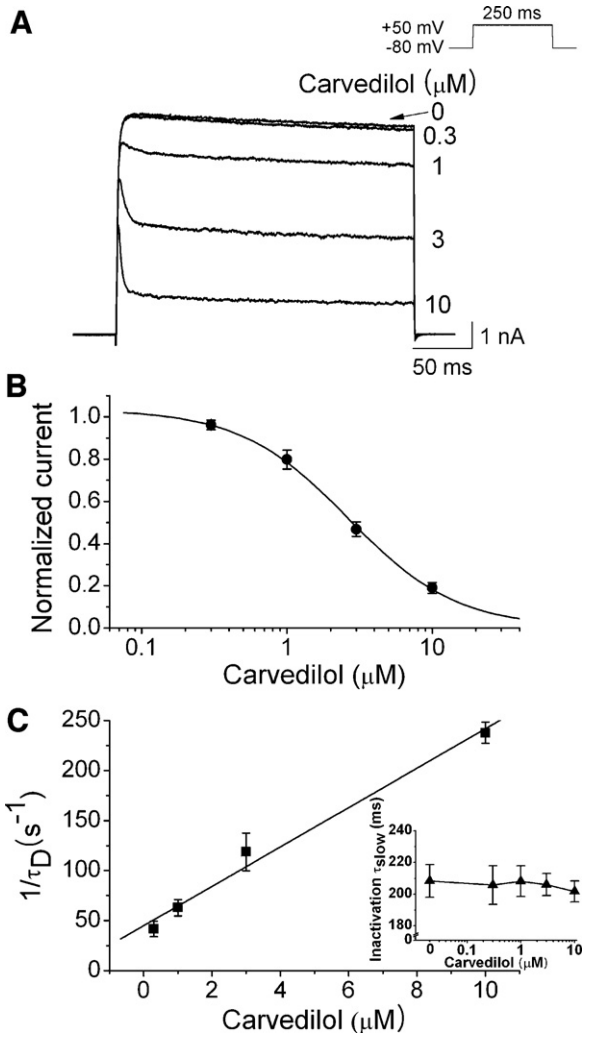

Fig. 1. Concentration dependence of carvedilol block for Kv1.5. (A) Currents were elicited by 250-ms depolarizing pulses from a holding potential of −80 mV to +50 mV at 10 s intervals in the absence and presence of 0.3, 1, 3 and 10 μM carvedilol. (B) Concentration–response curve for Kv1.5 block by carvedilol. The normalized currents were fitted to the Hill equation. (C) The drug-induced time constants were obtained from a biexponential fitting to the decaying traces of Kv1.5 currents. The reciprocal of drug-induced fast time constants (τ_D) was plotted versus carvedilol concentrations. The solid line represents the least-square fit of the data. The inactivation time constants of the slow component are shown in the inset. Data are expressed as the mean ± S.E.

3.2. Effects of other β-adrenergic blockers on Kv1.5

We tested whether the blocking of Kv1.5 by carvedilol is mediated through β-adrenergic blocking by using other β-adrenergic blockers (Fig. 3). Alprenolol and oxprenolol decreased Kv1.5 in a concentration-dependent manner, but complete block of Kv1.5 could not be achieved. A partial concentration–response curves for the action of alprenolol and oxprenolol on Kv1.5 tentatively yielded an IC_{50} value of 289.23 ± 18.02 μM with a Hill coefficient of 0.92 ± 0.05 (n = 6), and 429.67 ± 28.31 μM with a Hill coefficient of 0.98 ± 0.02 (n = 6), respectively. Carteolol had no effect on Kv1.5 currents, and even 300 μM of carteolol produced no more than about 1–5% block of Kv1.5 (n = 5) (data not shown). In addition, these drugs had no effects on the inactivation kinetics of Kv1.5 currents.

3.3. Voltage-dependent block of Kv1.5

Fig. 4A and B shows the representative Kv1.5 currents evoked by a series of 250-ms depolarizing pulses between −50 and +50 mV from a holding potential of −80 mV before and after application of carvedilol. The steady-state current–voltage relationship demonstrated that carvedilol significantly decreased the Kv1.5 currents throughout the entire voltage range over which the current was activated (Fig. 4C). For voltage-dependent block, the fractional block ($I_{Carvedilol}/I_{Control}$) was plotted against the membrane potential (Fig. 4D). Carvedilol-induced block increased from 29.4 ± 2.8% at −10 mV to 49.4 ± 3.1% at +10 mV, which corresponded to the voltage range of channel activation (n = 8, $P < 0.05$). However,

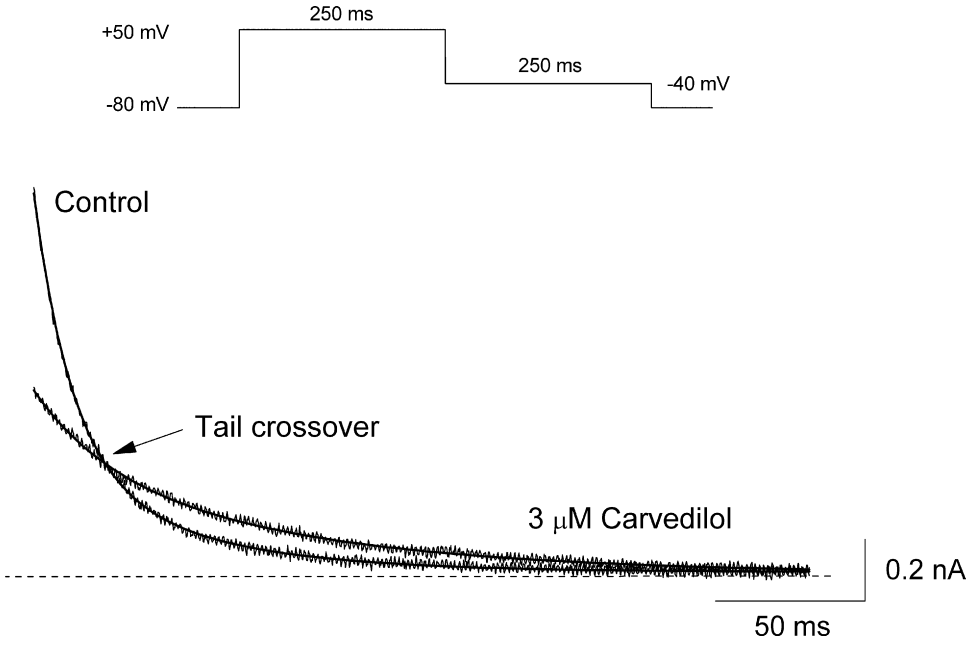

Fig. 2. Effects of carvedilol on the deactivation kinetics of Kv1.5. By superimposing the two tail currents in the absence and presence of carvedilol, a tail crossover phenomenon (indicated by arrow) was observed. The dotted line represents the zero current level.

the block in the voltage range between +20 and +50 mV, where the channels were fully activated, was not voltage-dependent.

3.4. Effects of carvedilol on the steady-state activation and inactivation of Kv1.5

The voltage dependence of steady-state activation of Kv1.5 was evaluated by tail current analysis with a double-pulse protocol in the absence and presence of carvedilol (Fig. 5A). Carvedilol decreased the tail currents of Kv1.5. The potentials of the half-activation point ($V_{1/2}$) and the slope factor (k) of the steady state activation curves were -5.23 ± 0.96 and 9.08 ± 1.30 mV for the control, and -6.98 ± 0.72 and 8.75 ± 1.38 mV for 3 μM carvedilol ($n = 7$). Although carvedilol slightly shifted the steady-state activation curve to a hyperpolarized potential, this shift was not statistically significant. The voltage dependence of the steady-state inactivation curve of Kv1.5 was also analyzed (Fig. 5B). Under control conditions, the potential for the half-inactivation point ($V_{1/2}$) and the slope factor (k) of the steady-state inactivation curve were -22.58 ± 1.40 mV and 5.10 ± 0.30 mV, respectively ($n = 7$). After drug application, the $V_{1/2}$

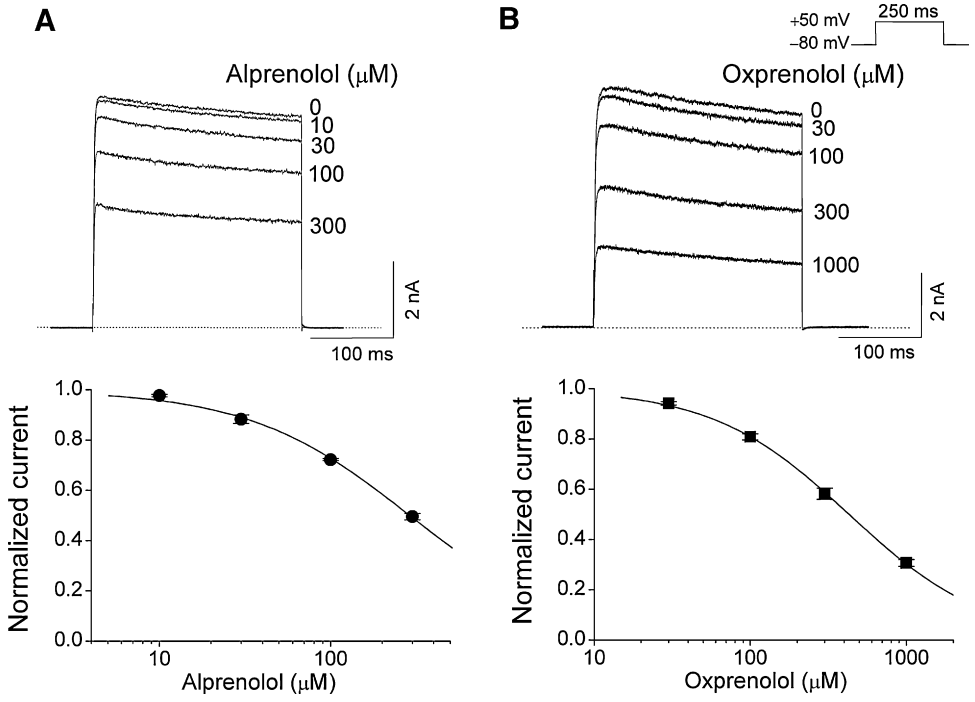

Fig. 3. Effects of other β-adrenergic blockers on Kv1.5. Currents were elicited by 250-ms depolarizing pulses from a holding potential of −80 mV to +50 mV at 10 s intervals in the absence and presence of alprenolol (A) and oxprenolol (B). Partial concentration–response curve for the blocking action of alprenolol and oxprenolol. Data are expressed as the mean ± S.E.

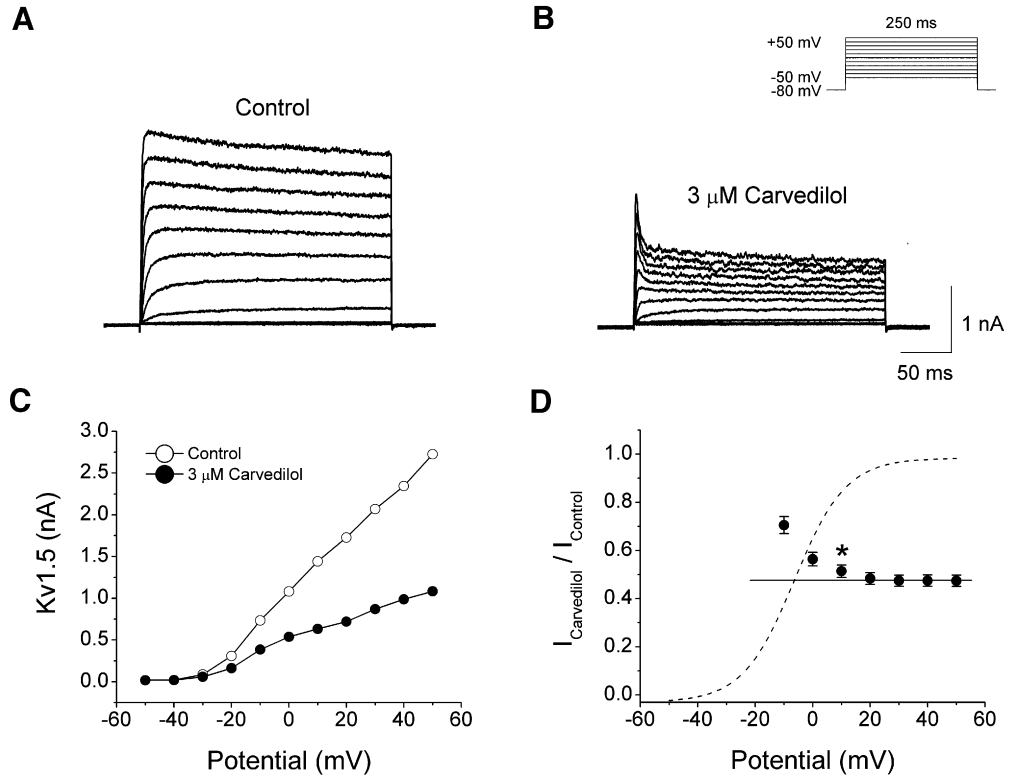

Fig. 4. Voltage dependence of Kv1.5 block. The whole-cell currents were elicited by 250-ms depolarizing pulses between −50 mV and +50 mV in 10 mV increments every 10 s from a holding potential of −80 mV under control conditions (A) and after addition of 3 μM carvedilol (B). (C) Resultant current–voltage relationships were taken at the end of the test pulses. (D) Normalized block shown as relative current ($I_{carvedilol}/I_{control}$) from data C. In the voltage range between −10 and +10 mV for channel opening, the block of Kv1.5 increased and was significantly different ($n = 8$; $^*P < 0.05$ versus data at −10 mV). The dotted line represents the Kv1.5 activation curve under control conditions. Data are expressed as the mean ± S.E.

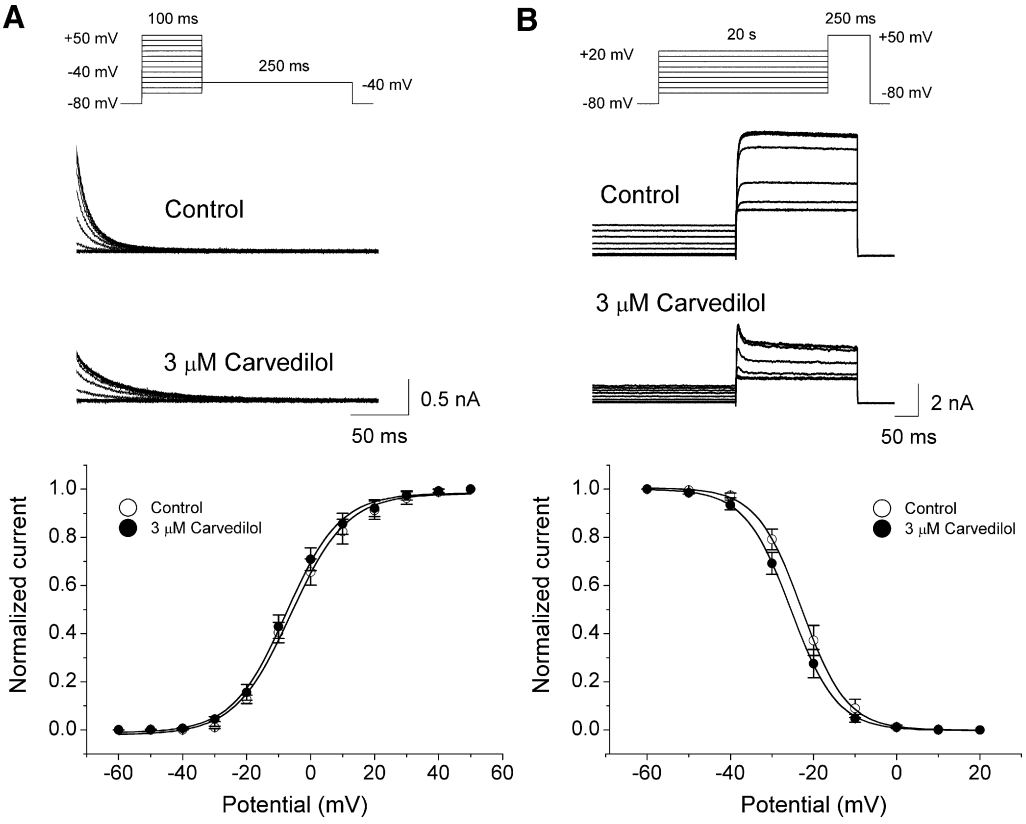

Fig. 5. Effects of carvedilol on the steady-state activation and inactivation of Kv1.5 channels. (A) Representative Kv1.5 tail current traces in the absence and presence of carvedilol. The normalized currents were plotted as a function of the test potentials, and the resulting curves were fit to the Boltzmann equation. (B) The steady-state inactivation curves in the absence and presence of carvedilol were obtained using a two-pulse protocol followed by normalizing and fitting each data point with the Boltzmann equation. Data are expressed as the mean ± S.E.

and k yielded -25.28 ± 1.28 mV and 5.19 ± 0.28 mV, respectively ($n = 7$). These changes of $V_{1/2}$ and k values were not statistically significant.

3.5. Use-dependent block of Kv1.5

Fig. 6A and B shows the effects of 1 and 2 Hz trains of 20 depolarizing pulses under control conditions and after the addition of carvedilol. The peak amplitudes of current at every pulse were normalized by the peak amplitudes obtained at the first occurrence of pulse, and then plotted against the pulse number. Under control conditions, the amplitude of Kv1.5 currents slightly decreased by $3.9 \pm 0.9\%$ and $6.7 \pm 0.4\%$ ($n = 10$) at 1 and 2 Hz, respectively. In the presence of carvedilol, the amplitude of Kv1.5 progressively decreased, reaching a steady-state at the end of 20 depolarizing pulses. The extent of the steady-state block was $11.4 \pm 0.8\%$ ($n = 10$, $P < 0.01$) and $20.5 \pm 1.4\%$ ($n = 10$, $P < 0.01$) at 1 and 2 Hz, respectively. These results suggest that carvedilol exhibited a strong use-dependent block of Kv1.5. We evaluated the effect of carvedilol at increasing stimulation frequencies to quantify the use-dependent block (Fig. 6C). The block by carvedilol was slightly increased with an increase in stimuli frequency with IC_{50} values of 2.56 ± 0.05 μM at 0.1 Hz, 2.38 ± 0.03 μM at 1 Hz and 2.03 ± 0.05 μM at 2 Hz ($n = 10$).

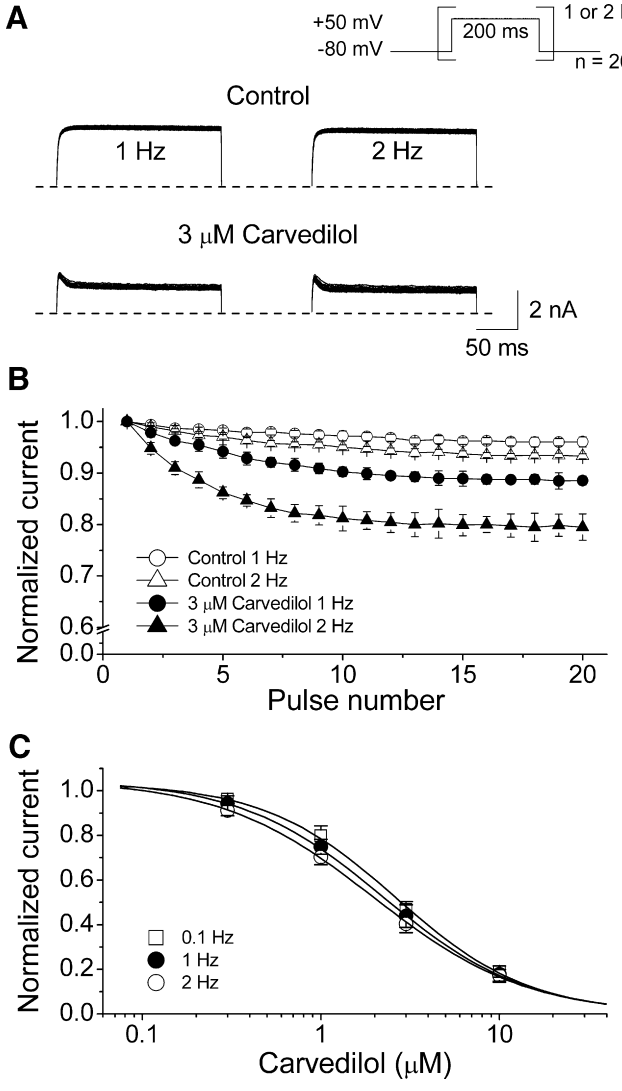

Fig. 6. Use-dependence of Kv1.5 block. (A) Twenty repetitive 200-ms depolarizing pulses of +50 from a holding potential of −80 mV were applied at 1 and 2 Hz under control conditions and in the presence of carvedilol. (B) The peak amplitudes of currents at every pulse were normalized by the peak amplitude obtained at the first pulse and then plotted versus the pulse number. (C) The drug-induced inhibition at each frequency was measured, normalized by current in the absence of the drug and then plotted against the concentration of carvedilol. Concentration–response curves at increasing stimulation frequencies (0.1, 1 and 2 Hz). Data are expressed as the mean ± S.E.

3.6. Recovery time course from inactivation of Kv1.5

The effect of carvedilol on the recovery of Kv1.5 from inactivation was measured with a double-pulse protocol. Fig. 7 shows a typical example of the kinetics of recovery from inactivation of Kv1.5 currents. Under control conditions, the recovery from inactivation could be described by a biexponential function that exhibited a fast time constant of 33.23 ± 4.18 ms and a slow time constant of 781.58 ± 29.01 ms ($n = 7$). In the presence of 3 μM carvedilol, the recovery process was also best fitted with a biexponential function (fast time constant of 36.9 ± 5.13 ms, slow time constant of 1315.06 ± 48.6 ms, $P < 0.05$, $n = 7$). The fast time constant obtained in the presence of carvedilol remained unchanged but the slow time constant was statistically different from that of recovery under control conditions.

3.7. Reversible block of Kv1.5

To examine the reversibility of the effect of 3 μM carvedilol, the time course for alteration in steady-state currents produced by the

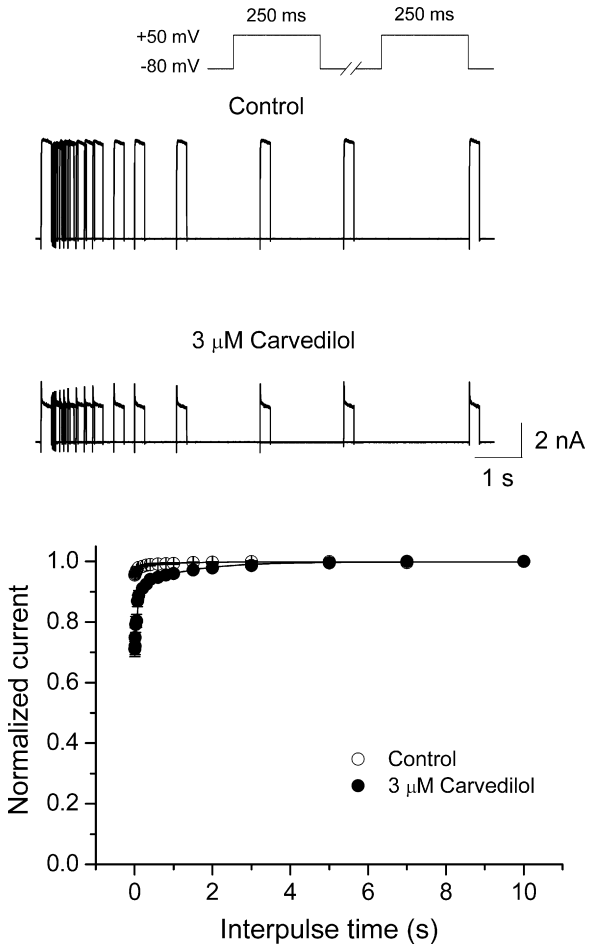

Fig. 7. Effects of carvedilol on the kinetics of Kv1.5 recovery from steady-state inactivation. A two-pulse protocol was used to characterize the time course of recovery from inactivation in the absence and presence of carvedilol. The solid lines represent the typical double exponential fit of the peak amplitude of Kv1.5 as a function of the interpulse interval.

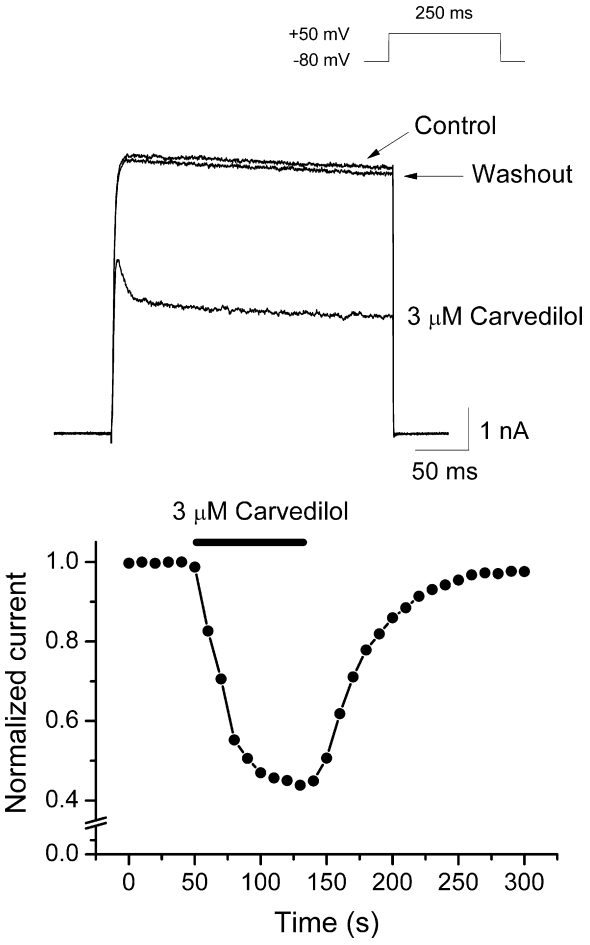

Fig. 8. Time course of Kv1.5 block in the presence of carvedilol. Maximal block occurred within 1 min after drug application. A slow recovery from block was observed after drug washout.

drug and by ensuing drug washout was analyzed (Fig. 8). When a drug-free solution was changed to a drug-containing solution, Kv1.5 block was elicited within 10 s, and steady-state block of Kv1.5 was reached within 1–2 min. The currents recovered to 97.9 ± 1.1% (n = 7) following a 2–3 min washout, indicating that the effect of carvedilol on Kv1.5 was completely reversible.

4. Discussion

Carvedilol was identified in the present study as a potent blocker of the Kv1.5 currents that are stably expressed in CHO cells. The results show that carvedilol causes a reversible, concentration-, time-, voltage-, and use-dependent block of Kv1.5 currents.

Carvedilol accelerated the macroscopic current decline of Kv1.5 currents in a concentration-dependent manner. It switched the Kv1.5 currents from a single exponential inactivation process under control conditions to a biexponential decay process with concentration-dependent acceleration of the rapid exponential component. These fast time constants can be considered a result of the interaction of carvedilol with Kv1.5 channels [24]. The estimated K_D (k_{-1}/k_{+1}) was 2.28 μM, which was in good agreement with the IC_{50} value of 2.56 μM calculated from the concentration–response curve. This is a characteristic feature of open channel block, and has previously been described for Kv1.5 block by several agents, such as loratadine, zatebradine, propafenone and bisindolylmaleimide [22,26–28]. Thus, the apparent acceleration of Kv1.5 inactivation in the presence of carvedilol reflects a relatively slow open channel block, indicating that the block of Kv1.5 current developed gradually during voltage steps. The voltage dependence of carvedilol block is composed of a steep phase of current activation. This voltage dependence of Kv1.5 block is also consistent with open channel block [29]. However, block induced by carvedilol was voltage-independent in the voltage range for full activation. Since the pK_a value of carvedilol is 7.8, carvedilol is about 70% charged at physiological pH [30]. Thus, the voltage-independent block indicates that the charged form of the drug interacts with the binding site outside the electrical field, or the uncharged form is predominantly responsible for the block. The lack of voltage dependence of steady-state inactivation also suggests that carvedilol interacts with the open rather than the inactivated state of Kv1.5 [31]. The recovery from block by carvedilol upon repolarization was slower than the recovery from intrinsic inactivation of Kv1.5. This finding may explain the increase in Kv1.5 block with repeated depolarization. Another finding that suggests open channel block is the tail cross-over observed under control conditions and in the presence of carvedilol [28].

Adrenergic stimulation is known to modulate Kv currents in different cell types. In guinea pig ventricular myocytes, catecholamines (isoproterenol and norepinephrine) increased the delayed rectifying K^+ currents [3]. Isoproterenol increased, and phenylephrine decreased, ultra-rapid delayed rectifier K^+ currents in human atrial myocytes, in a concentration-dependent manner [32]. These results suggest that β-adrenergic stimulation enhanced, and α-adrenergic stimulation decreased ultra-rapid delayed rectifier K^+ currents. In the present study, however, treatment with other β-adrenergic blockers (alprenolol, oxprenolol and carteolol) had little or no effect on Kv1.5 currents. Similarly, alprenolol and oxprenolol were either weak or virtually inactive inhibitors ($IC_{50} > 600$–1000 μM) of the outward K^+ current induced by KRN2391 (K^+ channel opener) in *Xenopus* oocytes [33]. Despite their similar structures, the structural difference in the side chain of carvedilol is thought to be responsible for its higher potency in blocking Kv1.5 currents. In addition, our results cannot be explained by an indirect effect of carvedilol on Kv1.5 expressed in CHO cells that lack endogenous adrenergic receptors [34]. Thus, our results suggest that the effect of carvedilol on Kv1.5 did not occur by block of the adrenergic effect and was due to the direct interaction of carvedilol with the Kv1.5 channel protein. However, recent study has indicated that carvedilol induced a potent inhibitory effect on Kir2.3 channels by interfering with phosphatidylinositol 4,5-biphosphate (PIP_2)–channel interaction [35]. Carvedilol, which is highly lipophilic, interacts with the lipid bilayers and may alter the biophysical properties of membranes [36]. Although our data suggest that carvedilol acts as a pore blocking agent, we cannot completely rule out the possibility that carvedilol modulated Kv1.5 by affecting the lipid environment of the membrane.

Carvedilol, a non-selective β-adrenergic blocker, was originally used as an antihypertensive agent [4]. It also has anti-ischemic, anti-proliferative, anti-oxidant, anti-inflammatory and antiarrhythmic properties [37–40]. Although the antiarrhythmic activity of carvedilol may be attributed to its β-blocking effects [6], many of its effects on cardiac rhythm may be attributable to its inhibitory effects on several ion channels involved in cardiac action potential. Carvedilol is a potent blocker of the transient outward and ultra-rapid delayed rectifier K^+ currents in human atrial myocytes [9]. It is also highly potent at blocking the rapidly activating component of the delayed rectifier K^+ current in rabbit ventricular myocytes, but blocks HERG K^+ currents expressed in *Xenopus* oocytes with a potency that is relatively low [5,8]. In addition, carvedilol produced a moderate prolongation of the action potential duration of rabbit ventricular myocytes by concomitant block of the repolarizing K^+ currents and L-type Ca^{2+} currents [8]. Indeed, carvedilol caused prolongation of action potential duration in rabbit sinoatrial and

atrioventricular node cells [13]. Block of cardiac Kv channels, which results in prolongation of ventricular repolarization and the refractory period, seems to be related to proarrhythmia [14]. Direct block of HERG or its native current, rapidly activating delayed rectifier K^+ current, is the commonly proposed mechanism of drug-induced QT interval prolongation and ventricular arrhythmia [41]. Thus, it is possible that carvedilol blocks the cardiac repolarizing K^+ currents including HERG, thereby prolonging action potential duration and causing proarrhythmic effects. However, carvedilol treatment reduces the incidence of ventricular fibrillation in the feline myocardium and the frequency of ventricular tachycardia in heart failure, thus producing a significant improvement in mortality [15–17]. Furthermore, a significant QT prolongation was not noted in patients undergoing treatment with carvedilol [15]. Ultra-rapid delayed rectifier K^+ current is particularly important in the early phase of cardiac repolarization [19]. This current activates very rapidly and inactivates slowly during the plateau phase of action potentials. Kv1.5 current is believed to underlie ultra-rapid delayed rectifier K^+ current [18,19]. This current is reported to be present in the atrium, but not in the ventricle, of the human heart. Indeed, blockade of ultra-rapid delayed rectifier K^+ currents had no effect on the QT interval, but it prolonged the atrial effective refractory periods [42]. Since selective block of Kv1.5 current that is exclusively expressed in the atrium did not prolong the duration of ventricular repolarization and is expected to be devoid of ventricular proarrhythmic effects, the Kv1.5 channel is regarded as a promising therapeutic target for safer antiarrhythmic drugs without the risk of ventricular proarrhythmic action [20,43].

Mean therapeutic plasma concentrations of carvedilol range from 0.1 to 0.6 μM [44,45]. Carvedilol inhibits ultra-rapid delayed rectifier K^+ currents and transient outward K^+ currents in human atrial myocytes with IC_{50} values (0.39–0.50 μM) similar to therapeutic plasma concentration [9]. The present study found that carvedilol inhibited Kv1.5 with an IC_{50} of 2.56 μM, which was slightly higher than therapeutic plasma levels. Similarly, IC_{50} values for carvedilol to block transient outward and slowly activating delayed rectifier K^+ currents in rabbit ventricular myocytes are higher than therapeutic plasma levels [8]. The pore-forming α-subunits of Kv1.5 coassemble with Kvβ subunits to form ultra-rapid delayed rectifier K^+ currents in human atrium [46]. In the present study, the IC_{50} value for the block of Kv1.5 by carvedilol is about 7 times higher than that of human ultra-rapid delayed rectifier K^+ currents [9]. Interaction with Kvβ subunits modified the cell surface expression, gating kinetics and pharmacology of the Kv1.5α subunit [47]. This factor may account, in part, for the difference in drug sensitivity of Kv1.5 and ultra-rapid delayed rectifier K^+ currents. Although we could find no published data concerning carvedilol's local concentrations in cardiac myocytes, carvedilol is a highly lipid soluble compound that would accumulate in tissue at concentrations higher than those observed in plasma [48,49]. Thus, it is possible that the local concentrations required to block Kv1.5 in our experiments could be achieved in various clinical settings. In addition, use-dependent block, which is a clinically important feature of antiarrhythmic drugs, could produce a strong suppression of beats occurring during arrhythmia without affecting beats occurring at physiological rates [50]. In the present study, the action of carvedilol on Kv1.5 was slightly increased at high stimulation frequencies, and this enhances its therapeutic value.

In conclusion, the present study demonstrated that, at concentrations slightly higher than therapeutic plasma concentrations, carvedilol might exert a potent Kv1.5 block in addition to a β-adrenergic antagonistic effect. These results are relevant to an understanding of the ionic mechanisms underlying the antiarrhythmic properties of carvedilol.

Conflict of interest

None declared.

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