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Carvedilol blocks the repolarizing K^+ currents and the L-type Ca^{2+} current in rabbit ventricular myocytes

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Abstract

Carvedilol ((±)-1-(carbazol-4-yloxy)-3-[[2-(*o*-methoxyphenoxy)ethyl]amino]-2-propanol), a β-adrenoceptor-blocking agent with vasodilator properties, has been reported to produce dose-related improvements in left ventricular function and reduction in mortality in patients with chronic heart failure. However, its electrophysiological effects have not been elucidated. We studied ion channel and action potential modulation by carvedilol in rabbit ventricular preparations using whole-cell voltage-clamp and standard microelectrode techniques. In ventricular myocytes, carvedilol blocked the rapidly activating component of the delayed rectifier K^+ current (I_{Kr}) in a concentration-dependent manner ($IC_{50} = 0.35$ μM). This block was voltage- and time-independent; a prolongation of the depolarizing pulses from a holding potential of −50 mV to +10 mV within the range of 100–3000 ms did not affect the extent of I_{Kr} block. Carvedilol also inhibited the L-type Ca^{2+} current (I_{Ca}), the transient outward K^+ current (I_{to}) and the slowly activating component of the delayed rectifier K^+ current (I_{Ks}) with IC_{50} of 3.59, 3.34, and 12.54 μM, respectively. Carvedilol (0.3–30 μM) had no significant effects on the inward rectifier K^+ current. In papillary muscles from rabbits pretreated with reserpine, action potential duration was prolonged by 7–12% with 1 μM and by 12–24% with 3 μM carvedilol at stimulation frequencies of 0.1–3.0 Hz. No further action potential duration prolongation was observed at concentrations higher than 3 μM. These results suggest that concomitant block of K^+ and Ca^{2+} currents by carvedilol resulted in a moderate prolongation of action potential duration with minimal reverse frequency-dependence. Such electrophysiological effects of carvedilol would be beneficial in the treatment of ventricular tachyarrhythmias.

Keywords: Adrenoceptor antagonist; Membrane current; Membrane potential; Ventricle, rabbit; Frequency-dependence reverse

1. Introduction

Carvedilol is a nonselective β-adrenoceptor-blocking agent with vasodilating properties exerted primarily through α_1-adrenoceptor-blockade (McTavish et al., 1993; Dunn et al., 1997). This agent has no intrinsic sympathomimetic activity, but has a number of other potentially beneficial pharmacological effects including antioxidant and antiproliferative activities (Moser and Frishman, 1998). This drug was originally designed and developed as a vasodilating compound for efficacious and safe treatment of hypertension and coronary artery disease (McTavish et al., 1993). Recently, interest in carvedilol has been focused on the potential utility of the drug in congestive heart failure. Many analyses have revealed that carvedilol produced a significant reduction in mortality of patients with heart failure, especially from progressive deterioration of left ventricular function and sudden death (Bristow et al., 1996; Packer et al., 1996).

Patients with severe congestive chronic heart failure have a high incidence of ventricular arrhythmias causing sudden death (Chakki and Gherghiade, 1985; Packer, 1985; Bigger, 1987; Kjekshus, 1990; Cohn et al., 1993). In patients who survive an acute myocardial infarction, over 50% of the deaths are due to fatal ventricular tachyarrhythmias (Underwood et al., 1997). The conventional antiarrhythmic drugs (apart from amiodarone) often fail to suppress the arrhythmias and may aggravate their occurrence because of their proarrhythmic properties (Pratt et al.,

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1989). In contrast, β-adrenoceptor-blocking agents were shown to reduce mortality in patients with post myocardial infarction as well as in chronic heart failure patients (Bristow et al., 1996; Packer et al., 1996; Underwood et al., 1997). Carvedilol has been shown to prevent ventricular tachyarrhythmias in various animal models including ischemia/reperfusion- and digitalis-induced arrhythmias (Hoher et al., 1989; Bril et al., 1995; Brunvand et al., 1996). Precise mechanisms underlying the antiarrhythmic action remain to be clarified. The present study has revealed that carvedilol is a potent blocker of the rapidly activating component of the delayed rectifier K^+ current (I_{Kr}). At high concentrations, it also blocked the L-type Ca^{2+} current (I_{Ca}), the transient outward K^+ current (I_{to}) and the slowly activating components of the delayed rectifier K^+ current (I_{Ks}). Such a balanced inhibition of K^+ and Ca^{2+} channels resulted in a moderate prolongation of action potential duration with minimal reverse frequency-dependence. This property would be beneficial in the treatment of ventricular tachyarrhythmias in patients with chronic heart failure or post myocardial infarction.

2. Methods

2.1. Current recordings in rabbit ventricular myocytes

2.1.1. Isolation of rabbit ventricular myocytes

This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1995).

Single ventricular myocytes from rabbit hearts were obtained by enzymatic dissociation (Yazawa et al., 1990). Rabbits of either sex weighing 1.5 to 2.0 kg were anesthetized with thiamylal sodium after being heparinized, and the hearts were rapidly excised and mounted via the aorta on a Langendorff retrograde perfusion apparatus. The hearts were first perfused with HEPES-buffered solution (gassed with 100% O_2 at 37°C) for 3–5 min then with Ca^{2+}-free HEPES-buffered solution for 10–15 min and finally with 0.12 mg/ml collagenase (Yakult, Japan) containing Ca^{2+}-free solution for 15 min. The hearts were subsequently washed with high-K^+ storage solution (Kraftbrühe solution: KB solution) for 5 min. The ventricles were separated and minced with a pair of surgical scissors, and the tissue was then passed through a 200 μm stainless steel mesh. The filtrate was washed twice with KB solution by centrifuging at a speed of 1000 rpm for 5 min. The cells obtained were stored in KB solution at 4°C before use.

2.1.2. Electrophysiological recordings

The single-pipette whole-cell voltage clamp method was used for recording membrane currents (Hamill et al., 1981). An aliquot of the cell suspension was placed in the recording chamber (with a volume of 0.5 ml) on the stage of an inverted microscope (Diaphoto, Nikon, Tokyo). A brief period was allowed for cell adhesion to the coverslip at the bottom of the chamber, and then the cells were superfused with HEPES-buffered solution at 3 ml/min. The bath temperature in all experiments was maintained at 34°C. The glass pipettes had a resistance of 3–5 MΩ after filling with the pipette solution. Each pipette was connected to a patch-clamp amplifier (Axopatch 200B, Axon Instruments, USA). Cell membrane capacitance was measured by calculating the area under the capacitive transient evoked by applying a hyperpolarizing pulse (5 mV) from a holding potential of −50 mV and then compensated. The series resistance was electrically compensated by about 40–70%. Command potentials generation and data acquisition were performed though a patch-clamp amplifier (Axopatch 200B) controlled by using pCLAMP software (Axon Instruments) and an IBM-compatible computer. Current signals were filtered at 1 kHz, and digitized at a sampling frequency of 2 kHz. Measurements of the membrane currents were performed after a stabilizing period of 10 min following the formation of the whole cell recording conformation.

2.2. Action potential recording in papillary muscles

Japanese white rabbits of either sex weighing 1.5 to 2.0 kg were pretreated with reserpine (1 mg/kg, i.p. for 3 days) before experiments in order to deplete endogenous cardiac catecholamines. The animals were anesthetized by intravenous injection of thiamylal sodium (30 mg/kg). The right ventricular papillary muscles (0.4 to 0.6 mm in diameter and 3 to 4 mm in length) were dissected from the hearts and were mounted in a tissue bath (0.5 ml) and superfused at 32°C with modified Krebs–Ringer solution gassed with 95% O_2/5% CO_2. The preparation was stimulated by a pair of 1.0-mm platinum wire electrodes placed 1.0 mm apart from both sides of the muscle. Pulses used for stimulation were 1 ms in duration and 20% higher than the diastolic threshold. Transmembrane action potentials were recorded through two glass microelectrodes filled with 3 M KCl, one intracellularly and the other extracellularly, placed close together. The electrodes were each connected by Ag/AgCl wire to an high-input impedance pre-amplifier in turn connected to a differential amplifier (SEN-7203, Nihon Kohden). Stimulation frequency was changed in steps from 0.1 to 3 Hz, and the steady-state action potentials were recorded 3–5 min after pacing at each frequency. The values of action potential duration were measured at 90% repolarization.

2.3. Solutions and drugs

The HEPES-buffered solution used for cell isolation and in the single myocyte experiments was composed of (in mM): NaCl, 143; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5;

NaH_2PO_4, 0.25; HEPES, 5.0; and glucose, 5.6, pH adjusted to 7.4 with NaOH. The Ca^{2+}-free solution was the same as above except that it lacked $CaCl_2$. The KB solution contained (in mM): KOH, 82; L-glutamic acid, 50; KCl, 40; KH_2PO_4, 20; taurine, 20; HEPES, 10; $MgCl_2$, 3; glucose, 10; EGTA, 0.5, pH adjusted to 7.4 with KOH. I_{to}

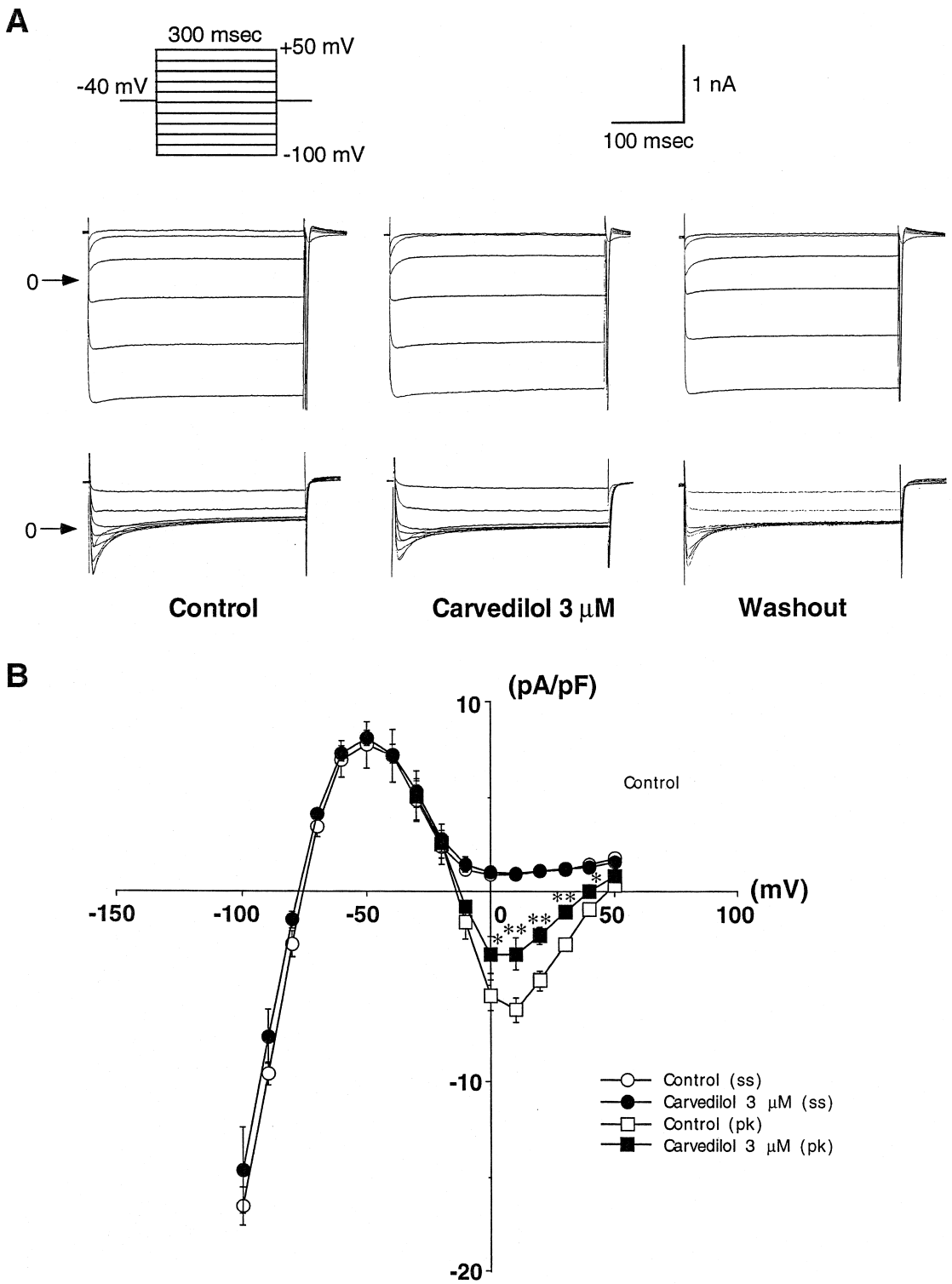

Fig. 1. Effects of carvedilol on I_{K1} and I_{Ca}. Currents were elicited by applying 300-ms hyperpolarizing or depolarizing voltage steps between −100 and +50 mV from a holding potential of −40 mV at 0.1 Hz. (A) The representative recordings obtained under control condition, in the presence of 3 μM carvedilol and upon 10 min of washout. (B) *I–V* relationships for the steady-state current (ss) and the peak inward current (pk) in the absence and presence of 3 μM carvedilol. Carvedilol showed no significant effect on the *I–V* relationship of I_{K1} while significantly inhibiting the density of I_{Ca}. $^*P < 0.05$; $^{**}P < 0.01$ vs. control; $n = 5$.

was measured in HEPES-buffered solution, in which 3 μM nisoldipine (or 2 mM $CoCl_2$) and 10 μM tetrodotoxin were added to block I_{Ca} and the Na^+ current (I_{Na}), respectively. I_{Kr} was recorded in the HEPES-buffered solution containing 3 μM nisoldipine and 30 μM chromanol 293B (an I_{Ks} blocker) (Busch et al., 1996). I_{Ks} was measured in the Na^+-free, K^+-free solution composed of the following (in mM): *N*-methyl-D-glucamine, 149; $MgCl_2$, 5; HEPES, 5; $CaCl_2$, 0.9 (pH adjusted to 7.4 with HCl). 3 μM nisoldipine and 10 μM E-4031 (an I_{Kr} blocker) were added in this solution. The internal pipette solution contained (in mM): KOH, 60; KCl, 80; aspartate, 40; HEPES, 5; EGTA, 10; MgATP, 5; sodium creatinine phosphate, 5; and $CaCl_2$, 0.65 (pH 7.2, pCa 8.0). The composition of the modified Krebs–Ringer solution used in the recording of action potentials from papillary muscles

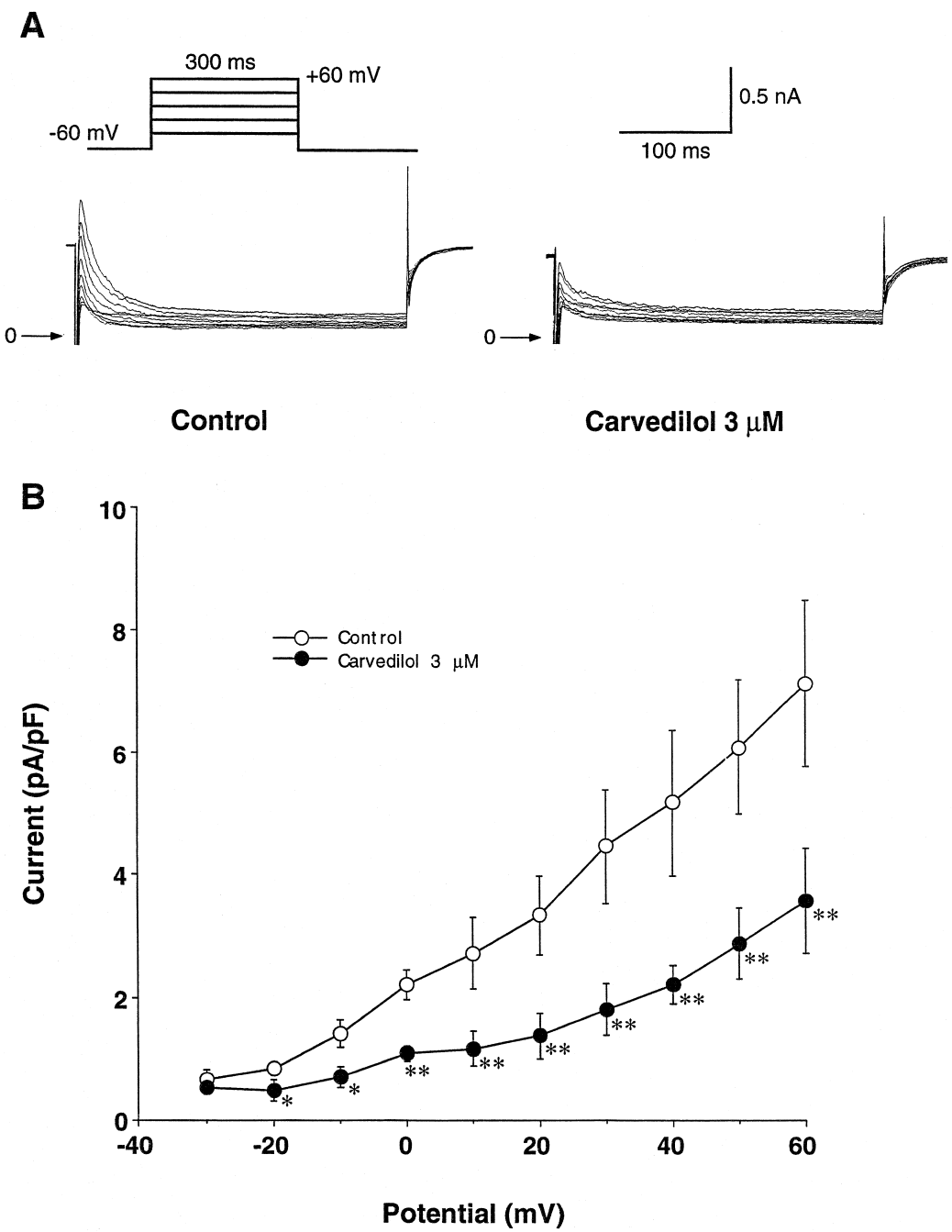

Fig. 2. Effects of carvedilol on *I–V* relationship of I_{to}. I_{to} were elicited by a series of 300 ms depolarizing pulses applied at 0.1 Hz from a holding potential of −60 mV to +60 mV. The external (HEPES-buffered) solution for the experiments included 3 μM nisoldipine and 10 μM tetrodotoxin. (A) Representative recordings obtained under control condition and in the presence of 3 μM carvedilol. (B) Effects of carvedilol on the *I–V* relationship of I_{to}. Carvedilol (3 μM) caused a significant decrease in the density of the current at most potentials. $^*P < 0.05$; $^{**}P < 0.01$ vs. control; $n = 5$.

was as follows: NaCl, 120.3; KCl, 4.0; $CaCl_2$, 1.2; $MgSO_4$, 1.3; NaH_2PO_4, 1.2; $NaHCO_3$, 25.2; and glucose, 5.8, pH adjusted to 7.4 with NaOH after gassing with 95% O_2/5% CO_2.

Carvedilol ((±)-1-(carbazol-4-yloxy)-3-[[2-(*o*-methoxyphenoxy)ethyl]amino]-2-propanol) was obtained from Daiichi Pharmaceuticals (Tokyo, Japan). It was dissolved in dimethyl sulfoxide to make a 10 mM stock solution. E-4031 (*N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl] methanesulfonamide dihydrochloride dihydrate) was a gift from Eisai Pharmaceuticals (Tokyo, Japan). It was dissolved in distilled water as a 1 mM stock solution. Chromanol 293B (*trans*-6-Cyano-4-(*N*-ethylsulphonyl-*N*-methylamino)-3-hydroxy-2,2-dimethyl-chromane) was a gift from HMR Chemical Research (Frankfurt, Germany). It was dissolved in ethanol to make a 10 mM stock solution. All the drugs were diluted in superfusates to the desired final concentrations immediately before each application. Carvedilol was added to the perfusing solution in a cumulative way. Each drug concentration was applied for 10 min in whole-cell voltage-clamp study and for 30 min in action potential study.

2.4. Statistics

Data were expressed as mean ± S.E.M. The curve-fitting program Igor (Wave Metrics, USA) was used in data analysis. Analysis of variance (ANOVA) with Dunnet's

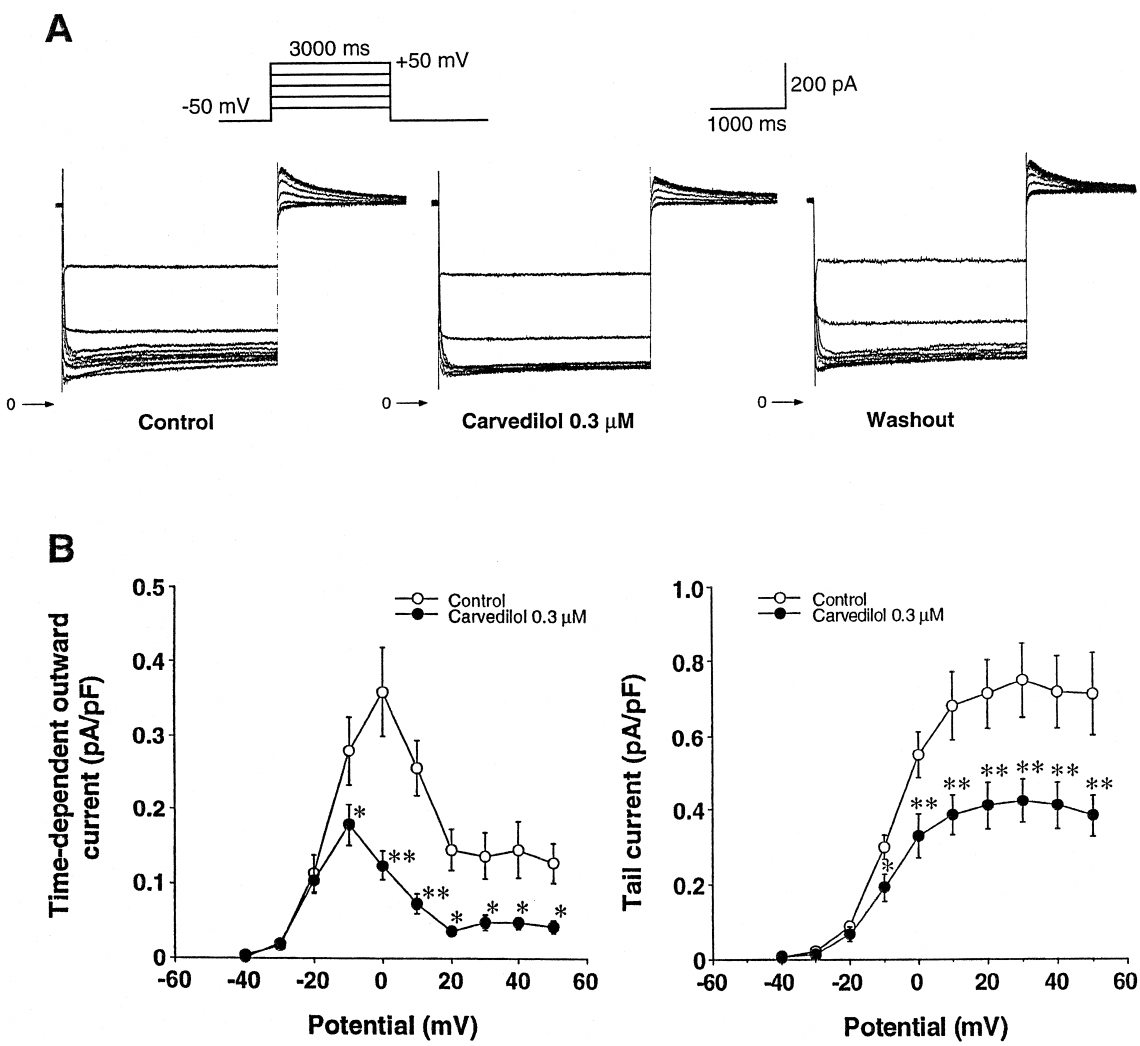

Fig. 3. Effects of carvedilol on the *I–V* relationship of I_{Kr}. Activation of I_{Kr} was elicited by applying the voltage clamp steps at 0.1 Hz from a holding potential of −50 mV to depolarizing potentials ranging from −30 to +60 mV. The external (HEPES-buffered) solution for the experiments included 3 μM nisoldipine and 30 μM chromanol 293B. (A) Representative recordings obtained under control condition, in the presence of 0.3 μM carvedilol and upon 10 min of washout. (B) Effects of carvedilol on the *I–V* relationships of I_{Kr}. The time-dependent outward current and the tail current densities were plotted as functions of the test potential. Carvedilol (0.3 μM) inhibited both the time-dependent outward current and the tail current of I_{Kr} significantly without greatly altering the voltage dependence of activation of the tail current. $^*P < 0.05$, $^{**}P < 0.01$ vs. control; $n = 5$.

t-test was used for critical differences among multiple means, and Student's *t*-test was used for comparison between two means. A P value of less than 0.05 was considered statistically significant.

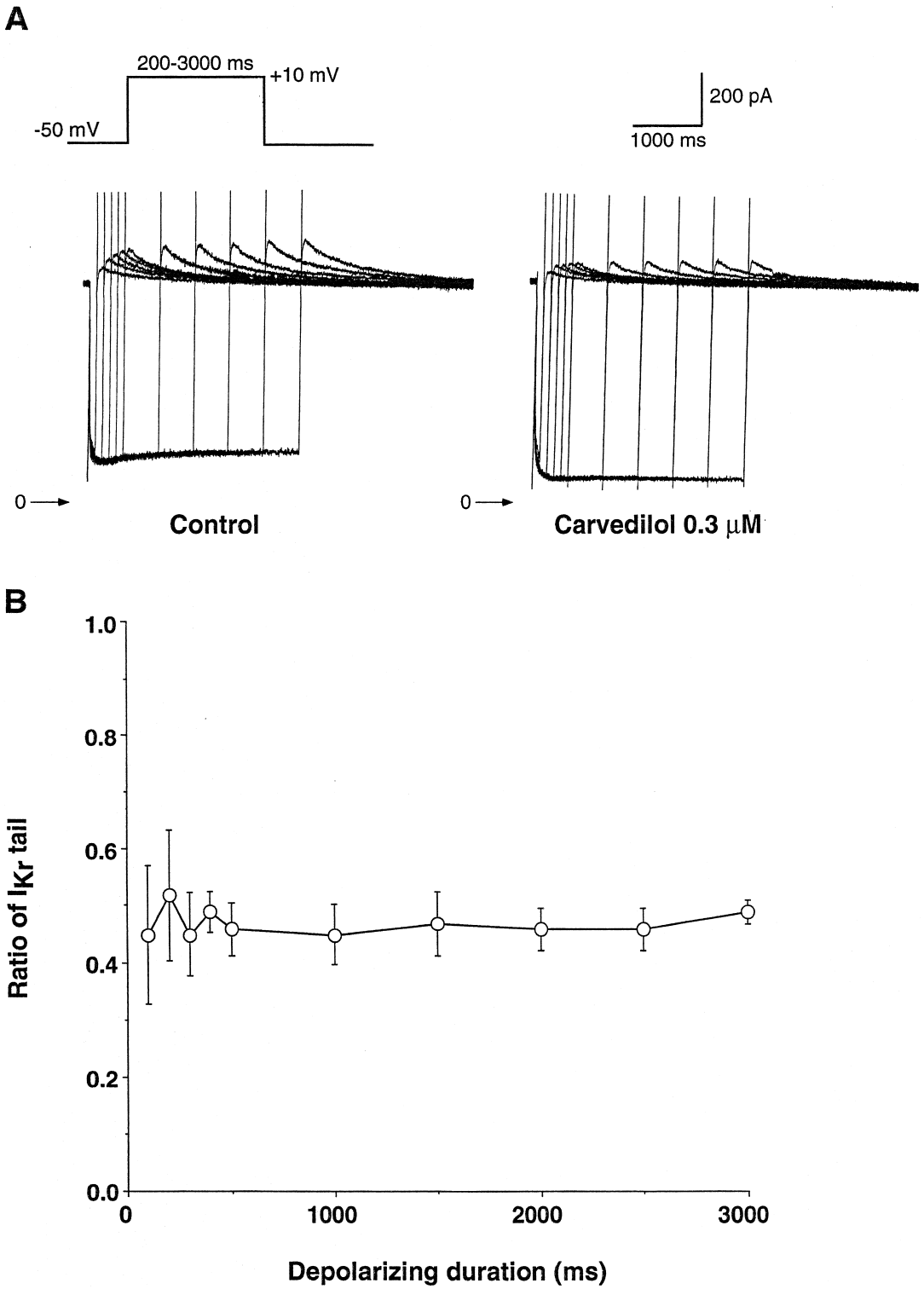

Fig. 4. Development of block on I_{Kr} by carvedilol. Envelopes of tail current were generated by applying depolarizing pulses of variable duration (from 0.1 to 3 s) from a holding potential of -50 mV at 0.1 Hz. (A) Superimposed tracings are envelopes of the current under control condition and after application of 0.3 μM carvedilol. (B) Ratios of tail current density in the presence of carvedilol to those in control were plotted as a function of the duration of the depolarizing pulse. Carvedilol reduced the tail current to a similar extent (45–49%, $n = 5$) at any tested duration of depolarizing pulses.

3. Results

3.1. Effects on the inward rectifier K^+ current and the L-type Ca^{2+} current

Firstly, the effects of carvedilol on the inward rectifier K^+ current (I_{K1}) and the L-type Ca^{2+} current (I_{Ca}) were examined (Fig. 1). Membrane current was elicited by applying 300-ms hyperpolarizing or depolarizing voltage steps between −100 and +40 mV from a holding potential of −40 mV at 0.1 Hz. The steady-state currents at the end of the clamp pulses up to 0 mV were measured as an index of I_{K1}, which is responsible for the terminal repolarization of action potential in rabbit ventricular cells (Shimoni et al., 1992). The peak inward current measured upon depolarization to potentials from −30 to +40 mV was measured as an index of I_{Ca}. Carvedilol (0.3–30 μM) had no significant effect on the current–voltage (I–V) relationship of the steady-state current, whereas it concentration-dependently inhibited the peak inward current with no appreciable influence on its voltage dependence of activation. The density of the peak inward current at +10 mV depolarization was decreased significantly from 6.27 ± 1.08 pA/pF ($n = 5$) in control to 3.35 ± 0.84 pA/pF ($P < 0.01$) at 3 μM and 1.51 ± 0.33 pA/pF ($P < 0.01$) at 10 μM of carvedilol. Washout of carvedilol for 10 min resulted in a recovery of the peak inward current to a level of 81–89% of the control. The representative recordings and the I–V relationships of the steady-state and the peak inward currents obtained before and after the application of 3 μM carvedilol are shown in Fig. 1.

In rabbits, unlike guinea pigs, both I_{Kr} and I_{Ks} are activated slowly with time constants of 300–400 ms and 460–560 ms, respectively (Carmeliet, 1992; Cheng et al.,

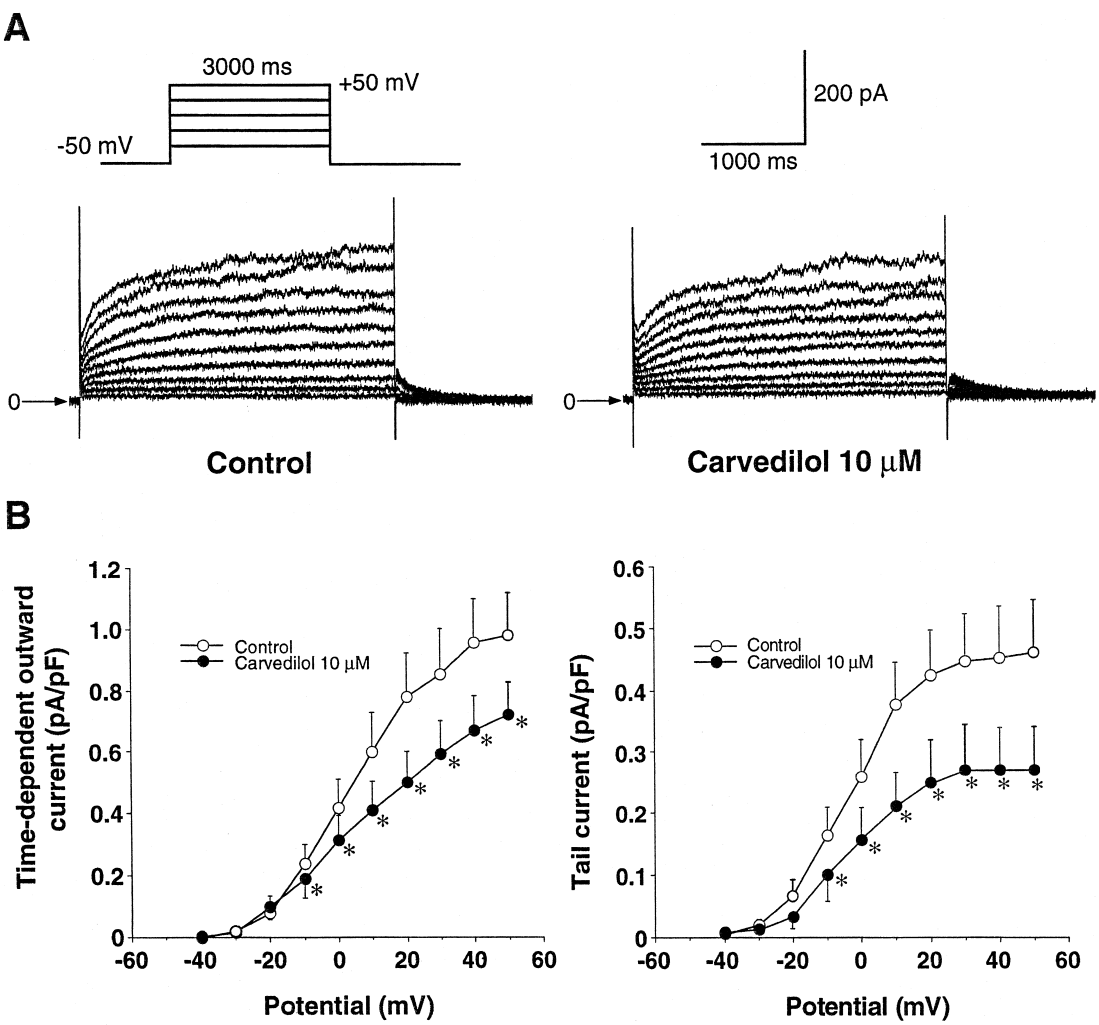

Fig. 5. Effects of carvedilol on the I–V relationship of I_{Ks}. Activation of I_{Ks} was elicited by applying the voltage clamp steps at 0.1 Hz from a holding potential of −50 mV to depolarizing potentials ranging from −30 to +60 mV. The external (Na^+-free and K^+-free) solution for the experiments included 3 μM nisoldipine and 10 μM E-4031. (A) The representative recordings obtained before and after the application of 10 μM carvedilol. (B) Effects of carvedilol on the I–V curve of I_{Ks}. The time-dependent outward current and the tail current densities were plotted as functions of the test potential. Carvedilol (10 μM) inhibited both the time-dependent outward current and the tail current of I_{Ks} significantly without greatly altering the voltage dependence of activation. $^*P < 0.05$ vs. control; $n = 5$.

1999). The depolarization pulse duration of 300 ms is therefore insufficient to activate I_{Kr} and I_{Ks}, causing very small tail currents. Moreover, I_{Kr} amplitude during the depolarization is reduced through voltage-dependent inactivation, giving rise to its minimal contribution to the steady-state current. Accordingly, the inhibition of I_{Kr} by carvedilol is not visible in Fig. 1.

3.2. Effects on the transient outward K^+ current

In rabbit ventricular myocytes, I_{to} is responsible for the early phase of repolarization, especially at low rates (Giles and Imaizumi, 1988; Hiraoka and Kawano, 1989; Fedida and Giles, 1991). The effect of carvedilol on the I–V curve of I_{to} was examined in HEPES-buffered solution containing 3 μM nisoldipine and 10 μM tetrodotoxin (Fig. 2). I_{to} was elicited by a series of 300 ms step depolarizing pulses applied at 0.1 Hz from a holding potential of −60 mV to test potentials between −50 mV to +60 mV. The difference between the peak outward current and steady-state current during depolarization was measured as the amplitude of I_{to}. The activation of the delayed rectifier K^+ currents (I_{Kr}, I_{Ks}) during the 300 ms depolarization was minimal because of their slow activation kinetics. Carvedilol (1–100 μM) caused a concentration-dependent inhibition of I_{to}. The density of I_{to} at +40 mV depolarization was decreased significantly from 5.18 ± 1.19 pA/pF ($n = 5$) in control to 2.22 ± 0.32 pA/pF ($P < 0.01$) at 3 μM and 0.81 ± 0.19 pA/pF ($P < 0.01$) at 10 μM of carvedilol. The representative recordings and the I–V relationships of I_{to} before and after the application of 3 μM carvedilol are shown in Fig. 2.

3.3. Effects on the delayed rectifier K^+ currents

In rabbit ventricular myocytes, I_K is composed of two kinetically different components: I_{Kr} and I_{Ks}, with differential sensitivities to block by methanesulfonanilide class III antiarrhythmic agents (Salata et al., 1996). The effects of carvedilol on the I–V relationship of I_{Kr} (chromanol 293B resistant component of I_K) were studied in HEPES-buffered solution containing 3 μM nisoldipine and 30 μM chromanol 293B (Fig. 3). I_{Kr} was activated by applying voltage clamp steps for 3000 ms at 0.1 Hz from a holding potential of −50 mV to different depolarizing levels from −30 to +60 mV. Under the control condition, the time-dependent outward current during depolarization showed strong inward rectification with the peak amplitude at 0 mV. The tail current upon repolarization showed a voltage-dependent increase in its amplitude and reached a steady-state level at about +10 mV. Carvedilol (0.03–3 μM) decreased the amplitude of both the time-dependent outward current and the tail current in a concentration-dependent manner. The time-dependent outward current at 0 mV was decreased significantly from 0.36 ± 0.06 pA/pF ($n = 5$) in control to 0.12 ± 0.02 pA/pF ($P < 0.01$) at 0.3 μM and 0.05 ± 0.01 ($P < 0.01$) at 1 μM of carvedilol. The tail current following depolarization to +10 mV was decreased significantly from 0.68 ± 0.09 pA/pF ($n = 5$) in control to 0.39 ± 0.05 pA/pF ($P < 0.01$) at 0.3 μM and 0.18 ± 0.03 pA/pF ($P < 0.01$) at 1 μM of carvedilol. Washout of carvedilol for 10 min resulted in a recovery of the both time-dependent current and the tail current to a level of 83–93% of the control. The voltage showing the peak of the time-dependent outward current was shifted to −10 mV, whereas the voltage-dependence of activation of the tail current was unaffected. The voltage of half maximum activation, which was obtained by fitting the average data to the Boltzmann equation, was −8 mV in control and −10 mV at 0.3 μM carvedilol. Panel A of Fig. 3 shows typical recordings in the control condition (left) and after application of 0.3 μM carvedilol (right). The corresponding I–V curves are presented in panel B.

The development process of I_{Kr} block by carvedilol was examined using the envelope tests for the tail current (Fig. 4). Envelopes of the I_{Kr} tail current were generated by applying depolarizing pulses to +10 mV at 0.1 Hz with durations ranging from 100 to 3000 ms from a holding potential of −50 mV. Tail currents elicited on repolarization to −50 mV were measured before and after the application of the drug. Typical recordings obtained in control condition and after application of 0.3 μM carvedilol are shown in panel A of Fig. 4, and the time course of the block development on I_{Kr} by carvedilol (0.3 μM) are shown in panel B. Carvedilol suppressed the tail current to a similar extent (45–49%, $n = 5$) across all the tested durations of the depolarizing pulses.

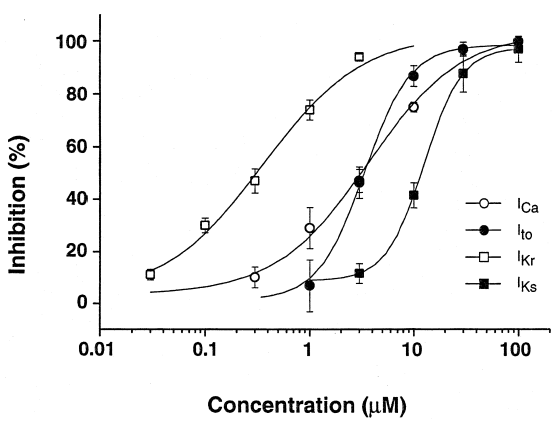

Fig. 6. Comparison of the blocking potencies of carvedilol on I_{Ca}, I_{to}, I_{Kr} and I_{Ks}. I_{Ca} was measured as the peak inward current during depolarization to 0 mV. I_{to} was measured as the difference between the peak outward current and steady-state current during depolarization to +40 mV. I_{Kr} and I_{Ks} were measured as the tail currents in response to depolarizations of +10 and +40 mV, respectively. $n = 5$ in each group. The current amplitudes in percentage of control were plotted as functions of drug concentrations, and the concentration–response curves were fitted according to Eq. (1).

The effects of carvedilol on the I–V relationship of I_{Ks} (E-4031 resistant component of I_K) were studied in Na^+-free and K^+-free solution containing 3 μM nisoldipine and 10 μM E-4031 (Fig. 5). I_{Ks} was activated by applying

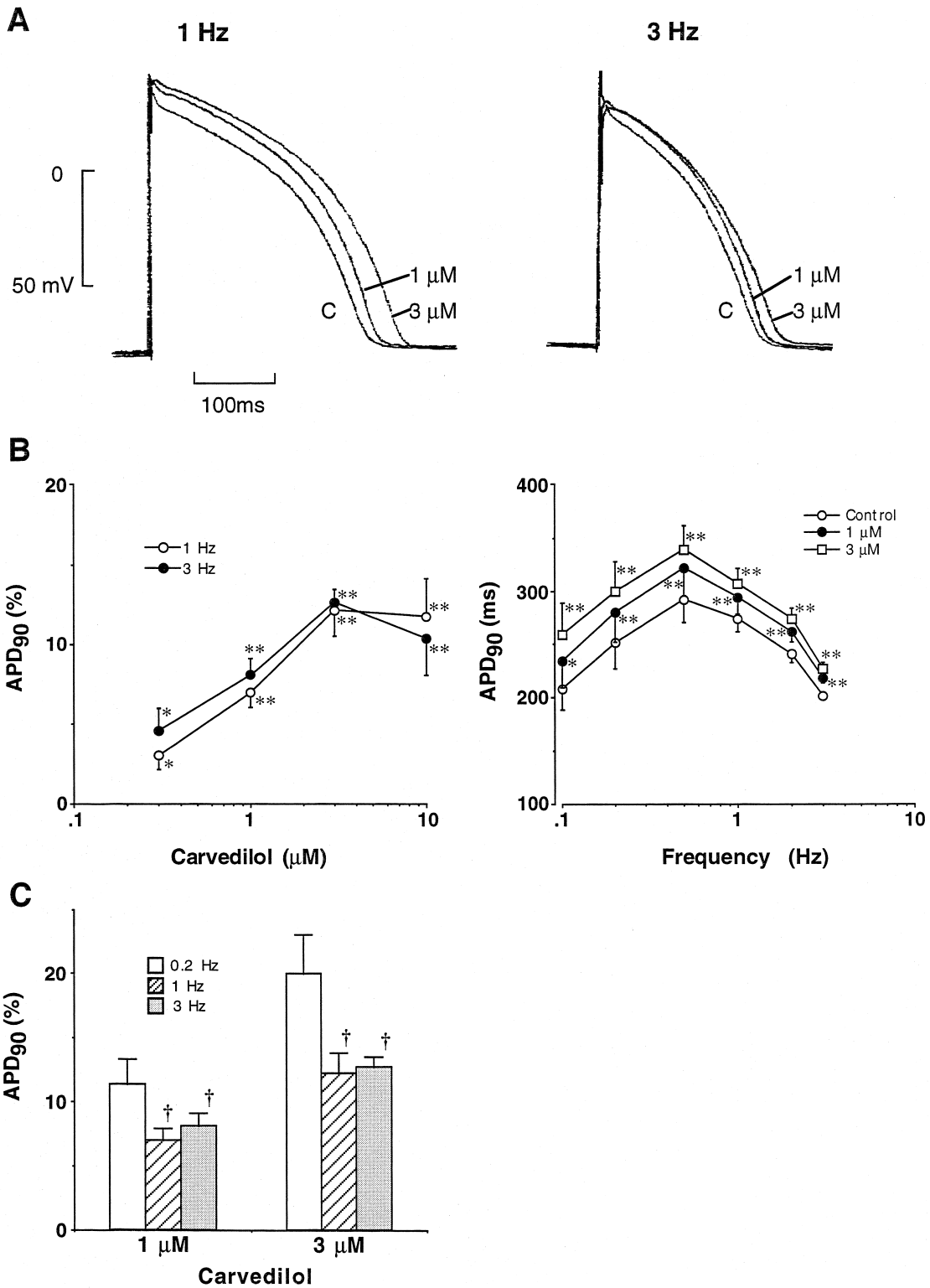

Fig. 7. Concentration and frequency dependence of action potential duration prolongation by carvedilol. (A) Examples of action potentials recorded before and after the exposure to 1 and 3 μM of carvedilol at stimulation frequencies of 1 Hz (left) and 3 Hz (right). (B) Concentration–response curves of carvedilol on action potential duration at 1 and 3 Hz (left) and frequency–response curves before and after application of 1 μM and 3 μM carvedilol (right). (C) Comparison of the percentage prolongation at 0.2, 1 and 3 Hz by 1 and 3 μM carvedilol. Carvedilol induced a moderate prolongation of action potential duration with minimal reverse frequency-dependence. $^*P < 0.05$; $^{**}P < 0.01$ vs. control value; †$P < 0.01$ vs. the value at 0.2 Hz; $n = 5$.

voltage clamp steps for 3000 ms at 0.1 Hz from a holding potential of −50 mV to different depolarizing levels from −30 to +60 mV. The time-dependent outward current during depolarization increased linearly at more positive potentials. The tail current upon repolarization showed a voltage-dependent increase in its amplitude and reached a steady-state level at about +40 mV. Carvedilol (3–100 μM) decreased the amplitude of both the time-dependent outward current and the tail current at much higher concentrations than those needed to block I_{Kr}. The time-dependent outward current at +40 mV was decreased significantly from 0.96 ± 0.14 pA/pF ($n = 5$) in control to 0.67 ± 0.12 pA/pF ($P < 0.05$) at 10 μM and 0.28 ± 0.07 pA/pF ($P < 0.01$) at 30 μM of carvedilol. The tail current following depolarization to +40 mV was decreased significantly from 0.45 ± 0.12 pA/pF ($n = 5$) in control to 0.28 ± 0.07 pA/pF ($P < 0.05$) at 10 μM and 0.05 ± 0.01 pA/pF ($P < 0.01$) at 30 μM of carvedilol. The voltage-dependence of activation was not significantly altered by the drug. The voltage for half maximum activation of the tail current, which was obtained by fitting the average data to Boltzmann equation, was −3 mV in control and −5 mV at 10 μM carvedilol. Typical recordings obtained in control condition and after application of 10 μM carvedilol are shown in panel A of Fig. 5, and the corresponding I–V relationships for the time-dependent outward current and the tail current of I_{Ks} are shown in panel B.

3.4. Comparison of the blocking potencies of carvedilol on I_{Ca}, I_{to}, I_{Kr} and I_{Ks}

The concentration–response relationships for the block on I_{Ca}, I_{to}, I_{Kr} and I_{Ks} by carvedilol are summarized in Fig. 6. I_{Ca} was measured as the peak inward current during depolarization to 0 mV. I_{to} was measured as the difference between the peak outward current and steady-state current during depolarization to +40 mV. I_{Kr} and I_{Ks} were measured as the tail currents in response to depolarizations of +10 and +40 mV, respectively. The current amplitudes in percentage of control were plotted as functions of drug concentrations. To characterize concentration–response effects of carvedilol, the averaged data were fitted to Hill's equation

$$Y = 1/\left[1 + (\mathrm{IC}_{50}/x)^{n}\right] \quad (1)$$

where x represents the concentration of carvedilol, Y the percentage of inhibition of the current, IC_{50} the concentration corresponding to a 50% current inhibition and n the Hill coefficient. Carvedilol inhibited I_{Kr} most potently with an IC_{50} of 0.35 μM. Block of I_{Ca}, I_{to} was about 10-fold less potent with IC_{50} of 3.59 μM and 3.34 μM, respectively. The inhibition on I_{Ks} by carvedilol was least potent with an IC_{50} of 12.54 μM. The Hill coefficients for the block on I_{Kr}, I_{Ca}, I_{to} and I_{Ks} were 0.91, 0.97, 1.97 and 2.39, respectively.

3.5. Concentration and frequency dependence of action potential duration prolongation

The effects of carvedilol on action potential duration were examined in rabbit papillary muscles by the microelectrode method (Fig. 7). The concentration-dependent effects of carvedilol (0.3–10 μM) on action potential duration were observed at 1 and 3 Hz. Typical recordings of action potential obtained in control and then after the application of 1 and 3 μM were shown in Fig. 7A. Carvedilol caused a concentration-dependent prolongation of action potential duration, but no further prolongation was observed when the concentration was increased to 10 μM ($n = 5$). The effects were similar at both stimulation frequencies of 1 and 3 Hz (Fig. 7B, left). The frequency-dependent effects of carvedilol (1 and 3 μM) on action potential duration was recorded in papillary muscles stimulated at 0.1–3 Hz. When the stimulation frequency was changed in steps from 0.1 to 3 Hz, the action potential duration in control condition initially increased and then decreased, showing a bell-shaped frequency–response curve with the longest action potential duration at 0.5 Hz. Carvedilol prolonged action potential duration by 7–12% at 1 μM and by 12–24% at 3 μM ($n = 5$) over the entire range of stimulation frequencies, and the bell-shaped frequency–response curve was well preserved (Fig. 7B, right). Washout of carvedilol for 60 min resulted in a full recovery of the action potential duration. To further analyze the frequency dependence of action potential duration prolongation by carvedilol, the percentage prolongation at 0.2, 1 and 3 Hz were compared (Fig. 7C). The drug-induced action potential duration prolongation at 0.2 Hz was appreciably larger than those at higher stimulation frequencies, but no significant difference was obtained between 1 and 3 Hz. Other electrophysiological parameters, such as the resting membrane potential, the action potential amplitude and the maximum upstroke velocity of depolarization (V_{max}), were unaffected by carvedilol at the concentrations tested (data not shown). Thus, in papillary muscles, carvedilol caused a moderate prolongation of action potential duration with minimal reverse frequency-dependence.

4. Discussion

The present study demonstrates that carvedilol has direct electrophysiological effect on ventricular cells: carvedilol is a multi-channel blocker with relative selectivity for I_{Kr}. Such a balanced inhibition of K^+ and Ca^{2+} channels resulted in a moderate prolongation of action potential duration with minimal reverse frequency-dependence. The prolongation of action potential duration by carvedilol at low concentrations (0.3–1 μM) appears to be primarily due to an inhibition of I_{Kr}. The K^+ channels blocking effects of carvedilol should have leaded to a profound prolongation of action potential duration at higher

concentrations. However, a concomitant block of I_{Ca} may have resulted in a limited increase in action potential duration at higher concentrations (≥ 3 μM).

Many studies have suggested that carvedilol possesses a Ca^{2+} channel blocking activities at concentrations (> 1 μM) much higher than that for its adrenoceptor antagonistic effects (in a nM order) (Ruffolo et al., 1990a). In pithed rats, carvedilol (1 mg/kg, i.v.) suppressed the vasopressor response induced by a Ca^{2+} channel agonist, BAY K8644 [methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate] (Nichols et al., 1991). Carvedilol (0.1–30 μM) also inhibited the canine coronary artery contraction induced by BAY K8644 (Hattori et al., 1989). In KCl (70 mM) depolarized rabbit aorta, carvedilol produced a 10-fold parallel rightward shift in the concentration–response curve to $CaCl_2$ (Nichols et al., 1989). Carvedilol increased the cutaneous blood flow by 64% and reduced the cutaneous artery resistance by 57% (Ruffolo et al., 1990b). The present study has provided direct evidence that carvedilol at concentrations above 0.3 μM causes a significant inhibition of the L-type Ca^{2+} current (I_{Ca}) in ventricular cells.

The potency of the block on I_{Kr} by carvedilol (IC_{50} = 0.34 μM) was similar to that of E-4031 observed in guinea pig ventricular cells (397 nM) (Sanguinetti and Jurkiewicz, 1990) and in rabbit ventricular cells (0.3 μM, unpublished data). Like E-4031 (Cheng et al., 1996), the development of I_{Kr} block by carvedilol exhibited no voltage- and time-dependence. This suggests that carvedilol may have already blocked I_{Kr} channel at the holding potential when the channel was in the closed state or that the block by carvedilol occurs very rapidly (during the first depolarizing pulse). Additional experiment is needed to discriminate the two possibilities.

The action potential duration prolongation of carvedilol differs notably from the methanesulfonanilide class III agents (such as d-sotalol, E-4031, dofetilide, etc.), which were targeted against single membrane current, in terms of their frequency-dependence. The action potential duration prolonging effects of these drugs were greatly enhanced at low but diminished at high stimulation frequencies (Hafner et al., 1988; Hondeghem and Snyders, 1990; Tande et al., 1990; Gwilt et al., 1991; Wettwer et al., 1991). For example, the percentage prolongation of action potential duration by 0.3 μM E-4031 in rabbit papillary muscles was only 38% at 3 Hz, but it was enhanced to 89% at 1 Hz and to 511% at 0.1 Hz (Toyama et al., 1997). Although these drugs have been shown to exert beneficial antifibrillatory effects in patients with atrial fibrillation, they also produced a variable incidence of torsades de pointes, resulting in either a neutral (e.g., dofetilide) or deleterious (d-sotalol) effect on mortality in survivors of myocardial infarction (Singh, 1998). Attention therefore has been focused on compounds that have the propensity to block more than one ion channel (e.g., tedisamil and azimilide). Such a property may not be associated with reverse use- or frequency-dependency of action on repolarization, and may therefore less ''torsadogenic'' compared with other specific I_{Kr} blockers (Singh, 1998). The present study shows that carvedilol caused a moderate prolongation of action potential duration with minimal reverse frequency-dependence. This mode of action of carvedilol resembles that of chronic treatment with amiodarone (Kodama et al., 1992), the only antiarrhythmic agent which has been proved to be effective in the treatment of tachyarrhythmias and to reduce mortality in patients with post myocardial infarction or chronic heart failure (Scheinman et al., 1995; Kowey et al., 1995; Amiodarone Trials Meta-Analysis Investigators, 1997). Therefore, such a frequency–response relationship of class III action shared by carvedilol and amiodarone might be important for their more antiarrhythmic and less proarrhythmic activities.

The measurement of the peak plasma concentration of carvedilol after a single oral dose or daily dose of 12.5, 25 or 50 mg revealed that it was in a range of 32–252 μg/l in patients with hypertension (McPhillips et al., 1988; Morgan et al., 1990). This corresponds to concentrations of 0.1–0.6 μM (the molecular weight of carvedilol is 406.48). Therefore, it is speculated that at therapeutic dosage, carvedilol may exert not only β- and α-adrenoceptor antagonistic effects, but also a potent I_{Kr} blocking effect as well as a moderate I_{Ca} blocking effect. In heart failure patients, the level of sympathetic drive to the heart is elevated (Esler et al., 1997). This preferential activation of cardiac sympathetic outflow contributes to arrhythmia development and probably to the progression of heart failure. Under such a pathological condition, treatment with carvedilol may produce an antiarrhythmic action through both its intrinsic class III antiarrhythmic activity associated with β-adrenoceptor-blockade and direct ionic channels modulating effects. Indeed, carvedilol has been reported to significantly reduce the frequency of the premature ventricular contractions in patients with mild to moderate essential hypertension, stable angina or chronic heart failure (Senior et al., 1992). Recently, carvedilol is reported to be effective in the treatment of ventricular tachyarrhythmia in a patient with dilated cardiomyopathy without causing significant deterioration of cardiac function or excise capacity (Wright et al., 1997). Furthermore, inhibition of Ca^{2+} and K^+ channels by carvedilol will reduce sinus-node firing, as is evidenced by a significant reduction in heart rate in patients of acute myocardial infarction (Basu et al., 1997). Both prolongation of action potential duration and reduction of sinus-node beating may facilitate cardiac pumping function and improve the prognosis of chronic heart failure. The possible negative inotropic action of carvedilol (resulted from β-adrenoceptor blockade and Ca^{2+} current blocking effect) in the treatment of chronic heart failure is supposed to be offset by the vasodilatory effects of carvedilol which resulted in a decrease in impedance to left ventricular ejection. As a consequence, stroke volume and cardiac output are maintained or even

increased in animals and in patients with congestive heart failure treated with carvedilol (Ruffolo and Feuerstein, 1997).

There are several limitations concerning on our study. Firstly, the existence of rundown of the K^+ and Ca^{2+} currents may cause an overestimation of the blocking potencies of carvedilol on those currents. In order to minimize the influence of rundown on the data interpretation, we deliberately selected the cells with good seals and stable recordings, and verified the drug effects by washout whenever possible. Secondly, the effects of carvedilol on I_{Ks} were observed in Na^+- and K^+-free solution rather than the more physiological HEPES-buffered solution. Because of the small amplitude of this current, it is technically difficult to record it in physiological solution when other overlapping currents are present. Finally, the effects of carvedilol on the action potential duration were only studied at frequencies up to 3 Hz. This frequency is just close to the normal frequencies of rabbit heart in vivo. It would be important to study the effects of carvedilol at increased heart rate. At stimulation frequencies higher than 3 Hz, however, action potentials were elicited without full repolarization, and this made it difficult to measure the action potential duration at 90% repolarization.

In conclusion, carvedilol induced a moderate prolongation of action potential duration with minimal reverse frequency-dependence through the blockade of both K^+ and Ca^{2+} currents. These electrophysiological properties of carvedilol may result in a higher efficiency in the treatment of tachyarrythmias and lower risk of proarrhythmic effects than those of the pure class III antiarrhythmic agents, providing an underlying pharmacological rationale for the use of the drug in the treatment of ventricular tachyarrhythmias in patients with coronary artery disease or congestive heart failure.

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References

Amiodarone Trials Meta-Analysis Investigators, 1997. Effects of prophylactic amiodarone on mortality after acute myocardial infarction and in congestive heart failure. Lancet 350, 1417–1424.

Basu, S., Senior, R., Raval, U., van der Does, R., Bruckner, T., Lahiri, A., 1997. Beneficial effects of intravenous and oral carvedilol treatment in acute myocardial infarction. A placebo-controlled, randomized trial. Circulation 96, 183–191.

Bigger, J.T. Jr., 1987. Why patients with congestive heart failure die: arrhythmias and sudden cardiac death. Circulation 75, 28–35.

Bril, A., Tomasi, V., Laville, M.-P., 1995. Antiarrhythmic effects of carvedilol in rat isolated heart subjected to ischemia and reperfusion. Pharmacol. Commun. 5, 281–300.

MOCHA Investigators, Bristow, M.R., Gilbert, E.M., Abraham, W.T., Adams, K.F., Fowler, M.B., Hershberger, R.E., Kubo, S.H., Narabara, K.A., Ingersoll, H., Krueger, S., Young, S., Shusterman, N., 1996. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. Circulation 94, 2807–2816.

Brunvand, H., Frlyland, L., Hexeberg, E., Rynning, S.E., Berge, R.K., Grong, K., 1996. Carvedilol improves function and reduces infarct size in the feline myocardium by protecting against lethal reperfusion injury. Eur. J. Pharmacol. 314, 99–107.

Busch, A.E., Suessbrich, H., Waldegger, S., Sailer, E., Greger, R., Lang, H., Lang, F., Gibson, K.J., Maylie, J.G., 1996. Inhibition of I_{Ks} in guinea pig cardiac myocytes and guinea pig I_{sK} channels by chromanol 293B. Pflügers Arch. 432, 1094–1096.

Carmeliet, E., 1992. Voltage- and time-dependent block of the delayed K^+ current in cardiac myocytes by dofetilide. J. Pharmacol. Exp. Ther. 262, 809–817.

Chakki, C.S., Gherghiade, M., 1985. Ventricular arrhythmias in severe heart failure: incidence, significance, and effectiveness of antiarrhythmic therapy. Am. Heart J. 109, 497–504.

Cheng, J., Kamiya, K., Kodama, I., Toyama, J., 1996. Differential effects of MS-551 and E-4031 on action potentials and the delayed rectifier K^+ current in rabbit ventricular myocytes. Cardiovasc. Res. 31, 963–974.

Cheng, J., Kamiya, K., Liu, W., Tsuji, Y., Toyama, J., Kodama, I., 1999. Heterogeneous distribution of the two components of delayed rectifier K^+ current: a potential mechanism of the proarrhythmic effects of methanesulfonanilide class III agents. Cardiovasc. Res. (in press)

Cooperative Studies Group, Cohn, J.N., V-HeFT, V.A., 1993. Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias, and plasma norepinephrine as determinants of prognosis in heart failure. Circulation 87, 5–16, Suppl. VI.

Dunn, C.J., Lea, A.P., Wagstaff, A.J., 1997. Carvedilol: a reappraisal of its pharmacological properties and therapeutical use in cardiovascular disorders. Drugs 54, 161–185.

Esler, M., Kaye, D., Lambert, G., Esler, D., Jennings, G., 1997. Adrenergic nervous system in heart failure. Am. J. Cardiol. 80 (11A), 7L–14L.

Fedida, D., Giles, W.R., 1991. Regional variations in action potentials and transient outward current in myocytes isolated from rabbit left ventricle. J. Physiol. 442, 191–209.

Giles, W.R., Imaizumi, Y., 1988. Comparison of potassium currents in rabbit atrial and ventricular cells. J. Physiol. 405, 123–145.

Gwilt, M., Arrowsmith, J.E., Blackburn, K.J., Burges, R.A., Cross, P.E., Dalrymple, H.W., Higgins, A.J., 1991. UK-68,798: a novel, potent and highly selective class III antiarrhythmic agent which blocks potassium channels in cardiac cells. J. Pharmacol. Exp. Ther. 256, 318–324.

Hafner, D., Berger, F., Borchard, U., Kullmann, A., Scherlitz, A., 1988. Electrophysiological characterization of the class III activity of sotalol and its enantiomers: new interpretation of use-dependent effects. Drug Res. 38, 231–236.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391, 85–100.

Hattori, Y., Nakaya, H., Endou, M., Nakao, Y., Kanno, M., 1989. Vascular effects of carvedilol, a new β-adrenoceptor antagonist with vasodilating properties, in isolated canine coronary artery. J. Cardiovasc. Pharmacol. 13, 572–579.

Hiraoka, M., Kawano, S., 1989. Calcium-sensitive and insensitive transient outward current in rabbit ventricular myocytes. J. Physiol. 410, 187–212.

Hoher, M., Friedrich, M., Sommer, T., Marten, A., Ehmer, B., Hombach, V., Hirche, H., 1989. Effects of carvedilol on left ventricular function and arrhythmias during repeated short-time myocardial ischemia in experimental pigs (in German). Z. Kardiol. 78, 7–15, Suppl. 3.

Hondeghem, L.M., Snyders, D.J., 1990. Class III antiarrhythmic agents

have a lot of potential but a long way to go. Reduced effectiveness and dangers of reverse use dependence. Circulation 81, 686–690.

Kjekshus, J., 1990. Arrhythmias and mortality in congestive heart failure. Am. J. Cardiol. 65, 421–481.

Kodama, I., Suzuki, R., Kamiya, K., Iwata, H., Toyama, J., 1992. Effects of long-term oral administration of amiodarone on the electromechanical performance of rabbit ventricular muscle. Br. J. Pharmacol. 107, 502–509.

The Intravenous Amiodarone Multicenter Investigators Group, Kowey, P.R., Levine, J.H., Herre, J.M., Pacifico, A., Lindsay, B.D., Plumb, V.J., Janosik, D.L., Kopelman, H.A., Scheinman, M.M., 1995. Randomized, double-blind comparison of intravenous amiodarone and bretylium in the treatment of patients with recurrent, hemodynamically destabilizing ventricular tachyarrhythmia of fibrillation. Circulation 92, 3255–3263.

McPhillips, J.J., Schwemer, G.T., Scott, D.I., Zinny, M., Patterson, D., 1988. Effects of carvedilol on blood pressure in patients with mild to moderate hypertension. Drugs 36, 82–91, Suppl. 6.

McTavish, D., Campoli-Richards, D., Sorkin, E.M., 1993. Carvedilol: a review of its pharmacological and pharmacokinetic properties, and therapeutic efficacy. Drugs 45, 232–258.

Morgan, T.O., Anderson, A., Cripps, J., Adam, W., 1990. Pharmacokinetics of carvedilol in older and younger patients. J. Human Hypertens. 4, 709–715.

Moser, M., Frishman, W., 1998. Results of therapy with carvedilol, a β-blocker with antioxidant properties, in hypertensive patients. Am. J. Hypertens. 11, 15S–22S.

Nichols, A.J., Sulpizio, A.C., Ashton, D.J., Hieble, J.P., Ruffolo, R.R. Jr., 1989. In vitro pharmacologic profile of the novel β-adrenoceptor antagonist and vasodilator, carvedilol. Pharmacology 39, 327–336.

Nichols, A.J., Gellai, M., Ruffolo, R.R. Jr., 1991. Studies on the mechanism of arterial vasodilation produced by the novel antihypertensive agent, carvedilol. Fundam. Clin. Pharmacol. 5, 25–38.

Packer, M., 1985. Sudden unexpected death in patients with congestive heart failure: a second frontier. Circulation 72, 681–685.

US Carvedilol Heart Failure Study Group, Packer, M., Bristow, M.R., Cohn, J.N., Colucci, W.S., Fowler, M.B., Gilbert, E.M., Shusterman, N.H., 1996. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. New. Engl. J. Med. 334, 1349–1355.

Pratt, C.M., Eaton, T., Francis, M., Woolbert, S., Mahmarian, J., Roberts, R., Young, J.B., 1989. The inverse relationship between baseline left ventricular ejection fraction and outcome from antiarrhythmic therapy: a dangerous imbalance in risk-benefit ratio. Am. Heart J. 118, 433–440.

Ruffolo, R.R. Jr., Feuerstein, G.Z., 1997. Pharmacology of carvedilol: rationale for use in hypertension, coronary artery disease, and congestive heart failure. Cardiovasc. Drugs Ther. 11, 247–256, Suppl. 1.

Ruffolo, R.R. Jr., Gellai, M., Hieble, J.P., Willette, R.N., Nichols, A.J., 1990a. The pharmacology of carvedilol. Eur. J. Pharmacol. 38, S82–S88.

Ruffolo, R.R. Jr., Sauermelch, C.F. Jr., Willette, R.N., 1990b. Hemodynamic differences between carvedilol and labetalol in the cutaneous circulation. Eur. J. Clin. Pharmacol. 38, S112–S114.

Salata, J.J., Jurkiewicz, N.K., Jow, B., Folander, K., 1996. I_K of rabbit ventricle is composed of two currents: evidence for I_{Ks}. Am. J. Physiol. 271, H2477–H2489.

Sanguinetti, M.C., Jurkiewicz, N.K., 1990. Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by class III antiarrhythmic agents. J. Gen. Physiol. 96, 195–215.

The Intravenous Amiodarone Multicenter Investigators Group, Scheinman, M.M., Levine, J.H., Cannom, D.S., Friehling, T., Kopelman, H.A., Chilson, D.A., Platia, E.V., Wilber, D.J., Kowey, P.R., 1995. Dose-ranging study of intravenous amiodarone in patients with life-threatening ventricular tachyarrhythmias. Circulation 92, 3264–3272.

Senior, R., Müller-Beckmann, B., DasGupta, P., van der Does, R., Lahiri, A., 1992. Effects of carvedilol on ventricular arrhythmias. J. Cardiovasc. Pharmacol. 19, S117–S121.

Shimoni, Y., Clark, R.B., Giles, W.R., 1992. Role of an inwardly rectifying potassium current in rabbit ventricular action potential. J. Physiol. 448, 709–727.

Singh, B.N., 1998. Antiarrhythmic drugs: a reorientation in light of recent developments in the control of disorders of rhythm. Am. J. Cardiol. 81 (6A), 3D–13D.

Tande, P.M., Bjornstad, H., Yang, T., Resfum, H., 1990. Rate-dependent class III antiarrhythmic action, negative chronotropy, and positive inotropy of a novel I_K blocking drug, UK-68,798: potent in guinea pig but no effect in rat myocardium. J. Cardiovasc. Pharmacol. 16, 401–410.

Toyama, J., Kamiya, K., Cheng, J., Lee, J.-K., Suzuki, R., Kodama, I., 1997. Vesnarinone prolongs action potential duration without reverse frequency dependence in rabbit ventricular muscle by blocking the delayed rectifier K^+ current. Circulation 96, 3696–3703.

Underwood, R.D., Sra, J., Akhtar, M., 1997. Evaluation and treatment strategies in patients at high risk of sudden death post myocardial infarction. Clin. Cardiol. 20, 753–758.

Wettwer, E., Scholtysik, G., Schaad, A., Himmel, H., Ravens, U., 1991. Effects of new class III antiarrhythmic drug E-4031 on myocardial contractility and electrophysiological parameters. J. Cardiovasc. Pharmacol. 17, 480–487.

Wright, D.J., Cooke, G.A., Tan, L.B., 1997. Intractable recurrent ventricular tachycardia in dilated cardiomyopathy controlled by a vasodilating β blocker. Heart 77, 581–582.

Yazawa, K., Kaibara, M., Ohara, M., Kameyama, M., 1990. An improved method for isolating cardiac myocytes useful for patch-clamp studies. Jpn. J. Physiol. 40, 157–163.